ANNALS OF BOTANY

EDITED BY

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The Proteases of Plants (VI).

BY

S. H. VINES, F.R.S.

Sherardian Professor of Botany in the University of Oxford.

THE latter part of my last paper (21) on this subject was devoted to an account of experiments upon ungerminated Hemp-seed (*Cannabis sativa*), made with the object of strengthening the evidence in favour of the view, to which I had given expression in several previous papers of this series, that 'vegetable trypsin' is not, as is commonly held, a single substance, but is a mixture of at least two proteases. Of these, the one is only capable of peptonizing the higher proteins (fibrin, albumin, &c.): the other has no action upon these proteins, but actively splits peptones and albumoses into amino-acids and other non-proteid nitrogenous substances. That is to say, that 'vegetable trypsin' is a mixture of a *peptase* (peptonizing protease) with an *ereptase* (peptolysing protease), and possibly of more than one of each of these two kinds of enzymes.

The method adopted in those experiments enabled me to prepare from Hemp-seed, solutions, of which some digested fibrin but were without action on peptones, that is, were merely peptonizing solutions; others were without action on fibrin, but split up peptones (as indicated by the tryptophane-reaction)—that is, were merely peptolysing solutions. Thus it was shown that two distinct proteases, having different solubilities, can be extracted from Hemp-seed; and there is no reason to doubt that these two proteases exist separately in the seed.

Having obtained this result with Hemp-seed, I proceeded to apply the same method to various structures and substances, in the hope of obtaining confirmatory results; but I soon found that it was only applicable to material which, like seeds, is rich in proteid substances. It became necessary, therefore, to find another method which should be of more general application; and, as the result of many attempts, the method sought for was found in the course of some experiments with papaïn, as described in the following pages.

Papaïn (or Papayotin).

The latex of the Papaw (*Carica Papaya*) has long been known to act proteolytically. The first record of the digestive action is to be found in Griffith Hughes's 'Natural History of Barbados' (1750); it is there

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stated that the milky juice of the fruit 'is of so penetrating a nature that if the unripe fruit, unpeeled, be boiled with the toughest old salt meat it will soon make it soft and tender; and if hogs are for any considerable time fed with it, especially raw, it is said that it will wear off all the mucous slimy matter which covers the inside of the guts, and would in time, if not prevented by a change of food, entirely lacerate them'. A similar account was given by Patrick Browne in his 'Civil and Natural History of Jamaica' (1756). He says: 'Water impregnated with the milky juice of this tree is thought to make all sorts of meat washed in it very tender; but eight or ten minutes' steeping, it is said, will make it so soft that it will drop in pieces from the spit before it is well roasted, or turn soon to rags in the boiling.'

The latex was first investigated scientifically by Wurtz and Bouchut (1). Having extracted the latex with distilled water, they mixed the aqueous extract with ten times its volume of alcohol: the considerable precipitate formed was collected in a filter and dried. To the white powder thus obtained they gave the name *papaïn*, and regarded it as the digestive enzyme of the latex. They observed that an aqueous solution (0·2 per cent.) of this powder readily digested fibrin at 40° C., whether the liquid were neutral, slightly alkaline (KHO) or acid (0·2 per cent. HCl). They found the ultimate products of the fibrin-digestions to be peptones. On this account they considered papaïn to be allied to animal pepsin, but to differ from it in that it digests in neutral, acid, and alkaline medium, whereas the action of pepsin is limited to an acid medium. Their ultimate view is expressed in the following words:—'Il circule réellement dans les différentes parties de cet arbuste un suc presque neutre qui a toutes les propriétés de la *pepsine*, sauf que celle-ci est acide, et n'agit qu'étant acide; et quelques propriétés de la *pancréatine*, ce qui en fait une sorte de *pancréatine végétale*.'

After a considerable lapse of time, the investigation of papaïn was resumed by Martin (2). From the analysis of 'commercial papaïn', he came to the conclusion that papaïn consists of a mixture of two proteids, a globulin and an albumose, and that the ferment-action is associated with the albumose. Having proved the formation of leucin and tyrosin in the course of digestion of fibrin and albumin, Martin concluded (3) that papaïn is the only vegetable ferment which has as yet been proved to act like trypsin, and then its normal action takes place in a neutral medium. Thus it was shown that papaïn not only digests the higher proteins, but also hydrolyses the peptones: it both peptonizes and peptolyses.

Several years after Martin's papers had appeared, I made some experiments on papaïn-digestion (14), and found, by means of the tryptophane-test, that papaïn actively digests peptones in neutral solution, more actively in the presence of acid (best in 0·5 per cent. citric acid), and less actively in

the presence of alkali (0·5 per cent. Na_2CO_3), results that were confirmed by some further observations which were published about a year later (16). Returning again to the subject (18), I was struck by the fact that the digestion of fibrin and that of peptone were not always similarly affected by changes in the experimental conditions; and consequently expressed the opinion that papaïn may be a mixture of two proteases, the one fibrin-digesting but not peptolytic, the other peptolytic but not fibrin-digesting. In a supplementary paper, containing some further facts concerning papaïn-digestion amongst others, I suggested that the fibrin-digesting enzymes should be classed as *peptases*, and the peptolysing enzymes as *ereptases*.

The mode in which I have endeavoured to establish these views, in the case of papaïn by separating the peptase and the ereptase, is based upon the observation frequently made in the course of my work, that extracts of plant-material made with 2 per cent. NaCl-solution digest fibrin more actively than similar extracts made with distilled water. This fact suggested the possibility of there being a fibrin-digesting enzyme (peptase) present, which is less soluble in distilled water than the peptone-digesting enzyme (ereptase). If this be so, it should be possible to wash out all the ereptase, by treating the material with relatively large quantities of distilled water, leaving some, at any rate, of the peptase behind. I tried this repeatedly with papaïn, as well as with other material, but without success; however much water I used, the washings, as shown by experiment, continued to contain ereptase: in fact the ereptase in the material seemed inexhaustible.

It occurred to me eventually that extraction of papaïn with NaCl-solution, instead of with water, might lead to the desired result, and this proved to be the case. I found that when papaïn was extracted with 20–25 times its volume of 2 per cent. NaCl-solution and the liquid filtered off, either the residue contained no ereptase, or any remaining in it could be washed out by means of water; and further, that this residue treated with 2 per cent. NaCl-solution, yielded, after filtration, a liquid which digested fibrin but was without action on peptone. By these means, however, only the fibrin-digesting enzyme is separated; the ereptase not being obtained in pure solution. The method is illustrated in detail by the following note of an experiment:—

10 grms. of commercial papaïn mixed thoroughly in a mortar with 200 cc. 2 per cent. NaCl-solution: after standing for an hour or so, the mixture was placed on a filter: filtration was slow (it would have been better if 250 cc. of the NaCl-solution had been used).

Filtrate 1. The filtered NaCl-extract was clear, light yellow, acid; it gave a dense ppt. on boiling, and on the addition of nitric acid (HNO_3): it also gave a distinct tryptophane-reaction.

Digestive properties. 40 cc. of the filtrate digested 0·2 grm. fibrin within five hours, and also digested Witte-peptone, as shown by the tryptophane-reaction.

The papaïn-residue (about 5 grms.) was removed from the filter, triturated in the mortar with 200 cc. dist. water, and the mixture filtered.

Filtrate 2. The filtered H_2O-extract was clear and neutral, gave no ppt. or turbidity on boiling, or on adding HNO_3, and only a slight xanthoproteic reaction.

Digestive properties. 40 cc. of the filtrate failed to digest 0·2 grm. of fibrin in forty-eight hours, and had no action on Witte-peptone.

Hence it appears in this case, that, since ereptase is readily soluble in water, the papaïn-residue contained none of it: that, in fact, the whole of the ereptase had been dissolved out in the extraction with NaCl-solution.

The papaïn-residue, after the extraction with H_2O, was extracted with 120 cc. of 2 per cent. NaCl-solution; the mixture was allowed to stand for a short time, and was then filtered.

Filtrate 3. The filtered NaCl-extract was colourless and neutral: it gave no turbidity on boiling, but a slight turbidity on adding HNO_3, a faint xanthoproteic reaction, no biuret-reaction, no tryptophane-reaction.

Digestive properties. 35 cc. of the filtrate were put into each of three bottles, Nos. 1, 2, 3, the liquid placed in No. 2 having been previously boiled; to each bottle was added 0·2 grm. fibrin; and to Nos. 1 and 2, 0·2 grm. of Witte-peptone.

Within twenty-four hours the fibrin had completely disappeared in Nos. 1 and 3, remaining unaltered in No. 2. The contents of No. 1 gave no tryptophane-reaction: those of No. 3 gave a ppt. on boiling and on adding HNO_3, and a good biuret-reaction, but no tryptophane-reaction.

It is clear that the NaCl-extract, filtrate 3, contains a fibrin-digesting (peptonizing) enzyme, but no peptone-splitting (peptolysing) enzyme.

A further experiment was made with the same papaïn-residue. It was extracted once more with 200 cc. 2 per cent. NaCl solution and filtered.

Filtrate 4. The neutral filtrate gave no turbidity on boiling or on adding HNO_3, and but a slight xanthoproteic reaction.

Digestive properties. An attempt was made to ascertain in what way, if any, the digestive properties of the fibrin-digesting enzyme are affected by the reaction of the medium.

35 cc. of the filtrate were put into each of five bottles, Nos. 1–5: to one bottle (No. 2), 0·2 grm. of Witte-peptone was added, just to make sure that no ereptase had been extracted; to all the others, 0·2 grm. of fibrin: the contents of No. 3 were acidified to 0·1 per cent. HCl, those of No. 4 to 0·5 per cent. citric acid, and those of No. 5 were made alkaline to 0·5 per cent. Na_2CO_3.

After twenty-four hours' digestion, the fibrin had disappeared in No. 5 (alkaline), and was much broken up in No. 1 (normal), whilst it was unaltered in Nos. 3 and 4 (acid). The contents of Nos. 1 and 5 gave ppt. on boiling and on adding HNO_3, a strong xanthoproteic and a good biuret-reaction, but no tryptophane-reaction.

After forty-eight hours' digestion, the contents of No. 2 gave no tryptophane-reaction, thus proving the absence of ereptase: the fibrin in Nos. 3 and 4 was still quite unaltered.

The foregoing experiments demonstrate the isolation from papaïn, by this method, of a solely peptonizing enzyme, peptase, in the form of an active NaCl.-solution. It is clear, however, that a great deal of this enzyme is lost in the preliminary treatment of the papaïn with 20–25 times its bulk of 2 per cent. NaCl-solution: for, as stated above, this first extract actively digests fibrin. This consideration led me to devise a supplementary method which makes use of this extract, as explained in the following experiment:—

10 grms. of 'raw papaïn', merely the dried latex as imported, were ground to powder, and were well triturated with 150 cc. of 5 per cent. NaCl-solution: the mixture was placed on a filter, and the filtrate dropped into 300 cc. of alcohol, where a dense ppt. was formed. The ppt. was then collected on a filter: it was subsequently extracted with 200 cc. of distilled water, and the mixture was put on a filter.

Filtrate 1. The filtered H_2O-extract is of a brown colour, the colour having developed during filtration; it is clear and neutral; it gives ppt. on boiling, dense ppt. on adding HNO_3, and strong xanthoproteic reaction: a portion of it, boiled and filtered, gives good biuret-, but no tryptophane-reaction.

Digestive properties. The filtrate was found to digest both fibrin and Witte-peptone within eighteen hours.

The residue was now extracted with 100 cc. 2 per cent. NaCl-solution, and the extract filtered.

Filtrate 2. The filtered NaCl-extract was clear, colourless, neutral, gave a turbidity on boiling and on adding HNO_3, and a distinct xanthoproteic reaction: a portion of it, after being boiled and filtered, gave no biuret- or tryptophane-reaction.

Digestive properties. The experiment is worth giving in full. 25 cc. of the filtrate were put into each of four bottles, the liquid put into No. 2 having been previously boiled; to each bottle were added 0·2 grm. fibrin and the same quantity of Witte-peptone; to No. 3 citric acid was added to 0·3 per cent.; and to No. 4, HCl to 0·05 per cent.

After twenty-fours' digestion, the fibrin had disappeared in No. 1 (neutral); it was unaltered in No. 2 (boiled) and in Nos. 3 and 4 (acid), and remained so during twenty-four hours' further digestion. No tryptophane-reaction was given by the contents of any of the bottles.

Hence the solution contained only a fibrin-digesting enzyme, the action of which was arrested by acidity.

Thus the results obtained by the investigation of the NaCl-extract of papaïn agree with those obtained by the investigation of the papaïn-residue.

The material used in these experiments was chiefly what is known as 'Commercial Papaïn'; the more important results were verified by experiments with 'Raw Papaïn'—that is, the dried latex as imported, kindly supplied to me by Messrs. Thomas Christy & Co., of London.

It was thus shown that the digestive properties of the latex, as here described, are not due to, nor are materially affected by, the processes of purification by which commercial papaïn is prepared from the raw latex.

I may add that neither form of papaïn is completely soluble in water or NaCl-solutions; after extraction, a considerable insoluble residue remains, which, in the case of commercial papaïn, consists largely of some form of coagulated protein, as Martin has already noticed. It should also be recorded that all the digestions took place at 39° C., and that HCN was the antiseptic.

Turning, now, to the consideration of the bearing of the new facts that I have described, the first effect of them will be the disuse, as being no longer necessary, of the name 'papaïn' or 'papayotin'). The name, in its original strict sense, was applied to what was considered to be a single substance; but now that this supposed tryptic protease has been shown to be a mixture of two proteases the name has lost its significance, and, if used at all, might be applied to the dried latex, whether raw or refined.

A point of great importance is the relation between the digestive activity of the papaïn-enzymes and the reaction of the medium. As already mentioned, Wurtz and Martin both found that digestion of fibrin or albumin (peptonization) was most active when the medium was neutral. Martin showed further that digestion is even more active in a feebly alkaline medium (0·25 per cent. Na_2CO_3), and continues, though with diminishing activity as the alkalinity is increased, even when the medium contains 1 per cent. Na_2CO_3; and that it is arrested in a medium which is acid to the small extent of 0·05 per cent. HCl. The few observations that I have made on this point in the course of these experiments confirm these results. Thus (p. 4) I found that the NaCl-solution of peptase digested fibrin when neutral or alkaline (0·5 per cent. Na_2CO_3), but did not do so in the presence of 0·1 per cent. HCl, or of 0·5 per cent. citric acid. In another experiment (p. 5) digestion was prevented by 0·3 per cent. citric acid, and by 0·05 HCl.

The results of some earlier experiments on this very point (19) had led me to form a rather different opinion from that just stated. The conclusion drawn from them was that the reaction-range for fibrin-digestion by papaïn extended from an alkalinity = at least 1·5 per cent. Na_2CO_3 to an acidity = 0·3—0·5 HCl. The discrepancy on this point between the results of my present and of my previous experiments is, I believe, to be accounted for on the ground that in the latter the solution used contained more protease; and further that it also contained a good deal of protein in solution, which would protect the protease to some extent from the action of the acid, whereas, in the former, the amount of protein was very small.

The question of the relation between the enzymes and the proteins of papaw-latex naturally arises here. It has been already mentioned (p. 2) that Martin considered the digestive action to be associated with the albumose. My experiments seem to show that the fibrin-digesting enzyme is associated with the globulin of the latex, inasmuch as it is more soluble in

NaCl-solutions than it is in water; probably the pure peptase would be insoluble in pure water. But it must be pointed out that in several experiments the active peptase-solution gave but a faint xanthoproteic reaction, indicating a very small quantity of protein.

The demonstration of the presence of two distinct proteases in papaïn would be more complete had I succeeded in preparing, not only extracts that were exclusively peptonizing, but also extracts that were exclusively peptolysing. I have endeavoured to obtain solutions of the latter kind at various stages of the methods here described for obtaining those of the former kind. For instance, I have treated the papaïn residue, after extraction with 2 per cent. NaCl-solution, with water, and obtained solutions which peptolysed Witte-peptone actively, but also had some digestive action on fibrin; similarly, the alcoholic precipitate of the NaCl-solution, when treated with water, gave a solution that peptolysed actively, but also peptonized. The only liquid that I have found to be exclusively peptolytic is the alcoholic liquid filtered off from the alcoholic precipitate of the NaCl-extract. I found that some of this liquid, diluted with an equal bulk of water, acted upon Witte-peptone so as to cause a distinct tryptophane-reaction, but had no effect upon fibrin. Ereptase was clearly present; but it is not so clear that peptase was absent, though probably it was; for the amount of alcohol present was sufficient, as I found by control-experiments, to prevent the peptonization of fibrin in liquids known to contain peptase. I anticipate, however, that it will be possible to make use of the solubility of ereptase in fairly strong (above 60 per cent.) alcohol in devising a method for its isolation.

Yeast (*Saccharomyces Cerevisiae*).

The investigation of the proteolytic activity of Yeast began in 1889 with Salkowski's observation that, if Yeast be kept in chloroform-water, the liquid eventually contains leucin and tyrosin which can only have been formed by the digestion of its own proteins. Ten years later (1898–1900) Hahn and Geret began the publication of a series of papers (22, 23) bearing upon the subject; they found that leucin and tyrosin were formed in the self-digestion of the expressed juice of Yeast; that the juice digested fibrin, egg-albumin, casein, with the formation of leucin, tyrosin, and tryptophane among the products; and that digestion was most active in acid medium (0·2 per cent. HCl). The name 'endotrypsin' or 'endotryptase' was given by them to the proteolytic enzyme, on account of the nature of its digestive activity. It was, and still is in fact, regarded as a form of 'vegetable trypsin'.

My own observations on Yeast began in 1901, when I tested its digestive action in the course of my search for tryptophane as a constant product of the proteolysis of plants (14). The result of these first experiments was that tryptophane was found in Yeast-digestions when the medium was neutral or acid, but not when it was alkaline.

A year later, I returned to the study of Yeast-digestion, and pursued it in greater detail. The material used was chiefly dried Yeast. The results which I obtained were these (17)—

(1) dilute watery extracts of Yeast digested Witte-peptone but not fibrin;

(2) dilute extracts of Yeast made with NaCl-solution (2 per cent.) readily digested both fibrin and Witte-peptone;

(3) peptolysis and peptonization were influenced in the same manner, but not in the same degree, by the addition of acid or alkali.

These results raised the important issue that may be best expressed in two questions: (1) Is there, as is generally held, a single protease in Yeast, or is there more than one? (2) In the latter case, how many proteases are there, and what is their nature?

My answer to these questions was that 'the two digestive processes —that is, the digestion of fibrin (peptonization) and the digestion of Witte-peptone (peptolysis)—are not effected by one and the same protease. On the contrary, the facts described in this paper indicate the presence of two proteases; the one exclusively peptolytic, readily soluble in water [ereptase]; the other exclusively peptonizing, less soluble in water, but readily soluble in 2 per cent. NaCl-solution [peptase]'.

After completing the investigation of the Hemp-seed and of papaïn, with the results already described, I turned yet once more to Yeast, to see if it might not be possible here also to effect the separation of the two enzymes, of which the distinct individuality had been so strongly suggested by my previous experiments.

These experiments had, in fact, already demonstrated that, on making a dilute extract of Yeast with distilled water, and filtering it, a solution is obtained which has no action upon fibrin, but readily digests Witte-peptone with the formation of tryptophane. Clearly such a solution contains only ereptase. This being so, what remained to be done in the new experiments was to obtain a solution from Yeast which would digest fibrin but not Witte-peptone, a solution which should, in fact, contain peptase.

I have now, after many and long-continued experiments, succeeded in preparing such a solution from both fresh and dried Yeast. In illustration of the method employed, I give a description of an experiment made with fresh Yeast.

About a litre of yeast was obtained from the brewery: it was placed upon a filter to allow the beer to drain off, and it was washed by running water through it. After allowing it to drain until no more water dropped from it, the solid residue was of the consistence of thick paste and amounted to about nine large table-spoonfuls. This was removed from the filter and thoroughly mixed with 500 cc. 5 per cent. NaCl-solution; to the mixture was added chloroform enough to give the strength of chloroform-water (0·5 per cent.), and it was left standing all night in a covered jar kept in a cold room. It was noticed next morning that the mixture in the jar had frothed up

considerably, as if it had been fermenting: this occurred in all experiments with fresh Yeast.

The mixture was put on a filter next morning, the filtrate dropping into a vessel containing 1 litre of alcohol. A portion of the filtrate was collected for examination, it was found to be clear, yellow, strongly acid, giving marked turbidity on boiling, less turbidity on adding HNO_3, strong xanthoproteic reaction, distinct biuret-, but no tryptophane-reaction: its digestive properties were that it acted quickly upon Witte-peptone, the digestion-liquid giving marked tryptophane-reaction in twenty-four hours, whilst its action upon fibrin was slower.

The filtrate dropping into alcohol gave rise to a copious precipitate: the mixture was put on a filter, and the alcoholic liquid drained off. When filtration was completed, the moist precipitate on the filter was found to weigh just over 15 grms.

The precipitate was now mixed with 200 cc. distilled water, and left standing for some hours. The mixture was then filtered: the water did not appear to have dissolved much, if any, of the precipitate, and the filtrate had no digestive action on either fibrin or Witte-peptone.

After having thus been washed with water, the precipitate was mixed with 150 cc. 2 per cent. NaCl-solution: after standing for some hours, the mixture was put on a filter.

The *filtered NaCl-extract* was neutral, opalescent, giving slight precipitate on boiling, and turbidity with HNO_3, a slight xanthoproteic reaction, but no biuret.

60 cc. of the filtrate were taken for experiment: 30 cc. were put into a bottle (*a*) with 0·2 grm. of fibrin and 0·2 grm. of Witte-peptone; the other 30 cc. were boiled and filtered, the liquid being made up to the original quantity by adding distilled water, and put into a bottle (*b*) with fibrin and Witte-peptone as before: a few drops of HCN were added to each.

After twenty-four hours' digestion, the fibrin had almost entirely disappeared in (*a*); the liquid contents of it gave a considerable precipitate on boiling, somewhat less on adding HNO_3, but no tryptophane-reaction: the fibrin in (*b*) was quite unaltered.

30 cc. of the filtrate were also put into each of three bottles; the contents of the first were acidified to 0·06 per cent. HCl, those of the second to 0·45 per cent. citric acid, and those of the third were made alkaline to 0·6 per cent. Na_2Co_3: 0·2 grm. of fibrin was placed in each bottle, with a few drops of HCN.

At the end of seventy-two hours in the incubator the fibrin was apparently unaltered in all three bottles. It appears, therefore, that the reaction range of the peptase lies within the limits of acidity and alkalinity indicated in this experiment—a result which differs, as regards acidity, from that obtained by Hahn and Geret (see p. 7), the discrepancy being due, no doubt, to the presence of a considerable amount of protein in their liquids; but agrees fairly with my previous results (17, pp. 302–3).

By this method, which is the same as the supplementary method applied to the investigation of papaïn (see p. 5), it was possible to prepare a liquid which digested fibrin, but had no action on Witte-peptone; a liquid, therefore, which contained only peptase in solution.

The conclusion to be drawn from my experiments upon Yeast is that the cells contain two proteases, peptase and ereptase. The so-called 'endo-

trypsin' of Yeast, is, therefore, not a single protease, but a mixture of two; consequently the term may now be dispensed with.

It will be seen that the results obtained with Yeast agree with those obtained with papaïn, and lead to the same conclusions.

Review of the Subject.

Now that my researches upon the proteases of plants have led to a definite conclusion, valid at least for the materials that have been subjected to experiments, I should like to retrace briefly the steps by which I have arrived at that conclusion.

Without attempting a complete history of the subject, the delivery of Sir Joseph Hooker's presidential address at the Belfast meeting of the British Association in 1874, and the publication of Darwin's book on 'Insectivorous Plants' in the following year, may be taken as the starting-point of the scientific investigation of proteolysis in plants. The first conclusion arrived at was that the secretions of insectivorous plants contain an enzyme similar to the pepsin of animals, inasmuch as it digests fibrin and the other higher proteins in acid medium.

The next step was the discovery by von Gorup-Besanez, about the same time, of the existence of proteolytic enzymes in ordinary, non-insectivorous, plants. The fact of the presence of leucin and asparagin in seedlings of a Vetch, when grown in darkness, suggested to him that these substances must be the products of changes in the reserve-protein of the seed effected by a digestive enzyme. Accordingly he examined the seeds of the Vetch, but whether germinated or ungerminated he does not say, and succeeded in extracting from them, by means of glycerin, an enzyme that converted fibrin into peptone in the presence of 0·2 per cent. HCl (4). He subsequently investigated the seeds of the Hemp (*Cannabis sativa*) and of the Flax (*Linum usitatissimum*), apparently ungerminated, as also Malt, arriving at much the same result as in the case of the Vetch (5). He sought in vain for leucin, trypsin, and asparagin among the products of the digestion of fibrin. Thus he failed to trace the leucin, asparagin, &c., found in seedlings to a digestive process; and though he did not call the enzyme that he found by the name 'pepsin', he described it as 'peptonbildendes ferment'.

The first general idea as to the nature of the apparently wide-spread protease of plants was, therefore, what may be termed the *pepsin-idea*: that is, it was considered to be a peptonizing enzyme acting only in acid medium. However, facts soon began to come to light which gradually made this view untenable. The investigation of papaïn by Wurtz, already mentioned on p. 2, showed that here was an enzyme which digested fibrin not only in acid, but also in neutral and even alkaline media. Although Wurtz found nothing but peptone in the products of digestion, he realized that enzyme could not be a pepsin, so he regarded it as a kind of

ERRATUM

Page 10, line 13 from bottom, *for* trypsin *read* tyrosin

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pancreatin (i. e. trypsin). Martin's investigation of papaïn carried the matter a good deal further on in the same direction. He found leucin and tyrosin among the products of digestion, and ascertained that the presence of acid retarded, whilst that of alkali, within certain limits, accelerated, digestion; consequently he associated it, though very cautiously, with animal trypsin.

In this way the second general idea, what may be termed the *trypsin-idea,* of plant-proteases originated, the idea that these proteases not only peptonize fibrin and other proteins, but further split up the albumoses and peptones into amino-acids, such as leucin and tyrosin, and other nitrogenous substances. It was soon supported by a considerable amount of experimental evidence. Thus, Green found that from germinating seeds (Lupin, Castor-oil) extracts could be obtained which digested fibrin, in the presence of 0·2 per cent. HCl, leucin, tyrosin, and asparagin being formed during digestion (6, 7). Soon afterwards he showed that the Kachree Gourd (*Cucumis Melo* var. *utilissimus*) contains an enzyme which digested coagulated egg-albumen actively in alkaline (1·5 per cent. Na_2CO_3) medium, less actively in neutral, and least actively in acid (0·2 per cent. HCl); he found leucin among the products of digestion (8). Hansen's observations on the latex of the Fig (*Ficus Carica*) may be mentioned, though they are inconclusive (9). The latex digested fibrin in both acid (0·2 per cent. HCl) and alkaline (2 per cent. Na_2CO_3) medium; the products of digestion were not fully examined, but leucin and tyrosin were not found in the alkaline digestion. Chittenden (10), investigating the juice of the Pine-apple (*Ananas sativus*), observed that it digested fibrin most actively at its own natural acidity, and that leucin and tyrosin were products of digestion. He also found that the juice digested coagulated egg-albumen most actively when neutral in reaction, less actively at natural acidity, or at an alkalinity of 0·1 per cent. Na_2CO_3, still less actively when made more alkaline or more acid (with HCl), and that digestion was arrested by an alkalinity = 1 per cent. Na_2CO_3, and (in an artificial solution) by an acidity = 0·1 per cent. HCl.

Further evidence of this kind was afforded by my observations upon the digestive properties of the pitcher-liquid of *Nepenthes* (11, 12, 13, 1897–8, 1901). In these three papers I showed that the liquid has a 'tryptic' action, inasmuch as it not only peptonizes fibrin, but also splits the peptones into amino-acids, such as leucin and tryptophane. It was in the last of these three papers that I introduced the chlorine-reaction for tryptophane into the study of the proteolysis of plants. Although digestion was found to take place only in acid medium, I considered that the enzyme of the pitcher-liquid should be regarded as belonging to the trypsin-group (tryptases), on account of the products formed in digestion; and in the last of the three papers I went so far as to express the opinion 'that all known proteolytic

enzymes of plants are tryptic'. I extended my observations (14, March 1902) to other parts and secretions of plants, such as the juice of the Pine-apple (*Ananas sativus*), the latex of the Papaw (*Carica Papaya*) and of the Fig (*Ficus Carica*), Malt (*Hordeum sativum*), and Yeast (*Saccharomyces Cerevisiae*), and found in every case that tryptophane was one of the products of digestion, a proof that the digestion had been 'tryptic' in character, and that all the enzymes were active in acid medium. The conclusion that I drew from these facts was 'that the proteolytic enzymes of plants in general are essentially tryptic'; adding that 'this statement will hold good until definite evidence is adduced to prove the existence of a "peptic" enzyme'.

So far my investigations had been confined to parts or secretions of plants which were already known to have more or less definite digestive activity. I now turned my attention to the question of the possible presence of proteolytic enzymes in plants in which they had not yet been detected (15, Jan. 1903).

In the first set of these experiments, Witte-peptone was the material for digestion; the production of tryptophane was effected as the result of the action of the following substances: tissue of the Mushroom (*Agaricus campestris*); watery extract of Green Peas (*Pisum sativum*); extract of 'germ' of Wheat (*Triticum vulgare*); the expressed juice of the Melon (*Cucumis Melo*), of the Cucumber (*Cucumis sativus*), and of the Vegetable Marrow (*Cucurbita Pepo* var. *ovifera*); watery extract of the Banana (*Musa Sapientum*); the juice of the Tomato (*Lycopersicum esculentum*); the rind of the Apple (*Pyrus Malus*), and of the Orange (*Citrus Aurantium*); the juice of the Grape (*Vitis vinifera*); extract of the laticiferous shoots of *Euphorbia Characias*; the laticiferous leaves of the Lettuce (*Lactuca sativa*); the stems of *Dahlia* and of *Mirabilis*; the leaves of *Spinacia oleracea*, of the Cabbage (*Brassica oleracea*), of a Grass (*Holcus mollis*), of a Fern (*Scolopendrium vulgare*), and of several other plants; the bulbs of the Tulip, the Hyacinth, and the Onion (*Allium Cepa*); the tubers of the Potato (*Solanum tuberosum*) and of the Jerusalem Artichoke (*Helianthus tuberosus*); the roots of the Turnip (*Brassica Rapa*), the Tomato, the Vegetable Marrow, the Carrot, the Beet, and others.

Having shown that these various parts of plants possessed the property of digesting Witte-peptone, I proceeded, in the next place, to ascertain if any of them also possessed the power of digesting fibrin. In this way I was able to add to the list of plants known, at that time, to digest fibrin, the following: the juice of the Cucumber and of the Melon; the tissue of the Mushroom; the bulb of both the Tulip and the Hyacinth, but only in alkaline medium. I entirely failed to obtain any evidence of the capacity of ordinary foliage-leaves to digest fibrin.

The conclusions that I drew at the time are as follows: 'I have

succeeded in demonstrating that a proteolytic enzyme is widely distributed in plants; and it may be inferred that it is much more generally present than I have shown it to be. The next point to be considered is the probable nature of the enzyme. In the previously known cases, the evidence goes to prove that the enzymes are allied to the trypsin of animals, since they both peptonize and proteolyse actively. Amongst the plants that I have recently examined, there are only two, the Melon and the Mushroom, which approach those previously known in their power of peptonization and proteolysis. Whilst all the others readily proteolysed Witte-peptone, their action on the higher proteins, so far as it was tested, was relatively feeble, and in some cases altogether wanting. It may be that the precise conditions favourable for peptonization were not afforded in the experiments: that is a point for future investigation. But taking the facts as they stand, it is an inevitable conclusion that if in some cases, such as the Melon and the Mushroom, the enzyme may be regarded as a vegetable trypsin, this view cannot be extended to the others. It seemed to me, at first, that I had come upon an altogether new type of enzyme, an idea that occasioned a certain amount of temporary misgiving as to the accuracy of my observations. But it was pointed out to me by my colleague, Professor Gotch, that within the last year (1901–2), Cohnheim had described an enzyme, formed in the mucous membrane of the small intestine, which actively proteolyses peptone and casein, but does not act upon the higher proteins. It is to this enzyme, termed 'erepsin' by Cohnheim, that the apparently new proteolytic enzyme of plants would correspond. It would appear, therefore, that plants form two distinct kinds of proteases, the one a trypsin, the other an erepsin; and so far as the facts go, they indicate that the former is generally associated with depositories of proteid nutriment, such as seeds, fruits, bulbs, laticiferous tissue, the latter with ordinary foliage-leaves, stems, and roots'.

The discovery of the 'erepsin' of plants, or 'ereptase', as I prefer to call it, and the wide and general distribution of this substance, necessarily involved a modification of the 'trypsin-theory' of the proteases of plants previously mentioned, and to this extent: those plants whose tissues or juices peptonized fibrin, were considered to contain 'trypsin', with possibly ereptase as well; those plants whose tissues or juices had no effect upon fibrin, but digested (peptolysed) Witte-peptone, as indicated by the tryptophane-reaction, were considered to contain ereptase only.

For some time (1903–4) I continued my researches from this point of view, adding to the number of plants that were found to have some kind of digestive activity, investigating also the conditions of the digestion-experiments with special reference to the effect of antiseptics upon the digestive processes, and giving the result of extracting the plant-material with NaCl-solution instead of distilled water. The experiments are described in two papers, published respectively in June 1903 (16), and in April 1904 (17).

Those in the latter paper were a more detailed re-examination of Yeast and of the Mushroom; and it is worth while to repeat here the conclusion to which I was led: 'It is suggested that the Yeast and the Mushroom contain two associated proteases, vegetable erepsin and vegetable trypsin . . .'

My next paper (18, Jan. 1905) contains an account of further experiments upon both new and old material, confirmatory of those already described. Perhaps the most important observation recorded here is the discovery that the leaves of *Phytolacca decandra*, unlike all the leaves (except those containing latex) previously examined, readily digested fibrin.

Another paper which followed closely (19, April 1905) upon the preceding, raised the question, 'What is the nature of the fibrin-digesting protease?' I had observed, when testing the action of various antiseptics upon the digestive processes, that sometimes the fibrin-digesting property of an extract or a tissue was destroyed by an antiseptic, whilst the peptone-splitting activity remained unimpaired, and *vice-versa*. This paper is a study of the action of acids and of alkalies upon the digestive processes in a variety of plant-material (papaïn, Pine-apple juice, Yeast, Mushroom, Malt, Hyacinth-bulb, pitcher-liquid of *Nepenthes*). My conclusions were as follows: 'The experiments detailed in the foregoing pages constitute a demonstration of the differential effect of varied reaction upon the proteolytic activities of the juices and extracts of certain representative plants. . . . On further consideration of these results, it will, I think, be generally admitted that the method employed does actually afford the means of realizing that separation of the proteolytic activities which I postulated as being essential to the investigation of the nature of the supposed "vegetable trypsin". I cannot interpret the evidence thus obtained otherwise than as indicating that peptolysis and fibrin-digestion are effected by two distinct proteases; that "vegetable trypsin" is, in fact, not a single protease, but a mixture of two; the one a peptolytic enzyme belonging to the ereptases, the other a peptonizing, fibrin-digesting, enzyme belonging to the peptases. . . . If it be admitted that two proteases, or two groups of proteases, exist in plants, the ascertained facts as to the distribution of the proteases in the vegetable kingdom may be succinctly stated in the following propositions: All plants that have been examined contain ereptase; in some of these plants the ereptase has been found to be associated with a larger or smaller proportion of a peptase; in no plant has a peptase been found to exist unassociated with ereptase'.

This altogether new view of the nature of the plant-proteases was a challenge to the idea of 'vegetable trypsin'; but the evidence was as yet insufficient to be convincing. The kind of evidence still required was the actual separation of the two kinds of enzymes, the peptase and the ereptase, so that from the same material there should be prepared extracts, one of which

would be purely peptonizing (i.e. containing peptase only), the other purely peptolysing (i.e. containing ereptase only).

Some further indirect evidence in favour of the new view was obtained by a series of experiments that I made with certain, chiefly starchy, seeds (*Phaseolus multiflorus* and *vulgaris*, *Vicia Faba*, *Lupinus hirsutus*, *Pisum sativum*, and *Zea Mais*). In discussing these experiments, I stated the position of the question as follows (20, April 1906): 'It must be admitted, however, that all the evidence that I have accumulated does not yet suffice to prove that there is no such thing as "vegetable trypsin". One point, at any rate, has become clear, namely, that "vegetable trypsin" is a mixture of enzymes, and that ereptase is one of the constituents. But the nature of the other constituent (or constituents), the fibrin-digesting protease, remains uncertain: it may be a tryptase, but it may also be a peptase. It is not, I think, going too far to suggest that the known facts make the latter suggestion the more probable—to transfer, in fact, the *onus probandi* to those who hold that the enzyme in question is a tryptase.'

Pursuing my researches on seeds, I next turned my attention to oily seeds, and found them to be much more proteolytically active than starchy seeds, even when ungerminated. Most of the experiments were made with ungerminated Hemp-seed (*Cannabis sativa*); but several others, such as those of the Mustard (*Sinapis alba*), the Hazel (*Corylus avellana*), the Castor-oil plant (*Ricinus communis*), and the Flax (*Linum usitatissimum*) were also examined.

In the course of my investigation of the Hemp-seed, I made an attempt, which eventually proved successful, to prepare solutions containing respectively the peptase and the ereptase which my experiments had shown to be present in the seed. It is unnecessary to repeat here the description of the process employed, which is fully given in the paper in which the account of the experiments was published (21, Jan. 1908). The really important result was the preparation, from the Hemp-seed, of a solution containing only a fibrin-digesting enzyme (peptase). I had frequently prepared extracts of various kinds of material which contained only ereptase; but never before had I, or indeed any one else, prepared from a part of a plant a solution of a peptase unmixed with ereptase.

This result seems to me to involve the final demolition of the 'vegetable trypsin' theory. But of course it requires reinforcing by similar results obtained with other material. This reinforcement is, to some extent, afforded by the results obtained with papaïn and with Yeast, described in the earlier part of this paper. It is, however, necessary that I should repeat most of the experiments that I have described in this series of papers, from the fresh stand-point and with the application of the new methods, so as to complete the confirmation of this view as to the nature of the proteases, and perhaps to come upon further developments of it.

The view that I propose in substitution for the 'vegetable trypsin' theory is that the proteases of plants belong to two groups, the *peptases* and the *ereptases*—a view that is now supported by a considerable body of evidence, both direct and indirect. It remains now to consider the respective properties of these proteases, so far as they are known.

The Ereptases are enzymes which are readily soluble in water, in watery solutions of neutral salts, and in alcohol up to over 65 per cent.; their digestive activity seems to be exclusively peptolytic, and to be especially associated with an acid medium. I am unable to give even an approximate reaction-range for plant-ereptase, because I have not yet made any experiments with it in pure solution; all my experiments, so far, have been made with extracts containing other substances in solution, the presence of which materially affects the action of acids or alkalies upon the enzyme. For instance, I have found (17) the reaction-range of peptolysis in a 5 per cent. Yeast extract to be from 0·6 per cent. HCl to 2 per cent. Na_2CO_3, a very wide range which could certainly be very much reduced were the experiments to be repeated with a liquid containing only the ereptase in solution.

I have not yet come across any facts to indicate that the ereptases of various plants are materially different from each other.

The Peptases are proteases of which the digestive activity is limited to the peptonization of the more complex proteins.

There is some ground for thinking that there are at any rate two kinds of peptases, which differ from each other (*a*) in the mode of their occurrence, and (*b*) in the relation between their respective digestive activities and the reaction of the medium. The one kind exists in the tissues of plants, fruits, seeds, latex, &c., and may therefore be designated *endopeptase*; the other kind is to be found in the excretions of plants, for example, in the pitcher-liquid of *Nepenthes*, and may be distinguished as *ectopeptase*.

(*a*) *Endopeptase* is an enzyme which can be readily extracted from the tissues, &c., in which it occurs by NaCl-solutions; and also, to a less extent, by water, and by 50 per cent. alcohol. The extraction by means of water is due to the presence of salts and other substances which are extracted with the peptase; for, when obtained as free as possible from foreign bodies, it is not soluble in distilled water (*vide* Yeast, p. 9), but is still soluble in 2 per cent. NaCl-solution. Its digestive activity, like that of ereptase, is greatest at the natural reaction, generally somewhat acid, of the plant-extract which contains it. Very slight addition of mineral acid (0·05 HCl) or of a rather larger quantity of organic acid (·3 per cent. citric acid) arrests the action of a pure solution of the enzyme, which digests fibrin actively when neutral or slightly alkaline; an increase of alkalinity retards and finally stops digestion.

(*b*) *Ectopeptase* is an enzyme which is in solution in the excretion of at

any rate one carnivorous plant, *Nepenthes*. This is the only one of the carnivorous plants which has been adequately investigated from the point of view of digestive activity; but it is not improbable that the facts ascertained with regard to this plant will be found again when the other carnivorous plants are examined.

The pitcher-liquid of *Nepenthes* is, under normal conditions, a clear colourless or yellowish liquid, either neutral or acid in reaction, containing very little organic matter and not more than 1 per cent. of mineral matter. It is, therefore, a fairly pure aqueous solution of the protease. The characteristic feature of its digestive activity upon fibrin and other complex proteins is that acid reaction is absolutely essential: neutral liquid has no action; digestion is rapid at natural acidity, and also in the presence of added acid, whether organic (citric acid) or mineral (HCl 0·3 per cent.). It is especially in its relation to HCl that ectopeptase differs essentially from endopeptase: for the activity of the latter, when in pure solution (thus corresponding nearly to the pitcher-liquid) is arrested by the presence of as little as 0·05 per cent. HCl.

In considering the relation of the peptases to the acidity of the medium, it may be pointed out that there is some evidence (observations of Weis and others on Malt, quoted in No. 17, p. 291, of the list of references) to prove that the acid reaction which is always given by extracts of parts of plants, is due, at any rate in Malt, and probably also in other seeds, to the presence, not of free acid, but of acid phosphates. It is in an acid medium of this kind that endopeptase seems to be most active. Ectopeptase, on the contrary, is most active in the presence of free acid; this is well established in the case of *Nepenthes*.

It is impossible to conclude this discussion of the nature of the proteolytic enzymes of plants without some reference to those of animals. Certain analogies are obvious. The enzyme that I have termed 'ectopeptase' agrees in all essential properties with animal pepsin; this agreement is particularly interesting because it justifies the original conclusion (see p. 10) that the excretions of carnivorous plants contain pepsin. The only modification of that original view that is necessary is that these excretions (at least that of *Nepenthes*) contain ereptase in addition. Then, again, the ereptase of plants differs from that of animals only in that its reaction-range is more extensive in the direction of acidity, and is, perhaps, less extensive in the direction of alkalinity.

But it is not so easy to find an animal analogue for 'endopeptase'. It does not correspond to trypsin, because that substance is held to be an enzyme that both peptonizes and peptolyses; it would, however, correspond fairly well with the peptonizing factor in trypsin, if that were regarded as separable; and it may prove to be separable after all.

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On the Organization of the Nuclei in the Pollen Mother-cells of Certain Plants, with Especial Reference to the Permanence of the Chromosomes.

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With Plates I–III.

RECENT cytological investigation tends to strengthen the view that the chromosomes are everywhere permanent cell-structures. Beside the evidence as to constancy in numbers, size, and form, many newer facts furnish indisputable proof that the chromosomes retain their permanency as individual units from one cell-generation to another. In common with Rosenberg and several more recent authors, I have presented considerable evidence to show that the chromosomes persist as individual structures in the germ cells of such plants as *Campanula grandis*, *Helleborus foetidus*, *Thalictrum purpurascens*, and *Calycanthus floridus*. In the last two plants I have described particularly the presence of definite chromatic collections, or 'prochromosomes', in the somatic and germ cells. Numerous countings and careful study show that these bodies usually correspond to the somatic chromosomes both in number and size. These collections furnish excellent proof of the permanence of the chromosomes as individual units during the so-called resting condition of the nucleus. I have continued the study of these plants, and shall describe how the structure of the resting nucleus at the period of its maximum chromatic distribution differs markedly from the usual reticulum. I find the formation of a pre-synaptic heterogeneous spirem which consists of a double series of chromosomes alternating with linin segments. I am able to follow the disappearance of these linin segments in the post-synaptic spirem, a process which separates the definite diakinetic chromosomes. Each member of the chromosome pair splits longitudinally in diakinesis, thus producing tetrads, which can be followed through all stages in the formation of the spindle until they are separated in the division. The chromosomes have also been traced through the various stages of the

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telophases and prophases of succeeding divisions, my studies thus confirming the recent conclusions of several authors that the chromosomes remain separate during these phases. These plants also show the arrangement and position of the chromosomes in the pollen grain, which is of interest as showing the chromosomes arranged in a single series. I have also studied the pollen mother-cells of *Richardia africana* in all stages of development, and shall describe the stages which bear upon the above points.

The structure of the resting nucleus usually described for plants and animals, in which there is a net-work consisting of a fine, rather uniformly distributed linin and chromatin reticulum, is quite unlike that found in these plants. The limits of the individual chromosomes are not lost to view in the resting nucleus. Neither does the structure of these resting nuclei correspond to the conception that the reticulum consists of a single substance, as urged by Grégoire and Haecker, who hold that it is arranged into a reticulum, which stains more densely at the junctures of the threads, to form so-called net-knots. I find thus that the behaviour of the chromosomes during the prophases of division of the pollen mother-cells may be followed in detail on account of their continuous presence as definitely limited bodies. This makes it possible to establish the existence of a pre-synaptic bivalent spirem extending back to the resting nucleus, as against the contention of Farmer and Moore and Mottier. In most plants and animals studied, the limits of each individual chromosome are not to be determined during the spirem stage, a fact which makes it difficult to follow the behaviour of each individual chromosome. The heterogeneous post-synaptic spirem of the plants consisting of chromosomes and intervening linin segments shows its double nature from its formation. The limits of each somatic pair of chromosomes may be determined, since at no time is there a continuous chromatic post-synaptic spirem formed. Several authors, Sargant ('96, '97), Guignard ('99), Grégoire ('99), Strasburger ('00), and Mottier ('03), have described a splitting of the daughter chromosomes in the prophases to form the tetrads. They describe the appearance of a double series of chromosomes, one in each half of the longitudinally-split spirem or daughter chromosomes of diakinesis.

Calycanthus floridus and *Richardia africana* show that the tetrads are definitely differentiated by the longitudinal splitting of each component of the diakinetic pairs of chromosomes, resulting in four elongated rodlets, lying parallel, two of which have arisen from one chromosome and two from the other. The further history of these tetrads has been followed throughout the formation of the spindle and division stages.

The fixing fluids giving the best results for the pollen mother-cells of *Thalictrum purpurascens* are the alcohol-acetic and the alcohol-acetic-chloroform mixtures of Carnoy, which proved to be good for all stages. I have thus been able to obtain good fixation for all post-synaptic stages, which

I was unable previously to study. Flemming's mixtures of chromic, osmic, and acetic acids, although very favourable for the early prophases, have proved almost worthless for the post-synaptic stages of this plant. Good results were, however, obtained by first wetting the anthers in Carnoy's solution, and then fixing in the usual manner in Flemming's strong solution. This solution was employed with success in fixing the other plants studied. A one-per-cent. aqueous solution of platinum chloride was also used to fix some stages of *Thalictrum*. The sections, when necessary, were cut thick enough to contain entire nuclei. Being first mordanted in a two-per-cent. solution of potassium permanganate, the sections were stained with Flemming's triple stain or Heidenhain's iron-alum-haematoxylin combination.

In common with Grégoire ('07), we may adopt, provisionally at least, the following scheme of phases for convenience of clearness in description. The prophases of division naturally fall into two periods, the pre-synaptic and the post-synaptic phases. The resting nucleus, according to some authors, is transformed into a mass of fine filaments. To this transformation period, Winiwarter ('01) gives the name 'leptonema'. The fine filamentous stage described by some writers, in which the elements conjugate in pairs, Grégoire ('07) designates as 'zygonema'. Synaptic contraction is characteristic of this period. The period of the thick, unsplit spirem is called 'pachynema'. During the stage of the thick spirem, which is of long duration, the so-called longitudinal splitting becomes evident. In other words the spirem distinctly shows its bivalent character at this time. Dixon ('96) has designated this phase as 'strepsinema'. Whenever a second contraction occurs it is during this period. The phase when the chromosomes are definitely separated by the segmentation of the spirem, and lie freely distributed in the nuclear cavity, usually on the periphery next the nuclear membrane, Haecker ('97) has called 'diakinesis'. We may also still retain the terms 'multipolar', 'polyarch,' and 'multipolar diarch' as stages in spindle formation.

DESCRIPTION OF OBSERVATIONS.

I. Thalictrum purpurascens.

In *Thalictrum purpurascens*, shortly after the last pre-meiotic divisions, the cells of the sporogeneous tissue and their nuclei assume the appearances characteristic of spore mother-cells. These cells undergo a growth-period in which they increase in size. Their nuclei also enlarge proportionately. The cells in one loculus of an anther show a remarkable uniformity in development, and usually all the mother-cells in all four loculi exhibit a striking uniformity as to their stages of development.

I have attempted to trace the processes of reconstruction of the nuclei of the pollen mother-cells from the last pre-meiotic division, and to compare

the structure of these nuclei with that of ordinary somatic ones, but have experienced considerable difficulty in identifying with certainty the last pre-meiotic divisions. After the formation of the nuclear membrane, and during the period of nuclear enlargement, the chromatic material becomes rather regularly distributed in the nuclear cavity, the greater portion of the stainable substance lying in the prochromosomes, each suggesting by its form and size that it is derived from a chromosome of the preceding telophases. I am not prepared to discuss the problem as to how the chromosomes of the telophases are modified in passing over into the resting nucleus. Whether the process in germ cells is different from that in vegetative divisions certainly needs investigation. Grégoire and Wygaerts ('03) have claimed that in vegetative divisions the nucleus possesses an alveolar structure, a 'réseaux de réseaux', which arises by a progressive and irregular alveolization and anastomosing of the chromosomes of the telophase. According to Grégoire and his students the chromosomes are again formed in the prophases of division by a progressive recondensation of the chromatic reticulum to form the chromosomes. Haecker ('04) also observes that in *Siredon* the chromosomes, during the telophases, possess alveoli, which increase in size and number, finally leading to complete alveolization.

In *Thalictrum purpurascens* there is present in the nucleus at this period of greatest chromatic distribution a fine, more or less reticulated substance, the structure of which is much like that of the cytoplasm of the cells (Figs. 1, 2, 3, 4, 5, Pl. I). If it were not for the presence of the prochromosomes it would often be difficult to distinguish between the structure of this framework in the nucleus and that of the cytoplasm (Fig. 4, Pl. I). The substance of this framework is never impregnated with stainable chromatin. Through this reticulated framework run coarser threads, in which the chromatin is aggregated into the prochromosomes. The substance of the framework and of the coarser threads may perhaps be linin. I have been unable to discover any evidence that the threads of the framework and the threads which contain the prochromosomes anastomose. They are certainly different in staining reaction. The framework stains yellow with the triple combination, while the fibres which contain the prochromosomes stain blue with Gentian violet. In all nuclei at this stage, the prochromosomes show as distinct, rather uniform bodies, arranged in pairs. They stain densely black with haematoxylin, and red with safranin. This pre-synaptic pairing in the germ cells must not be confounded with the paired condition which I have already described for somatic cells. There can be no doubt that the prochromosomes are paired in somatic cells. Although this condition is somewhat more difficult to observe in somatic cells, some of the prochromosomes are always to be found in pairs (Fig. 1, Pl. I). In my former paper ('05) I figured these prochromosomes in pairs in certain cases (Pl. VI, Figs. 1, 2, 3, 4, and 24) in somatic cells, although no emphasis

was placed upon the appearance at that time. I have since become convinced that these prochromosomes are severally arranged in pairs in somatic cells of *Thalictrum purpurascens.* This association of the chromosomes in pairs might be expected to lead to the formation of apparent tetrads during the prophases of division, which cannot, of course, be associated in any way with a reduction process such as is found in the germ cells. Moore and Miss Embleton ('06) have also described the presence of double rods in both somatic and pre-meiotic nuclei of *Triton*, and Farmer and Moore ('05) have described a similar condition in *Periplaneta.*

Strasburger ('05) also found that the chromosomes of *Galtonia* and *Funkia*, in which they are of different sizes, became arranged in homologous pairs during the prophases of the divisions in nuclei of embryonic regions. He ('07) has also reported a similar condition for the root-tips of *Pisum sativum.* Strasburger maintains that the parental chromosomes mingle, becoming closely associated but retaining their individuality in somatic cells, first becoming intimately associated or united in the germ cells during synapsis. Just how early and in what manner this intimate association occurs remains unsettled.

Since the pairs are so very closely associated, each pair often appears as a single body in both somatic and germ nuclei, which may easily lead to mistakes in counting. I find that in my previous counting I mistook the pairs of chromosomes for a single chromosome. Therefore the number of chromosomes both in somatic and germ nuclei should be twice as many as formerly stated. The reduced number of chromosomes in *Thalictrum purpurascens* is 24, instead of 12 as stated by Strasburger ('04) and myself ('04, '05). In certain other species of *Thalictrum,* as *Thalictrum minus*, which is not apogamous, the reduced number is 12. In young pollen mother-cells the nuclei have the appearance shown in Figs. 2, 3, 4, and 5, Pl. I. With ordinary magnification the prochromosomes often appear as 24 single, distinct bodies (Fig. 2, Pl. I), while with higher powers (Figs. 3 and 5, Pl. I) each prochromosome appears double, so that each of the apparently single bodies is composed of two, or 48 in all.

Faint linin threads, which are parallel, connect the different pairs of prochromosomes, so that the nucleus appears to have two separate and distinct spirems, each being composed of a series of prochromosomes with intervening linin segments. The nucleus at this period, therefore, has somewhat the appearance of a post-synaptic nucleus, in which the two component parts of the double spirem are more or less distinct. The linin portions of these parallel pre-synaptic spirems stain blue with the triple stain. Sometimes the prochromosomes appear slightly granular at this stage, and these granules may be somewhat distributed or spread out in the linin. The structure of this nucleus is, therefore, neither that of a reticulated chromatic substance, nor does it have a linin reticulum with chromatic granules evenly

distributed throughout it. There is rather a framework, probably of linin, through which run two heterogeneous spirems, arranged parallel.

By examining Figs. 3, 4, and 5, Pl. I, it may be seen that the two prochromosome series and linin intervals of *Thalictrum purpurascens* are never widely separated from each other before synapsis. As already described, the prochromosomes are arranged in pairs at the period of greatest chromatic distribution, with every indication of parallel linin intervals. In all the earlier stages which I have studied these bodies and their linin connexions are apparently arranged in two spirems. This pairing of the prochromosomes and the linin intervals may possibly occur in the telophases of the last pre-meiotic division, so that they are already paired when the mother-cell nucleus is formed. The so-called leptonema or zygonema stage of the nucleus, therefore, in *Thalictrum purpurascens* does not originate by the transformation of a reticulum. The leptonema spirems are already present in the nucleus at the period of its greatest chromatic distribution. Just previous to the time when the synaptic contraction first becomes evident, the substance of the prochromosomes often spreads out along the linin intervals (Figs. 6, 7, and 8, Pl. I).

The first indication of the synaptic contraction in *Thalictrum purpurascens* consists in a concentration of the paired spirem threads either in the centre or at one side of the nuclear cavity (Figs. 6, 7, 8, Pl. I), so that the nucleus appears much more open and clear than at earlier stages. During the earlier stages of this contraction the threads, which constitute the framework, are present (Figs. 6, 7, Pl. I), but these later disappear as the whole mass rounds up (Figs. 8, 9, Pl. I). Fig. 9, Pl. I, is an exceedingly highly magnified figure of an early phase of the contraction. In my former paper I have described the phenomenon of synapsis, which I held to be a normal process, in which a union in pairs of homologous elements is brought about, with a consequent pseudo-reduction. Although synapsis was described as a phase, during which a mutual interchange or interaction of parental elements might occur, I also expressed the opinion that the homologous chromosomes retained their identity during the process. A continuous bivalent linin spirem is thus produced, in which the bivalent chromosomes are present at more or less regular intervals. The paired chromosomes and linin threads are very closely associated, yet each prochromosome of each pair is distinctly visible except when one lies above the other. Fine linin threads run to the periphery of the nucleus apparently suspending the synaptic mass in the nuclear cavity. As this is only a section of the nucleus the nucleole is not present. Figs. 10 and 11, Pl. I, show complete contraction stages, in which the prochromosomes, as well as the linin threads, are still evidently in pairs. The nucleole may or may not be entangled in the mass, as these figures show. Fine beaded linin threads run from the contraction mass to the periphery of the nucleus. Whether or not they are

connected with the cytoplasm I have been unable to determine. In this plant I have not discovered any relation between the position of the synaptic mass and the side of the nucleus nearest the cell wall as observed by Marquette ('07 and '08). In some cases the nucleus lies close to the cell wall, and the mass may be on this side (Fig. 10, Pl. I), and again the reverse is true. There is no apparent polarity in these cells.

In my studies on *Campanula grandis*, *Helleborus foetidus*, and *Podophyllum peltatum*, I found that the young post-synaptic spirem began to extend into the nuclear cavity in the form of more or less regularly arranged loops, each loop being connected through or with the nuclear membrane by one or more finely-beaded, delicate linin threads, which appear by their contraction to aid in the distribution of the post-synaptic spirem. Little by little these loops extend into the nuclear cavity, and it is then that the bivalent character of the spirem becomes very evident, its halves often diverging widely from each other. In these plants at this stage the number of these loops bears no constant relation to the number of the chromosomes, although in certain cases these loops correspond in number to the number of chromosomes. At that time I was unable to follow the behaviour of the post-synaptic chromosomes of *Thalictrum* and *Calycanthus* for reasons already mentioned.

In *Thalictrum* I have been unable at any stage in the development of the pollen mother-cells to find a continuous chromatic spirem. The linin and chromatin pass as distinct elements into the synaptic contraction. There is then a general loosening up of this mass. Each prochromosome seems to lengthen somewhat, suggesting a distribution of its chromatin along the linin (Figs. 12 and 13, Pl. I), but forming no continuous chromatic spirem. The spirem at this stage is simply a continuous bivalent linin thread with the chromatin of the parallel prochromosomes somewhat more distributed than in earlier stages (Fig. 13, Pl. I). Figs. 14, 15, Pl. I, show the chromatin and linin as still distinct. We see, then, that in this plant the prochromosomes may be traced through the synaptic contraction as individual bodies, which are certainly the same as those found in the post-synaptic spirem. Any regular folding of the thick spirem to bring these bodies together is absolutely impossible. They are associated in parallel pairs during pre-synaptic stages, and remain so until they assume the form found in the pachynema stages.

On account of the limits of the chromosomes being always visible, I have been able to follow the exact history of each univalent element from its entrance into the heterotypic spirem until the diakinetic stage. Fig. 14, Pl. I, shows how the bivalent chromosomes are arranged and distributed in the spirem. The chromatin of each univalent chromosome is still somewhat distributed along the linin as has been described above (Fig. 13, Pl. I), but the line which separates the two is still very apparent. The univalent

chromosomes are usually straight, with the two parts lying very close together. Sometimes, however, they may be somewhat bent, but, as Fig. 14, Pl. I, shows, they are eventually straightened out by contraction. The substance of each univalent chromosome contracts upon its own linin thread to form shorter bodies, leaving a more or less distinct double linin thread behind. Such appearances as is shown in Fig. 16, Pl. I, proves that each univalent chromosome condenses in its own linin thread. (Compare also Figs. 18, 19, and 20, Pl. I.) At this stage (Figs. 16, 17, and 18, Pl. I) univalent chromosomes are strikingly like, in size, shape, and staining reactions, the prochromosomes which appear in earlier prophases. (Compare Figs. 3 and 5 and Figs. 14 and 18, Pl. I.) The contraction of the chromosomes to the form of parallel double rods, which they have on the equatorial plate, is very gradual. The bivalent chromosomes are thus formed by a contraction of the pairs of univalent chromosomes upon the linin threads, which are probably incorporated into the chromosomes together with the chromatin. It is thus clear that the bivalent chromosomes are not formed by a bending or folding of the spirem as maintained by Farmer and Moore ('05).

The bivalent heterogeneous spirem, which is shown in Figs. 14 and 15, Pl. I, segments, forming the bivalent diakinetic chromosomes. Since the two univalent portions of the bivalent spirem may often be separated from each other, the two portions of each bivalent chromosome are also often entirely separated after the spirem segments. The chromatic portion of each univalent part of these structures contracts somewhat, the bivalent chromosomes appearing as short, rather thick double rods (Figs. 19 and 20, Pl. I). Fig. 21, Pl. I, shows two bivalent chromosomes still bound together by the bivalent linin intervals. The segmentation of the spirem has apparently been delayed at this point, leaving the two bivalent chromosomes still held together by linin. The four univalent chromosomes are distinctly visible. Such figures, which are not uncommon, show that the line of approximation (so-called longitudinal split) is the same as is found in the thick spirem. It is also evident from such figures that the stage represented in Figs. 22 *a*, Pl. I, could not possibly arise by any process of bending. The line which separates the two univalent chromosomes is therefore exactly the same line which has been visible since the two prochromosomes became associated in pairs in the early prophases.

Beside the parallel double rods (Fig. 22 *a*, Pl. I), which are the most usual figures found in *Thalictrum purpurascens*, other forms of figures occasionally occur. Fig. 22 *b*, Pl. I, represents an 8-shaped figure. Figs. 22 *c*, 22 *d*, and 22 *e*, Pl. I, are chromosomes taken from a late diakinetic stage. In Fig. 22 *c*, Pl. I, the two univalent portions are so orientated as to form an O-shaped figure. In the portion of the O at the right of this figure a longitudinal split is indistinctly visible. Just before the

nuclear membrane disappears and the chromosomes become arranged in the equatorial plate, each portion of the bivalent chromosomes shortens and thickens to form double rods, which eventually lie parallel to each other (Figs. 22 *d* and 22 *e*, Pl. I).

In diakinesis I have observed beside the chromosomes in most nuclei a linin-like substance present, very similar to that which Miyake ('05) and I have already described. This linin-like substance binds chromosomes together or to the nuclear membrane. In many cases portions of the linin intervals of the spirem remain which have not entered into the chromosomes during their concentration. Fine cross-threads are also present, resulting in a sort of linin reticulum. This arrangement of linin is plainly visible in Figs. 16, 19, and 20, Pl. I.

The formation of the spindle has not been followed in this plant with any particular detail. The formation of a felted zone, the dissolution of the nuclear membrane, and the formation of a multipolar spindle, which later becomes bipolar, seems to agree, in so far as I have been able to observe the stages, with the more recent results of several authors on spindle formation in the heterotypic division of higher plants. Allen ('03) has thoroughly described spindle formation in the pollen mother-cells of *Larix*, and Berghs ('05) has studied the process in *Paris quadrifolia*.

In *Thalictrum purpurascens* the nuclear membrane disappears and the chromosomes are arranged by means of the spindle fibres into the equatorial plate in characteristic fashion. The spindle fibres are attached to the chromosomes in bundles at definite points. Figs. 23 and 24, Pl. I, illustrate the position of the chromosomes during the formation of the multipolar and bipolar spindle. Each chromosome may be seen in these figures to be bivalent, with each of its parts forming a rather straight rodlet. I have never observed that the bivalent chromosomes of this plant at this stage ever form any other figures. They are always double rods. If we compare the size of these chromosomes in the equatorial plate with that of the bivalent structures of diakinesis or with the size of the chromosomes found in the thick spirem or even earlier stages, it is seen that they all are of essentially the same size. (Compare Figs. 3, 5, 19, and 23, Pl. I.) The chromosomes may be easily counted at this stage. Figs. 23 and 24, Pl. I, are sections, and the whole number of chromosomes does not appear in them. It is evident that the chromosomes are the same double rods or bivalent structures which were present in the spirem and also in earlier stages as paired chromosomes.

The orientation of the chromosomes in the nuclear plate and their behaviour during metaphase and anaphase is the same as I found it in *Campanula rotundifolia*, *Helleborus foetidus*, and *Podophyllum peltatum*, and as it has been described by Strasburger ('00), Mottier ('03), Allen ('05), and Miyake ('05). Since my studies on *Thalictrum purpurascens* confirm my

earlier results a detailed account would be a needless repetition. In this plant the chromosomes become arranged about the periphery of the equatorial plate and have the spindle fibres attached at their inner ends. The substance of the plastic chromosomes is often drawn out into a small point at the place of their attachment to the spindle fibres. In case the attachment is at one end, the separation of the two component chromosomes is usually from within outward (Figs. 25, 26, 27 *a*, and 27 *c*, Pl. I).

At the time the portions of the bivalent chromosomes are separating, the longitudinal split in each part becomes very apparent (Figs. 27 *b* and 27 *c*, Pl. I). These figures show very clearly that the chromosomes are four-parted (tetrads). Allen ('05) believes the appearance of this split to be very sudden in *Lilium canadense*. In Fig. 22 *c*, Pl. I, which represents a chromosome from diakinesis, the two univalent chromosomes are widely separated except at the ends. In the portion at the right of this figure a split is seen to be just appearing, or at least is first evident here. That the chromosomes are really tetrads at this stage is shown still more conclusively in *Calycanthus* and *Richardia*. As the parts of the bivalent chromosomes pass toward the poles of the spindle, the two halves of the split chromosome become plainly visible. That the parts of each bivalent chromosome separate and pass to the poles as double V's, as described by Flemming ('87), has long been held to be characteristic of the heterotypic division. These portions, according to Flemming ('87) and Meves ('97), undergo a longitudinal splitting as they diverge towards the poles, which has also been held to be characteristic of heterotypic mitoses. In *Thalictrum* the form of each univalent chromosome as it passes toward the pole is that of a V, each arm of which is formed by a longitudinal splitting of a single rod (Fig. 27 *b*, Pl. I). All four ends of the separating chromosomes are usually turned outward, away from the long axis of the spindle (Figs. 25, 26, and 27 *a*, Pl. I). Other figures may arise by a failure of the halves of each univalent chromosome to separate, or by their partial separation (Fig. 27 *c*, Pl. I). No matter how widely separated the two halves of the univalent chromosomes may become in the metaphases, they usually eventually come to lie close together and pass to the poles as double rods, being more or less bent or curved (Figs. 28 and 29, Pl. I).

I have made a detailed study of the later division stages of *Thalictrum purpurascens*. Fig. 28, Pl. I, shows the form and position of the chromosomes as they approach the poles of the spindle. Each chromosome, as in the earlier anaphases, may be seen to be composed of two parts. Fig. 29, Pl. I, shows these two parts very distinctly. As the chromosomes approach the poles they do not crowd together, as is so often the case in many plants, but remain rather widely separated. They may even be counted with ease in many cases. This wide separation of the chromosomes also obtains when they come to lie in the newly-forming cavity of the daughter

nucleus (Fig. 29, Pl. I). They also remain distinctly separated even after the formation of the nuclear membrane (Fig. 30, Pl. I). In Fig. 30, Pl. I, the daughter nucleus to the right of the figure shows the short two-parted chromosomes becoming arranged into a sort of spirem. The chromosomes are united by linin fibres into a single series. This condition shows distinctly in Figs. 31 and 32, Pl. I. In Fig. 32, Pl. I, which is a polar view of a daughter nucleus, I was also able to trace a continuous linin spirem, in which the chromosomes are arranged at rather regular intervals. These stages show that the chromosomes of the daughter nuclei become arranged into a linear series during the reconstruction stages. This spirem, however, is not a continuous chromatic spirem, but is heterogeneous, composed of chromosomes with intervals of linin as has been described for the prophases of the heterotypic nuclei. Each chromosome remains distinct. They may even be counted here, as they never are lost to view by a process of reticulation or alveolization. Although the chromosomes may be identified in the resting daughter nuclei, their small size renders them unfavourable for a very detailed study of their structure, but they often become somewhat distributed along the linin as in the prophases of the first division. In this spirem each chromosome manifests the split which occurred in the prophases of the first division (Figs. 30 and 31, Pl. I). Each chromosome remains, therefore, two-parted in the spirem.

The daughter nuclei increase in size and the phenomena characteristic of this division apparently follow each other in rapid succession. The chromosomes appear in the same relative position and form in which they entered the nuclei. The segmentation of the heterogeneous spirem is the same as in the prophases of the first division. If the chromosomes are at all distributed they become condensed, losing what alveolar-reticulate structure they possess, and again appear as homogeneous bodies. Each chromosome, however, may be seen to be composed of two segments as in the preceding telophase (Fig. 32, Pl. I). As the nuclear membrane disappears the spindle fibres become attached to the chromosomes, arranging them in the equatorial plate (Fig. 33, Pl. I). The spindle fibres are attached at one end of the chromosomes separating the halves much as in most vegetative divisions (Fig. 34, Pl. I).

The reconstruction of the granddaughter nuclei is of interest, since the segments or chromosomes are visible as prochromosomes even in the nucleus of the pollen grain. Fig. 35, Pl. I, shows the four nuclei with the chromosomes still distinct. Fig. 36, Pl. I, shows how these univalent chromosomes are arranged into a spirem composed of chromosomes and linin portions. During the reconstruction of these nuclei the chromosomes become arranged into this heterogeneous spirem by means of linin connexions or intervals. Each chromosome is, however, distinct, nor are the chromosomes entirely reticulated or alveolized in these nuclei up to the

stage of the ripe pollen. The prochromosomes are visible as univalent structures in the pollen grain and are arranged in a single series (Fig. 37, Pl. I). There is every reason to believe that, since the chromosomes appear as unreticulated bodies in the mature pollen grain, as well as in all preceding nuclei, they probably persist as permanent structures in the nuclei of the generative cells.

II. Calycanthus floridus.

In my former study of *Calycanthus floridus* I presented evidence to show that the chromosomes were everywhere permanent structures, being represented both in resting somatic and germ nuclei by chromatic aggregation or prochromosomes, just as in *Thalictrum*. I was, however, at that time unable to trace these bodies through all the stages of the development and division of the pollen mother-cells of *Calycanthus*. I am now able to present the then missing stages, and to show that the chromosomes retain their permanence during all stages, as in *Thalictrum*.

The pre-synaptic nucleus of *Calycanthus floridus* resembles that of *Thalictrum purpurascens* in that there is a distinct parallel arrangement of chromatin and linin elements, as I have described in a former paper. The chromatin is usually massed into definite bodies (Fig. 1, Pl. II). The prochromosomes are arranged in pairs, with parallel linin intervals, as has already been described for *Thalictrum purpurascens*. These pre-synaptic heterogeneous spirems are connected by cross linin strands, which are usually much more numerous and finer or thinner than the linin intervals of the spirem. There is a tendency in this plant for many of the prochromosomes to lie close to the nuclear membrane. The nucleus is much larger than in *Thalictrum purpurascens* and the chromosomes are fewer in number (24), so that the nucleus presents a much more open appearance. In some cases the chromatin of the prochromosomes is not entirely massed at definite points (Fig. 2, Pl. II), but may be somewhat spread out along the linin threads, a condition which I have never observed in *Thalictrum purpurascens*. The parallelism of the pre-synaptic elements is maintained whether the chromatin is much or little distributed (Figs. 1 and 2, Pl. II). The prochromosomes are, however, perfectly distinct in such nuclei, and may be easily counted. The parallel linin threads are also distinct in such cases. If the chromatic substance of the prochromosomes were still more distributed along the parallel linin threads, so that the limits of the individual prochromosomes could not be distinguished so as to form an almost continuous chromatic spirem, we should have a structure essentially similar to that of the onion, as I have already described in a previous paper. *Podophyllum peltatum* shows a spirem between the lily-type and that of *Calycanthus* and *Thalictrum*.

In *Calycanthus floridus* the prochromosomes vary in form and size in the pre-synaptic nuclei (Fig. 2, Pl. II). Fig. 9 *a*, Pl. II, shows that the prochromosomes are made up of granules apparently embedded in the linin substance. These granules in the two components of a pair are different in form and size, and apparently bear no definite relation in position to each other. The fine linin threads which lead off from each prochromosome connect with corresponding threads in other parts of the nucleus. At this stage the two prochromosomes may be much more closely associated, so as to appear as almost fused (Fig. 9 *b*, Pl. II). The granular structure may be invisible and the prochromosomes may appear as homogeneous structures, showing no trace of granules (Fig. 9 *c*, Pl. II). In this case the prochromosomes are short, very dense bodies, resembling those of *Thalictrum purpurascens*. At this stage the prochromosomes may also be long, narrow structures (Fig. 9 *d*, Pl. II). In this case there is again a granular structure, with apparently similar groups of granules in the two prochromosomes often lying opposite each other.

Fig. 9 *e*, Pl. II, represents a condition found in very early synapsis, when the linin intervals of the spirem threads have somewhat shortened, bringing the chromatic portions closer together. There is an evident condensation of the chromatin, which continues until the prochromosomes appear all alike in size and shape and become homogeneous in late synapsis (Figs. 5 and 6, Plate II). Figs. 9 *f* and 9 *g*, Pl. II, show the form and structure of these bodies in late synapsis, which they also retain in the post-synaptic spirem (Figs. 7 and 8, Pl. II).

I have in a previous publication pointed out that the fine cross linin threads which connect the pre-synaptic elements gradually disappear in *Calycanthus floridus*, leaving two distinct parallel spirems, which pass gradually into the synaptic contraction (Fig. 3, Pl. II). These spirems approach each other very closely in the neighbourhood of the nucleole (Fig. 4, Pl. II). There is an apparent condensation or diminution of the chromatic substance of each prochromosome as this synaptic contraction proceeds (Figs. 3, 4, and 5, Pl. II), so that the stainable portion of each prochromosome is much less when complete contraction is reached than at earlier stages. The prochromosomes at this stage show no very distinct granular structure, but appear as homogeneous bodies, staining uniformly black with haematoxylin or red with safranin (Fig. 6, Pl. II). Further details of the behaviour of the prochromosomes have already been fully described in my former paper, and the reader is referred to that study for a complete account of synapsis in this plant.

The bivalent heterogeneous spirem, when it first begins to pass out of synapsis and to be distributed in the nuclear cavity, appears in the form of loops, as in *Thalictrum purpurascens*, and as has been described for other forms generally in this stage (Fig. 6, Pl. II). These loops are not

constant in number and bear no apparent relation to the number and arrangement of the chromosomes. The prochromosomes may be placed anywhere in the loops and are not at definite fixed points. The loops become the uniformly distributed spirem, which has much the same appearance as that of *Thalictrum purpurascens*, except that the bivalent chromosomes are smaller in proportion to the size of the nucleus. There is no continuous chromatic spirem present (Figs. 7 and 8, Pl. II). It is a bivalent structure consisting of bivalent chromosomes and bivalent linin intervals. Segmentation of the spirem and segregation of the bivalent chromosomes take place in exactly the same way as has been described above for *Thalictrum purpurascens*, and need no further description here.

Fig. 10, Pl. II, represents a section of the nucleus in late diakinesis, in which the bivalent chromosomes are scattered freely in the nuclear cavity. The figures from this stage onward I was unable to obtain in my former study, and I have, therefore, drawn a complete series in order to trace the chromosomes throughout the various divisions. The forms of the diakinetic chromosomes are shown in Figs. 11 *a*–11 *e*, Pl. II. The parallel double rods are the usual forms, but variations may occur, which are, however, exceptional. Fig. 11 *a* shows the two parts of a bivalent chromosome twisted closely about each other. In Fig. 11 *b* they form a figure 8, with the two portions united at the ends. In Fig. 11 *c* the two components are united at the ends and are wide apart in the middle, forming an open ring. The form shown in Fig. 11 *d* is that of the chromosomes in which the two univalent portions are united at one end to each other. Nearly all the chromosomes in Fig. 10, Pl. II, are of this type. Fig. 11 *e* shows the characteristic double rods, which are somewhat separated in the middle but loosely attached at the ends. It is interesting to observe that in this plant it is the chromatic aggregations of the bivalent spirem which form the same sort of figures as the entire segments of the bivalent spirems produce in forms like the lily. I have constantly observed at least one bivalent chromosome, which is apparently much longer than the others, and am of the opinion that it is this chromosome which forms the 8- and O-shaped figures described above, while the shorter chromosomes assume the shape of double rods with the portions either parallel or separated at one end.

In Figs. 11 *c* and 11 *d* the longitudinal splitting of each univalent chromosome, which has been regarded as a second longitudinal fission, is visible, thus forming four-parted structures. That this split occurs by a longitudinal cleavage in each univalent component of the bivalent chromosomes, these figures show beyond a doubt. Since each univalent chromosome of the diakinetic pairs has been identified from the earlier stages, and since the line of separation of the univalent portions of each pair has been found to be the line of approximation, the appearance of a fission in each component of the pairs must certainly be a new splitting, and is not

to be identified with the longitudinal approximation line of the thick spirem. It is an entirely new fission, which splits the univalent chromosomes in half, forming four-parted chromosomes. In plants in which the approximation line can be identified during the various stages in the development of the bivalent heterotypic chromosomes, there is absolutely no danger of confusing this split with the approximation line.

A multipolar spindle is formed which later becomes bipolar, and the four-parted chromosomes become oriented in the equatorial plate as in *Thalictrum purpurascens*. Fig. 12, Pl. II, is a polar view of an equatorial plate stage, in which nearly all of the chromosomes may be seen to be four-parted as viewed from the end. Fig. 13, Pl. II, shows a spindle with the chromosomes in the equatorial plate. In each of these figures one chromosome is considerably larger than the others. In the last-mentioned figure the chromosomes are shown also as four-parted structures, forming the characteristic double V's as I described them for *Thalictrum purpurascens*. Figs. 14 *a* and 14 *d*, Pl. II, illustrate the characteristic forms of the four-parted chromosomes as their portions are being separated preparatory to passing to the poles. These portions are split, and the halves diverge at the same time. In Fig. 15 *a*, Pl. II, which represents an anaphase stage of the division, the receding univalent chromosomes are distinctly two-parted, just as they are in *Thalictrum purpurascens*. Fig. 15 *b*, Pl. II, shows one of these receding chromosomes on a larger scale, with the two parts lying close together. Fig. 16, Pl. II, which is a drawing of one pole of the spindle, shows that the chromosomes approach each other very closely as they approach the pole. Only four of the twelve chromosomes appear in this section. The reduction division is thus completed. The reconstruction of the daughter nuclei follows rapidly. Fig. 17, Pl. II, represents a polar view of a daughter nucleus with the chromatin in as complete a condition of distribution as I have been able to find. In this figure the chromosomes are still visible, with the two portions lying parallel. No continuous chromatic spirem is formed. The phenomena of reconstruction of the daughter nucleus in *Calycanthus floridus* is identically the same as has already been described for *Thalictrum purpurascens*. Figs. 18 and 19, Pl. II, show both polar and side views of homeotypic division spindles, with the two-parted chromosomes arranged in the equatorial plate. Fig. 20, Pl. II, is a drawing of a pollen grain, in which the nucleus may be seen to contain twelve prochromosomes as single unpaired structures. Each prochromosome is exactly the same size as each prochromosome in Fig. 1, Pl. II. In the first case these bodies are paired. In the pollen grain nucleus they are present as unpaired structures. The phenomena have been described for *Thalictrum*, and the prochromosomes are also present in the pollen grain, arranged into a single series.

III. Richardia africana.

The Monocotyledons so far as at present described are not found to show prochromosomes in all stages of the resting vegetative and germ nuclei. I find, however, that in *Richardia africana* the prochromosomes are constantly present as visible, well-defined bodies, which correspond to the chromosomes in number as I have described for *Thalictrum purpurascens* and *Calycanthus floridus*.

I have investigated the pollen mother-cells of the common Calla (*Richardia africana*). The material, which was obtained from greenhouse plants, was fixed in Flemming's fluid. The chromosomes in this plant have been traced throughout the various stages in the development of the pollen mother-cell, in which they persist as permanent structures similar to those of *Thalictrum* and *Calycanthus*. *Richardia* is especially interesting on account of the early appearance of the longitudinal splitting of each component of the bivalent chromosomes. The material is also especially favourable as showing the permanence of the chromosomes during the reconstruction stages of the daughter nuclei, and the prophases of the homeotypic division.

The nuclei of the young pollen mother-cells of this plant are not as large as those of *Calycanthus*, but are considerably larger than those of *Thalictrum*. The pre-synaptic nuclei of *Richardia* show essentially the same conditions as have been described for *Calycanthus*. Fig. 1, Pl. III, shows a pre-synaptic nucleus, with the prochromosomes well defined. The linin framework is also very apparent, but the parallel arrangement of the linin connecting intervals, which is so markedly present in *Thalictrum*, is not so easily made out in *Richardia*.

The structure of the prochromosomes is shown in Figs. 9 *a*–9 *c*, Pl. III. The chromatin is densely massed (Fig. 9 *a*, Pl. III), or may be somewhat spread out along the linin intervals (Fig. 9 *b*, Pl. III) so that a row of chromatic groups may be distinguished. The chromatic groups are also often scattered along the linin threads, being thus somewhat separated from each other (Fig. 9 *c*, Pl. III). In this stage the chromatin and linin intervals are frequently parallel.

Figs. 2 and 3, Pl. III, represent the beginnings of the synaptic contraction, which condition is much more advanced in Fig. 3 than in Fig. 2. There is a distinct parallel arrangement of elements, but the limits of the prochromosomes are not easily made out (Fig. 2, Pl. III). In Fig. 4, Pl. III, the contraction is still more complete. Fig. 5, Pl. III, represents a complete contraction stage, in which the elements are shown massed together much more compactly than in either *Thalictrum* or *Calycanthus*. In the complete contraction stage the prochromosomes are still plainly visible, arranged in parallel pairs. The nucleole may or may not be entangled in the synaptic

mass as is shown in Figs. 5 and 6, Pl. III. The synaptic contraction is a gradual process as in the other plants studied.

Later stages of the synaptic contraction are shown in Figs. 7 and 8, Pl. III, in which the chromatin is grouped in masses on parallel linin threads, but it soon begins to spin out along these threads, until an almost complete homogeneous spirem is formed in synapsis (Fig. 10, Pl. III). Figs. 9 *a*–9 *c*, Pl. III, illustrate the behaviour of the chromatin as it passes through synapsis. In Fig. 9 *c*, drawn from a late synapsis stage, a spirem is beginning to be formed, which at first glance is homogeneous or continuously chromatic. This condition just immediately precedes the redistributed spirem (Fig. 10, Pl. III). As in *Thalictrum* and *Calycanthus*, I have been unable to find distinct, regularly-arranged chromosomes in the spirem as is often described for *Lilium* and *Allium*. Fig. 11, Pl. III, shows a completely distributed post-synaptic bivalent spirem, and needs no especial mention. The two univalent portions of such a double spirem are distinctly visible at this stage (Fig. 12 *a*, Pl. III). The bivalent spirem much resembles that of *Campanula grandis*, or the lily-type, which I have described in my former paper. Although the chromosomes appear so evenly distributed in the spirem, I have been able to distinguish the individual limits of the chromosomes by the fact that the places in this spirem where two adjacent chromosomes unite end to end are always marked by thinner, less chromatic regions, as is shown in the above figures. I have been unable to discover the existence of a second synapsis, or any regular arrangement of the spirem into loops corresponding to the number of the chromosomes, as has been described for *Lilium* and *Podophyllum*. The post-synaptic spirem remains distributed in the nucleus until its segmentation. Since the chromatin of the chromosomes is more distributed along the linin threads of the spirem, the segmentation of the spirem is exactly like the process described for *Thalictrum* and *Calycanthus*, the details of which are represented in Figs. 12 *a* and 12 *b*, Pl. III. Since the chromosomes of *Richardia* are larger than in the other two plants, some phases of the process might possibly be interpreted as due to bending, but a careful study shows that the segmentation and consequent separation of each univalent portion of the spirem to form the corresponding univalent portions of the diakinetic chromosomes does not occur at the points of bending (Figs. 12 *a* and 12 *b*, Pl. III). The parts of the bivalent chromosomes often become twisted about each other (Fig. 12 *b*, Pl. III). Just after the segmentation of the spirem the chromosomes lie rather uniformly scattered in the nuclear cavity (Fig. 13, Pl. III). Figs. 14 *a*–14 *e*, Pl. III, are drawings of single chromosomes taken from diakinesis, and show the characteristic shapes of the chromosomes. The usual arrangement is that of parallel double rods (Figs. 14 *a* and 14 *b*, Pl. III), but there are some exceptions (Figs. 14 *c* and 14 *d*, Pl. III). Fig. 15, Pl. III, represents a late diakinetic nucleus just before the nuclear

membrane disappears, in which the chromosomes are all short parallel double rods. Figs. 17 *a*–17 *c*, Pl. III, show some conditions rarely observed in which these rods are arranged endwise or crossed to form an X-shaped figure. The most usual arrangement is that of parallel double rods (Fig. 17 *b*, Pl. III).

In diakinesis the longitudinal splitting of each component of the bivalent chromosomes occurs or becomes at first evident. This split apparently takes place before the diakinetic chromosomes become much shortened and thickened (Fig. 14 *e*, Pl. III). Each component of the parallel double rods splits lengthwise throughout its whole length, at right angles to the plane of approximation, giving rise to four parallel rods, or the so-called four-parted chromosomes (Fig. 16 *a*, Pl. III). When the diakinetic chromosomes become completely shortened and thickened, this split in each component is apparently obliterated for a time (Fig. 17 *b*, Pl. III). In Fig. 16 *b*, Pl. III, the four parts are also distinctly visible, but are differently oriented with reference to each other, forming an X-shaped figure with each arm of the X consisting of two parallel portions. In some cases the halves of each univalent portion may become more or less separated from each other at this period, so that various figures may arise depending upon the relative positions of the two split portions of the chromosomes. If these are placed end to end (Fig. 17 *a*, Pl. III), the four portions may spread apart at their inner juncture, forming a double V-shaped figure with the two halves facing each other (Fig. 16 *c*, Pl. III).

The arrangement of the chromosomes in the equatorial plate and their separation during metaphase are similar to those which I have described for *Thalictrum* and *Calycanthus*. The multipolar spindle (Fig. 18, Pl. III) later becomes bipolar (Fig. 19, Pl. III), and its poles are rather more pointed than in *Thalictrum*. Fig. 20, Pl. III, shows a polar view of the equatorial plate, with the sixteen chromosomes placed in different planes. In Fig. 19, Pl. III, which is a side view of the same, the majority of the chromosomes are shown in late metaphase. Some chromosomes in this figure are shown as having already separated, and as just beginning to pass to the poles. Figs. 21 *a*–21 *e*, Pl. III, show the various forms of the heterotypic chromosomes during metaphase. As the chromosomes pass toward the poles of the spindle, they show distinctly as two-parted structures (Figs. 23 *a*–23 *d*, Pl. III).

In studying *Richardia* I have especially focussed my attention upon the arrangement of the chromosomes and their behaviour during the reconstruction of the daughter nuclei. This plant furnishes especially favourable material for the study of the nuclear structure during these stages. As the sixteen, short, rather thick, two-parted chromosomes approach the poles, they are widely separated from each other. They keep this arrangement as they arrive at the poles (Fig. 24, Pl. III). As development proceeds

each chromosome of the daughter nuclei may be followed as an individual entity, but I have not been able to follow the origin of the generic structure during the reconstruction stages. Each chromosome undergoes very little change, except to become slightly elongated and somewhat thinner (Fig. 25, Pl. III). At this stage the nuclear membrane is present. The chromosomes appear to be irregularly distributed in the nuclear cavity. One may often find chromosomes so closely associated as to appear united at their ends, while others lie apparently free in the nuclear cavity (Figs. 25 and 26, Pl. III). Ultimately, however, all the chromosomes are arranged into a spirem, which is not entirely chromatic, but consists of chromosomes and linin intervals, just as I have described for the prophases of the first division. Each chromosome may have its chromatic portion somewhat distributed, but it is always seen to consist of its two daughter segments arranged side by side in this spirem. These daughter segments never separate or open apart as has been described for *Lilium*. In this stage the chromatin material appears somewhat reticulated (Figs. 26, 27, and 28, Pl. III), but the chromosomes are still plainly distinguishable at the period of their greatest distribution. Anastomoses connect the various chromosomes. No matter how vacuolated or reticulated a daughter segment may become, a portion is always present, which is a denser central portion by which each chromosome may be identified (Fig. 28, Pl. III). From this study it is certain that the chromosomes do not lose their identity, but persist as permanent structures in the reconstruction of the daughter nuclei of the first maturation division as they do in all resting stages.

During the above described processes, the daughter nuclei increase in size. In the prophases of the homeotypic division the chromosomes again appear more condensed, occupy the same relative position, and have the same form as they entered the daughter nucleus. They lose whatever alveolar-reticulate structure they have and again appear as homogeneous bodies. Each daughter segment of each chromosome is, however, distinct in the prophases. Fig. 29, Pl. III, shows the chromosomes lying free in the nuclear cavity just before the nuclear membrane breaks down. In Fig. 30, Pl. III, a pollen mother-cell wall is shown with two daughter cells in the process of division. One spindle shows the equatorial plate as seen from the side, and the other shows a polar view of the plate. Sixteen two-parted chromosomes are present. Fig. 31, Pl. III, represents an anaphase of the second division. Each chromosome is single. These single chromosomes form a univalent spirem (Fig. 32, Pl. III), during the reconstruction processes, as I have described for *Thalictrum*. I have also followed these chromosomes during the development of the young pollen grain, in which they appear as distinct, separate, univalent bodies or prochromosomes (Fig. 33, Pl. III), such as are found in the vegetative cells of the anther wall.

DISCUSSION.

The Permanence of the Chromosomes.

The majority of botanical as well as zoological writers recognize two essential parts of the nuclear reticulum. The linin, consisting of fine granules or thready substance, forms the general framework of the nucleus. My observations in general confirm these conceptions. I find a substance staining like portions of the cytoplasm present in the nucleus. This substance I have called the linin framework. It is in all probability a linin-like substance, although it seems to be quite distinct from the linin intervals, which connect the prochromosomes.

Rosen ('92) describes two sorts of nucleoles, eunucleoles and pseudonucleoles, the latter of which are identical with the chromatin. Zacharias ('95) also distinguishes two kinds of nucleoles in *Cucurbita Pepo*, one of which is chromatic in nature.

Rosenberg ('04) in studying the resting nuclei of *Capsella*, *Zostera*, and *Calendula* came to the very important conclusion that the chromosomes are represented in the extreme resting condition of the nucleus as definite bodies, which are to be identified, as he noted, with the pseudonucleoles of Rosen and Zacharias. He comes to the conclusion, 'dass die Chromosomen nicht etwa im Ruhestadium im Kern aufgelöst werden, sondern noch weiter bis zuletzt, wenn in etwas modifizierter Form, ihre Selbständigkeit beibehalten und also einen immer vorhandenen Teil, ich möchte sagen, Organ des Kerns ausmachen.' There can be no question, as Rosenberg ('07) in his work on *Hieracium* notes, that the prochromosomes, which I ('05) have found and described both in somatic and pollen mother-cells and which he has found in the pollen mother-cells of *Hieracium*, may well be likened to these bodies.

Laibach ('07) has repeated Rosenberg's work on *Capsella*, and also found chromatic collections corresponding in number to the chromosomes in several other Crucifers, but believes that each such collection represents a centre, about which the greater part but not all of the material of the chromosomes is collected. Not all nuclei among the Cruciferae show these centres or the chromatic collections.

Yamanouchi ('06) observed that in the nuclear reticulum of *Polysiphonia* chains of chromatin granules appear in irregular rows, which he regards as the 'beginnings of the chromosomes', and believes these structures are similar to the prochromosomes which I have described. These prochromosomes gradually become more pronounced and homogeneous to form the chromosomes as they appear in division stages.

In his investigations on pollen mother-cells of certain Monocotyledons

Miyake ('05) found certain chromatic collections often present, but determined that they did not always correspond in numbers to the number of chromosomes, and came to the conclusion that each chromosome was represented, not by a single chromatic mass, but by several such masses. Strasburger ('05) also found that similar chromatic collections were present in the somatic cells of *Galtonia*, and that there was an apparent correspondence in number to the chromosome number, a condition which did not obtain in *Funkia*. Tischler ('06) observed in *Bryonia* chromatic collections which varied in number and size, but came to no conclusion as to their relation to the chromosomes. The more deeply staining portions in the resting vegetative nuclei of *Phaseolus* and *Solanum* are regarded by Mano ('04) as portions of the chromosomes. Cardiff ('06) figures chromatic masses in resting meiotic nuclei which, however, do not correspond in number to the chromosome number. Norén ('07) also finds chromatic masses in *Juniperus*, which are more numerous than the chromosomes. Kirwood ('07) finds that the chromatin during the early prophases of division in the pollen mother-cells of certain Cucurbitaceae becomes distributed in masses, which he regards as representing chromosomes.

Prochromosomes, or bodies corresponding thereto, which bear some definite relation to the chromosomes either in number, form, or size, have been described for a rather large number of plants. We may summarize these results for convenience as follows:—*Capsella bursa pastoris* (Rosenberg, '04; Laibach, '07); *Zostera marina* (Rosenberg, '04); *Calendula* sp. (Rosenberg, '04); *Thalictrum purpurascens* (Overton, '05); *Calycanthus floridus* (Overton, '05); *Helleborus foetidus* (Overton, '05); *Campanula grandis* (Overton, '05); *Galtonia candicans* (Miyake, '05); *Polysiphonia violacea* (Yamanouchi, '06); *Hieracium auricula* (Rosenberg, '07); *H. venosum* (Rosenberg, '07); *Sisymbrium strictissimum* (Laibach, '07); *Brassica Napus* (Laibach, '07); *Stenophragma Thalianum* (Laibach, '07); *Alyssum Wierzbikii* (Laibach, '07); *A. argentum* (Laibach, '07); *Iberis pinnata* (Laibach, '07); *Lunaria biennis* (Laibach, '07); to which list we must now also add *Richardia africana.*

Moore and Miss Embleton ('06) are thoroughly convinced that in somatic and pre-meiotic cells the chromosomes may be identified by chromatic rods or masses, which resemble chromosomes of division both in their number, form, and size. 'In *Triton* each anlage (or prochromosome) becomes gradually enlarged and thickened into a long pre-meiotic chromosome of the spirem figure, while in *Periplaneta* they gradually assume the form of dense, short rods characteristic of pre-meiotic division figures of that arthropod. In both cases the chief interest of these bodies lies in the fact that they obviously represent the chromosomes of division during rest; and we may say without reserve that their presence at all stages of rest between the successive pre-meiotic divisions seems to con-

clusively prove the permanence of the chromosomes from one cell generation to another.'

Farmer and Moore ('05) have also observed in the cockroach that the limits of the chromosomes are usually visible even when the nucleus is in a condition of complete rest, although these bodies are not quite so definitely shown as in *Triton*. Moore and Walker ('05) have reported that similar chromosome primordia are also present in the meiotic cells of the guinea-pig.

Recently Dublin ('05) has observed that the chromosomes of *Pedicellaria* persist from the telophases of the oogonia to the prophases of the ovocyte.

Additional evidence of the permanence of the chromosomes has been brought out by the observations of Grégoire and Wygaerts ('03), Kowalski ('04), and Mano ('04); and the observations of Grégoire ('06, '07) tend to show that in nuclei in which there is an apparent disappearance of each chromosome during rest, they do, nevertheless, persist as individual or autonomous structures. The reticulum is formed by a progressive vacuolization and alveolization of each chromosome and union of these alveolized chromatic bands by lateral anastomoses. The chromosomes are, therefore, present in the nucleus as autonomous bodies, but in a different form from that in the division stages. The resting nucleus is then an 'association de chromosomes alvéolisés et réticulisés', each of which again becomes homogeneous during the prophases. These prophases appear to Grégoire and his students to strongly support the hypothesis of chromosome permanence and individuality. Haecker ('04) also believes that the resting reticulum is formed in a similar manner. In the epidermal cells of *Siredon* larvae he observed that the chromosomes of the telophase become alveolized from without inward, so that he was able to distinguish a peripheral 'grosswabigen Alveolenmantel' and an axillary 'gekörnelt erscheinenden Chromatinstrang', which later undergoes further alveolization. If these central granular strands had remained unalveolized a structure would remain by which one could absolutely identify each chromosome in the resting reticulum.

The organization of the nucleus as described by Rabl ('85) involves directly the permanence of the chromosomes. A comparison of the position and form of the chromosomes during reconstruction stages in the epithelial cells of *Salamandra*, with their position and form during the prophases of division, convinced Rabl that the chromosomes do not lose their individuality in the resting nucleus, and that they appear in the same relative position and forms in which they entered the reticulum. In the resting nucleus Rabl believed he could find traces of the chromosomes, and described a distinct polarity of the nucleus, in which the chromosomes converge toward a definite point. Out of the resting nucleus the chromosomes again

come into view due to the chromatic substance flowing back along predetermined paths into the primary chromosome bodies.

The fact that the chromosomes persist as distinct bodies (the prochromosomes), suggests that they may have definite positions and attachments to each other as noted above. One of the first observations as to the organization of the cell related to the definite orientation of the chromosomes with reference to a 'polar field'. I have not been able to find any connexion of the chromosomes with the cytoplasm, and it seems probable that such connexions are wanting, or much less definite in cells without centrosomes. Still Marquette ('07, '08), as noted, has observed in *Equisetum* and *Marsilia* that this synaptic contraction bears a definite relation in position to a mass of starch in the cytoplasm. A. and K. E. Schreiner ('05, '06), and Farmer and Moore ('05), and other zoologists have observed a certain definite relation between synapsis and the centrosome, and Harper ('05) has observed the same in the mildews. Synapsis may, perhaps, be an expression of one phase of the mechanics of division.

Montgomery ('03, '04) has called attention to the polarity of the nucleus in *Desmoganthus* during synapsis, and a large number of figures of recent workers, especially among zoologists, which relate to the synaptic contraction, strongly support Rabl's conception of polarity and permanence of the chromosomes. By an examination of the following figures one may observe that the chromatic threads converge thickly on the side of the nucleus on which the centrosome lies: see especially Eisen ('01), *Batrachoseps*, Figs. 12–15, Pl. II; Janssens ('01), *Triton alpestris*, Fig. 3, Pl. I; *Triton punctatus*, Fig. 32, Pl. I, Figs. 49, 50, and 60, Pl. II; Janssens ('02), Text-figs. 5, 8, 10, and 11; Schoenfeld ('02), Spermatogenesis in the bull, Figs. 3, 4, and 23 b, Pl. I, which show a less distinct polarity; Meves ('02), *Paludina*, Figs. 17, 18, and 19, Pl. I; A. and K. E. Schreiner ('04), *Myxine*, Text-figs. 2, 3, and 4, *Spinax*, Text-fig. 13; Farmer and Moore ('05), *Periplaneta*, Figs. 54–60 and 62, Pl. XXXIX; Figs. 68–72, Pl. XL; Moore and Robinson ('05), *Periplaneta*, Figs. 9, 10, 11, and 14, Pl. XLIV; A. and K. E. Schreiner ('05), *Myxine*, Figs. 48–54, 56, and 59-66, Pl. VIII; Figs. 69–72, Pl. IX; Figs. 168–70, Pl. XIII; Janssens ('05), *Batrachoseps*, Figs. 6, 7, 8, 14, and 15, Pl. III; Fig. 36, Pl. IV; Maréchal ('05), *Trigla hirundo*, Text-figs. 3, 5, and 7; *Gasterosteus aculeatus*, Text-figs. 6 and 7; *Amphioxus lanceolatus*, Text-figs. 21–23; A. and K. E. Schreiner ('06), *Tomopteris*, Figs. 16–23, Pl. I; Figs. 27–31, Pl. II, *Salamandra*, Figs. 8, 9, and 10, Pl. XXIII; Figs. 32 and 34, Pl. XXIV; *Spinax*, Figs. 48–51 and 53, Pl. XXIV; Fig. 56, Pl. XXV; *Myxine*, Figs. 90–94, Pl. XXVI; Maréchal ('07), *Pristiurus melanostomus*, Figs. 8–12, Pl. I; *Scyllium canicula*, Figs. 54, 56, and 57, Pl. III; *Gasterosteus aculeatus*, Fig. 64, Pl. IV; Van Mollé ('07), Spermatogenesis in the squirrel, Figs. 6, 7, and 8, Pl. I; Wassilieff ('07), *Blatta germanica*, Figs. 26–29, Pl. I, Figs. 30 and 31, Pl. II;

Popoff ('07), *Paludina vivipara*, Figs. 34 and 35, Pl. V, *Helix pomatia*, Figs. 88–91, Pl. VIII.

The studies of Harper ('05) and of Miss Sands ('07) on certain mildews, in which the chromatic strands are in continuous connexion with a permanent central body, furnish conclusive direct evidence that the chromosomes are permanent cell structures in these plants.

As the result of a series of experimental studies on *Ascaris*, Boveri ('04) has, perhaps, given the most complete statement of what he terms the 'Hypothesis or Theory of the Individuality of the Chromosomes'. He conceives the chromosomes as individual, elementary organisms, 'die in der Zelle ihre selbständige Existenz führen', a conception which Rabl ('06) has also adopted. Harper ('05) points out that it is, perhaps, questionable whether Boveri is justified in combining the conception of the permanence of the chromosome and the doctrine that they are individual or elementary organisms, which lead a relatively independent existence in the cell. He further doubts if permanence in number, form, and position in the nucleus even suggests such a conclusion, any more than that the cytoplasm is an individual organization because it grows and divides. Apparently no more is gained in support of the hypothesis of chromosome individuality by regarding the chromosomes as elementary, relatively independent organisms, which bear a symbiotic relationship to the cell than that they are definite permanent parts of a cell mechanism, having a permanent but not necessarily independent existence in the cell.

One of the strongest arguments of the individuality of the chromosomes is the fact that the size of the nucleus is dependent upon the number of chromosomes which it contains. Boveri ('05, '07) has shown that in sea-urchin larvae the surface area is proportional to the number of chromosomes which the nucleus contains. In nuclei which contain an abnormal number of chromosomes, he also finds that the abnormal number prevails during the subsequent divisions.

Němec ('04) found that in chloralized roots of *Pisum* many cells contain large nuclei with twice or four times the normal number of chromosomes. Strasburger ('07) has repeated these experiments, and obtained similar results. He finds evidence that the quadruple number, once established, persists. Evidence that the number of chromosomes determines the size of the nucleus is also furnished in the development of supernumerary pollen grains and nuclei of certain plants especially hybrids, as has been found by Strasburger ('82) and Juel ('97) for *Hemerocallis*, and by Cannon ('03), Tischler ('06, '08), and others for certain plants.

Results similar to those of Boveri, showing that the number of chromosomes determines the size of the nucleus, were obtained by Zur Strassen ('98) in the giant embryos of *Ascaris*. Boveri ('92, '95) in his studies on fertilized enucleated eggs of Echinoderms showed that exactly

the same number of chromosomes issues from a nuclear reticulum as enters it, which conclusions have also been supported by Herla ('95), Morgan ('95), and Zoja ('95), in their studies on *Ascaris*.

One of the principal objections raised against the hypothesis of chromosome individuality is the usual apparent loss to view of each chromosome, as Mottier ('03) claims for *Podophyllum* and *Lilium*, in the resting nuclei of many plants and animals. Even in such cases this objection is not entirely well founded, as Haecker ('04) and Grégoire ('07) have pointed out that the chromosomes are present as such in a more distributed form. They point out that during the telophases of division each chromosome by itself becomes transformed into a reticulum, so that the reticulum of a nucleus may be conceived of as being made up of a definite number of unit reticula.

Although Haecker ('04) admits the truth of the hypothesis of chromosome continuity, he holds that in most cases only portions of the old chromosomes ('Idomeren' or 'Kernbezirke') are present in the differentiation of the new ones. He believes that the disappearance of the stainable material can be harmonized with the doctrine of continuity by regarding the achromatic portion or linin, and not the chromatin, as the continuous substance. This hypothesis is at least superfluous in such cases as *Thalictrum*, *Calycanthus*, and *Richardia*, in which the stainable material does not entirely disappear during rest.

The Double Nature of the Somatic Nucleus.

The acceptance of the doctrine that the chromosomes are permanent and that the parental elements remain separate throughout the life cycle of the organism, involves the conception that every nucleus derived from the nucleus of the fertilized egg is double, as first pointed out by Haecker ('92), containing two separate sets of chromosomes. It is of especial interest to determine, as far as possible, the fate of the parental chromatins in the offspring, and questions at once arise concerning the arrangement and grouping of the parental chromosomes in both somatic and germ nuclei, and also concerning the qualities of these elements. Acceptance of the doctrine of chromosome individuality also means that, at some period in the life cycle, these parental elements must be segregated, sorted out, or newly arranged, preparatory to being distributed to the gametes, and thus handed on from one generation to another. The facts thus established as to the behaviour of the chromosomes and their constancy in number in the sporophyte generation, show that each cell of the offspring, which develops from a normally fertilized egg, will contain elements one-half of which are purely paternal and one-half purely maternal in origin, and suggests the theoretical conclusion that the offspring will inherit equally from both

parents, as a result of the apparent persistence of the chromosomes as individuals from one generation to another. The final behaviour of the chromosomes during maturation of the germ cells, though they are not as numerous as the Mendelian characters are generally assumed to be, nevertheless, on the basis of the facts of symixis can be brought into accord with the Mendelian principles of heredity.

Although the earlier students of fertilization observed what was an apparent complete fusion of the germ nuclei, later workers have shown that in normal development fusion of the germ nuclei involves no fusion of the homologous parental chromosomes. Van Beneden ('83) showed that the germ nuclei of *Ascaris* give rise to two individual groups of chromosomes whose halves pass independently into the daughter nuclei. Rückert ('95) and Haecker ('96, '02) have shown that in *Cyclops* the parental chromosomes do not mingle, but persist as individuals and retain their parental grouping through several cell generations, possibly persisting through the entire life-cycle, which Conklin ('02) has shown to probably obtain in *Cripidula.*

Haecker ('02, '07), as a result of his and Rückert's ('95) studies in *Cyclops*, maintains that the more important parental nuclear substances do not commingle but remain distinct until the prophases of the first division, when the conjugation of the parental chromosomes occurs. Haecker ('07) has recently further developed his conception of the autonomy or the separate existence of the paternal and maternal nuclear parts ('gonomeres'), believing that this independence of the gonomeres is general in sexually produced animals and plants.

Haecker ('02) further maintains that the nuclei show their double nature by the presence of two nucleoles and two spirems, as well as by their two-parted or two-lobed form. Boveri ('04), however, points out that it is not essential in view of the theory of chromosome individuality, whether the parental nuclear portions have a spacial separation or not. The work of Moenkhaus ('04), however, on hybrid fishes, in which the parental chromosomes are of different sizes, points to a final commingling of the chromosomes, although they retain their individuality and the nuclei keep their two-lobed form with a single nucleole in each lobe.

The investigations of Blackman ('04) and his students, and of Christman ('05) on the rusts, show that the parental nuclei may remain perfectly distinct in the same cell for the entire period of the sporophyte. Hamburger ('04) maintains that the nuclei of conjugating *Paramaecia* do not entirely fuse.

Blackman ('98), Chamberlain ('99), and especially Miss Ferguson ('04), have furnished evidence that the maternal and paternal chromatins remain distinct during fertilization, observing that during the first division of the fertilized egg-cell of *Pinus*, the parental chromatins form two separate spirems. Similar results have also been reported by Murrill ('00), in *Tsuga*,

and by Shaw ('98), in *Onoclea*. Dublin ('05) reports a similar condition for the bryozoan *Pedicellina*.

In testing Haecker's ('02) hypothesis of the independence of the gonomeres, Strasburger ('05) finds that in Guignard's ('91) figure of *Lilium Martagon* a double spirem stage appears, which may perhaps represent independent parental parts. He further examined the fertilized eggs of *Iris* and *Triticum*, without discovering any such separation of parental chromosomes.

Although from my own studies upon the vegetative nuclei of plants showing prochromosomes I believe the parental elements remain distinct, I am thoroughly convinced that there is no such spacial separation of the parental nuclear parts as Haecker maintains, not even of the nucleoles. In my former studies I ('05) concluded that the 'Chromosomen der Urmutterzelle bleiben in der Pollenmutterzelle als Prochromosomen kenntlich' and 'Diese Prochromosomen zeigen sich parallel zueinander in Paaren angeordnet' and further 'Es ist sehr wahrscheinlich, dass die eine Hälfte der präsynaptischen Chromosomen väterlichen und die andere mütterlichen Ursprunges ist'. By an examination of Figs. 1–4 and 24, Pl. VI, of my former work, which represent somatic cells, one may distinguish in many instances that the prochromosomes are arranged in pairs. Fig. 1, Pl. I, of the present study shows this condition distinctly, and I am very strongly convinced that the arrangement of the prochromosomes in somatic and young germ-cells is the same, that is, they are in parallel pairs. If this be so, there can be no constant independence of the entire pronuclei as Haecker maintains. Even though the parental parts may remain distinct for a while a final mixture occurs in such a manner, as I believe, that the homologous parental elements are brought side by side.

I have very carefully examined root-tips of *Calycanthus floridus* and have observed that in the resting nuclei the prochromosomes are arranged in pairs. The prochromosomes in these root-tips are quite as large as the ordinary somatic chromosomes and have the same shape, and I think there can be no question whatever that they represent the greater portion of the chromosomes. In these root-tips I have also found the chromosomes associated in pairs just as Strasburger ('05) has described them for *Funkia* and *Galtonia*, in which plants he found the chromosomes of different sizes associated in homologous pairs during the prophases. Strasburger ('07) further reports that in the root-tips of *Pisum*, the chromosomes are arranged in pairs during the equatorial plate stages.

The nucleus is, therefore, not only double in the sense that it contains two sets of parental chromosomes, but these chromosomes are so placed that there may be an interaction between homologous pairs. Allen ('05) points out that there is nothing in the evidence now at hand which absolutely negatives the possibility of an interaction between the two sets

of chromosomes present in each somatic nucleus. Evidence is now increasing which seems to me to greatly favour the existence of interaction. This interaction could best be accomplished if the homologous chromosomes are arranged in parallel pairs as Strasburger and I have described them, and not in different parts of the nucleus as Haecker would seem to have them.

Bateson ('07) suggests that the discovery of the possibility of an interaction between chromosomes involving the opportunity for an interaction between corpuscles representing distinct 'allelomorphic' pairs of characters constitutes one of the most important advances in genetics since the discovery of Mendel's work. It seems to me that the arrangement of homologous parental chromosomes carrying more or fewer characters in pairs in the somatic cells of a hybrid and the consequent interaction, which could thus occur, would greatly aid us in explaining the puzzling complexities of heredity.

The Conjugation of the Chromosomes in the Prophases of the First Division.

All recent studies tend to show that it is during the maturation of the germ-cells that the parental chromosomes conjugate. The origin of the bivalent heterotypic chromosomes by the association of a parental and a maternal one as first advanced by Henking ('91) and later by Montgomery ('00, '04), Winiwarter ('01), and Sutton ('02) has been shown to be general in all plants and animals studied in respect to this point, and many authors have agreed with Moore ('95) that synapsis is a most important phase in this process.

From what he considers the strongest evidence available, Montgomery finds the following series of changes in the spermatogenesis of animals. The normal number of univalent chromosomes persists through a number of successive generations of spermatogonia, the last generation of which produce the spermatocytes of the first order. At an early period in these spermatocytes a pairing of homologous parental chromosomes occurs and the bivalent chromosomes, by a junction end to end or side by side, become densely grouped in synapsis during the growth period. Montgomery ('05), however, recently argues that the homologous chromosomes, which unite later in synapsis, lie next to each other during the spirem stage of the spermatogonia of *Syrbula*.

Recently a number of zoological investigators are inclined to believe that the formation of the bivalent chromosomes occurs earlier than the prophases of the first maturation division. Montgomery ('00, '01) in his studies on *Peripatus* and *Hemiptera* maintains that the association in pairs occurs during the telophases of the last spermatogonial divisions, with which results those of Nichols ('02) on *Oniscus*, of Sutton ('02) on

Brachystola, of Blackman ('03, '05) on *Scolopendra,* and of Downing ('05) on *Hydra* agree. Dublin ('05) holds that in *Pedicellina* the chromosomes unite in pairs during the anaphases of the last spermatogonial division. Ancel ('02) and Miss Stevens ('03) find in *Helix* and *Sagitta* that the chromosomes are united in parallel pairs in the spermatogonia.

I desire also in this connexion to call attention to the fact that not only in somatic cells, but also in all the pollen mother-cells of the dicotyledons investigated by me in my former study, I was able to distinguish paired elements in all the pre-synaptic nuclei. In such plants as *Helleborus, Podophyllum* and *Campanula,* in which an apparent continuous chromatic post-synaptic spirem is found, and in which the individual limits of the post-synaptic chromosomes cannot be distinguished, the paired condition in the very youngest pre-synaptic nuclei is evident. See Figs. 25–27, 39 and 40, 53 and 54, Pls. VI and VII, of my 1905 communication. These observations convinced me that the chromosomes of the penultimate meiotic cells appear in the mother-cell nuclei in homologous pairs.

In order to emphasize the conception that the homologous chromosomes are already paired when they enter the mother-cell, the reader is referred to Figs. 2 and 3, Pl. I, which represent exceedingly young nuclei of *Thalictrum.* In these nuclei the prochromosomes as well as the linin intervals are unquestionably paired. That they remain so until synapsis or later is shown in Figs. 4–12, Pl. I. In Figs. 1, 2, and 3, Pl. II, the paired pre-synaptic elements are quite distinct in the cells of *Calycanthus.* Although this pre-synaptic pairing is less distinct in *Richardia,* yet Figs. 2 and 3, Pl. III, show that this condition exists in this plant.

It appears from my observation that this association of parental chromosomes in pairs occurs much sooner even than in the immediately preceding pre-meiotic cells. I have always observed the prochromosomes in pairs in all of the cells of the anther, as well as in all other somatic cells. It is highly probable that this association occurs during fertilization, and that the actual conjugation occurs during synapsis or associated stages.

That the association of parental nuclear parts may occur during the pre-synaptic stages is further shown by the recent observations of many students, especially among botanists,[1] many of whom present evidence to show that conjugation occurs by a side-to-side union of the pre-synaptic chromosomes. Differences, however, exist as to the exact period, when this union occurs. Allen ('04, '05) finds the pairing beginning before synapsis, but fusion occurs in synapsis. My own account in '05[2] is

[1] Grégoire ('04), Berghs ('04, '05), Allen ('04, '05), Rosenberg ('05, '07), Kirkwood ('07), Strasburger ('05), Miyake ('05), Overton ('05), Tischler ('06), Lagerberg ('06), Norén ('07), and Yamanouchi ('08).

[2] See also Cardiff's ('06), Lagerberg's ('06), Norén's ('07), and Yamanouchi's ('08) results.

essentially similar to that of Allen. Some authors[1] find that the threads, although perhaps present in pre-synapsis, first pair during the synaptic contraction. According to Grégoire and Berghs there is not a complete fusion even in synapsis, but an association of spirems.

A. and K. E. Schreiner ('05) express the opinion concerning the results of Strasburger ('05), Allen ('05), Miyake ('05), and Overton ('05) that these authors have not yet entirely established, either by their descriptions or figures, the standpoint taken, viz. that the conjugation is bound up with the contraction stage and that the chromosomes do not lose their identity, but appear as well formed and well determined structures. Above all, the Schreiners contend, one misses the representation of parallel threads in all the figures. Certainly one is compelled to admit that the Schreiners have clearly and beautifully represented parallel threads for *Myxine glutinosa*, *Tomopteris onisciformis*, *Salamandra maculosa*, and *Spinax niger* in certain stages, but the very earliest nuclei do not show such a parallelism of elements. It is only later in the history of these nuclei that the threads become parallel. To refer to only one point in this connexion, it is difficult to understand how a more distinct parallel arrangement of threads could be imagined than is shown by Allen's, Figs. 14 and 15, Pl. II, for *Lilium canadense*, and which is shown for a whole series of plants by my own figures, a parallelism so distinct as to even appear diagrammatic to Mottier ('07). In comparing the Schreiners' figures with my own I am of the opinion that the plants studied and described by me show the parallel threads much more distinctly than do the animals studied by them.

Mottier ('07) maintains that the pre-synaptic nucleus of *Podophyllum* shows no such distinct parallel arrangement of the elements as I have described, and cites his figures 1–4, Pl. XXVII, to support his views. Distinct masses of chromatin are present, with parallel linin threads often apparent, and closer examination might very well bring out the condition which I have represented. Mottier further believes that there is no definite or reliable evidence 'to support the prochromosome theory' as emphasized by Rosenberg, Miyake, and myself. He bases his conclusions upon studies of *Podophyllum* and *Lilium*. I have distinctly stated in my former paper that *Podophyllum* did not show such chromatin collections, and that the individual limits of the prochromosomes could not be distinguished in this plant. In this respect Mottier's conclusions agree with my own. One cannot, however, maintain that, because such collections are not present in *Podophyllum*, they do not exist in other plants. I see no reason whatever for a change of view, I am still convinced that in *Podophyllum*, although the prochromosomes are not distinguishable, there is a distinct parallel arrangement of the linin and chromatin just as I have described it in my former paper.

[1] Grégoire ('04, '07), Berghs ('04, '05), Miyake ('05), and Tischler ('06).

With minor variations the accounts of several zoologists concerning the pairing and fusion of the chromosomes during maturation show a substantial agreement with those of the above-mentioned botanists.[1] Complete agreement of opinion, however, does not exist concerning the manner of chromosome association or pairing or the time of fusion or interchange. According to most of those authors who advocate an end-to-end union of somatic chromosomes, the spirem becomes longitudinally split during contraction stages. Fusion has already occurred. Why the contraction stage should be favourable to the longitudinal splitting of the spirem has not been made clear.

There seems to be a tendency on the part of most cytologists to consider synapsis as meaning the massing of the chromatin and linin in the nuclear cavity, and since there is also entire uncertainty as to when the association, conjugation or interchange of influence or material occurs, Haecker ('04) has proposed the word *Syndesis* to apply to the conjugation or association of the homologous parental chromosomes. He would also use the word *Symmixis* to signify a chromosome pairing, in which there is an actual interchange of chromosome parts if such occurs.

The massing of the nuclear elements has been found to occur in nearly all plants and animals studied with reference to this point. To be sure there are some authors in common with McClung ('02, '05), Schaffner ('06, '07), Janssens ('04, '05) and Haecker ('07), who still maintain that it is either an artifact or else has no significance in the reduction process, but the best recent results seem to indicate that it is a natural phenomenon associated in some way with the process of maturation of the germ cell.

In addition to the investigations relating to the Angiosperms synapsis has been recently described for several other groups of plants. Cardiff ('06) has observed it in *Botrychium obliquum*, Farmer and Moore ('05) and Grégoire ('07) in *Osmunda regalis*, Yamanouchi ('08) in *Nephrodium molle*, Burlingame ('07) in certain Ophioglossiales, Farmer and Moore ('05) in *Psilotum triquetrum*, Marquette ('07, '08) in *Isoetes lacustris*, *Marsilia quadrifolia* and *Equisetum hyemale* and Strasburger ('07) in a large number of species of *Marsilia*. Cardiff ('06) describes synapsis in *Ginkgo biloba* and Norén ('07) in *Juniperus communis*, Farmer and Moore ('05) describe synapsis in *Aneura pinguis*. Among the Algae, Allen ('05) finds it in *Coleochaete*, and Yamanouchi ('06) in *Polysiphonia violacea*. Blackman ('06) and his students in their studies on the rusts find synapsis. Harper ('05) and Miss Sands ('07) describe it in the mildews, and Olive ('07) has found it in the Myxomycetes.

[1] See among others Winiwarter ('01), Schoenfeld ('02), A. and K. E. Schreiner ('04, '05, '06), Maréchal ('04, '05, '07), Marcus ('04), Tretjakoff ('05), Lerat ('05), Bonnevie ('05), Janssens ('05), Grégoire and Deton ('06), Schleip ('06), and Van Mollé ('07).

Probable Separation in Time of Association and Interchange.

It is quite apparent from a survey of the recent literature of reduction phenomena that there is a strong tendency to the view that the association in pairs of the homologous chromosomes and an actual interchange or influence, in so far as any may occur, preparatory to reduction division may be widely separated phenomena. The view is quite general that the possible interchange is associated with synapsis.

Although Juel ('05) believes that it is not necessary for chromosome union and fusion to occur uniformly at the same period in the maturation of the germ cells, I believe the facts of reduction as we now know them indicate a uniformity of the phenomenon of actual fusion in point of time. I am also inclined to agree with Rosenberg ('05) and Juel ('05) that the actual fusion occurs in the plants which I have studied during the early spirem stage rather than during synapsis or pre-synapsis.

Although the prochromosomes have always been found in parallel pairs during pre-synapsis, as is shown in Figs. 2–8, Pl. I, 1–3, Pl. II, and 1–3, Pl. III, the members of each pair are no more closely united during this period than they are in the somatic or pre-meiotic cells. Compare Figs. 9–11, Pl. I, 4–6, Pl. II, 4–7, Pl. III. During the early post-synaptic stages there is a close association of the homologous components of the pairs, so that each univalent chromosome is somewhat difficult to recognize.

In Fig. 12, Pl. I, *Thalictrum purpurascens*, the post-synaptic spirem is shown as just beginning to be distributed in the nuclear cavity. At this stage the two parallel portions are still quite distinct. In Fig. 13. Pl. I, which represents a portion of a much more distributed spirem, the univalent parts are very close together. Fig. 14, Pl. I, shows that the univalent portions of a completely distributed spirem are very much more closely associated than they are during pre-synaptic stages. Figs. 16–19, Pl. I, show how very closely the parallel portions are associated during post-synaptic and diakinetic stages. In Fig. 6, Pl. II, the synaptic contraction in *Calycanthus floridus* is shown as loosening up. Here the parallel portions are more closely associated than in the earlier stages (Fig. 5, Pl. II). Figs. 7 and 8, Pl. II, show the parallel portions of the distributed spirem as very closely associated. That the parallel portions of the bivalent post-synaptic spirem are more closely associated than during pre-synaptic stages in *Richardia africana* is shown by Figs. 8–11, Pl. III.

From my own observations it is evident that there certainly exist in pre-synapsis parallel pairs of chromosomes or chromosome primordia, which do not fuse during the synaptic stages, but become most intimately associated during the post-synaptic spirem stages. If symmixis is to occur, it is

apparent then that this period is most favourable for any interchange of discrete particles, or even for chemical influence.

According to some authors[1] the two filaments of each pair in the spirem fuse by means of their linin substratum, and an interchange of particles according to Allen and Strasburger could probably occur. If such an interchange does occur, it must take place in the spirem according to my observations. Berghs ('04, '05) and Grégoire ('04, '07) maintain that no such fusion occurs. 'Ces filaments demeurent, dans chaque paire, parfaitement distincts l'un de l'autre,' but they remain as distinct 'que le seraient deux doigts de la main entrelacés l'un autour de l'autre.' The persistent duality of the spirem has also been admitted by several other investigators,[2] but these authors have figured the two univalent portions most closely associated during the spirem stage, thus perhaps admitting of a possible symmixis.

Absence of a Continuous Chromatic Spirem.

Many authors have recently come to the conclusion that a continuous chromatic spirem does not exist. Strasburger ('05) was unable to find such a spirem in the somatic cell of *Funkia* and *Galtonia*. Grégoire and his students are also of the opinion that such a spirem is not present in vegetative and germ nuclei. Although a continuous spirem is found in *Podophyllum peltatum*, *Campanula grandis*, and *Helleborus foetidus*, I was unable to find one in *Calycanthus floridus* and *Thalictrum purpurascens*. My latest studies confirm my former observations on these two plants. *Richardia africana*, however, forms an apparently continuous chromatic spirem. In such cases as *Richardia*, *Allium*, &c., in which there is an apparent continuous chromatic spirem, there probably exist individual segments or chromosomes very closely united, without any very extensive linin intervals as described for *Thalictrum* and *Calycanthus*. Grégoire ('07) has shown that the individual chromosomes of the spirem converge toward a definite point in *Osmunda*, much as has been described for many animal cells, so that the absence of a continuous spirem seems out of the question in this plant.

Second Contraction Figure.

The second contraction figure, in which the chromosomes are arranged in a more or less regular fashion about a central point,[3] has been nowhere observed in these plants, and cannot be held to be characteristic of maturation divisions in general, although it may occur in some plants. Grégoire ('07)

[1] Allen ('04, '05), Strasburger ('05), and Lagerberg ('05).

[2] Overton ('05), Cardiff ('06), Maréchal ('04, '05, '07), Bonnevie ('05), Grégoire and Deton ('06), and Schleip ('06).

[3] This has been described as a second synaptic contraction by some authors. Compare Miss Sargant ('96, '97), Tretjakoff ('04), Farmer and Moore ('05), Allen ('05), Overton ('05), Schaffner ('06), Mottier ('05, '07), Griggs ('06), Grégoire ('07).

has observed that it may or may not be present in *Lilium speciosum*, and believes that it has no special significance. It appears that the phenomena of second contraction may occur in plants like *Lilium* and *Podophyllum*, which possess comparatively long chromosomes. It certainly does not occur in the plants with short chromosomes which I have considered, and I do not believe that any importance can be placed upon this phase.

Résumé.

1. In the somatic nuclei of *Thalictrum purpurascens* and *Calycanthus floridus*, the chromosomes are represented during rest by definite visible bodies, the prochromosomes, which are arranged in parallel pairs, with apparent linin intervals. These heterogeneous spirems, the homologous portions of which have become early associated in pairs, probably remain distinct throughout the life-history of the sporophyte.

2. Prochromosomes are also present in the resting nuclei of the germ cells of these plants and *Richardia africana* in exactly the same arrangement and form as in the somatic nuclei. The homologous parental elements are, therefore, already associated in pairs when they enter the reconstruction stages of the germ nuclei.

3. During the prophases of the heterotypic division these parallel parental heterogeneous spirems become more distinct, and the general linin framework of the nuclei disappears.

4. The synaptic contraction, or massing of the nuclear elements, which is regarded as a natural phenomenon, is a gradual process, during which the homologous parental spirems may become more closely associated. During synapsis the parental spirems remain distinct.

5. Since the homologous portions are most closely associated during post-synaptic stages, conjugation, interchange, or mutual influence probably occurs during the spirem stages.

6. Association of homologous chromosomes probably occurs during fertilization or shortly thereafter, but the actual interchange of parental parts or influence occurs during synapsis or related stages (see 5).

7. There is no continuous chromatin spirem at any stage.

8. There is no second contraction figure, which is regarded as having no significance in the reduction process.

9. The chromosomes persist as definitely limited visible bodies throughout all the prophases of the heterotypic division.

10. Each of the two parts composing each diakinetic chromosome represents a somatic chromosome. Homologous somatic chromosomes have become associated in parallel pairs very early in development of the sporophyte. No folding process to form these bivalent chromosomes seems possible.

11. Each univalent portion of each bivalent diakinetic chromosome undergoes a longitudinal fission, forming apparent tetrads.

12. The first or heterotypic division separates entire somatic chromosomes.

13. The chromosomes remain distinct between the telophases of the first and the prophases of the second division (i. e. during interkinesis).

14. The somatic chromosomes or prochromosomes appear in the pollen grain, arranged in a single univalent series.

MADISON, WISCONSIN,
July, 1908.

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DESCRIPTION OF FIGURES IN PLATES I, II, AND III.

Illustrating Prof. Overton's paper on the organization of the nuclei in the pollen mother-cells of certain plants.

All the figures were drawn with the aid of a camera lucida, and with a Bausch and Lomb 1/12 oil immersion or with a Zeiss apochromatic 2 mm. objective.

PLATE I.

Thalictrum purpurascens.

Fig. 1. Cell from anther-wall showing prochromosomes in pairs, linin framework and linin intervals.

Fig. 2. Very young pollen mother-cell showing prochromosomes.

Fig. 3. More highly magnified nucleus of young pollen mother-cell, in which prochromosomes are shown arranged in pairs with parallel linin intervals.

Fig. 4. Somewhat older pollen mother-cell than is shown in Fig. 2. Prochromosomes in pairs.

Fig. 5. More highly magnified nucleus of pollen mother-cell in about same stage as shown in Fig. 4. Paired prochromosomes, linin intervals and linin framework.

Fig. 6. Nucleus just previous to synaptic contraction.

Fig. 7. About same stage as shown in Fig. 6. The prochromosomes are arranged more on one side of the nucleus.

Fig. 8. Synaptic contraction has begun. The chromatin of the prochromosomes is somewhat spread out along the linin intervals.

Fig. 9. Highly magnified nucleus in early stage of synaptic contraction.

Fig. 10. Complete synaptic contraction.

Fig. 11. Complete synaptic contraction viewed more from above than Fig. 10. Prochromosomes and linin threads still in pairs.

Fig. 12. Synaptic mass loosening up. The chromatin of paired prochromosomes is somewhat spread out along the linin intervals.

Fig. 13. Portion of very young post-synaptic spirem, showing the relation of two consecutive pairs of prochromosomes to each other.

Fig. 14. Early stage of uniformly distributed spirem. Bivalent chromosomes with linin intervals still distinct.

Fig. 15. Somewhat older spirem stage than is shown in Fig. 14.

Fig. 16. Early diakinetic stage showing bivalent chromosomes with parallel linin intervals.

Fig. 17. Diakinetic stage. Somewhat older nucleus than is shown in Fig. 16.

Fig. 18. Still older diakinetic stage. Linin connexions still present.

Fig. 19. Diakinetic chromosomes distinctly shortened, with linin intervals less distinct.

Fig. 20. Diakinetic nucleus during early stage of spindle formation.

Fig. 21. Highly magnified figure of two bivalent diakinetic chromosomes still connected by linin intervals.

Fig. 22. Diakinetic chromosomes: 22 *a*. The univalent portions lie parallel. 22 *b*. The univalent portions are slightly twisted about each other. 22 *c*. Univalent portions separated in the middle, but still attached at the ends. Longitudinal splitting is visible in chromosome at the right of this figure. 22 *d*. The two univalent chromosomes have shortened, forming parallel double rods. 22 *e*. Same as Fig. 22 *d*, but the two rods have somewhat separated at one end.

Fig. 23. Multipolar spindle stage, showing position and form of several chromosomes.

Fig. 24. Spindle becoming bipolar. Chromosomes appear as short double rods.

Fig. 25. Equatorial plate stage. Chromosomes mostly in metaphase. Chromosomes shown as double V's.

Fig. 26. Equatorial plate stage. Chromosomes in late metaphase and in early anaphase.

Fig. 27. Bivalent chromosomes in various stages of division: 27 *a*. Side view of early metaphase; parts separating from within outward. 27 *b*. Late metaphase showing the double V-shaped figure. 27 *c*. Late metaphase, showing the position of the chromosome when the two halves of each univalent portion remain closely applied to each other.

Fig. 28. Spindle with chromosomes in late anaphase. Each chromosome shows a longitudinal splitting.

Fig. 29. Single chromosome in anaphase; each chromosome showing longitudinal splitting as it diverges toward the pole.

Fig. 30. Telophase of first division. Chromosomes still distinct but becoming arranged into a pseudospirem.

Fig. 31. Daughter nuclei formed. Pseudospirem present in each nucleus.

Fig. 32. Polar view of daughter nucleus showing pseudospirem.

Fig. 33. Equatorial plate stage of second division.

Fig. 34. Anaphase of second division.

Fig. 35. Pollen mother-cell with four granddaughter nuclei.

Fig. 36. Nucleus of young tetrad, showing arrangement of chromosomes into a single series with linin intervals.

Fig. 37. Young pollen grain showing prochromosomes in single series with linin intervals.

PLATE II.

Calycanthus floridus.

Fig. 1. Young pollen mother-cell, showing prochromosomes, linin intervals and linin framework.

Fig. 2. Young pollen mother-cell, showing chromatin not entirely massed in prochromosomes but somewhat spread out along linin intervals.

Fig. 3. Very early synaptic contraction, showing two distinct heterogeneous pre-synaptic spirems.

Fig. 4. Synaptic contraction somewhat more advanced with nucleole in the centre of the mass.

Fig. 5. Complete synaptic contraction showing the prochromosomes in pairs.

Fig. 6. Late synaptic contraction. Loops of the bivalent spirem begin to extend into the nuclear cavity.

Fig. 7. Post-synaptic distributed bivalent heterogeneous spirem in which chromosomes are visible.

Fig. 8. Post-synaptic spirem, showing distinct bivalency. Chromosomes and linin intervals are distinctly paired.

Fig. 9. Pairs of single prochromosomes drawn before and during synapsis, showing various forms and sizes: 9 *a*. Prochromosomes composed of granules embedded in linin. 9 *b*. Two components of a pair very closely associated. 9 *c*. No trace of granules. 9 *d*. Apparently similar groups of granules in the two components lying opposite each other. 9 *e*. Prochromosome pair in early synapsis, linin and chromatin somewhat contracted. 9 *f*, 9 *g*. Prochromosome parts from late synapsis. Chromatic substance much condensed.

Fig. 10. Late diakinesis stage, showing chromosomes much scattered in nuclear cavity.

Fig. 11. Chromosomes from early and late diakinesis, showing various forms: 11 *a*. Univalent portions closely applied and twisted about each other. 11 *b*. The two portions forming an 8-shaped figure. 11 *c*. The two portions forming an O-shaped figure. 11 *d*. The two portions placed endwise, also showing indication of longitudinal splitting. 11 *e*. Characteristic parallel double rods. Longitudinal split visible in portion at left of figure.

Fig. 12. Polar view of equatorial plate, nearly all chromosomes seen from end, showing four parts. One chromosome much larger than others.

Fig. 13. Side view of spindle. Chromosomes in late metaphase.

Fig. 14. Chromosomes from equatorial plate, showing method of separation:—14 *a*, 14 *b*. Lateral view of chromosomes during metaphase. 14 *c*. Later metaphase, longitudinal split in each daughter chromosome evident. 14 *d*. Metaphase with the four parts of the chromosomes entirely separated at their outer ends.

Fig. 15 *a*. Spindle with chromosomes in anaphase. Each chromosome may be seen as being two-parted as it passes to pole.

Fig. 15 *b*. Single two-parted chromosome in anaphase.

Fig. 16. One pole of spindle, chromosomes crowded together at pole.

Fig. 17. Polar view of daughter nucleus; chromosomes somewhat distributed in linin substance.

Fig. 18. Polar view of homeotypic division spindle, showing arrangement and form of chromosomes.

Fig. 19. Homeotypic division figures, one spindle viewed laterally and the other from the pole.

Fig. 20. Pollen grain showing prochromosomes as single unpaired chromatic bodies.

PLATE III.

Richardia africana.

Fig. 1. Very young pollen mother-cells, showing linin intervals and linin framework.

Fig. 2. Early synaptic contraction stage; prochromosomes and linin somewhat more distinctly parallel.

Fig. 3. Synaptic contraction still more advanced. Distinct parallel arrangement of elements. Limits of prochromosomes not distinct.

Fig. 4. More complete contraction stage. Chromatin rather more uniformly distributed in the spirem.

Fig. 5. Complete contraction stage. Nucleole entangled in the mass. Elements compactly massed.

Fig. 6. Complete contraction stage. Nucleole not entangled in the mass. Threads distinctly parallel.

Fig. 7. Synaptic mass loosening up. Bivalent chromosomes and linin intervals distinct.

Fig. 8. Still more complete post-synaptic bivalent spirem. Chromosomes and linin intervals distinct.

Fig. 9. Showing structure of prochromosomes: 9 *a*. Chromatin grouped in dense masses in parallel linin threads. 9 *b*. Chromatin spread out along linin. Two parallel series of chromatin masses visible in linin. 9 *c*. Chromatin masses still more scattered along linin.

Fig. 10. Nearly homogeneous chromatic post-synaptic spirem.

Fig. 11. Completely distributed post-synaptic spirem. Bivalent chromosomes and linin intervals distinct.

Fig. 12. Portions of the bivalent post-synaptic distributed spirem. 12 *a*. Showing limits of individual bivalent chromosomes. 12 *b*. Segmentation of post-synaptic spirem.

Fig. 13. Early diakinetic stage. Chromosomes widely scattered in nuclear cavity.

Fig. 14. Single chromosomes from various diakinetic stages: 14 *a*. Usual parallel double rods. 14 *b*. Same with each component somewhat contracted. 14 *c*. The two components crossed, forming an X-shaped figure. 14 *d*. Same with components attached nearer one end. 14 *e*. Parallel double rods slightly separated.

Fig. 15. Late diakinetic nucleus. Chromosomes all in form of short double rods.

Fig. 16. Diakinetic chromosomes showing four-parted structure: 16 *a*. Each component of parallel double rods shows longitudinal splitting. 16 *b*. Each component of X-shaped figure shows longitudinal splitting. 16 *c*. Each component shows longitudinal splitting. Parts separated to form distinct double V-shaped figures.

Fig. 17. Late diakinetic chromosomes: 17 *a*. Two components placed end to end. 17 *b*. Parallel double rods: usual arrangement. 17 *c*. Components attached at middle with ends separated. X-shaped figure.

Fig. 18. Multipolar spindle stage becoming bipolar. Arrangement of chromosomes also shown.

Fig. 19. Side view of equatorial plate stage showing chromosomes in late metaphase as early as anaphase.

Fig. 20. Polar view of equatorial plate, showing the sixteen chromosomes in different planes.

Fig. 21. Single bivalent chromosomes from equatorial plate, showing forms and method of separation of parts during metaphase: 21 *a*. Chromosomes being separated from within outward. 21 *b*. Separation nearly complete. Four parts showing distinctly. 21 *c*, 21 *d*. Four parts of the chromosome showing as double V's. 21 *e*. Double V's nearly separated.

Fig. 22. Side view of spindle showing chromosomes during anaphase. Each chromosome is two-parted as it passes to the poles.

Fig. 23. Single anaphase chromosomes. Each chromosome two-parted: 23 *a*. Spindle attached at one end. 23 *b*. Spindle attached at one end, completely contracted. 23 c. Spindle attached at middle. 23 *d*. Chromosomes near the poles.

Fig. 24. Showing arrangement of two-parted chromosomes at the spindle pole.

Fig. 25. Polar view of daughter nucleus. Chromosomes very slightly changed in form.

Fig. 26. Daughter nuclei. Chromosomes somewhat elongated, but still distinct.

Fig. 27. Daughter nucleus showing chromosomes arranged into a pseudospirem. Each chromosome may, however, be distinguished.

Fig. 28. Single segment of pseudospirem from daughter nucleus. Each chromosome may be seen to be a two-parted structure.

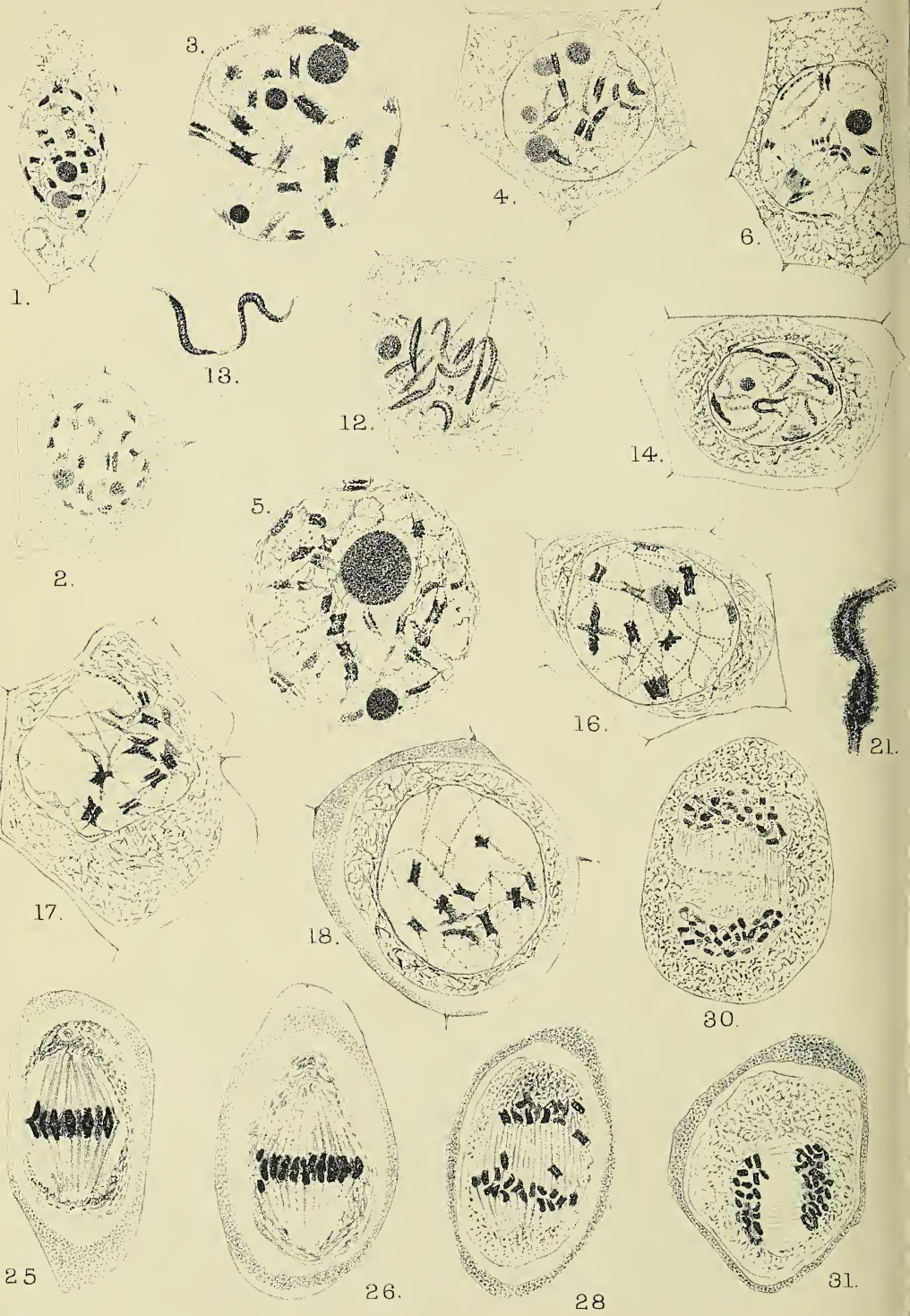
Fig. 29. Daughter nucleus in prophase of division. Chromosomes lie free in nuclear cavity.

Fig. 30. Two daughter cells in division within mother-cell wall. One spindle seen from side and one from pole.

Fig. 31. Anaphase of second division.

Fig. 32. Single chromosomes forming univalent spirem in granddaughter nucleus.

Fig. 33. Young pollen grain, showing single chromosomes in the resting nucleus as distinct bodies (prochromosomes) arranged into a single series.



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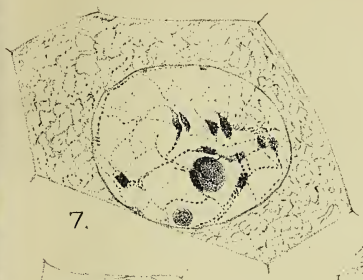
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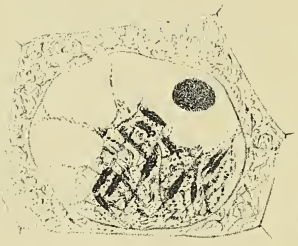
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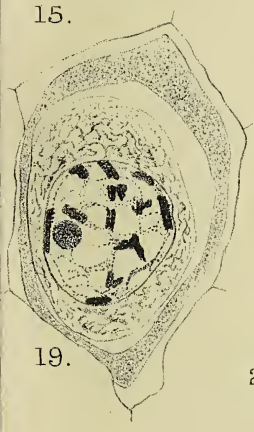
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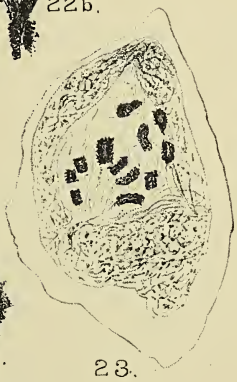
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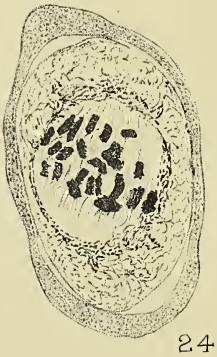
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27b.


27c.


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22d.


22e.


36.


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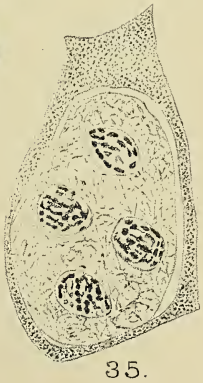
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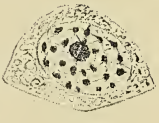
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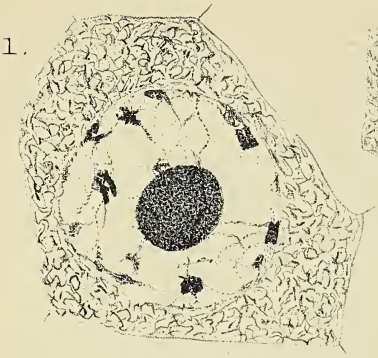
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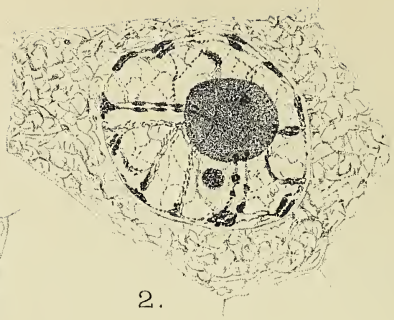
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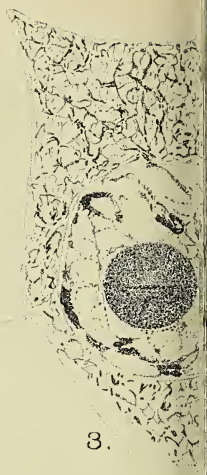
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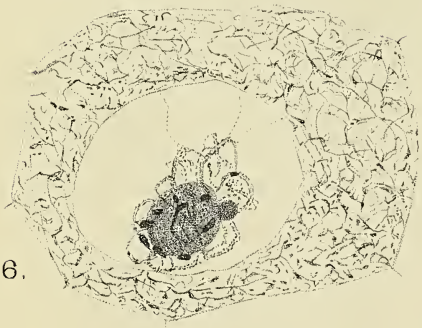
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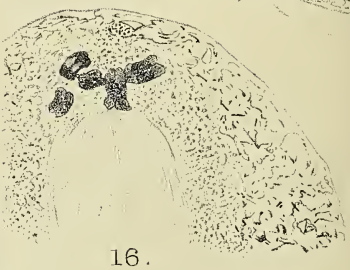
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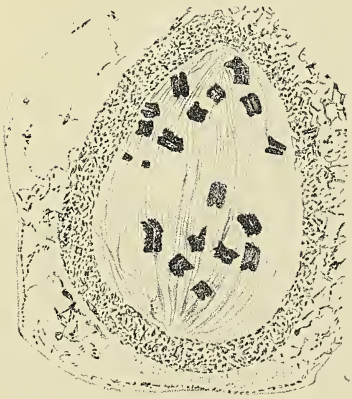
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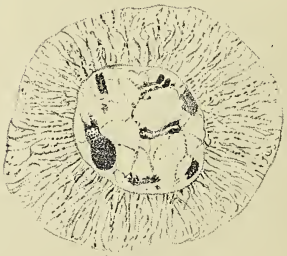
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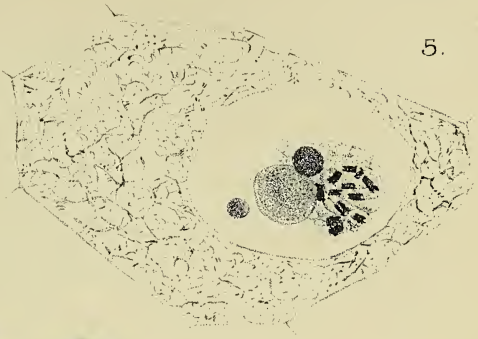
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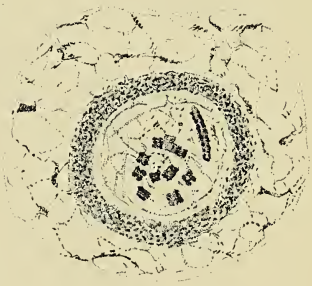
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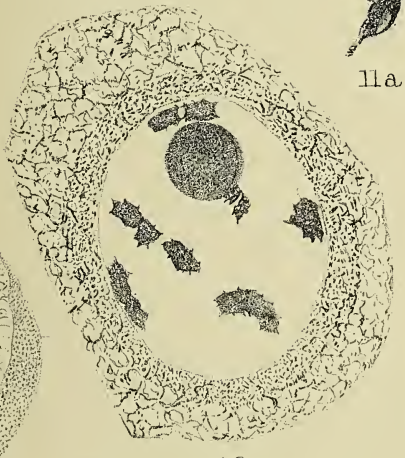
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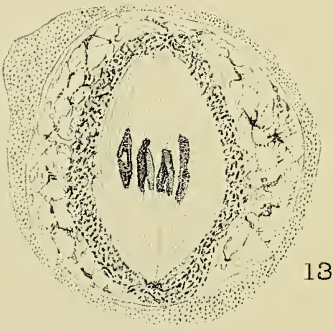
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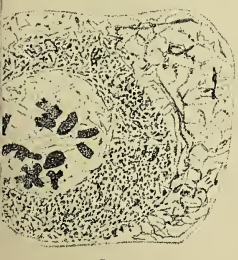
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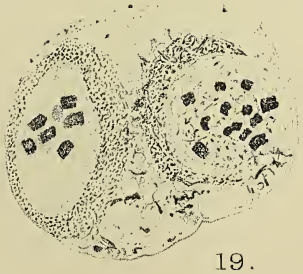
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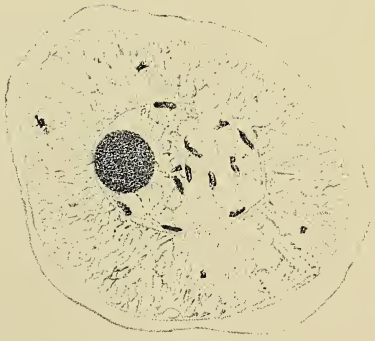
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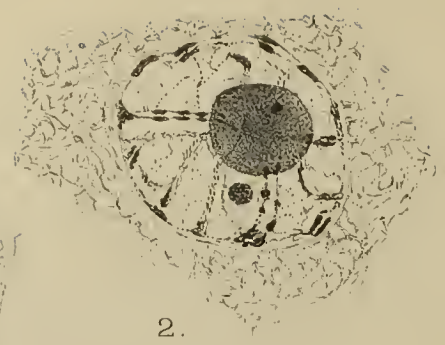
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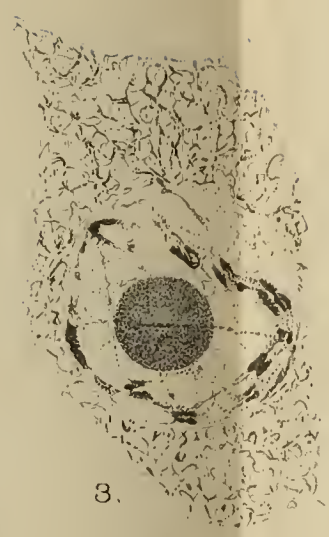
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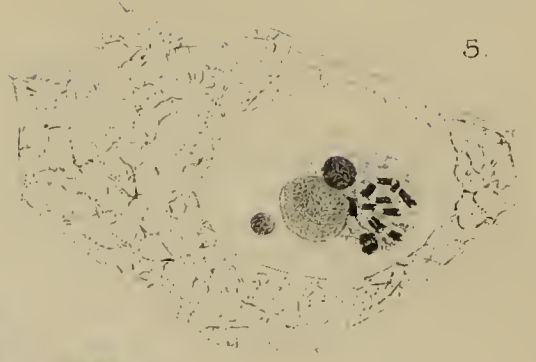
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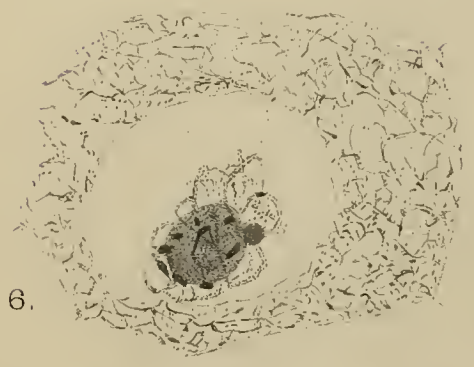
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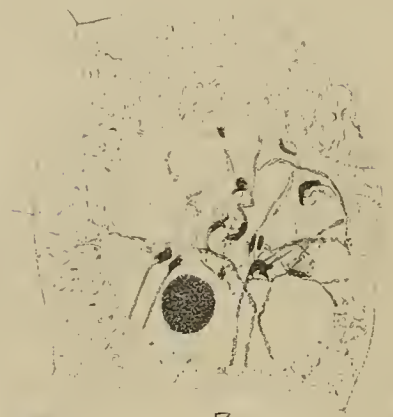
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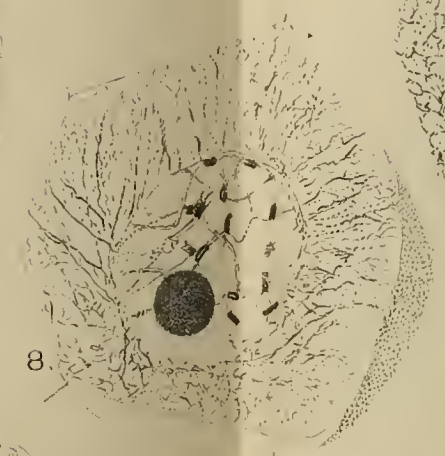
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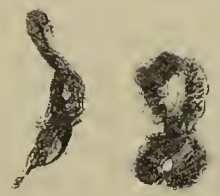
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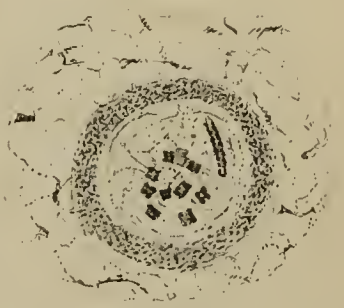
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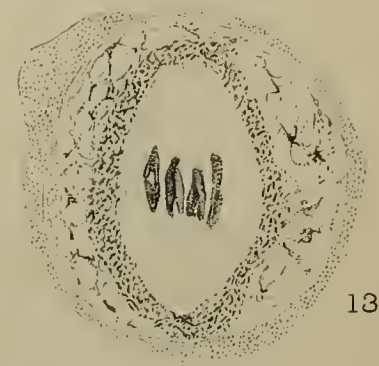
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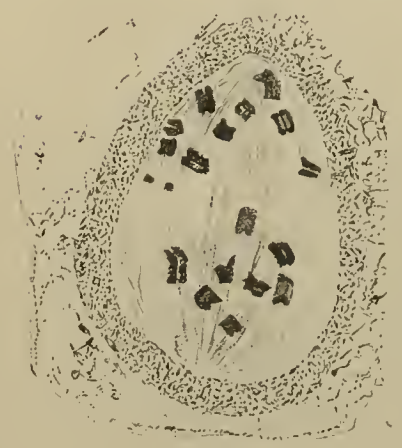
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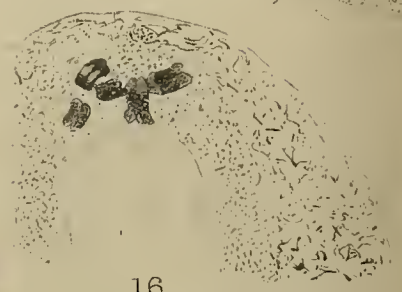
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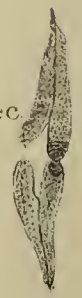
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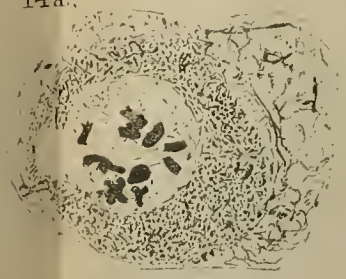
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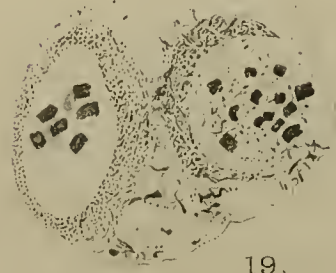
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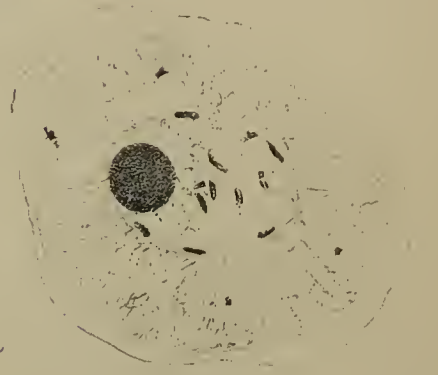
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J. B. O. del.

Huth, hth. et. imp.

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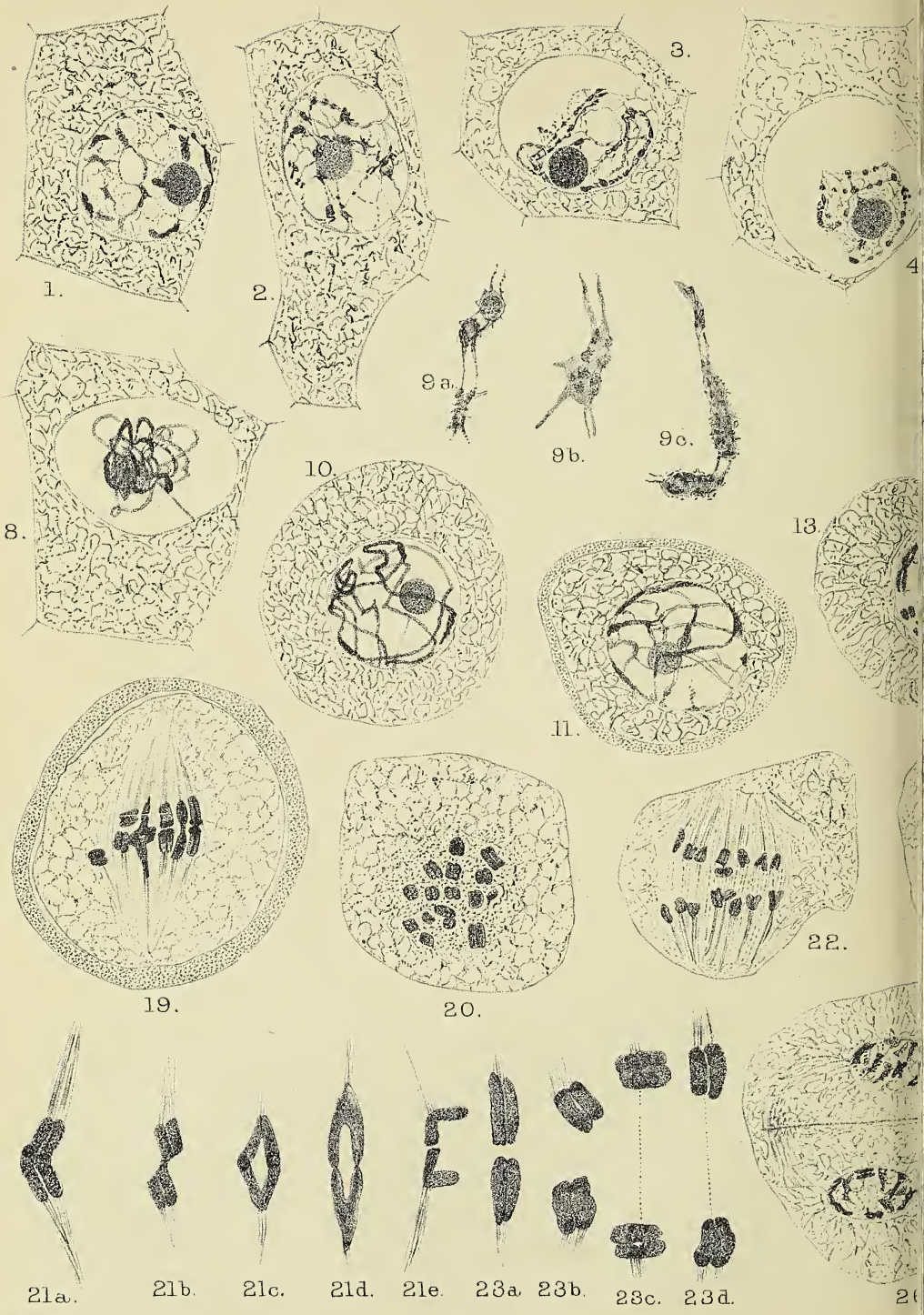
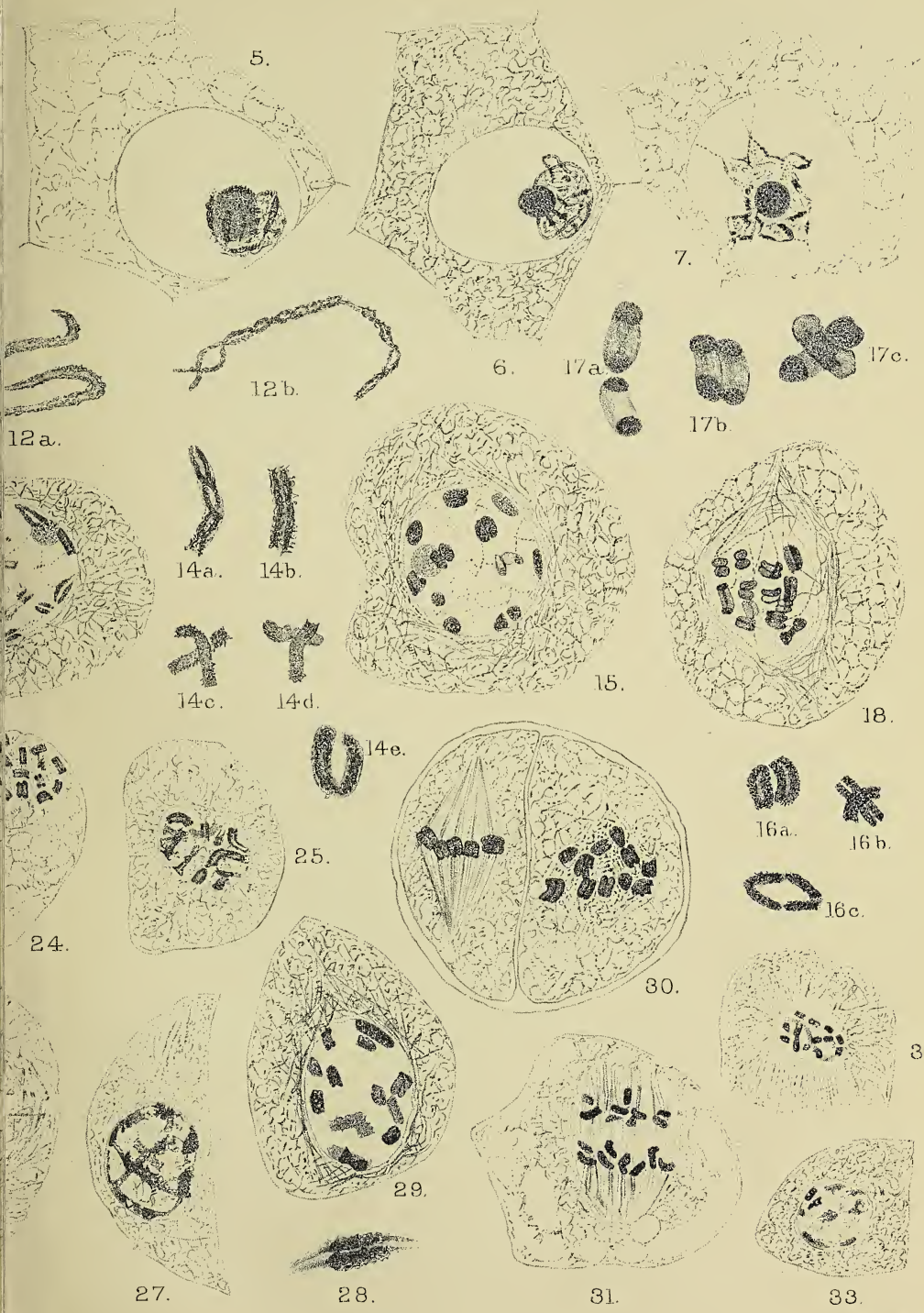


OVERTON — ORGANIZATION OF NUCLEI.



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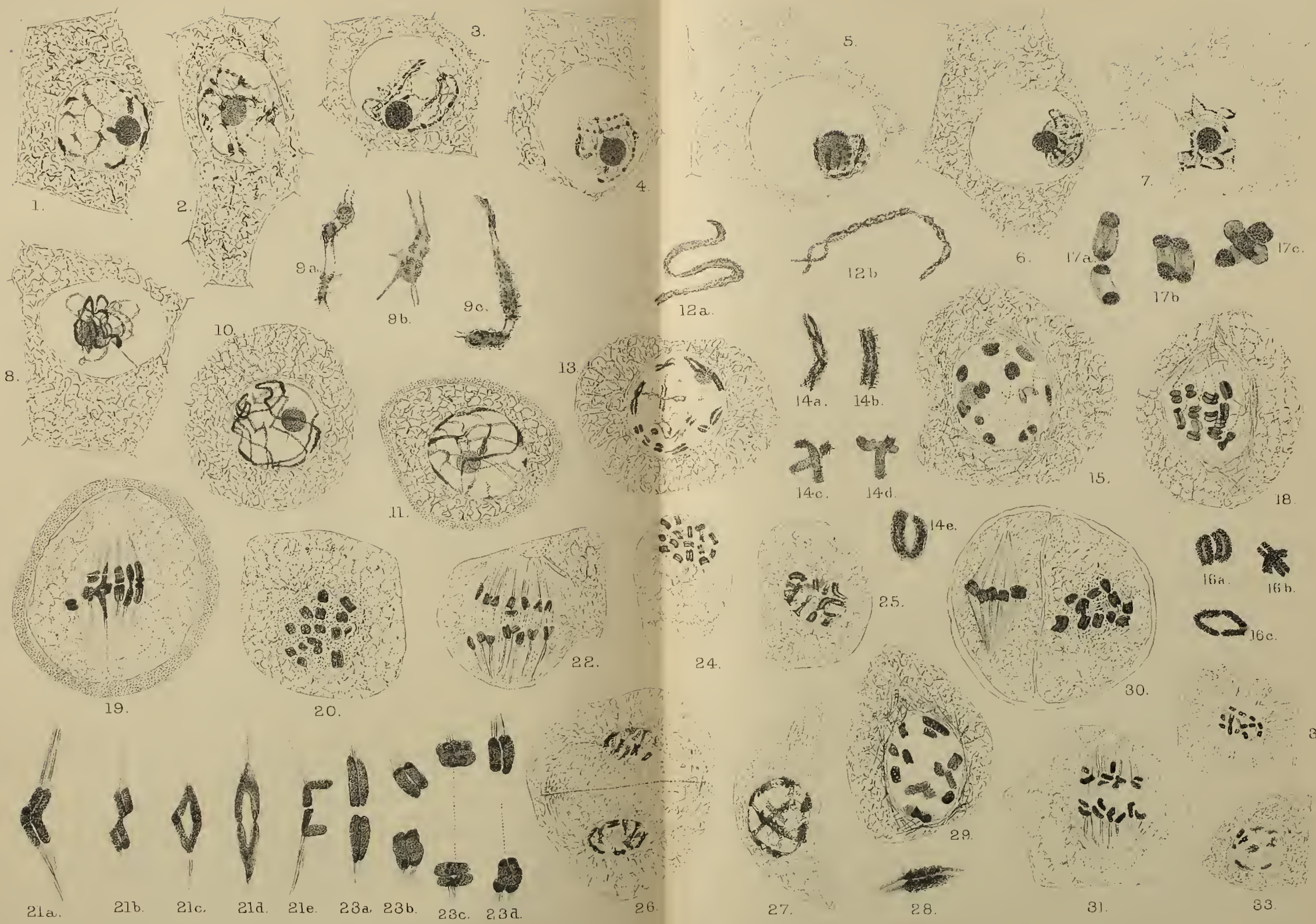

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Bathlith et imp

OVERTON — ORGANIZATION OF NUCLEI.

On Elaioplasts.

BY

RUDOLF BEER, B.Sc., F.L.S.

With Plate IV.

IN 1888 Wakker (1) described the occurrence of a conspicuous body lying in the epidermal cells of the leaves and in the superficial tissues of the root and stem of *Vanilla planifolia*. This body is somewhat larger than the nucleus and considerably larger than the amyloplasts; it possesses a sharply-defined outline and a peculiar, somewhat yellowish colour. In each plate-like epidermal cell one such body occurs, and it often lies near the nucleus, although in other cases it may occupy a different position in the cell. As this body consists of protoplasmic material and contains oil, Wakker named it *Elaioplast*, or oil-former.

He made a careful study of the effect of reagents upon the elaioplast, and briefly described the gradual disappearance of these bodies in older cells. Wakker was unable to study the origin of the elaioplasts as he had no suitable material for this purpose. He found elaioplasts also to occur in the cells of another species of *Vanilla*, known to him under the name of *Vanilla aromatica latifolia*. In 1893 Zimmermann (2) found similar bodies to occur in *Funkia coerulia*, *F. lancifolia*, *F. Sieboldiana*, *Dracaena sp.*, *Ornithogalum scilloides*, *Agave americana*, *A. Mitis*, and in *Oncidium suave*.

Raciborski (3) in the same year described elaioplasts in the tissues of various species of *Ornithogalum*, *Albuca*, *Funkia*, and *Gagea*.

Zimmermann further found these bodies in the internal cells of the stem of *Psilotum*, and in the perianth leaves of *Maxillaria picta*. The shape of the elaioplasts differs in various plants, but it is usually constant in the same species. Spherical forms, grape-like bodies, irregular plasmodium-like masses, have all been described.

Usually only one elaioplast occurs in a cell, but in some cases they may be more numerous (e. g. *Ornithogalum*). The finer structure of the elaioplast has been carefully examined both by Zimmermann and by Raciborski. It has a finely granular appearance due to the occurrence of a number of tiny, highly refractive spherical bodies lying in its substance. One or more less refractive spots often occur within the elaioplast. The elaioplasts of

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the species of *Gagea* are said by Raciborski to possess a somewhat different structure. In these plants the spherical elaioplasts are characterized by the feeble development of the stroma; they are surrounded by a plasmatic envelope and contain within this an oily substance like that found in other elaioplasts. The micro-chemical observations of Wakker and Zimmermann have shown that the elaioplasts consist of a plasmatic matrix or stroma in which are embedded numerous minute oil drops. Zimmermann points out that these oil drops show a close similarity in their reactions with the oil drops obtained from plastids.

The origin of the elaioplasts has been very little studied. Raciborski states that in *Ornithogalum umbellatum* they arise as small, highly refractive spherules which always lie at one pole of the usually elongated cell-nucleus.

Although Wakker had no material with which to work out the development of the elaioplast, he ventures an interesting suggestion at the conclusion of his account of the oil-bodies of liverworts. 'Leider ist durch diese Mittheilung der Ursprung der Elaioplasten nicht ausgemacht, es ist mir aber äusserst wahrscheinlich, dass es bei den Lebermoosen metamorphosirte Chlorophyllkörner sind. Vielleicht ist dieses auch bei *Vanilla* der Fall.'

Garjeanne's work (5) on the oil-bodies of the Jungermanniales clearly indicates that these bodies have a different origin to that suggested by Wakker; my own observations on elaioplasts recorded below will, however, show that his guess was nearer the truth in the case of some Phanerogamic elaioplasts.

The function of the elaioplast is quite unknown. Wakker believed that they might be oil-formers much as leucoplasts are starch-formers. Zimmermann offered the suggestion that they might prove to be parasitic or symbiotic fungi living within the cells of the higher plant.

Raciborski, however, considers them to be normal organs of the cell in which they occur, and classes them with oil-bodies, tannin-vesicles, and ordinary vacuoles.

In the liverworts oil-bodies have been known to occur since the time of Gottsche, and even earlier. The first really fundamental description of these bodies is due to Pfeffer (4), and quite recently their development has been fully worked out by Garjeanne (5). Oil or fat bodies of a somewhat similar appearance to those of the liverworts have been described by Radlkofer (6 and 7), Monteverde (8), Solereder (9), and others, in the tissues of a number of Dicotyledons and Monocotyledons. Opinion varies very much as to the relation of these oil-bodies of liverworts and Phanerogams to the elaioplasts. Some believe the two structures to be closely allied, whilst others are of opinion that they are radically different from one another.

From this brief survey of the literature it will be seen that elaioplasts,

corresponding to those discovered by Wakker in *Vanilla*, have up to the present been found only in Monocotyledons, with the single exception of *Psilotum* reported by Zimmermann.

Moreover, apart from a few quite insufficient observations we are still entirely in the dark regarding their true nature, their significance, their origin, and their fate.

During some observations upon the pollen-grains of Compositae I was interested to find bodies which resembled the Monocotyledonous elaioplasts occurring in the tissues of the floral region of *Gaillardia Lorenziana*[1].

This would extend the distribution of elaioplasts to a member of the Dicotyledons.

My first endeavour was to make sure that I was dealing with true elaioplasts. In the hairs upon the corolla of young flowers, where I first observed these bodies, they occur as more or less spherical, highly refractive, granular structures usually somewhat larger than the nucleus, and in most cases lying singly in a cell, although two or even more such bodies were occasionally met with.

Their reactions were found to be as follows:—

1. Heated gently upon the slide (whilst lying in a physiological salt solution), drops of oil are exuded from their surface (Fig. 13).

2. Osmic acid (as this occurs in Flemming's stronger solution) turns them black or brown.

3. Potassium bichromate after twenty-four hours' action leaves them quite colourless, but causes the extrusion of oil globules as in 1.

4. Alkannin solution in 60 per cent. alcohol colours the bodies deeply red.

5. Iodine (in KI) colours them brown and causes the extrusion of oil-globules (Fig. 12).

6. Absolute alcohol dissolves out the oil from their interior and leaves them vacuolated.

7. In strong HNO_3 (warmed), followed by NH_3, they give the Xanthoproteic reaction (viz., deep yellow coloration).

8. Glacial acetic acid after twenty-four hours' action causes great extrusion of oil drops but no solution.

9. 10 per cent. KOH. after twenty-four hours' action dissolves neither stroma nor oil drops.

These reactions, combined with their general appearance, show that the bodies occurring in the hairs of *Gaillardia* are in all respects similar to the elaioplasts described by previous authors.

In very young hairs from capitula which were still quite small and

[1] *Gaillardia Lorenziana* is a German variety of *G. picta*, which itself appears to be only a garden variety of *G. pulchella*. I have not yet had an opportunity of examining any other form or species of *Gaillardia* except the one mentioned above.

immature and entirely enclosed within the involucral bracts, no elaioplasts were yet to be seen. The cell contained a nucleus and cytoplasm which partly formed a peripheral layer and partly extended in strands and bars through the cell cavity (Figs. 1 and 2). Embedded in the cytoplasm was a number of small, highly refractive grains which had all the appearances of ordinary leucoplasts. That these refractive grains are really leucoplasts is confirmed by two facts.

Firstly, the resemblance between the unquestionable, starch-forming leucoplasts occurring, for example, in the hairs which cover the very young leaves, and the highly refractive grains contained in the corolla-hairs, is complete, although starch is not found in the latter under the usual conditions of growth.

Secondly, if the enveloping bracts be removed from a young inflorescence without detaching it from the parent plant, and the corolla-hairs exposed to a strong insolation, starch can be seen to have developed in some of these refractive grains.

For these reasons I believe the highly refractive grains occurring in the cells of the corolla-hairs to be leucoplasts, some of which, however, may have lost the power of starch-formation.

In somewhat older hairs these plastids, a number of which show signs of undergoing degeneration, tend to aggregate together at one or more spots within the cell. Not infrequently this aggregation of the plastids is in the neighbourhood of the nucleus, but in many instances it is found to occur at other regions of the cell (Figs. 3 and 4).

At first the aggregation of the refractive grains is a very loose one, but it gradually grows closer and closer (Fig. 5) until the compact, highly refractive bodies are formed, which we have already recognized as elaioplasts (Figs. 6, 7, and 8). The elaioplasts in the corolla-hairs of *Gaillardia* are, therefore, formed by the aggregation of plastids and their degeneration products at one or more spots in the cell. Within the elaioplast the plastids soon appear to undergo further degeneration with the production of an oily material. That Zimmermann should find a close similarity between the oil of the elaioplasts studied by him and the oil obtained from plastids is no longer surprising.

All the plastids of the cell have not clumped together within the elaioplast. A certain proportion still remain scattered through the cell (Fig. 7).

For some time there is little alteration within the cell. The conspicuous elaioplast may lie in almost any part of the cell, but often it takes up a position near the nucleus. In some instances it entirely envelops the nucleus, as I have represented in Fig. 8.

In much older hairs we find the elaioplast undergoing a change. Its outline becomes less regular, and in some cases it becomes drawn out

and elongated in form (Fig. 9). In favourable cases one can see that the faintly yellowish drops or granules of which it now chiefly consists are becoming detached from the periphery of the main body of the elaioplast, and that these drops or granules are gradually scattered through the cell cavity (Fig. 10). Here they deepen their yellow tint, and in association with the red pigment developed in the cell-sap they produce the yellow, orange, or red coloration of the mature corolla-hairs, according as the one or the other pigmenting material predominates (Fig. 11).

The constituent plastids of these elaioplasts, therefore, undergo quite a similar series of changes as the chlorophyll grains in autumn leaves, which were first described in detail by Sachs in 1863 (10), or in ripening fruits, also studied by Sachs (1865). By the time the hairs are fully matured the elaioplast has entirely resolved itself into the scattered yellow pigment of the cell.

The corolla-hairs are not the only place in which elaioplasts occur in *Gaillardia*. They are also to be found in the stigmatic hairs, or in the more internal cells of the stigma and of the style, in the vegetative cells of the anther, and in the cells of the young pappus (calyx).

I sought for them in vain in the root-hairs or in the tissues of the root, in the leaf and the hairs which cover it, in the stem, and its clothing of hairs.

After I had completed my observations on the elaioplasts in the corolla-hairs, and drawn from them the conclusions which I have expressed above, I received a beautiful confirmation of the correctness of these views from the study of the elaioplasts in the other floral regions of *Gaillardia*.

In the cells of the connective of the young stamen, bodies occur which resemble the elaioplasts of the corolla-hairs in every respect except that they are coloured more or less deeply green. They are mostly spherical, although sometimes elongated in shape (Fig. 14). Moreover, in neighbouring cells of the connective we find every transition between deeply green bodies of this description, and others which are almost colourless and differ in no way from the elaioplasts of the corolla-hairs. On the addition of Iodine solution the occurrence of starch within the *green* bodies is readily demonstrated (Fig. 12). After remaining in the Iodine solution for some hours these intensely black-stained bodies form a most conspicuous feature in the otherwise yellow cells.

The appearance and reactions of these green bodies, no less than the transitions which occur between them and the ordinary elaioplasts, leave no doubt that they also are elaioplasts which contain chlorophyll, and which have retained the power of starch-formation. The cells of the young style and stigma also possess green, starch-producing elaioplasts.

Another very interesting case of green elaioplasts is furnished by the cells of the flattened basal plates or wings of the young pappus. In

many of these cells scattered chloroplasts occur, arranged as in ordinary assimilating tissue (Fig. 15). These chloroplasts are large, and many of them contain droplets of an apparently oily nature embedded within their substance. Probably these oil-drops mark the first stage of degeneration, although the power of starch-formation has not yet been lost. I was at first inclined to believe that the oily drops within these chlorophyll corpuscles represented the normal grana of these bodies developed to a rather unusual extent. The fact, however, that the chloroplasts of the other organs of *Gaillardia* (e. g., of the leaf) do not show any distinct grana of this kind, coupled with the further fact that the chlorophyll bodies of the pappus soon show undoubted signs of degeneration, has led me to conclude that the oil-drops are associated with the degradation of these chloroplasts.

In other cells of the pappus-plates the chlorophyll corpuscles tend to hang, more or less loosely, together. In yet other cells the aggregation of the chloroplasts is closer, although the outlines of each separate plastid is still maintained (Fig. 16). A further step in this aggregation of the chlorophyll bodies is seen in other, neighbouring cells in which they become so closely clumped together that the outlines of the individual chloroplasts can no longer be distinguished, and we obtain a typical green elaioplast in which the oil-drops of the plastids produce the finely granular appearance characteristic of these structures.

All these stages may be observed in adjoining cells of one and the same pappus-plate. They are best studied at about the time when the young pollen-grains are still without a membrane of their own and are enveloped in the special-wall (special mother-cell stage).

In older pappus-plates the green colour of the elaioplasts gives place to yellow, and other degeneration processes become evident.

Now that the development of the elaioplast has been followed in at least one species we are in a better position to compare this body with the oil-bodies of Hepaticae. Wakker evidently believed in the identity of the two structures, whilst other authors—such as Von Küster (11)—held an opposite opinion. On comparing what has been written above regarding the elaioplasts of *Gaillardia* with Garjeanne's careful account of the development of the oil-bodies of several Jungermanniales, it will be seen that the two structures have a very different origin. In the latter the oil-bodies arise as vacuoles in the cytoplasm, whilst we have seen that the elaioplasts of *Gaillardia* are formed by the aggregation of plastids and their degeneration products. Whilst, therefore, we cannot draw general conclusions until other species have been examined more fully, we may say that the developmental history of the elaioplasts of *Gaillardia* is essentially different from that of the oil-bodies of the Hepaticae.

External conditions seem to exert very little influence on the appearance of the elaioplasts.

I have kept the young capitula in total darkness for several (3–6) days without altering the development or structure of the elaioplasts of the corolla-hairs in the least.

The only deviation which I have ever found in the behaviour of the plastids of the corolla-hairs occurred in a very young capitulum from which the protecting bracts had been dissected away so that the tiny flower-buds were exposed to the full effect of the light.

Here the aggregation of the plastids into elaioplasts had been retarded in a number of cells.

The clumping together of the plastids of a cell into a more or less close mass is by no means an unusual occurrence. Kraus (12) many years ago described the effect of cold upon the chlorophyll-grains of winter leaves. Here these bodies were found to have passed from the walls to the interior of the cells and were there aggregated in clumps. Charles Darwin (13), in 1882, observed a very close massing of the chloroplasts in the cells of certain insectivorous plants under the influence of ammonium carbonate (a solution of 4–7 parts of ammonium carbonate in 1,000 parts water).

The work of Stahl (14), as well as of others, has shown that an irregular aggregation of chloroplasts is produced under the influence of intense illumination.

Pfeffer (15) mentions that similar results are induced by injuries and various mechanical agencies.

The close massing of the plastids into compact elaioplasts is most probably connected with their degeneration, and may very likely be compared to the aggregation of these bodies produced by the injurious agencies enumerated above. That the elaioplasts have any particular function to perform which is of direct significance to the life of the cell is most unlikely.

A secondary use for the degeneration products of the plastids—massed into elaioplasts—certainly does occur in the case of the corolla-hairs of *Gaillardia*, for here they give rise to the yellow pigment which forms an important part of the attractive apparatus of the mature flower.

In other situations, however, the elaioplasts seem to disappear, without having even this secondary biological significance.

It will be interesting to examine the Monocotyledonous elaioplasts again more closely in the light of what has been learnt of these bodies in *Gaillardia*, to see whether they possess the same nature and history. I hope to obtain material for this purpose during the next season.

In conclusion, I must express my indebtedness to a Government grant for assistance in carrying out this research.

Summary.

1. Elaioplasts which hitherto had only been met with in Monocotyledons (and *Psilotum*) have now been found to occur in a Dicotyledon—*Gaillardia*.

2. The elaioplasts occurring in the corolla-hairs of *Gaillardia* are found to agree in their appearance and in their reactions with the elaioplasts described by Wakker and Zimmermann in Monocotyledons.

3. They have been found in the corolla-hairs, the pappus, the connective of the stamens, the style and the stigma of *Gaillardia*. They are absent from the tissues of the stem, the root, and the leaf of this plant.

4. They are formed by the aggregation of plastids and their degeneration products at one or more spots in the cell.

5. In the corolla-hairs of *Gaillardia* they give rise to the oily, yellow pigment which, in association with the red cell-sap, gives the mature hairs of the flower their characteristic colour.

6. The elaioplasts occurring in the stamens and in the style and stigma of *Gaillardia* agree in all respects with those of the corolla-hairs except that they are coloured green with chlorophyll, and can form starch within their substance. In neighbouring cells of these tissues all transitions occur between elaioplasts, which are coloured brightly green, and those which are almost colourless like those of the corolla-hairs.

7. In the tissues of the young pappus every transition can be found in neighbouring cells between those which contain scattered chloroplasts entirely free from one another, and those in which the chloroplasts have clumped together to form a green mass identical with the green elaioplast of the stamen or the stigma.

8. The elaioplasts of *Gaillardia* (and *probably* of the Monocotyledons also) differ essentially in their development from the oil-bodies of the liverworts.

9. External conditions were found to exert very little influence upon the appearance of the elaioplast, although rather strong, direct illumination seemed in one case to have somewhat retarded the aggregation of the plastids.

10. The close massing of the plastids into compact elaioplasts is probably connected with their degeneration, and may be compared to the aggregation of the plastids under the influence of various (mostly injurious) agencies described by several previous writers.

11. It is most unlikely that the elaioplasts perform any function of direct importance to the life of the plant, although they may in some cases (corolla-hairs of *Gaillardia*) serve a secondary, biological purpose.

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15. Pfeffer, W.: Physiology of Plants (Eng. trans.), iii, 1906, p. 329.

EXPLANATION OF FIGURES IN PLATE IV.

Illustrating Mr. Beer's Paper on Elaioplasts.

All the figures refer to tissues of *Gaillardia Lorenziana*. Preparations examined and drawn in ·6 per cent. NaCl solution unless otherwise stated.

Fig. 1. Very young cell of corolla-hair before elaioplasts have developed. Leucoplasts distributed in the cytoplasm. × 1075.

Fig. 2. Apex of another corolla-hair showing scattered leucoplasts. × 650.

Fig. 3. Young cell of corolla-hair showing an early stage in the aggregation of plastids. × 650.

Fig. 4. Corolla-hair showing a stage in aggregation of plastids. × 650.

Fig. 5. Corolla-hair with later stage of elaioplast development. × 650.

Figs. 6, 7, 8. Elaioplast completely developed in corolla-hairs. × 650.

Fig. 9. Cell from older corolla-hair. Elaioplast elongated and showing first indication of disintegrating. × 650.

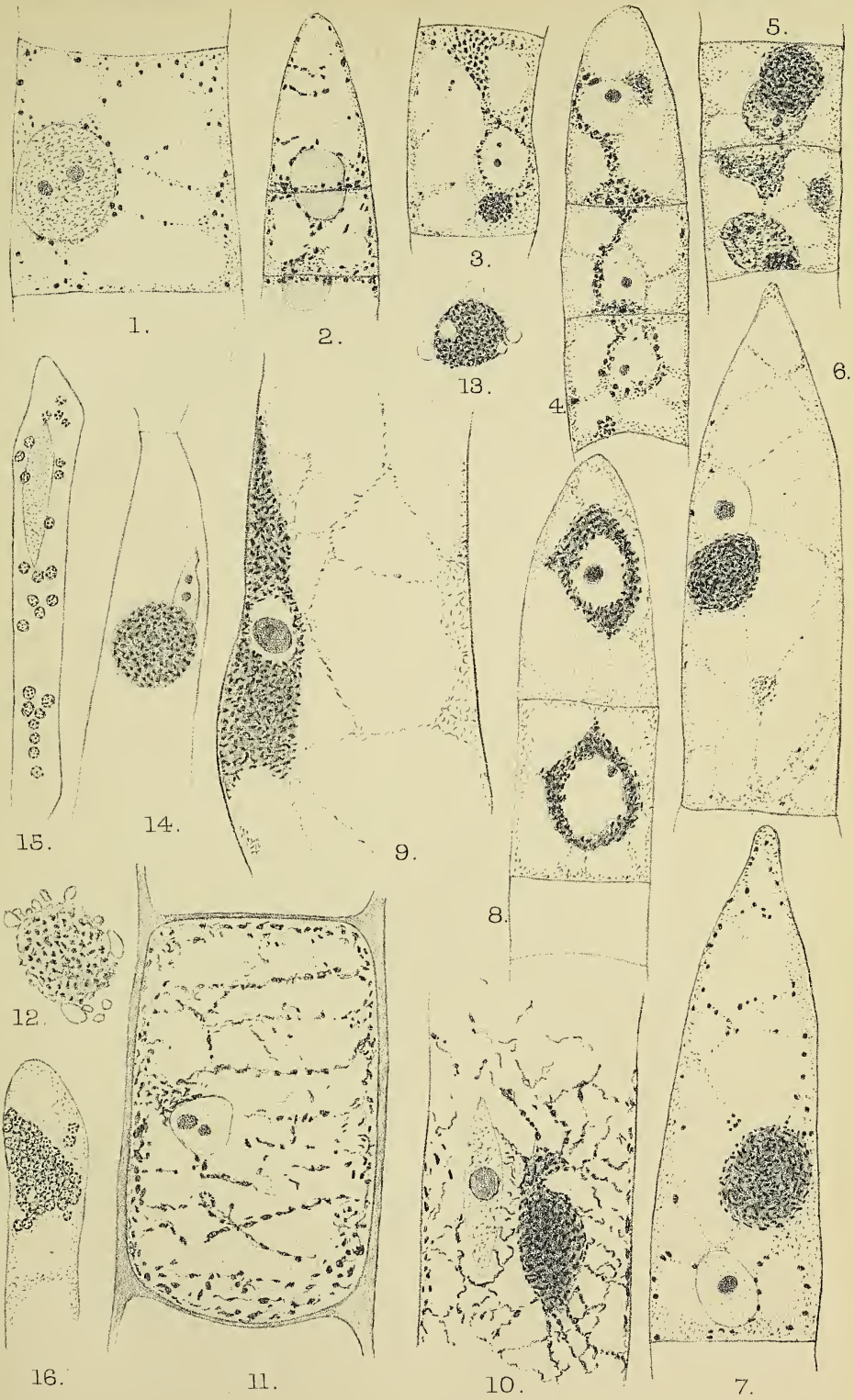
Fig. 10. Later stage; substance of elaioplast becoming distributed through the cell. × 600.

Fig. 11. Mature corolla-hair. Yellow pigment-material derived from elaioplast completely distributed through the cell. × 650.

Fig. 12. Green elaioplast from anther after two days in dilute Iodine solution. Oil-drops have been exuded at the surface. Starch (shown in the figure as black grains) contained in the yellow-stained matrix. × 600.

Fig. 13. Elaioplast from corolla-hair in ·6 per cent. NaCl after gently warming on slide over spirit-lamp. × 650.

Figs. 15 and 16. Two neighbouring cells from plate of pappus. In Fig. 15 the chloroplasts are scattered, whilst in Fig. 16 they are massed into a loose clump. Note oil-drops within the chloroplasts (represented as black dots). × about 600.



Huth, lith et imp.

BEER — ELAIOPLASTS

On Physostoma elegans, Williamson, an archaic type of Seed from the Palaeozoic Rocks.

BY

F. W. OLIVER, F.R.S.

With Plates V, VI and VII and ten Figures in the Text.

CONTENTS.

I. Introduction.

At the meeting of the British Association at Bristol in 1875, the late Professor Williamson gave some account of a 'very distinct' petrified seed from the Lower Coal-Measures of Lancashire, for which he proposed the

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name *Physostoma elegans*.[1] A year or two later, a description of this seed was included by Williamson in his eighth Memoir,[2] but, owing to the imperfect character of the material at that time available for study, he placed it provisionally in his new genus *Lagenostoma*, under the name *L. physoides*—the other member being *Lagenostoma ovoides*. To these he added in his manuscript catalogue a third species, *Lagenostoma Lomaxii*, a seed which recent investigation proves to have been borne by *Lyginodendron*.[3]

In addition to these three species of *Lagenostoma*—all petrified seeds from the Lower Coal-Measures of Lancashire and Yorkshire—additional species have been discovered preserved as casts. These include the fully-described *L. Kidstonii* and *L. Sinclairii*, of Arber,[4] and one or more species discovered by Grand'Eury,[5] and referred to *Sphenopteris Dubuissonis*, and other forms. To these casts further reference will be found at p. 112 of the present paper.

With these seeds must be associated one or more of the species of Williamson's genus *Conostoma*,[6] more particularly *Conostoma oblongum*, one of the rarer of our English Coal-Measure seeds.

The present communication deals with *Lagenostoma physoides*, the further study of which has entirely corroborated Williamson's opinion as to its ' very distinct ' character.

As the material for a detailed description is now adequate and abundant, the time has arrived when effect may be given to Williamson's original intention in the matter of nomenclature. In pursuance of this it is proposed to revive for this seed the abandoned generic name *Physostoma*, a course which seems free from serious objection. As regards the specific name, alternative courses seem open. The name *physoides* might be retained, or Williamson's original name, *Physostoma elegans*, which is both euphonious and appropriate, might be revived. It is proposed to follow the latter course.

Since the appearance of Williamson's description in 1877, based on two imperfect longitudinal sections of a single specimen,[7] a few additional facts regarding the structure of *Physostoma* have been placed on record. Under the name of *Sporocarpon ornatum*, Williamson described and figured poorly preserved transverse sections of *Physostoma* in his tenth and twelfth

[1] W. C. Williamson, On some ossil seeds from the Lower Carboniferous beds of Lancashire, Brit. Ass. Reports (Bristol) 1875, p. 159.

[2] W. C. Williamson, On the Organization, &c., pt. viii, Ferns, Gymnospermous Stems, and Seeds. Phil. Trans., 1877, p. 241.

[3] Oliver and Scott, On the structure of the palaeozoic seed *Lagenostoma Lomaxii*, &c., Phil. Trans. B., vol. cxcvii, 1904, p. 193.

[4] E. A. N. Arber, On some species of *Lagenostoma*, Proc. Roy. Soc. B., vol. lxxvi, 1905.

[5] C. Grand'Eury, Sur les graines de Sphénoptéris, etc. Comptes Rendus, t. cxli (1905), p. 812.

[6] Williamson, eighth Memoir, p. 243.

[7] The sections are in the Williamson Collection, now in the Department of Geology, Natural History Museum, and correspond to the numbers 1439 and 1440.

Memoirs.[1] More recently, the late John Butterworth called attention to the ribbed character and hairy covering of the seed, and published photographs of a tangential longitudinal, and of an oblique transverse section.[2] These photographs are sufficient to establish the identity of Williamson's *Sporocarpon ornatum* and *Physostoma*.[3]

II. Organization of the Seed.

1. *General Features.*

Physostoma elegans is a small seed showing considerable resemblance to the Lagenostomas in the general features of its organization. It is a straight, ribbed seed, with a free integument in the apical region; its length from the chalaza to the tip of the multipartite integument reaches $5\frac{1}{2}$–6 mm. —or occasionally a trifle more when the arms are fully extended. In transverse section it is circular, the longitudinal ribs, usually ten in number, giving the outer surface of the testa a sinuous contour. The seed is broadest about one-third up from the base, where its diameter (excluding the hairs which adorn the ribs) just exceeds two millims. The seed is thus a narrow one, broadest somewhat below the middle, and tapering gently towards the apex (Text-fig. 1). At the base it is bluntly rounded, so that a median longitudinal section recalls a cuttlefish in miniature.

The central body or nucellus of the seed, which has a length about five-sixths the entire seed, ends in a large apical pollen-chamber. The longitudinally ribbed integument is coalescent with the central body for a distance of about four millims. from the seed-base. Just below the pollen-chamber the integument, as a whole, becomes free from the central nucellar body, as in the Lagenostomas, and at once breaks up into about ten arms or tentacles to form a whorl or circlet which loosely surrounds the pollen-chamber. The free arms are the direct prolongation of the convex ribs of the testa, or, stated in a slightly different way, the testa forms a ribbed envelope coalescent with the body of the seed throughout its lower two-thirds, but free above where the ribs part to form the tentacles.

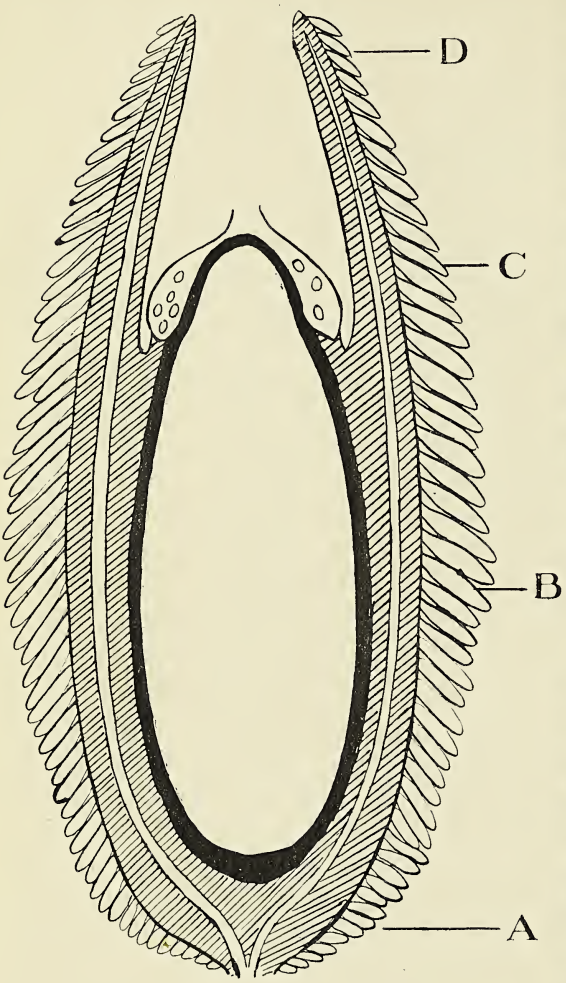
This replacement of the usual micropylar tube of the integument by separate arms is a distinctive character, in which this seed differs from all other known seeds, fossil or recent. These relations will be understood from a glance at the diagrammatic representations of a longitudinal section in Text-fig. 1, taken in conjunction with the series of transverse sections

[1] Phil. Trans., 1880, p. 510, and Pl. XVIII, Fig. 39; Ibid. 1883, p. 469, and Pl. XXXI, Fig. 27. In the latter the name is inadvertently cited as *Sporocarpon anomalum* in the description of the figures on p. 474.

[2] J. Butterworth, Some further investigation of Fossil Seeds of the genus *Lagenostoma*. Mem. and Proc. of the Manchester Lit. and Phil. Soc., vol. xli, pt. iii (1897).

[3] This identification received independent corroboration in a note in the New Phytologist, vol. ii, p. 18 (1903).

A–D cut at different levels (Text-fig. 2) The photograph of an almost median longitudinal section of the upper portion of a seed (Pl. V, Fig. 4) shows two of the tentacles cut in the direction of their length (t_0 and t_5), the insertion of the left-hand tentacle being particularly clear. Four other tentacles, cut transversely or obliquely, are shown at t_1, t_2, t_3, and t_4, immediately above the pollen-chamber (p). Nor should reference be omitted to Williamson's original figure. Published thirty years ago, and based on a rather thick section, this drawing shows the relations of the upper part of the nucellus and tentacles with unerring accuracy, and could hardly be improved upon.[1]



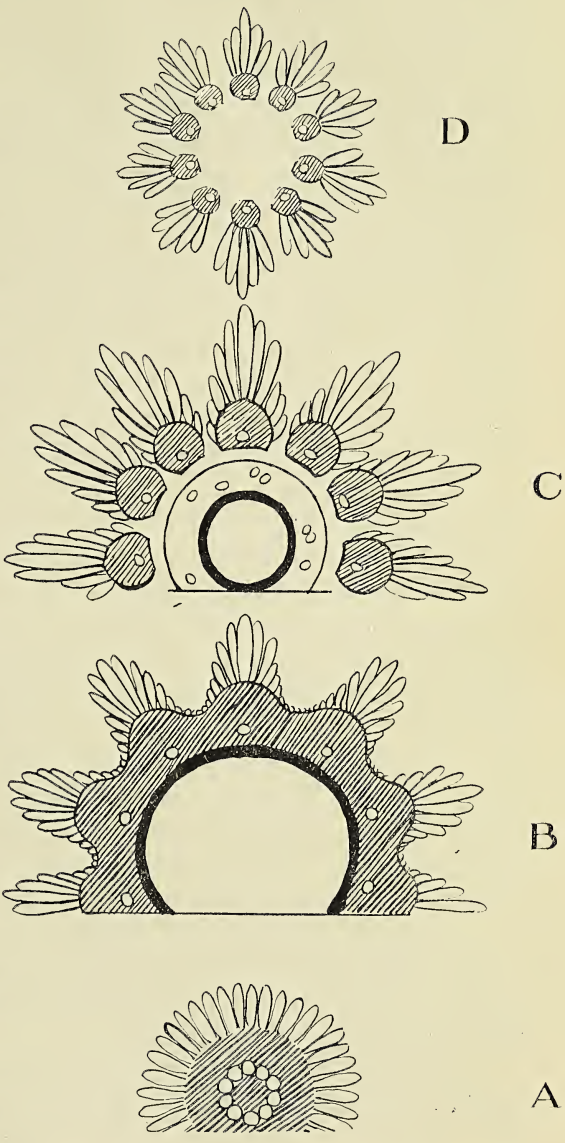
TEXT-FIG. 1. Diagrammatic median longitudinal section of *Physostoma elegans*. The integument is obliquely shaded: the secretory zone is omitted for the sake of clearness, whilst the tapetum is represented as a single layer in black; the vascular strands are left unshaded. The letters A, B, C, and D refer to the heights at which the transverse sections in Text-fig. 2 are cut.

A very striking feature about *Physostoma*, and one which makes even the smallest fragments easy of identification among the heterogeneous assortment of petrified plant-remains that go to form a coal-ball, is the conspicuous investment of long, club-shaped hairs which clothe the summits of the ridges on the body of the seed, and the outer (abaxial) faces of the tentacles. These hairs are commonly to be found with excellent preservation, and reach a length of ·5 mm. or more in the neighbourhood of the median line of ridge and tentacle respectively. No doubt, during life, they almost completely enveloped the seed (see the oblique section of an apex, Pl. V, Fig. 8).

[1] Williamson, eighth Memoir, Phil. Trans., 1877, Pl. XI, Fig. 77.

This indeed they still do in the petrifactions in the region of the free arms of the integument, but on the body of the seed they tend to stick together—thus exposing the grooves between the ribs—instead of diverging fan-wise, as occasional examples indicate to have been the position during life (Pl. VI, Fig. 13). That the investment by the hairs was complete is shown by the very tangentially cut section from the base of a seed given at Pl. VI, Fig. 22 (*t. h.*).[1] The upper third of the drawing represents the section where, after leaving the tissues of the seed proper, it traverses the covering of hairs in an oblique, upward direction. At the top of the figure, where the section runs through the outskirts, only the longest hairs reach the plane of section.

The vascular system of the seed has essentially the same distribution as in *Lagenostoma Lomaxii*. A supply bundle enters at the rather exiguous seed base, and at once separates into as many strands as there are ribs. The strands run up the seed deep down in the substance of the ribs—at about the level of the intervening grooves—and pass out into the tentacles. No vascular connexions have been traced to the pollen-chamber.



TEXT-FIG. 2. Series of diagrammatic transverse sections of *Physostoma elegans*. The letters A, B, C, and D correspond with the heights, in Text-fig. 1, to which the sections belong. Shading, &c., as in Fig. 1.

The general ground-substance of the testa is a rather thin - walled, closely - fitting tissue of prismatic cells, elongated in a direction parallel to the axis of

[1] The plane of section is plotted in Text-fig. 3, p. 80, R. 72, *a*.

the seed. There is no indication of any general sclerosis, nor is there any localized sclerotic layer such as characterized many of the seeds of the same period. The study of the histological character of the seed-wall gives the impression that, without being succulent, the seed was far from being nut-like. In this respect *Physostoma* was rather exceptional; perhaps the comparative softness of the tissues is to be correlated with the remarkable enveloping layer of hairs to which reference has been made.

Another feature relating to the testa merits passing comment, though it is mainly a question of preservation. In the case of the Lagenostomas (*L. Lomaxii* and *L. ovoides*) it is very rare to find the vascular strands *in situ*; they break away with a connecting sheath of tissue from the testa (the 'bundle ring') and this ring is generally to be found lying contracted some distance away from the outer part of the testa.[1] In *Physostoma* such a separation has not been met with, and the bundles, or in their absence—which is not infrequent—the lacunae marking their position are always found *in situ* (Pl. V, Figs. 2, 5, and 6, *vb.*). The possible explanation of this peculiarity will be discussed later on.

The central body of the seed (nucellus) conforms to the Cycadean type, and is largely occupied by the long embryo-sac or megaspore. The free apex is, as usual, modified to form a pollen-chamber which is very large in relation to the size of the seed. The internal cavity of the nucellus or megaspore-chamber attains a length of over four millims., and is sharply delimited by a uniform layer which is generally preserved as a black, opaque, structureless border, continuous from end to end. A characteristic feature in the form of the embryo-sac is the conical papilla at the apex (Pl. VI, Fig. 18, *mg.p.*) which projects or bulges for a distance of $\frac{3}{4}$ millim. into the pollen-chamber, like the incurved bottom of a wine-bottle.

The pollen-chamber itself is a bell-shaped crevice embracing the projecting apex of the embryo-sac 'as if it were a soft bladder half full of water, allowed to rest on one of the old-fashioned soda-water bottles.'[2]

This marked overlapping of the pollen-chamber and megaspore regions of the nucellus is a very unusual feature in the seeds that have so far come to light. It recalls on a grand scale the tent-pole mechanism of *Ginkgo*, and will require further consideration.

The orifice of the pollen-chamber is to be found at the summit, where it takes the form of a circular opening situated on a low papilla (Pl. VI, Fig. 18, *o.p.c.*). The diagrammatic longitudinal and transverse sections, given on pp. 76–77, will serve to epitomize the main relations in the organization of *Physostoma*.

[1] Oliver and Scott, *loc. cit.*, Pl. VII, Fig. 2, *i.s.* and Pl. X, Fig. 31, *i.s.* and *b.r.*

[2] Williamson, eighth Memoir, Phil. Trans., 1877, p. 242.

2. *Series of Transverse Sections.*

Before proceeding with the detailed consideration of the various regions of *Physostoma*, the general relations of its organization may be illustrated conveniently by the photographs of a series of three transverse sections cut from a single seed (Pl. V, Figs. 1, 2, and 3). That I have had the advantage of studying this series is due to the courtesy of my friend, Professor C. E. Bertrand, of Lille, who, hearing that I was engaged upon *Physostoma*, placed the preparations in my hands for investigation. The sections, which are numbered M.H. 369, 370, and 371, traverse the chalaza, the middle of the seed, and the apical circlet of tentacles, respectively. They belonged to the collection of the lamented Maurice Hovelacque, a botanist of marked versatility, who had already made valuable contributions to the knowledge of our English Coal-Measure plants at the time of his death in 1898. The nodule was doubtless derived from one of our Lancashire localities, whilst the preparation of the sections (carried out with exquisite perfection of technique) was entrusted by the collector to the lapidary, E. Rousseau, of Paris.

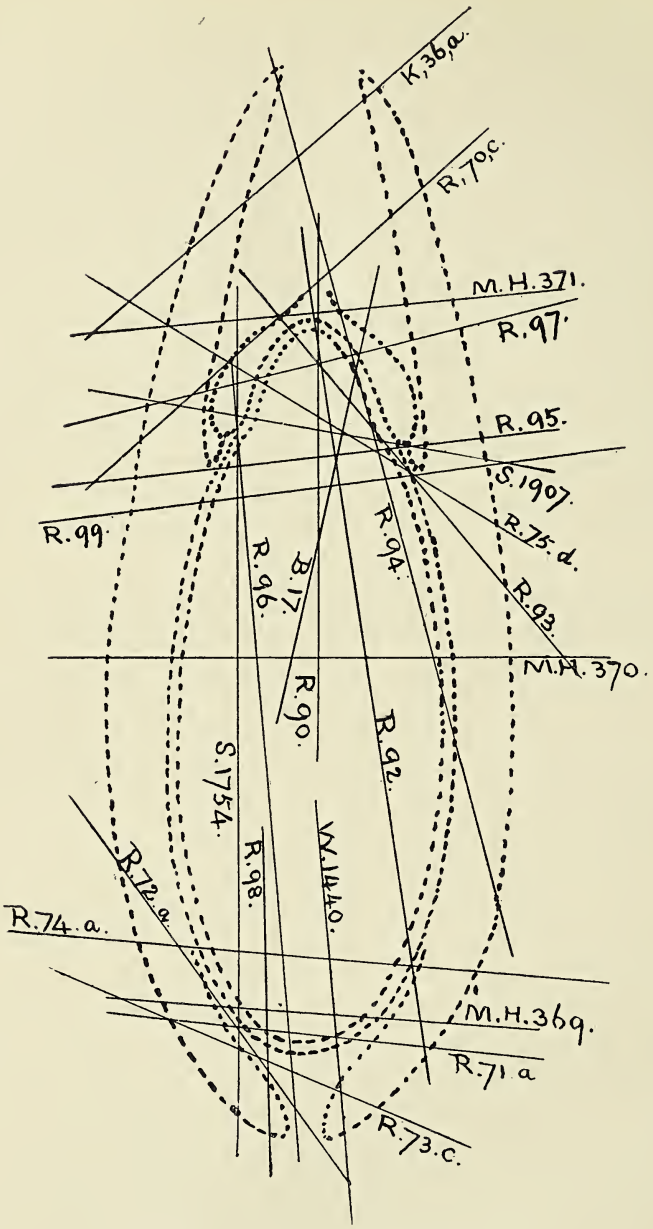
The value of the series depends on the fortunate conjunction of excellence of tissue-preservation with a minimum of displacement in the several zones. It is true the layer of epidermal hairs is indifferently preserved, but this defect can be repaired from a host of other sections.

The wall of the seed shows three conspicuous zones, viz.: (1) The integument, extending down to, and including, the vascular strands; (2) the nucellus, chiefly characterized by the zone of secretory sacs; (3) the tapetal lining, abutting on the embryo-sac.

The main features of the three sections are as follows:—

The lowest section (M.H. 369, Pl. V, Fig. 1), cut at a distance of ·75 millim. from the base, has a diameter of 1½ millims. (the epidermal appendages being excluded). The diameter of the internal cavity is ·6 millims. It shows the three layers so characteristic of the seed, viz.:—

(1) The integument, some five or six layers in width. This zone is completely separated by a tangentially orientated lacuna (Fig. 1, *l*) from the subjacent zone throughout the upper half of the section, whilst on the lower half the separation is incomplete in the radii corresponding to the grooves between the ridges. In all, ten lacunae are represented—four below and six above—the latter being tangentially confluent. Where the lacunae are distinct (i.e. below), the surface of the seed is ribbed, where they are confluent the ribbing is obscure (i.e. above). The inner margin of each lacuna contains the vascular strand belonging to its rib (Pl. V, Fig. 1, *v. b.*). Moreover, the tangential intervals between the neighbouring bundles are somewhat smaller on the lower than on the upper half of the section. Evidently the section is slightly tilted, the lower half in Fig. 1 corresponding



TEXT-FIG. 3. Diagrammatic sketch of a median longitudinal section of *Physostoma elegans*, upon which are plotted the approximate positions of the planes of section of the principal sections figured in the present paper. The reference letters and numbers given with each section on the figure are the designations under which the preparations are cited in the explanation to the plates W. = Williamson Collection. M.H. = Prof. Bertrand's Collection. S. = Scott Collection. R. and K. = University College, London, Collection. B. = Prof. Bottomley's Collection.

to a slightly higher level than the upper half. The probable position of this section is plotted in the diagram (Text-fig. 3, p. 80, M.H. 369), the right-hand side of the diagram corresponding to the upper side of the photograph as it is orientated in Pl. V, Fig. 1.

This separation of the integument at the seed-base would appear to be of practically universal occurrence in the specimens of *Physostoma*. The phenomenon is perhaps analogous to the splitting often shown by cut flower-stalks standing in water, which depends on the unequal swelling of the different tissues (cf. Pl. VI, Fig. 21, *l*).

(2) Below the plane of splitting is the zone of secretory sacs (*s.z.*), consisting of a delicate parenchyma in which are scattered great numbers of thin-walled secretory cells, with black, structureless contents. These sacs generally show stretching in the tangential direction. Their longest dimension, however, coincides with the axis of the seed. They are not distributed homogeneously throughout their zone, but have a maximum occurrence midway between the radii occupied by the bundles. At these points the sacs are about five deep, whilst opposite the bundles the number falls to about two.

(3) Within the secretory zone, and bordering the central cavity, is the conspicuous tapetal zone, ·1 millim. across, of thick-walled elements with marked radial flattening (Pl. V, Fig. 1, *tp.*). Towards the inner limit the cell-cavities are barely visible in consequence of the running together of the thick, black membranes; further out, where the layer abuts on the zone of secretory sacs, the large, tangentially elongated elements become conspicuous.

The second section of the series (M.H. 370, Pl. V, Fig. 2) has a mean diameter of $2\frac{1}{4}$ millims., whilst the internal cavity has expanded to $1\frac{1}{2}$ millims.

The three principal zones are recognizable, though their aggregate width shows a slight diminution when compared with the previous section. Of the three, it is the middle (secretory) zone that shows the most conspicuous thinning. The ribbing at the surface is very characteristic. Ten ribs are present lying in the same radii as the ten vascular strands. Separation between the outer and middle seed-zones is restricted to the immediate neighbourhood of the xylem-strands, so that each rib overlies a canal of crescent-shaped or semi-circular section. These canals evidently owe their origin to the breaking down of a delicate tissue that lay just on the peripheral side of the xylem-strands. Between the bundles, the tissues of the integument are in continuity with those of the secretory zone, and at these spots the deeper-seated layers of the integument—and occasionally the more superficial ones as well—show a radial seriation of the cells (Pl. V, Fig. 5, *r.f.*). In general, it may be remarked that the cells of the outer zone that underlie the furrows show less radial expansion than do those below the ridges.

The secretory zone, with its now extended perimeter, has thinned out considerably. In the inter-fascicular portions of its course, it shows only a single layer of secretory sacs, whilst beneath each bundle it expands to form a longitudinal cushion in which two ranks of secretory sacs are usually present. It is thus evident that the sacs undergo a rearrangement in the body of the seed; in the previous section their maximum occurrence coincided with the inter-fascicular radii; here, the reverse is the case.

The elements of the *tapetal zone* are seen more clearly in this section than in any other that has come under observation. As in the preparation M.H. 369, the cells are thick-walled and tangentially elongated, but there is less radial compression of the layers as a whole. The outmost of the five layers which it comprises shows clear continuity with the dark rim which marks the inner border of the secretory zone (Pl. V, Fig. 2, *tp.*). The successive layers of the tapetum show evidence of having been laid down in radial series, though, perhaps in consequence of encroachment by the prothallus within, the radial files have undergone a certain amount of displacement.

What may be regarded as the delicate prothallus—very rarely preserved in *Physostoma*—is seen in this section lying contracted in the cavity of the seed (Fig. 2, *ps.*). If the membrane which clothes it be the true megaspore-membrane, our seed was very different in this respect from *Lagenostoma Lomaxii* and *L. ovoides*, in both of which the spore-wall was robustly developed.

The third section of the series cuts the seed at the apex and is slightly tilted in the same sense as its fellow-sections (diagram, p. 80, M.H. 371). The plane of section falls above the conical apex of the prothallial cavity, the central body of the photograph (Pl. V, Fig. 3, *p.*), being the epidermal shell of the pollen-chamber. The arms towards the lower side of the photograph—of which one (on the left) has slipped out of the circlet—are cut transversely, and show the epidermal hairs on the peripheral side in approximate longitudinal section. In the upper part of the photograph the arms have somewhat greater radial and tangential dimensions, and the epidermal processes have a multiseriate arrangement—appearances that arise from the fact of the tentacles on this side being cut obliquely, and somewhat nearer to the level of their insertion (cf. diagram, p. 80).

Except when clothing the outwardly directed (abaxial) surfaces of the tentacles, the epidermal cells are without tubular processes and have black contents; the epidermis reaches its minimum thickness as it traverses the inside (adaxial) faces of the arms.

The filling tissue of the tentacles consists of a thin-walled, closely-fitting parenchyma which is incomplete towards the inside in consequence of the breaking-down of the vascular strands. Secretory sacs are absent from the tentacles.

It will be seen from the foregoing general sketch of the organization of *Physostoma* that, in its fundamental features, it is modelled on such a seed as *Lagenostoma Lomaxii*. Like *Lagenostoma*, *Physostoma* has nucellus and integument coalescent throughout the greater part of their extent; at the apex only, where the nucellus ends in a pollen-chamber, are these organs free. The relations of the vascular supply to the integument are identical in the two cases, and the pollen-chambers have much in common.

The outstanding features of difference in the gross structure of the two seeds are to be found in the free portion of the integument. In *Lagenostoma* this consists of nine arcs joined together to form a chambered dome—the 'canopy' of Williamson—through the midst of which the tubular orifice of the pollen-chamber projects to the surface. In *Physostoma*, on the other hand, the canopy is represented by the whorl of free arms; the spatial relations are the same, but fusion is lacking. It is evidently quite inappropriate to apply the term micropyle here, a term designed for an integumental tube leading down to a nucellus and giving access either to pollen-grains or to a pollen-tube. This remark applies with almost equal force to the case of *Lagenostoma*, where the orifice of the pollen-chamber is produced beyond the surrounding canopy, and must have received the pollen-grains without any assistance from that structure. In dealing with the seeds of such remote ages, the possibility cannot be ignored that features may be present belonging to a period anterior to the evolution of what is termed a micropyle (cf. Text-figs. 1, 2, and 10).

The main point to be recognized here is that the tentacles of *Physostoma* represent the units of the *Lagenostoma*-canopy in an unfused and, it may well be, in a more primitive condition.

Another point may be mentioned here. *Lagenostoma Lomaxii* is occasionally found still enclosed in a free, lobed sheath or *cupule*—like a hazel-nut in its husk. No structure of this kind has been detected in any of the specimens of *Physostoma elegans*, though the existence of a cupule is not of course excluded. *Lagenostoma*, with its fairly broad seed-base, had a very effective abscission layer and was shed quite early: *Physostoma*, with its tapering base, may well have been even more readily detached.

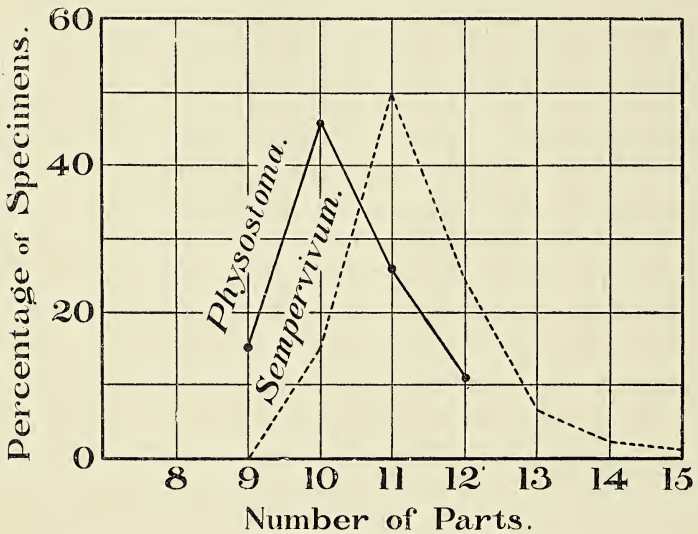
3. *Numerical variation.*

During the course of the present investigation, sections of about fifty specimens of *Physostoma* have come under observation in which it was possible to count the number of ribs or tentacles.

The result is as follows: Seeds with 9 ribs, 8; 10 ribs, 25; 11 ribs, 14; 12 ribs, 6. Total seeds counted, 53. Two seeds apparently showing 8 ribs were rejected as doubtful.

The frequency curve embodying these data expressed as percentages has been plotted in Text-fig. 4.

A similar curve based on countings of the petals of 1350 flowers of *Sempervivum Funkii*[1]—which vary round eleven—is placed beside it for comparison. The *Sempervivum*-curve also represents percentages.



TEXT-FIG. 4. Frequency curves, representing numerical variation of the ribs in *Physostoma* (on the left) and of the petals in *Sempervivum Funkii* (on the right). The numbers are expressed as percentages in both cases.

The close agreement in type between these curves points to the essential identity of the phenomena of numerical fluctuation in palaeozoic times and at the present day.

. . . . . .

The various organs and tissue-systems of *Physostoma* will now be described in detail.

III. Details of Structure.

In setting out the structural details of *Physostoma*, two principal regions are distinguished—the central or sporangial body of the seed and the envelope or integument by which the former is surrounded. These two regions are separate at the apex only; throughout the rest of the seed they are completely confluent.

In a good many seeds of this type the fusion is so complete that no anatomical feature stands out which can serve to indicate the position of the true boundary. In cases of this kind it is convenient to have recourse

[1] The Sempervivum-countings have been taken direct from Klebs's 'Ueb. künstliche Metamorphosen', Stuttgart, 1906, p. 13.

to an arbitrary boundary such as is obtained by producing down to the chalaza the plane of separation of the free regions of nucellus and integument. But in *Physostoma*, as we shall see, the anatomical characters come to our aid and leave us no choice in the matter. The following classification of regions is followed, whilst the grounds on which it is based will be stated in the discussion at the end (p. 106).

The Integument includes the tentacles and the ribbed investment of the seed as far as (but excluding) the secretory zone. The vascular strands belong to the integument.

The Nucellus includes the free apex (of which the peripheral portion forms the pollen-chamber) together with that part of the central body of the seed which lies within the outer limit of the zone of secretory sacs.

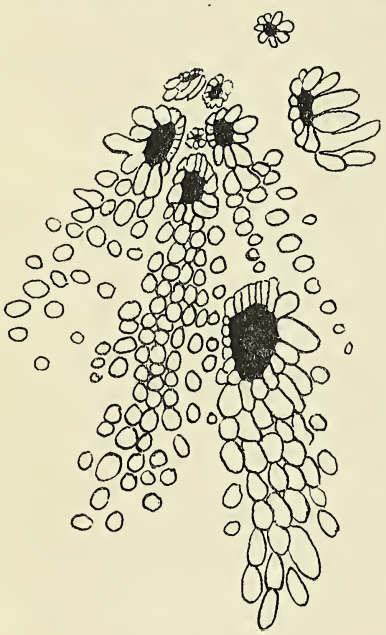
1. *The Integument.*

In the foregoing account we have seen that throughout the body of the seed the integument is a ribbed structure fused with the nucellus, whilst level with the pollen-chamber it becomes free, at the same time breaking into a ring of about ten tentacles arranged to form a loose cone around the pollen-chamber. Or, starting at the apex, and tracing it downwards, we have a whorl of separate arms which unite laterally with one another at the base of the pollen-chamber. The common tube thus formed almost at once becomes coalescent with the nucellus, and the two run in continuity to the base of the seed. The free arms, however, so far maintain their individuality as to be represented by very distinct ribs traversing the seed in the longitudinal direction. The grooves between the ribs are at first deep, but become shallower as the seed-base is approached—probably dying out at the insertion where the seed tapers to a blunt point (cf. Pl. V, Figs. 2 and 1; Pl. VI, Fig. 13). The common, free tube of joined tentacles—the zone of the seed where there is a continuous free integument—is excessively short and practically negligible. It is only in the case of sections cut horizontally, or all but horizontally, that the existence of such a tube becomes apparent at all. Such a one is Dr. Scott's section through a compressed seed (Scott Collection, 1907), which passes not quite horizontally through the region in question. At the top of the figure (Pl. VI, Fig. 15)—the actually highest part of the section—five arms are already entirely free, whilst at the bottom two are still confluent with each other and with the nucellus. On either side, however, are seen two pairs of tentacles still in tangential continuity, though free from the body of the seed, from which they are separated by a sinus (Pl. VI, Fig. 15, *sn.*). The figure shows the sinus in open communication with the exterior between the free tentacles.

Another section from a slightly lower plane is shown in Pl. VI, Fig. 13. At the top of the figure (*sn.*) the beginnings of the sinus are apparent. The extreme vertical depth of the sinus is found by reference to Pl. VI, Fig. 18.

It is at this region, were the section cut in the true horizontal plane, that the relations might be regarded as perfectly comparable to the 'canopy' of a *Lagenostoma*. Though practically insignificant here, attention is drawn to the fact, as it serves to show how very slight is the readjustment necessary in the mutual relations of the parts to pass from the *Physostoma*- to the *Lagenostoma*-condition so far as this particular feature is concerned.

At the level where they are still joined tangentially (Pl. VI, Fig. 13), the tentacles have a radial diameter of ·36 millim., and a tangential diameter of ·4 millim. The radial dimension, however, falls almost at once in correspondence with the expansion of the nucellus to form the pollen-chamber, whilst the tangential dimension more nearly holds its own for some little distance, as the bulging wall of the pollen-chamber prevents the whorl of tentacles in this region from contracting its perimeter. Above the pollen-chamber the arms gradually taper, the smallest transverse section of the distal part of an arm that has come under observation having a diameter (inclusive of the epidermis) of 77 μ.



Text-fig. 5. Oblique section across the tip of a seed, above the pollen-chamber. Nine tentacles are shown. Four of the lower ones, cut somewhat tangentially, are subtended by numerous cross-sections of epidermal hairs which were inserted at a lower level. The body of each tentacle is drawn black. × 34. U. C. L., K. 36, *a*.

The study of numerous longitudinal and oblique sections of the seed shows that the arms may attain a length of 2 millims. from the zone of their insertion—that is to say, their extension beyond the summit of the pollen-chamber is about 1 millim.

As regards their position, the majority of preparations show the proximal parts of the arms to have formed an enclosure to the pollen-chamber, one deep. Occasional specimens, however, show that one or another of the arms have slipped out of rank as in Prof. Bertrand's section (M.H. 371, Pl. V, Fig. 3, t^1).

With the distal portions of the arms, more irregularity of arrangement obtains. Sometimes they are found in an untidy bunch—as is well illustrated by an oblique section cut well above the pollen-chamber (Text-fig. 5), the highest that has come under observation. In this figure the interior tissue of the arms is blackened, whilst the epidermis and its appendages are drawn in outline. Four of the nine tentacles—distinguished by their

trains of satellites—are cut obliquely more or less in the tangential plane, whilst three are cut more nearly at right angles. It is evident that geometric symmetry is lost.

Again, in Pl. V, Fig. 4, the positions of the right and left longitudinally cut arms are quite asymmetrical, whilst the sections of four other tentacles above the pollen-chamber ($t_1 \ldots t_4$) show these also to have been in disorder. In contrast to these, others are met with in which the arms preserved a conical posture above the pollen-chamber, as in Dr. Scott's section S. 1753, W.C. 1440, and a beautiful preparation in Mr. Watson's Collection, prepared with his own hands (Text-fig. 7, p. 92).

The matter need not be pursued further: it is evident the arms afforded adequate protection for the pollen-chamber after pollination had taken place, and it may be conjectured that at an earlier stage they had diverged, permitting access to its orifice. Of this, however, no direct evidence has been found, as almost all our seeds appear to be, approximately, at the same stage of development.

2. *The Epidermis.*

The epidermal system of *Physostoma* merits detailed consideration on account of the remarkable tubular prolongations into which its cells are drawn out along the convex, outwardly directed faces of the tentacles and ribs—a feature which gives to almost every section of the seed a unique and beautiful appearance.

If a transverse section of a tentacle be examined at the level of the pollen-chamber, such, for instance, as *l.* in Pl. V, Fig. 7, the flat, centrally-directed face is found to be clothed with a close-fitting layer of small, almost cubical, cells having a radial diameter of about 30 μ.

Passing round to the flanks, the height of the cells increases. Many of the cells of intermediate height interlock with corresponding cells on the adjacent tentacles (cf. Pl. V, Fig. 7), thus giving the ring of tentacles in this region much of the character of a united tube. That real fusion is lacking is evident from the readiness with which the tentacles separated without injury to the cells. The interlocking, though less in degree, may be compared to the well-known case of the syngenesious anthers of a Composite flower. In not a few cases among the numerous transverse sections at the level of the pollen-chamber that have come under observation, close contact between the adjacent tentacles did not persist till fossilization, though the close correspondence of the indentations of the epidermal layers show this displacement to be the result of some post-mortem change. Directly the region of contact is passed, the epidermal cells, now much broader tangentially, are found expanded in their full width as large, cylindrical, unicellular hairs or processes with substantial walls which stand out at right angles to the surface of the tentacle. It is along the middle line

of the convex (abaxial) face of the tentacle that the hairs attain their greatest radial extension ; those on the flanks getting shorter and shorter as the region of contact between neighbouring tentacles is approached (Pl. V, Fig. 7).

The relations just described are shown on a larger scale in the sketch of a portion of a transverse section of a tentacle (Pl. VII, Fig. 26, where only the proximal portions of the hairs are given). It is usual to find about seven or eight hairs abreast on the transverse section of a tentacle in its thickest part, but where the section is obliquely cut (as in Pl. V, Fig. 8) this number is of course exceeded.

Although in a majority of cases the adjacent hairs on a rib or tentacle have a tendency to stick together, it is improbable that there was real fusion for a greater distance from the place of insertion than about 75 μ. The *form* of the hairs is subject to some variations. In a fair number of specimens they are club-shaped—the hair tapering to its base. It seems possible that the clavate type corresponds more nearly with the form during life.

As regards their *distribution*, the hairs are arranged in longitudinally running lines—on the tentacles and seed-body alike. This is clearly shown by an inspection of tangential or very oblique sections through the piliferous layer. In these the successive sections of the individual hairs follow one another in linear series, as shown in Pl. V, Fig. 11, and in Pl. VI, Fig. 16, for the tentacles, and in Pl. VI, Fig. 22, for the body of the seed. In this feature the hairs resemble the cells of the palisade-layer of the testa of *Lagenostoma Lomaxii*.[1]

With the tapering of the tentacles—as at the level of the summit of the pollen-chamber—first one and then another row of hairs dies out. Those that persist undergo no appreciable contraction as they pursue their way to the tip, though the length of the hairs diminishes. The fine points in which the tentacles end seem to have been without hairs (Text-fig. 5, three of the upper tentacles).

As already stated, the hairs formed an ample covering to the seed, so that its surface during life would at best have been dimly visible along the lines of the grooves.

The *contents* of the epidermal cells do not always show the same type of preservation. The non-elongated cells of the grooves on the seed-body and of the adaxial sides of the tentacles have, as a rule, blackened contents, but this is not invariable. The hairs, on the other hand, whilst occasionally showing blackened contents, are, in many cases, filled with an opaque yellow matter, which may be homogeneous or vacuolated, or fragmented into granules or larger masses. In the cases in which the secretion does not fill the hairs it occupies a peripheral position. The nature of the secretion that was present in these hairs must remain a matter of con-

[1] Oliver and Scott, On *Lagenostoma Lomaxii*. Phil. Trans. B., vol. 197, p. 205.

jecture: analogy suggests that it might have been of a mucilaginous character, but at present the investigation of fossilized secretions has been too slight for any confident expression of opinion. It seems probable, however, that we have to deal in this case with a genuine secretion of the protoplasm, rather than with a degradation product of the cell-membrane.

3. *The Ground-tissue of the Integument.*

The Ground-tissue of the Integument consists of closely-fitting, thin-walled parenchyma cells of elongated, prismatic form, the longer dimension being parallel to the axis of the seed. The approximate dimensions of these cells are 200 μ × 25 μ.

In the case of the tentacles, this tissue forms the whole of the filling substance. It was traversed in the median plane, just below the small-celled inner epidermis, by a vascular strand, but in the great majority of specimens the strand has broken down, and is represented by an intercellular space (Pl. V, Fig. 7, *l.*).

In the body of the seed, where integument and nucellus are confluent, there are from five to six layers of these cells, forming an undulating belt (Pl. V, Figs. 2 and 5). The outer layer (upon which the hairs are inserted) and the layer beneath it consist of somewhat larger cells than the deeper-lying regions; they often show a slight radial extension.

The cell-contents. The whole of these cells—alike in the tentacles and on the seed-body—show an interior contracted tube, light brown in colour, which on cursory inspection might be taken for a slightly plasmolysed and petrified protoplasmic body (Pl. V, Fig. 5, and Pl. VII, Fig. 26, *f.t.* and Fig. 28). These inner tubes are well seen in most specimens, though, as we shall see, the appearance is not invariable. However striking the resemblance to protoplasmic bodies, it is impossible to accept this interpretation without reserve in the absence of much more critical histological investigations than have yet been carried out upon fossilized cells and tissues. In the present instance this reserve is justified because a certain number of specimens have come under observation which show no inner tubes; moreover, such specimens have the further point in common that the walls of the cells are unmistakably thicker than in the case of the more usual type of preservation (Pl. V, Fig. 9). Hence the possibility is by no means excluded that the inner tubes or vesicles may not be protoplasmic vesicles at all, but rather the lining layers of the cell-wall which have become separated from the main reticulum of cell-walls. In view of these facts it would seem probable that the vesicles in question are really derived from the membrane and not from the protoplasm.

Throughout the body of the seed the interior border of the parenchyma-zone of the integument abuts upon the very delicate zone in which the secretory sacs are situated—the region usually least well preserved of any

part of the seed-wall. The salient ribs, of course, overlie the vascular bundles, or, more correctly, the lacunae which are constantly found on the abaxial side of these strands (Pl. V, Fig. 6; Pl. VII, Fig. 28). It is only at the base of the seed that these lacunae become confluent tangentially, so that the parenchyma-zone of the testa shows complete separation from the subjacent tissues. (Cf. p. 81 and Pl. V, Figs. 1 and 2, for trans. sections; Pl. VI, Figs. 21 and 22, for longitudinal sections.)

Passing from without inwards, except at the seed-base, the tissues follow one another as follows: epidermis, with hair-like prolongations on the ribs; parenchyma of integument; vascular strands; secretory zone; and finally the conspicuous black-walled zone lining the seed-cavity, which may be designated tapetum.

. . . . . .

At the base of the pollen-chamber, where the integument becomes free from the nucellus and separates into tentacles, the vascular strands, accompanied by their lacunae, bend out slightly and pass into the tentacles. The secretory zone does not enter the tentacles—indeed, secretory sacs have not been observed either in a tentacle or in the very short united tube above the level at which the nucellus becomes free. The secretory layer and tapetum, both with much diminished radial depth, pass up to the pollen-chamber, clothed externally, of course, by the epidermis which lines the sinus (Pl. VI, Fig. 13).

These layers form the narrow belt or plinth upon which the pollen-chamber is seated. This zone measures ·15–·2 millims. across; it is simply a zone of nucellar tissue intercalated between the place of separation of the tentacles and the bottom of the pollen-chamber. A similar zone is present in the full-sized seeds of *Lagenostoma Lomaxii*, where it is considerably more extensive than in *Physostoma* (·5 millims. across), whilst the slope up to the pollen-chamber is quite gentle, instead of being steep, as in the present case.[1] (Pl. V, Fig. 12, *pl*; Pl. VI, Fig. 18.)

4. *The Pollen-chamber.*

The general features of this organ have already been outlined in the introduction (p. 78). It is a circular chink-like cavity at the apex of the nucellus, formed by the separation of the outer layer (epidermis) which communicates with the exterior by a small circular orifice situated on a low papilla in the axis of the seed (Pl. VI, Figs. 17 and 18, *o.p.c.*). The extreme dimensions of the pollen-chamber are: Horizontal diameter (outside wall to outside wall) 1·2 mm.; height (from insertion to orifice) 1 mm. The corresponding dimensions in *L. Lomaxii* are ·75 mm. and ·7 mm.

The effective cavity of the chamber is restricted to a chink about 37 μ

[1] Oliver and Scott, loc. cit., p. 200.

wide, owing to the projection into it from below of the apical prolongation of the megaspore and surrounding tissue of the nucellus (Pl. V, Fig. 8, *p.c.*).

In most specimens the chink widens in its lower half (Pl. VI, Fig. 17), but this condition is probably not the natural one. In Williamson's original section,[1] and in a very few other specimens,[2] the central projecting column is buttressed below by a horizontal belt or cushion of tissue—which forms the actual lining of the chamber on its inner side—in such a manner that the width of the chink or lumen remains appreciably uniform throughout (Pl. VI, Fig. 18, *cu.*).

As a rule both the inner and outer walls of the pollen-chamber are preserved as black, structureless crusts, so that reliance must be placed on occasional specimens for anatomical details. The outer wall seems to have consisted of cubical cells with rather thick walls (Pl. V, Fig. 3, *p*, the lower side), which doubtless represent the epidermis; whilst in the inner wall, which was several cell-layers in depth, traces of the tapetum overlaid by secretory sacs may occasionally be recognized. The projecting cushion of tissue of the Williamson specimen (W. C. No. 1439) consisted of parenchyma, and that is all that can be said of it. No doubt in life it must have had assigned to it the double function of secreting a collecting drop and of providing for the nutrition of the pollen-grains during their development in the pollen-chamber. Morphologically, this tissue would be the equivalent of the prominent central cone of the Lagenostomas. If the megaspore-chamber of a *Lagenostoma* be conceived as invading the central cone tissue, so as to occupy almost the entire space except a residue on the flanks, the resulting relations would closely correspond with the *Physostoma*-condition.

The orifice of the pollen-chamber is situated on a low papilla at the apex. It is about 150 μ across (Pl. VI, Figs. 17 and 18, *o.p.c.*). In none of the specimens does the papilla project further than here shown, nor do horizontal sections cut above the pollen-chamber afford any reason for supposing that it is merely the persistent base of a longer beak. If this seed stood alone, it would be quite superfluous even to suspect that a caducous beak had been present; but when regard is had for the structure of the pollen-chamber in the allied seeds such a possibility must be recognized (cf. p. 92).

5. *Pollination.*

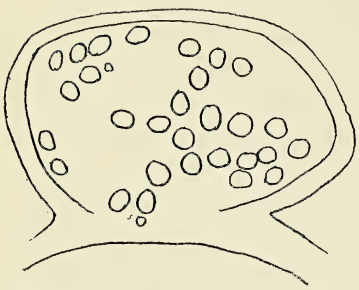
To judge from the quantity of pollen found in the pollen-chamber, the arrangements for pollination in *Physostoma* must have been unusually efficient. A section across an empty pollen-chamber is the exception. The specimen showing the largest number of pollen-grains that has yet

[1] Williamson Collection, No. 1439: figured in his eighth Memoir, Pl. XI, Fig. 77, and Pl. XII Fig. 79, *h*. The position occupied by the cushion here seems to correspond more closely with the natural condition than in our Fig. 18.

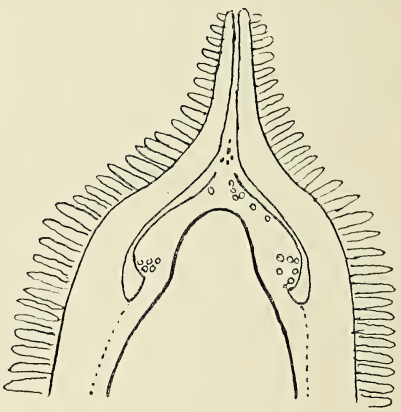
[2] e. g. Scott Collection, No. 1798.

come under observation came from the collection of Mr. D. M. S. Watson—prepared by his own hands. To him I am indebted for the opportunity of examining the two longitudinal sections, one median, the other tangential. The former shows seventeen grains in the pollen-chamber, the latter thirty (see Text-figs. 6 and 7). These two sections alone thus retain close on fifty pollen-grains; but the total number present must have been far in excess of this, when it is borne in mind how much of the pollen-chamber has been lost in the operations of cutting and grinding.

The questions at once arise: How did the pollen-grains get there? What was the agent of transport, and what were the arrangements at the orifice of the seed for the reception of pollen? As to the last, perhaps we may accept the position of the tentacles shown in the accompanying illustration (Text-fig. 7), based on the median longitudinal section in Mr. Watson's possession. In that case the tentacles behaved collectively



TEXT-FIG. 6. Tangential section through the pollen-chamber showing about thirty pollen-grains. Sketched from Mr. Watson's preparation. × 50.



TEXT-FIG. 7. Sketch of Mr. Watson's median section through the pollen-chamber of *Physostoma*, showing two arms approximated above the orifice. The dotted lines below the sinus mark where the integument has broken away from the nucellus. × 20.

as a closed tube, a micropyle in the making. No doubt a drop excreted from the pollen-chamber played an essential part in the mechanism, as in recent Gymnosperms. That the tentacles formed a close-fitting tube around the pollen-chamber and its orifice is also consistent with the rarity of pollen in the sinus at the base of the plinth.[1] Whether the 'micropyle' was occupied by a tubular extension of the pollen-chamber—which thus gained direct access to the exterior—remains uncertain. No traces of any such passage have been detected, but in view of the fact that the pollen-chambers of both *L. Lomaxii* and *L. ovoides* had direct access to the

[1] Notwithstanding the notable exception described on p. 94.

exterior, whilst that of the allied seed, *Conostoma oblongum*, was provided with a long tube, constricted or articulated at its insertion, the possibility of the existence of a caducous or non-permanent structure in *Physostoma* cannot be absolutely dismissed.

If the actual structural details are obscure, so, too, is the agent of transport. The quantity of pollen found in the pollen-chamber suggests an agency of greater precision than mere wind-dispersal, unless the collecting drop at the tip was much more persistent or the load of pollen brought by the wind far greater than is generally the case at the present day. The suspicion is difficult to resist that insects may have been attracted to these seeds in search of the mucilage or nectar or whatever it was that formed the drops.[1] In the contemporary flora the Pteridosperms must have been relatively important from this point of view, for the Angiosperms, with their superior attractions for insects, had not yet invaded the field. Still, even should this be conceded—that the ovules were visited for their drops—there would be little result unless the insects had the regular habit of frequenting the pollen-bearing organs, the Telangiums and Crossothecas, of which we know so little. Perhaps, when existing Cycads, *Welwitschia*, and other Gymnosperms have been more fully studied in respect of their relations to insects, it may become profitable to consider the pollination-methods of the Pteridosperms. The present discussion will have served its purpose if it lead to attention being directed to the living forms.

6. *The Pollen-grains and Spermatozoids.*

The pollen-grains of *Physostoma* are ellipsoidal in form, with average dimensions of $55 \times 45\,\mu$. They are the smallest sized pollen-grains met with in the four species of related seeds found as petrifactions in the Lancs-Yorkshire Coalfield. The dimensions of the others are as follows: *Lagenostoma Lomaxii*, $70 \times 55\,\mu$; *Lagenostoma ovoides*, $70 \times 55\,\mu$; *Conostoma oblongum*, $73 \times 55\,\mu$.

TEXT-FIG. 8. Pollen-grain of *Physostoma* showing the internal cells. The dotted area is the exospore where it has not been ground away. The grain occurs in Mr. Watson's specimen (Text-fig. 6). × 480.

Many of the grains of *Physostoma* show distinct traces of an internal cellular reticulum which recalls that of the pollen of the French seed *Stephanospermum akenioides*.[2] An example is represented in the accompanying Text-fig. 8. In view of the observations to be described below, many or most of these internal cells, and especially the larger ones, probably produced each a spermatozoid. Whether the residuum of smaller cells (of which there is some indication) was prothallial in nature, must remain undecided.

[1] The suggestion first came from Sir Joseph Hooker; see Oliver and Scott, On *Lagenostoma Lomaxii*, Phil. Trans. B., vol. 197, p. 214 (footnote).

[2] Oliver, On *Stephanospermum*. Trans. Linn. Soc., 2nd Ser., Botany, vol. vi, Pl. XLIV, Fig. 33.

We now come to the question of spermatozoids in connexion with the pollen-grains. Already, in 1904, Miss Benson expressed the conviction that the pollen-grains in a specimen of *L. ovoides* in her possession were 'in the act of yielding antherozoids, like those of Cycads and *Ginkgo*'.[1] In a recent note, the same writer has described this preparation in some detail.[2] Four pollen-grains are figured, of which one contains a supposed spermatozoid, whilst another is described as having germinated to form a protruding endospore—something of the nature of a rudimentary pollen-tube. Free in the pollen-chamber there are at least two bodies which the author regards as isolated spermatozoids.

To this example of *Lagenostoma ovoides*, the present investigation of *Physostoma* contributes a further instance which merits description.

The specimen is in the collection belonging to the Botanical Department, King's College, London, and I am indebted to the courtesy of Prof. Bottomley for the opportunity of examining it. Here the group of pollen-grains is contained, not in the pollen-chamber, but, as occasionally happens, at the bottom of the sinus between the tentacles and the pollen-chamber. Though analogy would indicate that the products of such misplaced grains must fail of their mission to accomplish fertilization, there is no evident reason why the pollen-grains should not continue to develop normally enough, provided the necessary protection and nutriment are forthcoming. In the present case three complete pollen-grains are present (Pl. VI, Fig. 30, *a*, *b*, and *c*), and what appears to be the remains of the internal cell-reticulum of a fourth (*d*). The pollen-grain *b* contains two flattened oval bodies, *c* contains one; two others are present, one in contact with the reticulum *d*, and another between *d* and *b*. These five bodies are all clearly defined, show the same type of preservation, are of approximately identical dimensions (20 × 15 μ), and have the same sub-reniform outline. In addition, four other bodies are present which are probably of the same nature, but are less well placed for observation. Two are in contact with, and are partly obscured by, the pollen-grain *a*, whilst two lie on the open edge of *c*.

No appendages or cilia have been detected in connexion with these bodies, nor is there any indication of their being limited by cell-membranes. Assuming that the bodies figured are spermatozoids, it would appear from this preparation that they arise within the pollen-grains themselves (Fig. 30, *b* and *c*). The reticulum *d* would then probably be the remains of a pollen-grain from which the exospore had disappeared.

Turning to Miss Benson's specimen of *L. ovoides*, one of the pollen-grains is found to contain a spermatozoid (loc. cit., p. 410, Fig. 2, p^1). So far the two seeds are in substantial agreement. But if the sperms arise in

[1] *Telangium Scottii*, Annals of Botany, vol. xviii, p. 168.

[2] M. Benson, On the Contents of the Pollen-chamber of a specimen *Lagenostoma ovoides*. Bot Gaz., vol. xlv, p. 409 (June, 1908).

this way, it is difficult to understand the significance of the 'protruding endospore' as figured for *L. ovoides* (Miss Benson, loc. cit., Fig. 2, p^2e), unless, as seems possible, the full history of these tubes has not yet been recovered. In the case of *Physostoma* it is, of course, just possible that the cell reticulum *d* (Fig. 30) may have been carried clear of the grain by the escaping sperms, though there is nothing in the preparation to indicate that *d* has any relation, except juxtaposition, to the pollen-grain *a*. These, however, are matters on which a decision is impossible till further data are forthcoming.

The point of immediate interest is the detection in two related seeds of bodies whose presence in and about the pollen-grains is difficult to account for except as spermatozoids. The two sets of bodies show a close agreement in occurrence, form, and preservation, whilst the large dimensions of the supposed sperms of *L. ovoides* (40×30 μ, as compared with 20×15 μ in *Physostoma*) accords with a similar relation in the size of the pollen-grains in the two cases.

Apart from some quite abnormal product of disintegrating protoplasm, which cannot be profitably discussed, these bodies must be regarded either as organisms parasitic on the pollen-grains, or as petrified spermatozoids, the normal product of pollen-grain development. These and other contemporary seeds are commonly attacked by fungi, and often show the mycelia and vesicles or spores with admirable preservation.[1] But careful comparison with numerous fungal vesicles that have come under my own observation has failed to convince me that these bodies are to be explained away as fungal productions. Some day, let us hope, expert mycologists may turn their attention to the fungal remains that abound in the petrified tissues of Palaeozoic plants. Till such critical studies are forthcoming a final decision on the nature of these supposed sperms is perhaps not possible. Subject to this reserve, I have no hesitation in expressing the opinion that the case for regarding these naked, sub-reniform bodies as spermatozoids is a strong one. As Miss Benson justly remarks, the discovery of spermatozoids in *Ginkgo* and the Cycads has paved the way for their detection in the seeds of Pteridosperms.[2] Now that attention has been drawn to the matter from several quarters, it may be hoped that additional and even more convincing specimens may come to light.

7. *The Vascular System.*

In its fundamental features the vascular system shows agreement with that of *Lagenostoma Lomaxii*. It may be recalled that in this seed the vascular supply is derived from the chalazal bundle, which enters at the seed-base, by its subdivision into a whorl of nine bundles which range themselves symmetrically around the nucellus, and pass distally into the loculi

[1] Cf. Oliver, Notes on Fossil Fungi, New Phyt., vol. ii, p. 49.

[2] Miss M. Benson, loc. cit., p. 412.

of the 'canopy' (cf. p. 109).[1] Owing to the contraction of the zone of cells along which they run, the bundles in *Lagenostoma* are very rarely found *in situ*; commonly they occur attached to a loose sheath which lies about midway between the surface and the axis of the seed. In *Physostoma* no such displacement is found; the bundles lie in a zone at the inner limit of the tissue of the integument where it abuts on the zone of secretory sacs (nucellus).

Another characteristic feature of our seed is afforded by the course followed by the individual strands as they pass from the chalaza. Instead of arising from the chalazal bundle, relatively high up and near the base of the embryo-sac, these strands are already recognizable at a point not far removed from the actual seed-base. No critical section has come under observation to settle the point whether the vascular supply entered the seed as a single strand, but there are several which show that a ring of contiguous strands surrounding a 'pith' was present very close to the abscission-zone (Pl. VI, Figs. 19, 20, and 21). These strands, usually ten in number, gradually diverge and make their way to the apex of the seed at the inner limit of the integument. The funnel-shaped 'pith' below the embryo-sac was filled with secretory sacs (see p. 97). At the summit of the seed the strands pass out into the tentacles—one to each.

A great feature in this seed is the system of lacunae that constantly accompanies the strands. At the base, the divergent ring of bundles is surrounded by a continuous annular chink or lacuna (Pl. V, Fig. 1). From $\frac{1}{2}$ to $\frac{3}{4}$ of a millim. higher up—where the bundles have reached their full peripheral extension—a single lacuna for each strand replaces this common ring (Fig. 2). These spaces, which are semi-circular or crescent-shaped, lie immediately outside the bundles, and pass with them into the tentacles. They probably originate from post-mortem contraction or decay.

Histologically, the bundles consist of xylem only: no phloem elements have been detected, but it is possible the phloem may have broken down to form the lacunae just described. The xylem strands are extremely delicate and rarely show more than six tracheal elements in any transverse section (Pl. V, Fig. 6; Pl. VII, Fig. 28). The largest tracheal elements do not exceed 10–12 μ in diameter, except at the chalaza, where they are somewhat tub-shaped, whilst 50 μ × 30 μ are usual dimensions for the strands. The smallest elements generally occur at or near the outside, so that it is probably correct to describe the xylem-strands as exarch or mesarch. The tracheal elements are for the most part of the delicate, scalariform type (Pl. VII, Fig. 27), though traces of pitted elements have been seen in the chalazal region. An occasional fine spiral element has been found, but the relation of these to the rest of the wood has not been ascertained.

The bundles, after they enter the tentacles, become much attenuated,

[1] Oliver and Scott, On *Lagenostoma Lomaxii*, p. 208.

and are very prone to break down without leaving any traces (Pl. VII, Fig. 26). As a matter of fact, tracheal elements are very rarely seen in the tentacles at all, and never, so far as our experience goes, in the more distal parts. By far the most striking feature about the vascular system is its extreme delicacy and liability to break down. From the phylogenetic point of view, the maintenance of the separateness of the bundles to a point very close to the seed-base is of interest. Taken in connexion with the ribs and tentacles of the integument, it is reasonable to regard this peculiarity in the arrangement of the vascular supply as relatively archaic. For if the integument originated as a whorl of separate outgrowths beneath the nucellus, these outgrowths would have, primitively, each its separate vascular supply. The point is further discussed at p. 105.

8. *The Zone of Secretory Sacs.*

This layer would seem to have been the most delicate region of the seed, and the details of its structure can be studied only in the best preserved specimens. Lying immediately within the ring of vascular strands, and abutting upon the tapetum within, this zone stretches from the chalaza to the apex of the megaspore cavity. It is broadest at the seed-base where it may reach 120 μ across (Figs. 1 and 14), whilst over the body of the seed it hardly exceeds 60–70 μ beneath the ridges (Fig. 2). At the apex it closely invests the megaspore cavity, and appears never to run out into the free arms or tentacles of the integument. For reasons given at p. 106, the secretory zone is regarded as representing the nucellus.

Histologically, the zone consists of delicate thin-walled cells, flattened in the tangential plane, the dark sacs mingled with clear parenchyma. On the transverse section the number of sacs on any radius ranges from one or two to five or six. Radial sections of the seed-wall show that the sacs often run in longitudinal seriation. The sacs are thin oblong cells of tabular form with the following average dimensions: length 60 μ; radial diameter, 8–10 μ; tangential diameter, 50–60 μ. They contain a black, structureless secretion, recalling that of the similar sacs in *Lyginodendron*.

As already stated, the zone of sacs lies within the vascular system of the seed. At the chalaza the entire funnel-shaped space—limited above by the embryo-sac, and on the flanks by the divergent vascular strands—is occupied by crowded sacs (Fig. 19, *s.s.*). The edge of this funnel is continued so as to enclose the embryo-sac, and for a short distance the secretory zone is seen at its maximum width (Fig. 14, *s.z.*). The principal accumulations of sacs in this region lie between, and usually project somewhat beyond, the vascular strands. At these spots the sacs may be counted as many as six deep (Pl. V, Fig. 1), whilst beneath the strands they are not more than two deep. These relations only obtain, however, to a distance of about a millim. from the seed-base, giving place gradually to the arrangement typical of the

greater part of the seed-body. There is a marked thinning out of the sacs between, and a corresponding accumulation beneath, the bundles. The secretory zone thus forms for the most part a thin mantle with thicker ribs or cushions underlying the bundles (Pl. V, Fig. 5, *s.z.*). These relations hold till the zone is reached at which the integumental arms break away from the nucellus. As the arms separate, their tissues become quite free of secretory sacs (Pl. VI, Fig. 13). The zone of sacs is continued in the nucellus right round the tip of the embryo-sac, though it cannot as a rule be separated from the tapetum, with which, in this region, it forms the black, structureless crust, so characteristic of the generality of specimens (Pl. V, Fig. 8). Occasional specimens show, however, that several layers of sacs are present immediately outside the tapetum. The narrow zone which tapers up to the pollen-chamber (the 'plinth') is clothed by a small-celled epidermis which separates at a slightly higher level from the tissue within, to form the wall of the pollen-chamber.

9. *The Tapetum.*

Within the zone of secretory sacs the megaspore-cavity is limited in most specimens by a broad, black, structureless crust which reaches a width of 100 μ. This layer, which lines the embryo-sac throughout, is so intimately consolidated with the secretory layer in the free apex of the nucellus, that the two together form the structureless, carbonized cone which serves as the hollow, dome-shaped core upon which the pollen-chamber is seated (Fig. 17).

In other specimens, however, the black layer is often frayed out on the inner concave side, so as to present the appearance of a number of black rods inserted at right angles to the direction of the layer as a whole, whilst in yet others this condition co-exists with a definite cellular structure on the side abutting on the secretory layer (Pl. VI, Fig. 23, tp^3, and Pl. VII, Fig. 28).

Transitional cases such as these lead up to the condition found but rarely and only in the best preserved specimens, of which Professor Bertrand's series affords an admirable example. Here the zone referred to consists of some six or more radially compressed layers, of which the innermost show the maximum compression, whilst the outermost—that abutting on the secretory zone—has resisted, or not been subjected to, compression (see Pl. V, Figs. 2 and 5, *tp.*, Pl. VII, Fig. 24).

This zone when preserved is composed of rather large cells with blackened walls but without contents. Though the successive layers have undergone displacement in the process of compression, it is probable that they stood in radial files, as suggested by Professor Bertrand's section, M. H. 370 (Pl. V, Figs. 2 and 5) and several others. The U. C. L. sections R, 71 *a*, and R, 74 *a* (Pl. VI, Figs. 14 and 23, and Pl. VII, Fig. 24) afford corroboration to this view.

Sections such as these prove that both the usual structureless condition

and that showing radially arranged black rods, are degradation products of a layer comparable, for descriptive purposes, to a zone of corky periderm that has undergone collapse.

Transverse sections near the base of the seed, where the embryo-sac is tapering, show the layer as a broader zone (as in M. H. 369, Pl. V, Fig. 1) than is the case higher up; whilst in appropriate sections still nearer the chalaza, the tapetum appears to fill the whole cavity of the seed (Pl. VI, Fig. 14, *tp.*).

It is very rare indeed to find this layer with structure preserved in the region of the pollen-chamber; the specimen figured in Pl. VII, Fig. 25, is an exception to the general rule. It is a somewhat oblique transverse section across the apical cone of the nucellus and pollen-chamber; and although the tapetum (which alone is figured) shows a long tangential rift, the layer shows substantial agreement in structure with that found in other parts of the seed.

The compression and poor preservation of the tapetum afford some ground for the supposition that its full functional activity corresponded to an earlier stage in the development of the seed than the one to which the majority of specimens of *Physostoma* belong. This supposition finds support in certain small-sized seeds or ovules of *Physostoma*, of which two specimens have come under observation. In these specimens the dimensions are about three-quarters the normal size, and the general preservation has something in common with the interesting little seed of *Lagenostoma Lomaxii*.[1]

A transverse section of one of these small specimens is represented in Pl. VII, Fig. 29, and the principal feature shown is the tapetum (*tp*), which, partly separated from the outer wall and contracted in stellate manner, is an extraordinarily conspicuous object. It is reasonable to suppose that this specimen, which shows other peculiarities (cf. p. 100), represents a considerably younger stage in development than the generality of specimens.

It seems probable that a tapetal layer may have been a common feature in the seeds of the *Lagenostoma*-group. For in addition to *Physostoma*, occasional specimens of another seed, the rare *Conostoma oblongum* of Williamson, now undergoing reinvestigation, show a well-developed tapetal zone resembling that of *Physostoma*, whilst a reexamination of the young seed of *Lagenostoma Lomaxii* raises the question whether the black-walled 'pipes' (there regarded as the shrivelled nucellus and megaspore wall) may not represent a tapetal layer in a state of collapse.[2]

The available information as to the occurrence of tapetal layers in the *Lagenostoma*-group of seeds may be shortly summarized as follows:— *Physostoma* and *Conostoma oblongum* had a many-layered tapetal zone,

[1] Oliver and Scott, On *Lagenostoma Lomaxii*, Phil. Trans., B., vol. 197, p. 212.

[2] Oliver and Scott, loc. cit., Pl. X, Fig. 34, *i.s.*

which, though it often persisted to a fairly late stage in the history of the seed, probably culminated functionally at a somewhat younger stage than that which most of our specimens have reached (as in R. 99, see Pl. VII, Fig. 29). Its persistence may have been facilitated by suberization of the membranes, as Thomson has shown in the case of recent Cycads and other Gymnosperms.[1] In *Lagenostoma*, on the other hand, a tapetum has not been detected in the ordinary pollinated specimens; so that, if one were present at all, its functional culmination must have occurred at a much earlier stage of seed-development.

10. *The Megaspore-Membrane and the Prothallus.*

Throughout the investigation of *Physostoma* careful search has been made for traces of the megaspore-membrane. In the majority of specimens this structure is conspicuous by its absence, and it is only in the rarest examples that a very delicate membrane has been found delimiting the prothallus (as in Professor Bertrand's specimen, M. H., 370, Pl. V, Fig. 2, *mg*). Though the evidence is mainly negative, the conclusion is difficult to resist that no robust membrane such as occurs in *Lagenostoma* (both species) was yet present in these seeds when they dropped and were petrified. In this connexion it is of interest to note that in *Conostoma oblongum* also—which like *Physostoma* has a many-layered tapetum—a megaspore-membrane has eluded observation. Under these circumstances it would appear probable that the megaspore-membrane in these seeds did not thicken so long as the tapetum was in a state of functional activity.

As regards the prothallus, there is nothing to add to the statement that it was a very delicate tissue in the few cases that have come under observation (as in M. H. 370, Pl. V, Fig. 1, *ps*.). Archegonia have not been seen.

11. *Undersized or abortive seeds.*

Two sections of what appear to be specimens of small seeds or ovules have come under observation during the course of the present investigation. As these sections belong to the middle regions of the seed, and their preservation is indifferent, no full account is possible as in the corresponding specimens of *Lagenostoma Lomaxi*.[2] There are certain features, however, worth placing on record. The better specimen of the two is contained in the preparation U. C. L., R. 99 (see Pl. VII, Fig. 29). This section seems to be cut a little below the height at which the tentacles were given off. Eleven ribs are present, and they are crowned by tubular hairs (the latter are not shown in the figure). The mean diameter of the section, excluding

[1] R. B. Thomson, The Megaspore-membrane of the Gymnosperms, Univ. of Toronto Studies, Biol. Ser., No. 4, 1905.

[2] Oliver and Scott, loc. cit., p. 211.

the hairs, is 1·6 millim., as compared with 2 millims. or more in normal specimens. The most striking feature is the tapetum (Fig. 29, *tp.*) which was unusually conspicuous and well-developed, and has been referred to on p. 99. The other feature of interest relates to the position occupied by the vascular strands, which is relatively far out in the ribs (Fig. 29, *v.b.*) as compared with the usual position in full-grown specimens (cf. Fig. 2, *v.b.*). The most obvious explanation of this apparent anomaly is to be found in the probable immaturity of the deeper layers of the integument. Now if we suppose the layers with radial seriation, that are present in full-grown specimens, especially below the furrows (Pl. V, Figs. 5 and 6, Pl. VII, Fig. 28, *r.f.*), to be the last portion to differentiate, this, combined with a general expansion of the seed, would bring about the necessary readjustment for the shallowing of the grooves; in this way the ribs would become less prominent and the bundles would come to lie relatively deeper as the seed approached maturity. Were the preservation adequate in the specimen under discussion, traces of this inner meristem ought to be visible.

12. Diagnosis.

Physostoma (Williamson).

Straight, ribbed seeds, radially organized, the ribs separating at the summit into a whorl of free arms which surround the pollen-chamber.

The ribbed integument is coalescent with the nucellus as far as the level at which the arms separate.

The summit of the embryo-sac tapers into a papilla which projects into the pollen-chamber as a hollow core, like the bottom of a wine-bottle.

A vascular system enters at the chalaza, dividing at once into separate strands which run along the ribs and out into the arms.

The nucellus contains numerous secretory sacs; a tapetal zone is also present.

1. *Physostoma elegans* (Will.).

'On some fossil seeds from the Lower Carboniferous beds of Lancashire', Brit. Association Reports (Bristol), 1875, p. 159.

Lagenostoma physoides, Will., 'Organization of the Fossil Plants of the Coal-Measures,' pt. viii, Phil. Trans., 1877, p. 241, Figs. 77, 78, and 79. *Sporocarpon ornatum*, Will., loc. cit., pt. x, Phil. Trans., 1880, p. 510 and Pl. XVIII, Fig. 39; pt. xii, Phil. Trans., 1883, p. 469, and Pl. XXXI, Fig. 27. *Sporocarpon anomalum*, Will., loc. cit., pt. xii, Phil. Trans, 1883, p. 474.

Localities: Moorhouse; Dulesgate; Bacup; Shore-Littleborough (abundant); Ashton-under-Lyne; Halifax; always in the seam-nodules.

Horizon: Lower Coal-Measures.

Length, 6 millims.; broadest diameter, $2\frac{1}{4}$ millims.; the convex faces of ribs and tentacles, which are usually ten in number, are densely covered with tubular hairs reaching a length of $\frac{1}{2}$ millim.

2. *Physostoma Kidstonii*, Arber.

Lagenostoma Kidstonii, E. A. N. Arber, 'On some new species of *Lagenostoma*,' Proc. Roy. Soc., B., vol. lxxvi, 1905, p. 245, and Pl. I and II.

Length 6 millims.; broadest diam., $2\frac{1}{2}$ millims.; ribs and apical lobes usually six in number. Known only as casts.

Locality: Stonehill Colliery, Stonehouse, Lanark.

Horizon: Lower Coal-Measures.

IV. General Discussion.

The Multiple Integument.

More than one writer on Palaeobotany has drawn attention to the very exceptional condition presented by the free part of the integument of *Physostoma*.[1] Comparison with such a seed as *Lagenostoma Lomaxi* shows that the united canopy finds its homologue in the circlet of free tentacles of *Physostoma*. The chambered structure of the former becomes intelligible when viewed in the light of the latter, and leads to the irresistible conclusion—finding support in the widest possible range of analogous cases—that priority in this line of descent must be given to an integument of separate, free segments. The numerical excess of the free tentacles of *Physostoma* over the united chambers of the known Lagenostomas points in the same direction, i.e. to the relatively primitive condition represented by our seed.[2]

If we were fully informed of the range of structure of the integument in the seeds of the *Lagenostoma*-group, we should doubtless recognize stages of coalescence intermediate between *Physostoma* and *Lagenostoma Lomaxii*. Such an intermediate condition may be accepted without much risk of error for the case of *L. Kidstonii* of Arber, whilst this author's *L. Sinclairii* may be a second example of the same condition. Both of these were ribbed seeds with notched apices, and their recognition tends to remove *Physostoma* from the very isolated position it would otherwise occupy.

When we come to the question of the *origin* of such an integument as that of *Physostoma*, the only point that is obvious is that here, and in the *Lagenostoma*-group generally, this origin has been a multiple one. In the absence of direct evidence, the morphological nature of the unit structures

[1] Miss M. Benson, On Telangium Scottii, Annals of Botany, vol. xviii, p. 169. D. H. Scott, Progressus Rei Bot., I, p. 211.

[2] The usual numbers are as follows: *Physostoma*, ten: *Lagenostoma Lomaxi*, nine; *Lagenostoma ovoides*, eight; *Conostoma oblongum*, seven; *Lagenostoma Kidstonii*, about six.

which collectively form the integument is susceptible of the most diverse interpretation. Two views have already been put forward, and as these are fundamentally opposed, and illustrate quite distinct tendencies in morphological interpretations, the matter is of some little interest.[1] Many morphologists hold that all new structures are really fashioned out of old ones that have undergone a functional change. The petal is a sterilized sporophyll; the paraphyses of mosses are sterilized antheridia; the cortex of the aecidial fruit in the Uredineae is the reduced product of fertile hyphae. Of this order is Miss Benson's suggestion that the integument of *Physostoma* has been elaborated by the sterilization of the peripheral sporangia of an ancestral synangium, the central member of which is represented by the nucellus of the seed—a suggestion which is embodied in her synangial theory of the seed. That sporangia may undergo sterilization and persist as paraphyses scattered through the sori is illustrated by many present-day ferns, more particularly by representatives of the Polypodiaceae, as in *Polypodium verrucosum*, *Vittaria rigida*, *V. Forbesii*, and *V. angustifolia*, *Acrostichum aureum*, &c.[2] Among palaeozoic Protofilices somewhat similar structures have been attributed by Renault to *Botryopteris forensis*.[3] In the absence of special investigations elucidating the nature of these curious structures, it may be admitted provisionally that their sporangial derivation seems probable, and that their existence gives plausibility to the synangial theory.

When we turn to cases offering a closer parallel to the seed condition, such as *Lepidocarpon* and the megasporocarp of *Azolla*, the facts that have been ascertained are less favourable to the theory. In *Lepidocarpon* it is difficult to interpret the integument as other than a special production or enation of the supporting bract or sporophyll, whilst in *Azolla* the non-functional sporangia, which are present immediately below the megasporangium, are found to abort during development, whilst the indusium or sporocarp-wall arises as an independent annular upgrowth below the point of insertion.[4]

Cases such as these show clearly enough that the possibility of integuments arising as new or special formations (where seeds and similar structures are involved) cannot be lightly dismissed. The synangial theory though no doubt tenable, is, after all, no more than a hypothesis, which presupposes in the ancestor the existence of the exceptional condition of a synangium in which the peripheral members were ranged symmetrically around a central sporangium, and in which—unlike *Azolla*—they persist as a sterilized envelope to form the seed-coat.

[1] Miss M. Benson, loc. cit., p. 161; Oliver and Scott, loc. cit., p. 232.

[2] See Hooker's Genera Filicum, Tab. 14, 68 B, 76 B, 77 A, 81 A, &c.

[3] See Renault, Bassin houiller et permien d'Autun et d'Épinac. Flore fossile, pt. 2, p. 54.

[4] See Goebel's Organography of Plants, Pt. 2, p. 488, Fig. 325.

It is undeniable that extreme reluctance is frequently shown in interpreting a given structure as a new formation, and no doubt this conservative attitude has much to justify it. But no one will be prepared to maintain that there have never been new departures involving fresh productions in evolutionary history, though he may incline to relegate them to a remote and prehistoric past. Before such a view can be seriously advanced in any given instance it is necessary to establish a strong case on general grounds that such a production is not merely useful—for that could only explain its survival not its origin—but that its apparition is inherently probable under the circumstances.

In considering the special case of the origin of a seed-envelope we do well to bear in mind what commonly happens in analogous cases. Now where there is localized reproductive activity—especially when centred in organs of a more or less persistent character—nothing is more usual than to find associated with it great vegetative vigour involving the parts round about. These manifestations may take the form of sterile, sheath-like upgrowths or pullulations which enclose the reproductive products, or the whole platform upon which the latter stand may be so permeated with growth that the reproductive organs become overarched or immersed. Examples of the former method are furnished by *Coleochaete*, *Chara*, numerous Fungi, Red Seaweeds, Liverworts, and the arils of Angiosperms; of the latter by Fucaceae, Pteridophyte prothalli with immersed archegonia, the perigynous and epigynous flowers of Angiosperms.

The capacity to form such enclosures is of such wide occurrence that Solms-Laubach was inclined to regard it as an indication of a means at the disposal of the plant for reaching a continually increasing complexity of structure.[1] To emphasize this 'principle', to which he attached importance, Solms-Laubach proposed for it the term 'cupular formation', or, as we may say *encasement*. Though it may be premature to attempt to define in terms of stimulus and response the precise sequence of events that leads up to encasement, it will be readily admitted that a new departure such as the inception of the seed habit (where provision has to be made for the increased nutritive drain involved by the retention of the gametophyte would be accompanied by nutritive disturbances that might easily favour the appearance of 'new formations'. Once streams of food become diverted to spots where reproductive bodies are to be fed, it is difficult to see how the associated vegetative tissues (which are usually still in an embryonic condition when the reproductive bodies are laid down) are to be excluded from a share of these supplies. If this be granted, we see that the conditions are favourable for encasement, whilst these encasements, if they serve a useful purpose, will tend to be perpetuated.

[1] Solms-Laubach, On the Fructification of *Bennettites Gibsonianus*, Ann. of Bot., vol. v, p. 451.

In view of these considerations it is suggested that the integument of *Physostoma* may be an example of encasement, a structure, that is to say, whose origin is contemporary with the inception of the seed habit. Here, as in other members of the group, it has become coalescent with the central sporangium. Those seeds which possess cupules, like *Lagenostoma Lomaxii* and *L. Sinclairii*, according to this view, have undergone a second encasement, though the envelope is much less specialized than in the case of the integument proper. In later groups, such as the Angiosperms where, in addition to two 'normal' integuments and a carpel, an aril is often present, we have further phases of the same function.

. . . . . .

Assuming the view to be well-founded that the integument of the seeds of the *Lagenostoma*-group was a lobed structure at its inception, it is not without interest to see how far this primitive feature has impressed itself upon the several seeds.

In *Physostoma*, as we have seen, the tentacles and ribs are the conspicuous feature at the surface, whilst deeper down the vascular strands are in perfect correspondence. In addition to the integument the tissues of the nucellus in the body of the seed are slightly involved, for the secretory zone *below* the ribs shows more numerous ranks of secretory sacs than occur *between* the ribs.

Conostoma oblongum stands next to *Physostoma*. The free part of the integument is united around the pollen-chamber, it is true, but the unit-portions show a distinct tendency to separate, and the lines of fusion are often marked by shallow grooves. The body of the seed is smooth and circular in section and without trace of ribbing save at the chalaza, where sharp-angled ridges appear overlying the vascular strands.

In *Lagenostoma Lomaxii* and *L. ovoides* the free part of the integument is perfectly united, and the body of the seed smooth throughout. The multiple origin of the integument, however, is well seen in the chambered, free integument—Williamson's 'canopy'—each chamber representing the apex of an original integumental lobe. The vascular strands correspond in number with the chambers of the canopy, though in very occasional specimens their number may be reduced by fusion lower down the seed-wall. The little ridges which surround the micropyle, and are conspicuous in some sections—especially in *L. Lomaxii*—should not be mistaken for the unit-lobes of the integument. They overlie the well-marked septa between the chambers of the canopy, and it is quite possible they owe their prominence to post-mortem contraction or collapse of the filling-tissues of the chambers.

The course pursued by the vascular strands at the chalaza may be recalled here. Whilst in the Lagenostomas a common supply bundle runs up almost to the embryo-sac before the peripheral strands separate out,

in *Physostoma* the individual bundles begin to diverge much lower down. It is difficult to resist interpreting the former as the derived, the latter as the relatively primitive condition.

2. *The Nucellus.*

The investigation of *Physostoma* has brought to light a number of features connected with the structure of the nucellus which cannot be dismissed without consideration.

These include (*a*) the zone of secretory sacs; (*b*) the tapetum; (*c*) the form of the megaspore cavity, and the pollen-chamber.

(*a*) *The Zone of Secretory Sacs.* That the secretory zone should be referred, morphologically, to the province of the nucellar wall rather than to that of the integument, seems to follow clearly from the non-continuation of the secretory zone into the free integumental arms. At the place of separation, the bundles and ground-parenchyma pass out into the arms, whilst the secretory zone and tapetum pursue their course into the free nucellar apex.

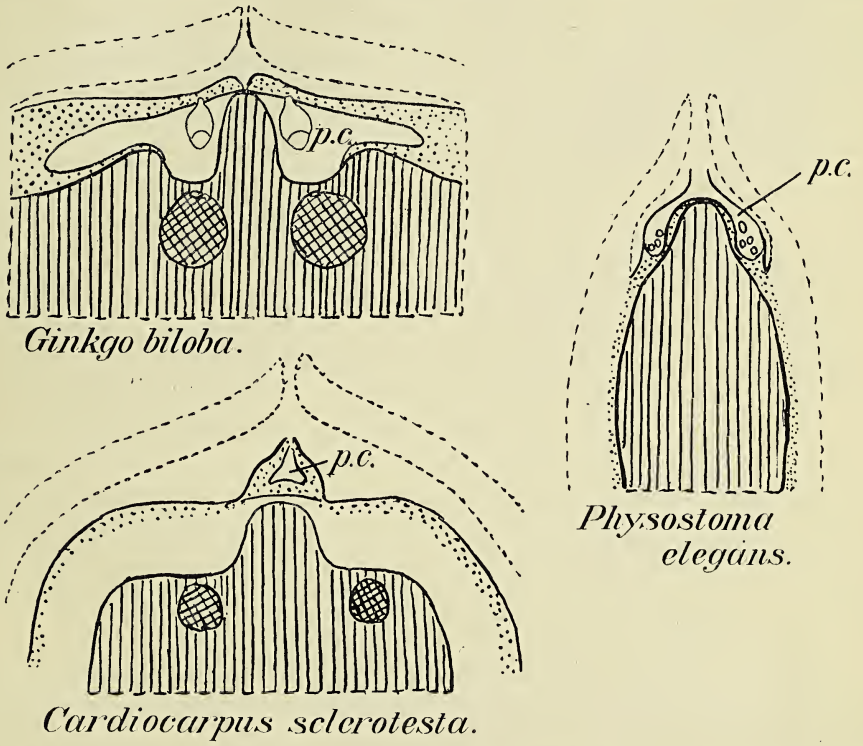
In view of the general similarity in organization that obtains between *Physostoma* and the other members of the *Lagenostoma*-group of seeds, it is of some interest to note that in none of the latter have secretory sacs been observed in the nucellus. For if it be true, as we conjecture, that the whole of this group of seeds belonged to separate but as yet undiscriminated members of the Lyginodendreae, we have in *Physostoma* the sole representative of the group of seeds which retains in a noticeable way a character that is invariably present in the vegetative organs of the hypothecated seed-bearers. As an isolated feature, not much significance would attach to the point; but it assumes a different complexion when we find it associated with an *ensemble* of peculiar and primitive characters, as we do in *Physostoma*.

(*b*) *The Tapetum.* As regards the tapetum, so striking a feature in *Physostoma*, little need be said. Layers of this kind, occupying a position between the sporangial wall and the sporogenous complex, are generally present in the dehiscent sporangia of the archegoniate or spermophytic series, whilst they have been frequently met with in the indehiscent sporangia (ovules) of both Gymnosperms and Angiosperms. Notwithstanding the doubt that has been expressed as to whether these structures can be regarded as really homologous, in view of the diversity of their development[1] in different cases, the presence of an extensive tapetum in an early seed like *Physostoma* raises the question whether it may not be a structure inherited from some non-spermophytic ancestor. Data bearing on this point in the oldest known Pteridophytes—the Botryopterideae—are meagre in the extreme, but so far as they go they point to the presence of a considerable

[1] Cf. Goebel in Organography of Plants, pt. 2, p. 596.

zone of tissue interposed between the sporangial wall and the spore-complex. Of these perhaps Grand' Eury's *Schizostachys grandosus* is the most notable example.[1]

(*c*) *The Form of the Megaspore-cavity and Pollen-chamber.* The conical prolongation of the megaspore-chamber at the apex is another curious feature which must not be passed over. It vividly recalls in somewhat generalized form the beak-like process of the embryo-sac in *Ginkgo*,



TEXT-FIG. 9. Diagrammatic longitudinal sections of the apices of the ovules of *Ginkgo*, *Cardiocarpus*, and *Physostoma*, showing the 'tent-pole'-like papilla of the megaspore. The gametophyte is represented by vertical lines, the nucellus is dotted, and the integument given in broken outline. *p.c.*, pollen-chamber.

which Hirasé compared with a tent-pole, holding up the roof of the pollen-chamber.[2] As Scott was the first to point out, a similar structure is found in several Cordaitean seeds.[3] The interest attaching to this correspondence

[1] Flore Carbonifère, p. 201, and tab. xvii. *d* and *d*[1], Other cases are enumerated in my 'Vascular Sporangium', New Phytologist. vol. i, pp. 62–3.

[2] S. Hirasé, Études sur la Fécondation, etc. du *Ginkgo biloba*, Journ. of the Coll. Science, Japan, vol. xii, p. 113, and Pl. IX, Figs. 35 and 36.

[3] Studies in Fossil Botany, 1900, p. 440. Numerous 'tent-poles' are figured in Brongniart's 'Les graines silicifiées', Pl. II, Fig. 2; Pl. V, Fig. 5; Pl. VI, Fig. 7; Pl. XI, Fig. 4; Pl. XII, Figs. 1 and 2; Pl. XV, Fig. 5. See also Coulter and Chamberlain, 'Morphology of Spermophytes,' pt. i, Gymnosperms, p. 140.

in *Physostoma* arises from the possibility that this seed presents us with the primitive structure of which the ordinary 'tent-poles' (*Ginkgo* and *Cordaites*) are surviving representatives—that the apical prolongation of the megaspore-chamber is the primordial tent-pole. In the accompanying Text-fig. 9, the three cases referred to are represented, as far as the relations at the apex of the nucellus are concerned.

If the conjecture prove well-founded that the extension of the megaspore-cavity right up to the apex was an archaic trait, derived from a very remote past, it would be intelligible that this part of the megaspore should show relative arrest or atrophy in its capacity for expansion; for the necessity for the pollen-chamber to become effective at an early period in the development of the ovule is quite obvious.[1] And if the pollen-chamber matured at an early stage in ontogeny, the stage being accompanied by cuticularization of the pollen-chamber wall, the immediate result would be an arrest of expansion at the apex, such as we find in *Physostoma*. The cuticularization, no doubt, may have been related to the imperfect protection afforded by the integument in the seeds of the *Lagenostoma*-group.[2]

If it be conceded that functional necessity determined an arrest of the nucellar apex, the final disappearance or abortion of the megaspore papilla ('tent-pole') would follow in its train, in those cases in which its persistence would be without significance. For in the *Lagenostoma*-group the wall of the pollen-chamber was probably sufficiently rigid to resist collapse without assistance from a 'tent-pole', and it is not very likely that the portion of the female gametophyte that lay within it was concerned in the production of archegonia. If this were the case, the disappearance of the apical process and its replacement by nucellar tissue, as we find it in *L. Lomaxii* and *L. ovoides*, is hardly surprising.

Closely bound up with the features discussed in the last section, is the question of the relation of the pollen-chamber to the pristine mode of dehiscence of an ancestral megasporangium. In the present state of our knowledge no data are available that throw any light upon this very important stage in seed-evolution, and under the circumstances discussion would be unprofitable.

3. *The Systematic Position of Physostoma.*

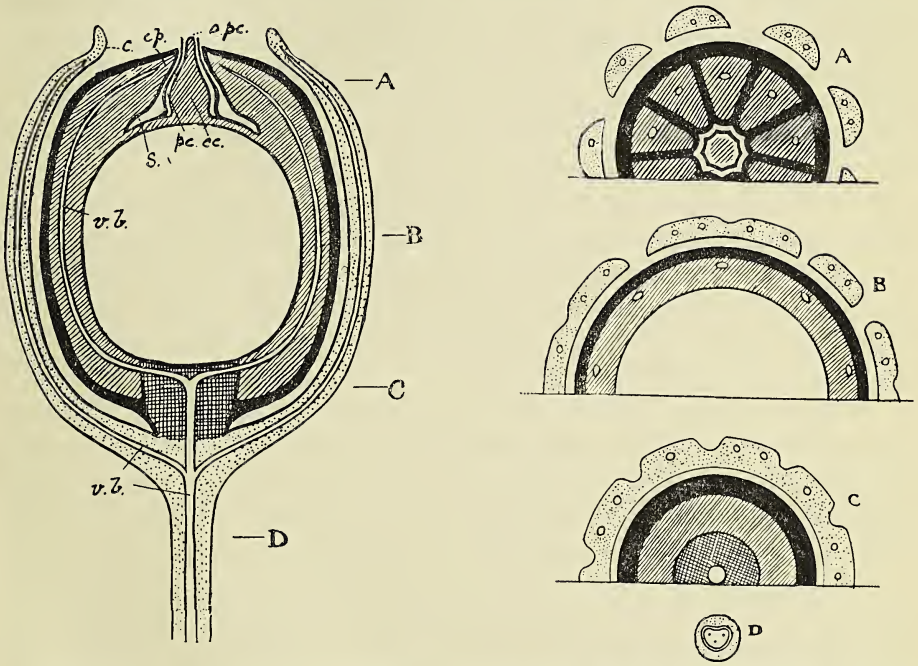
Throughout the preceding account of *Physostoma* a close relation to the seeds of the *Lagenostoma*-group has been assumed. Little remains to be said under this head beyond bringing together the scattered references in the text which emphasize the affinity. It should be stated at the outset

[1] The small-sized seeds of *Lagenostoma Lomaxii* possess fully developed pollen-chambers. Cf. Oliver and Scott, loc. cit., p. 212.

[2] The tip of the pollen-chamber protrudes beyond the integument in *L. Lomaxii*. Cf. Oliver and Scott, loc. cit., Pl. IX, Figs. 21 and 24.

that no direct evidence of continuity with vegetative organs has come to light during the course of the investigation, nor have any traces of such structures as a cupule or stalk been detected which might have served indirectly, as in the case of *L. Lomaxii*, to establish such a connexion. The relationship with the *Lagenostoma*-group depends upon essential similarity in organization between *Physostoma* and those seeds.

This agreement was evident to Williamson, who thirty years ago had



TEXT-FIG. 10. Diagrammatic longitudinal and transverse sections of *Lagenostoma Lomaxii*. The letters A, B, C, and D show the heights at which the transverse sections are cut. The hard outer layer of the seed-coat is drawn in black; the soft interior tissues obliquely shaded; the chalazal cushion cross-hatched; the vascular strands are left white; the cupule and stalk are dotted. *p.c.*, pollen-chamber; *o.p.c.*, orifice of pollen-chamber; *c.c.*, central cone; *s.*, sinus; *cp.*, canopy; *c.*, cupule; *v.b.*, vascular strands.

only a single imperfect specimen at his disposal, and further detailed study has served only to strengthen the bonds of resemblance.

The points of agreement between *Physostoma* on the one hand, and the *Lagenostoma*-group (which includes *L. Lomaxii*, *L. ovoides*, and *Conostoma oblongum*) on the other, are as follows:—

1. All the seeds are of that type in which the apex of the nucellus alone is free from the integument.

2. In all cases the free apex is transformed into a crevice-like pollen-chamber through the separation of the epidermis from the subjacent tissue of the nucellus. The orifice of the pollen-chamber is apical in position, and is borne at the extremity of a tube of varying length.

3. The characters of the free portion of the integument or 'canopy'. In *L. Lomaxii* the free portion of the integument forms a chambered shell which surrounds the pollen-chamber. The number of the chambers is nine, and each is penetrated by a vascular strand. In *L. ovoides* the structure is essentially the same, excepting that the number of chambers is usually eight. At the level at which the integument merges with the nucellus its chambered character is lost.

In *Physostoma* this canopy is represented by the whorl of, usually, ten tentacles, which are approximated—but never fused—to form a conical investment around the pollen-chamber. Comparison of these seeds points to the equivalence of the tentacles of the one with the fused chambers of the others. *Physostoma* might be described as a *Lagenostoma* with unfused chambers (cf. Text-figs. 1, 2, and 10 on pp. 76, 77, and 109).

4. The mucilage hairs of the integument. The whole series of seeds agree in showing the 'mucilage-habit' which they exhibit in differing degrees.

In *L. Lomaxii* the palisade layer of the integument bears little pegs, the summits of which are sometimes found curiously displaced, as though raised up by an emission of mucilage from the prismatic cells below or from the wall upon which they are seated.[1]

Though the pegs have not been observed, evidence of a similar emission of mucilage has been detected in the case of *L. ovoides*,[2] whilst in *Conostoma oblongum* the common outside membrane of the very conspicuous palisade-cells of the testa is sometimes found 'blown off', as though a number of these cells had emitted a quantity of mucilage.

In *Physostoma* a differentiation into separate prismatic cells and pegs is not met with, but the superficial cells on the ridges and tentacles are expanded into the well-known tubular processes which may well have had a mucilaginous secretion as contents.

The agreement between the several seeds is thus a striking one, and, taken in connexion with the other points of resemblance, is consistent with a derivation of the group from some common antecedent form.

5. The epidermal cells (prismatic cells in *L. Lomaxii* and *ovoides*, and the epidermal cells in *Physostoma*) were arranged in longitudinally-running linear series.

6. The vascular system in all these seeds has essentially the same distribution.

7. The many-layered tapetum of the nucellus or 'megaspore-jacket', so characteristic a feature in *Physostoma* is also met with in *Conostoma*; there is also some slight grounds for the suspicion that it may have been present at early stages of development in *L. Lomaxii*.[3]

[1] Oliver and Scott, loc. cit., Pl. X, Figs. 28, 28 A, 28 B, and Pl. V, phot. 12, and p. 206.

[2] In the specimen R. 22 in the University College Collection, the appearance is identical with that shown for *L. Lomaxii*, in Oliver and Scott, loc. cit., Pl. V, Fig. 12.

[3] Cf. p. 99.

8. The pollen-grains show a general agreement, and internal cells have been detected in all four species.

In *Physostoma* and *L. ovoides*, bodies have been found both within the pollen-grains and also associated with them, which there are grounds for regarding as spermatozoids. The form of the supposed spermatozoids is identical in the two cases, and recalls that of *Cycas*, except that the sperms in the fossils are much smaller, and no trace of the band of cilia has been detected.

The difference in size between the sperms of *Physostoma* and *L. ovoides* accords with the difference in the dimensions of their pollen-grains.

These several points of agreement, comprehending all the regions of the seed, appear to be too numerous and striking to admit of interpretation except as the outcome of close affinity, i.e., community of descent.

Whilst it seems evident that in this series of seeds of the *Lagenostoma*-group, the existence is disclosed of a number of closely-related species of Pteridosperms all occupying the same habitat, a difficulty is encountered when we try to point with any confidence to vegetative remains in our coal-nodules that could have belonged to the plants that bore these seeds. Up to the present time *Lagenostoma Lomaxii*, alone of the petrified seeds, has been definitely correlated with *Lyginodendron oldhamium*.[1] Among those preserved as impressions, several cases have come to light. Mr. Arber has referred *Lagenostoma Sinclairii* and *L. Kidstonii* to fronds of the Sphenopteris-type;[2] and Monsieur Grand' Eury has found strong grounds for referring other seeds of the same general character to *Sphenopteris Dubuissonis*.[3]

The position, therefore, is as follows: One petrified seed (*L. Lomaxii*), and at least three impressions, superficially in agreement with the seeds of the *Lagenostoma*-group, have been referred to the frond-type Sphenopteris. We are still left with *Physostoma*, *L. ovoides*, and the Conostomas, all petrifactions from the same group, and—excepting for *Heterangium*—there are no species of *Sphenopteris* yet separated from *Lyginodendron* by anatomical characters to which they could be assigned.

The close structural resemblance shown by *L. ovoides* and *L. Lomaxii* clearly points to *Lyginodendron* as their common source—indeed it has long been recognized that a vigorous analysis of the anatomical material that goes by the name of *Lyginodendron oldhamium* should lead to the discrimination of a group of related forms. The Burntisland species of *Conostoma* may be dismissed for the moment as probably the seed of *Heterangium*, in view of its common association in the nodules with the vegetative organs of this plant—to the total or almost total exclusion of

[1] Oliver and Scott, loc. cit.

[2] E. A. N. Arber, 'On some New Species of *Lagenostoma*', Proc. Roy. Soc., B., vol. lxxvi, p. 245.

[3] Grand'Eury, loc. cit.

Lyginodendron. Whether *Conostoma oblongum* should be referred to the same quarter must remain in suspense till it has been more fully investigated.

As to the parentage of *Physostoma*, we remain completely in the dark. It is just possible that its vegetative organs are not represented in our nodules. The delicacy of its vascular strands, and the readiness with which the tissues surrounding them gave rise to lacunae, may be significant of a general organization that was unable to resist decay.

Apart from this possibility, the organization of the seed—showing as it does close relationship with the *Lagenostoma*-group—seems to point to some form with Sphenopteris-foliage as its source. This conjecture is consistent with the facts available regarding *L. Kidstonii*, which, of all the Lagenostomas, shows the closest approach to *Physostoma*.[1] Whether the petrified remains of the *Physostoma*-fronds are included under the comprehensive *Lyginodendron oldhamium*, and if so whether they are anatomically distinguishable, must remain for future investigation to determine. The Systematist who handles recent plants finds the reproductive and vegetative organs in continuity, and thus escapes much of the perplexity to which the Palaeobotanist is liable. The predicament outlined above is perfectly comparable to that which would obtain with certain genera of recent Cupressineae or Umbelliferae were they only known in a fragmentary state.

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In conclusion, it remains to acknowledge help from many quarters. My thanks are due to Prof. C. E. Bertrand for the loan of the unique Maurice Hovalacque series, whilst Prof. Bottomley, Mr. R. Kidston, Dr. Scott, Mr. D. M. S. Watson, and Prof. F. E. Weiss have all placed preparations at my disposal. To Dr. Smith Woodward, of the Geological Department of the British Museum, I am indebted for facilities in examining the preparations of the Williamson Collection. Mrs. D. H. Scott kindly counted the ribs and tentacles of a number of Physostomas in the Scott Collection, thus swelling materially the numbers available for the curve on p. 84. In addition to supplying me with sections of the seed during a number of years, Mr. James Lomax, of Bolton, has spent much labour in the endeavour to trace the plant that bore these seeds. Were it not that good fortune is the determining factor for success in quests of this kind, I am confident that his patience and good judgement would have been rewarded long ago. Finally, I have to acknowledge grants towards the purchase of specimens from the Fossil Botany Committee of the British Association for the Advancement of Science.

[1] A resemblance not overlooked by Mr. Arber (loc. cit.). This seed, in view of the correspondence of characters, has been here assigned to the genus *Physostoma* (see p. 102).

SUMMARY.

The paper gives a full description of the Coal-Measure seed *Physostoma elegans*. It is a small straight seed about 6 millims. long by $2\frac{1}{4}$ millims. across the widest part, and shows many points of agreement with *Lagenostoma*. The integument, which is ribbed, is coalescent with the nucellus, except at the apex, where the ribs separate to form a whorl of ten tentacles surrounding the pollen-chamber. Ribs and tentacles alike are adorned with long tubular hairs reaching a length of nearly $\frac{1}{2}$ millim.; these hairs give all sections of the seed a very characteristic appearance. It is thought probable that this multiple character of the integument is an archaic feature of which traces are discernible in *Lagenostoma* and allied seeds. The vascular system, which is very delicate, agrees generally with that of *Lagenostoma*. The nucellus is represented by a zone rich in secretory sacs, and a well-marked tapetum is also present. The megaspore is peculiar in possessing an apical papilla which protrudes into the floor of the pollen-chamber. It may be compared with the 'tent-pole' found in *Ginkgo*, and many Cordaitean seeds. The pollen-chamber is rich in pollen-grains which show traces of an internal cell-reticulum. Inside the pollen-grains, and associated with them, bodies have been detected which may be regarded as fossilized spermatozoids.

A comparison of *Physostoma* with seeds of the *Lagenostoma*-group shows numerous common features. Reasons are given for regarding *Physostoma* as the most primitive seed that has yet come to light. The plant that bore it has not been traced, but it may be referred provisionally to the Lyginodendreae.

UNIVERSITY COLLEGE, LONDON,
October 1908.

EXPLANATION OF PLATES V, VI, AND VII.

Illustrating Professor F. W. Oliver's paper on *Physostoma elegans*.

W. = Williamson Collection. S. = Scott Collection. U. C. L., R. and K. = University College, London, Collection. B. = Professor Bottomley's Collection. M. H. = Professor Bertrand's Collection.

PLATE V. Figs. 1–12 (Photographs).

Figs. 1, 2, and 3. Series of three transverse sections of one seed, from preparations lent by Professor Bertrand. × 37. For explanation of references see under Fig. 4.

Fig. 1, near the chalaza, shows the ribs of the integument in the lower half only; the lacunae accompanying the vascular strands are distinct below, confluent above; within follow the secretory zone and the tapetum; the circular space in the centre is the embryo-sac. M. H. 369 (see p. 79).

Fig. 2, across the middle of the seed, shows clearly the ribs of the integument, the vascular

strands, and the lacunae; the secretory zone is much narrower than in the previous photograph, and the tapetum is unusually distinct; lying in the central space is the contracted prothallus, which is limited by a very delicate membrane (*m.g.*). M. H. 370 (see pp. 81 and 97).

Fig. 3. Across the apex, showing ten tentacles (with tubular hairs) surrounding the top of the pollen-chamber. The lower tentacles are cut transversely, the upper series somewhat obliquely and nearer to their insertions. *l.* lacuna accompanying vasc. strand; *p.* pollen-chamber; *ps.* female prothallus; *t.* tentacle; *t.* tentacle out of place; *t.h.* tubular hairs; *tp.* tapetum; *s.z.* secretory zone; *v.b.* xylem strand. M. H. 371 (see pp. 82, 91).

Fig. 4. Median longitudinal section through the seed-apex. The insertions of two tentacles are shown on the right and left (t_0, t_5); t_1, t_2, t_3, and t_4, other tentacles seen in section; numerous tubular hairs are present on the tentacles—well shown to the left of t_0; *p.* pollen-chamber—with a pollen-grain on the right-hand side; *sn.* space or sinus between pollen-chamber wall and inner side of tentacle; *x.* fracture in wall of nucellus below pollen-chamber; the large central cavity is the embryo-sac or megaspore chamber, which tapers into a papilla above. U. C. L., R. 92 (Shore). × 37 (see p. 87).

Fig. 5. A portion of the wall of the seed on the right of Fig. 2, enlarged. Two ribs, r^1 and r^2, are shown, the cells of the filling-tissue with contracted vesicles. Corresponding with the ribs are two lacunae (*l.*) and vascular strands (*v.b.*). Between the bundles the cells of the integument show a well-marked arrangement in radial files (*r.f.*). Within the bundles is the secretory zone (*s.z.*), with special development on the bundle radii, and within this the tapetum (*tp.*), about five cells deep. M. H. 370. × 64 (see pp. 89, 98).

Fig. 6. A single rib from the wall of the seed, from the top right-hand side of Fig. 2, enlarged. The xylem strand (*v.b.*), which is slightly displaced, is well shown. Other references as in Fig. 5 (see p. 96).

Fig. 7. Several tentacles from the lower side of Fig. 3, enlarged. The interlocking of the epidermal cells where the tentacles are in contact is well shown. *t.h.* tubular hairs of the epidermis; *e.* small-celled epidermis; *l.* lacuna. No traces of the vascular strands are preserved. M. H. 371. × 64 (see pp. 87, 89).

Fig. 8. An oblique section across the pollen-chamber region of a seed. Above are six tentacles entirely free; below four ribs not yet separated. The tubular hairs (*t.h.*) with dark contents are especially well shown on the tentacles. *p.c.* pollen-chamber (the upper edge of the section passes below its orifice); *ms.* apical papilla of megaspore; *s.z.* sacs of the secretory zone crowded together below the cavity of the megaspore; *v.b.* xylem strands of the ribs—the dark spots near them are detached secretory sacs. U. C. L., R. 93 (Shore). × 37 (see pp. 88, 98).

Fig. 9. Part of transverse section from about the middle of a seed showing ribs, lacunae (*l.*) and tubular hairs (*t.h.*). The tapetum (*tp.*) has separated from the zone of secretory sacs (*s.z.*). The cells of the integument show the 'thick' type of preservation. U. C. L., R. 91 (Dulesgate). × 40 (see p. 89).

Fig. 10. An oblique tangential section through the upper part of a seed. The plane of section, starting from a point well below the middle of the seed, proceeds obliquely upwards through the embryo-sac (*mg.s.*); at the apex it strikes the wall of the megaspore papilla (*mg.p.*), which it cuts tangentially. It emerges close to the orifice (*o.p.c.*) of the pollen-chamber (*p.c.*). U. C. L., R. 94 (Dulesgate). × 10

Fig. 11. Part of an oblique section across the apex of a seed. The circular space at the top is the pollen-chamber (*p.c.*); four tentacles are cut very tangentially, the plane of section traversing the investment of tubular hairs (*t.h.*) close to the body of the tentacles. U. C. L., R. 70, *c* (Shore). × 37 (see p. 88).

Fig. 12. A tangential section through the apex of a seed. The plane of section falls outside the apical papilla of the megaspore, so that the pollen-chamber (*p.c.*) appears as a broad, oval cavity. Several tentacles (*t.*) and groups of tubular hairs (*t.h.*) are cut through. *s.* sinus between pollen-chamber and tentacles; *pl.* plinth. S. 1753 (Dulesgate). × 15 (see p. 90).

PLATE VI. Figs. 13–23 (Drawings).

Fig. 13. Transverse section of a seed with twelve ribs just below the level at which the tentacles separate. At the top of the figure the sinus (*sn.*) is visible, and in this region the secretory sacs have disappeared from the tissue of the ribs. *s.z.* secretory zone; *tp.* tapetum; *l.* lacuna; *v.b.* xylem strand. U. C. L., R. 95 (Shore). × 34 (see pp. 85, 90, 98).

Fig. 14. Portion of transverse section cut low down in seed, so that the embryo-sac appears filled with tapetal tissue (*tp.*); *s.z.* zone of secretory cells, projecting at places beyond the vascular strands (*v.b.*); *l.* lacuna between integument (*int.*) and secretory zone: it is continuous at this level of the seed. U. C. L., R. 71 *a* (Shore). × 66 (see pp. 97, 99).

Fig. 15. Transverse section of a somewhat compressed seed. Seven of the tentacles are free, whilst the four ribs at the bottom of the drawing are still confluent. The section is cut through the lower part of the pollen-chamber (*p.c.*), which contains several pollen grains, but to the right and below it passes out through the floor. *sn.* sinus; *mg.* cavity of megaspore. The section is cut slightly above that given in Fig. 13. S. 1907 (Dulesgate). × 36 (see p. 85).

Fig. 16. An oblique tangential section through the outer side and hairy covering of a tentacle. The upper two-thirds of the figure represents the sections of the tubular hairs belonging to the distal part of the tentacle. The hairs stand in longitudinal rows—well shown by the third row from the left-hand edge. *t.* filling tissue of tentacle; *ep.* epidermal cells; *t.h.* tubular hairs. U. C. L., R. 70, *c* (Shore). × 60 (see p. 88).

Fig. 17. Median longitudinal section of the apex showing the orifice of the pollen-chamber (*o.p.c.*). On the left the insertion of the pollen-chamber wall has broken away from the nucellus. Five tentacles are shown above cut through in various directions. *tp. s.z.* black crust representing the tapetal and secretory zones. U. C. L., R. 90 (Shore). × 20 (see pp. 90, 91).

Fig. 18. Median longitudinal section through an entire seed. The outer part of the integument is wanting except on the right, above. The pollen-chamber and its orifice (*o.p.c.*) are well shown, also the projecting papilla of the megaspore (*mg.p.*). On the left of the concave floor of the pollen-chamber traces of the cushion are visible (*cu.*); *sn.* sinus. Above the pollen-chamber are portions of tentacles. S. 1798 (Dulesgate). × 14 (see pp. 85, 90, 91).

Fig. 19. Oblique section between seed-base and embryo-sac, showing ring of xylem-strands not yet separated (*vb.r.*); within this ring are crowded secretory sacs (*s.s.*) and a few stray ones outside. U. C. L., R. 73, *c* (Shore). × 46 (see pp. 96, 97).

Fig. 20. Tangential section of the base of a seed. *v.b.* four vascular strands diverging from point of entry; *s.z.* zone of secretory sacs. W. 1440 (Moorside). × 16 (see p. 96).

Fig. 21. Longitudinal section of seed-base—almost median. The divergent bundles (*v.b.*) enter at the base; around them the well-marked lacuna (*l.*); *int.* tissue of integument; *s.z.* secretory zone. U. C. L., R. 96 (Bacup). × 40 (see pp. 81, 90, 96).

Fig. 22. Oblique tangential section through the base of a seed just grazing the embryo-sac. *tp.* tapetal layer; *s.z.* zone of secretory sacs; *v.b.* three xylem strands diverging; *l.* lacuna; *int.* tissue of integument; above are traces of three ribs and tubular hairs (*t.h.*) cut transversely. U. C. L., R. 72, *a* (Shore). × 32 (see pp. 88, 90).

Fig. 23. Transverse section across lower part of seed showing curiously preserved tapetum. $tp.^1$ outmost layer of tapetum showing cellular characters as in the best preserved specimens; $tp.^2$ inner structureless zone of tapetum, where the cells have collapsed; $tp.^3$ peculiar striated type of preservation—very commonly fringing the embryo-sac: the striae are readily mistaken for tracheal sculpturing; *s.s.* secretory sacs. U. C. L., R. 74, *a* (Shore). × 200 (see p. 98).

PLATE VII. Figs. 24–30 (Drawings).

Fig. 24. Section through the tapetal zone from near the base of a seed: the radial files of cells, though distorted, are manifest. *i.s.* inner margin bordering on the embryo-sac; *o.s.* outer margin bordering on the secretory zone. U. C. L., R. 98 (Dulesgate). × 180 (see p. 98).

Fig. 25. Oblique transverse section across a pollen-chamber showing the tapetum of the apical papilla of the embryo-sac in a dilapidated state. *p.c.* pollen-chamber; *tp.* tapetum, torn and distorted; *c.a.p.* cavity of apical papilla. U. C. L., R. 97 (Shore). × 86 (see p. 99).

Fig. 26. Transverse section of a portion of a tentacle showing the epidermis and filling tissue—the cells of the latter with contracted vesicles. *i.e.* epidermis of inner (adaxial) side; *th.* proximal ends of tubular epidermal hairs on convex (abaxial) side; *f.t.* filling tissue; *l.* lacuna. The vascular strand is not preserved. U. C. L., R. 75, *d* (Shore). × 200 (see pp. 88, 89, 97).

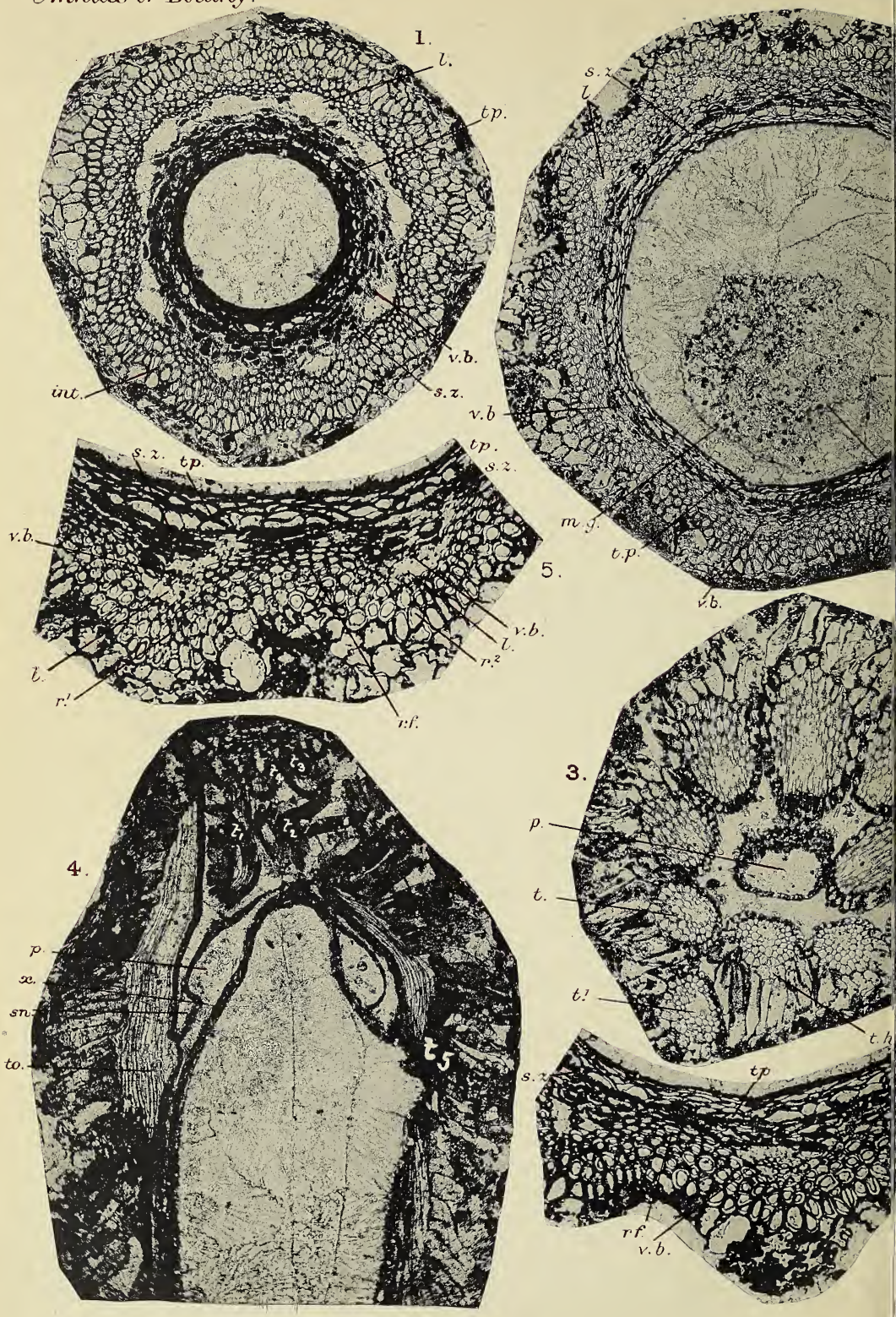
Fig. 27. Part of scalariform tracheal element. S. 1753 (Dulesgate). × 400 (see p. 96).

Fig. 28. Transverse section of a vascular strand with the accompanying lacuna and tissues—from the mid-height of a seed. *tp.* tapetum; *s.z.* secretory zone; *v.b.* xylem strand; *l.* lacuna; *r.f.* cells

of integument below a furrow showing radial seriation. U. C. L., R. 100 (Shore). × 100 (see pp. 96, 98).

Fig. 29. Transverse section of an undersized seed cut about two-thirds up from the base. The most striking feature is the tapetum (*tp.*), which has almost entirely separated from the external tissues. The right-hand end of the figure shows the ribs at a somewhat higher level than the left-hand end. *v.b.* vascular strands, which stand well away from the tapetum. U. C. L., R. 99 (Shore). × 32 (see pp. 99, 100).

Fig. 30. Group of pollen-grains and bodies supposed to be spermatozoids. Three pollen-grains (*a*, *b*, and *c*) are shown, and a cell-reticulum *d*, presumed to be the remains of a fourth pollen-grain; *b* contains two spermatozoids and *c* one: two others are present, one in contact with *d*, the other between *d* and *b*. B. 17 (Shore). × 380 (see p. 94).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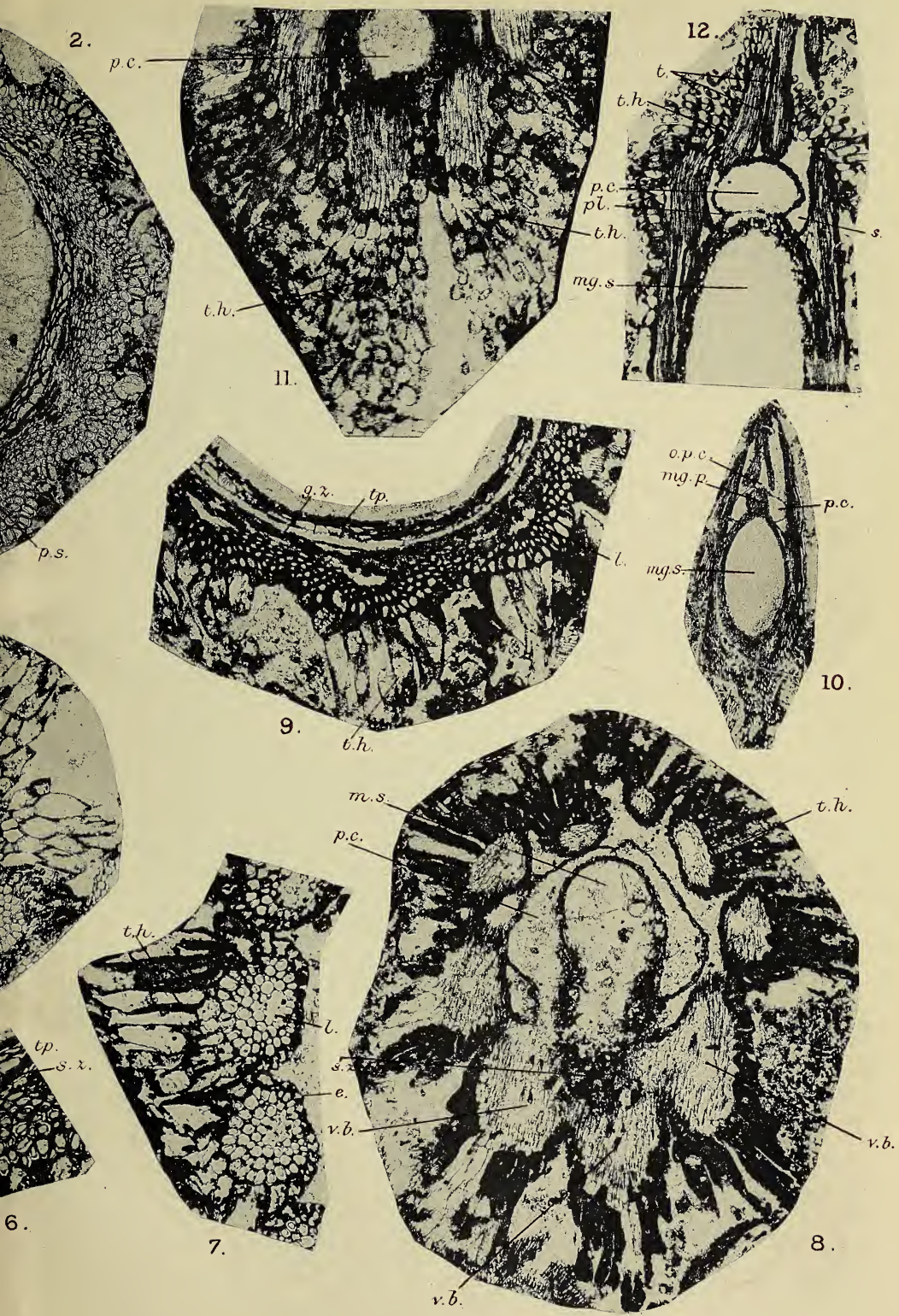
W. Tams, phot.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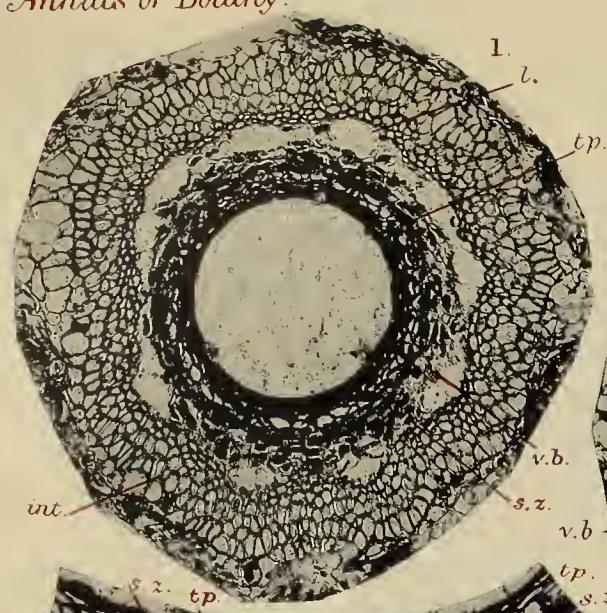

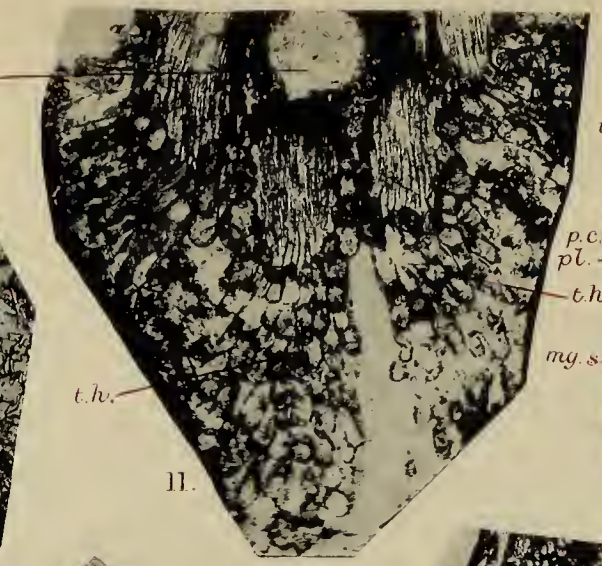

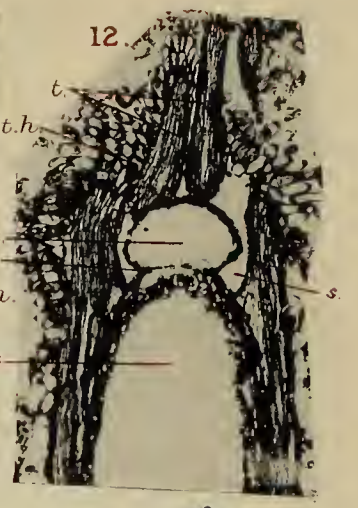

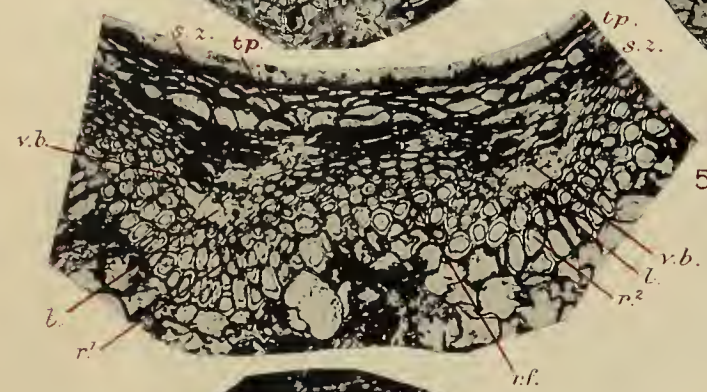

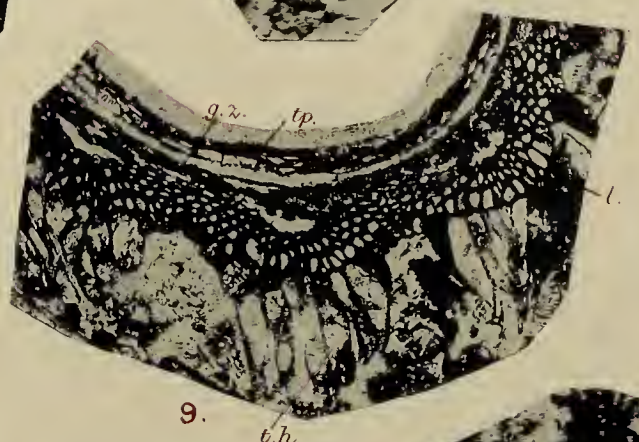

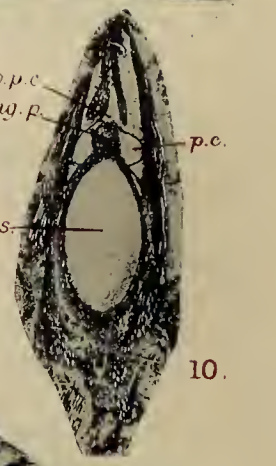

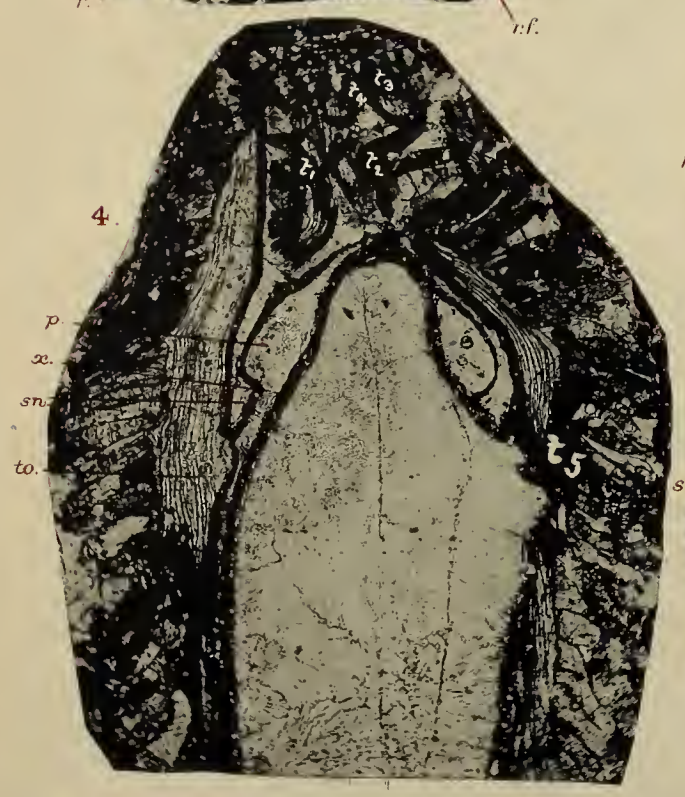

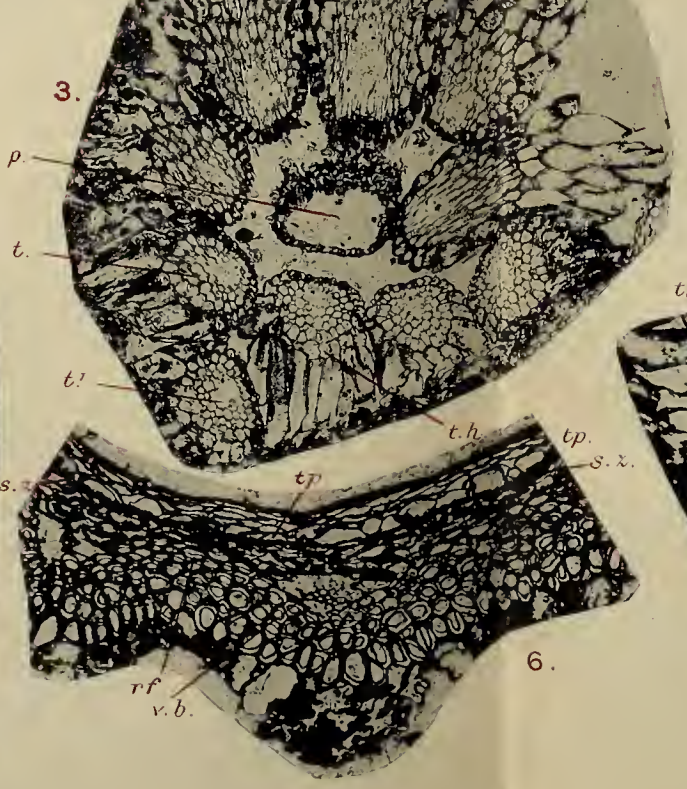

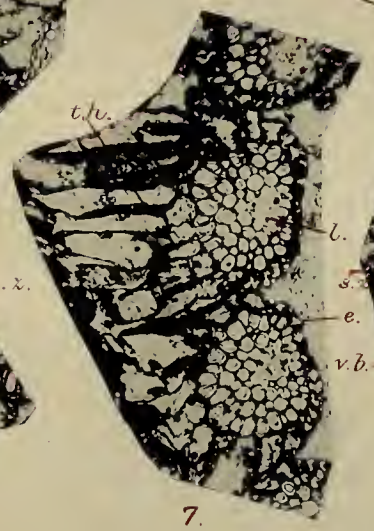

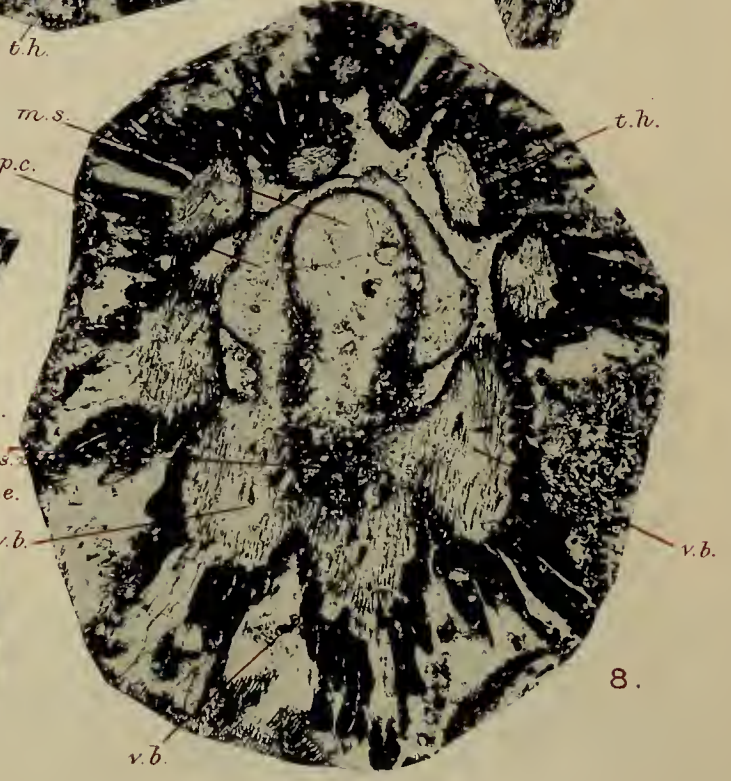

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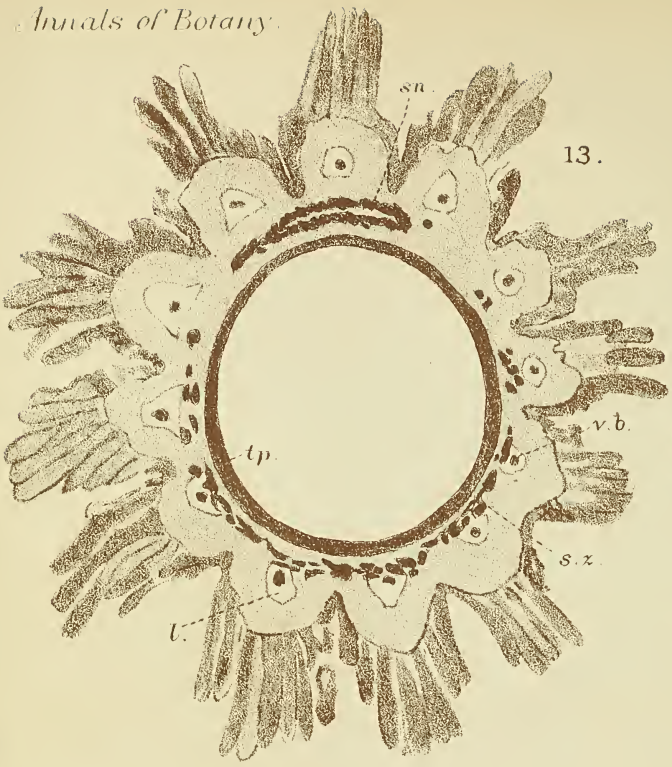
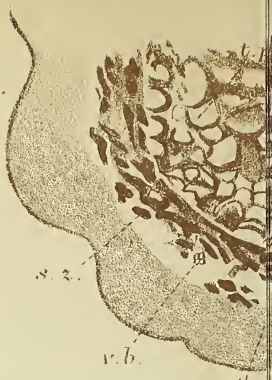
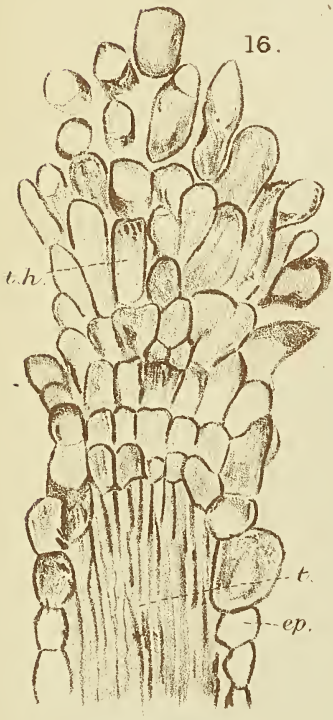
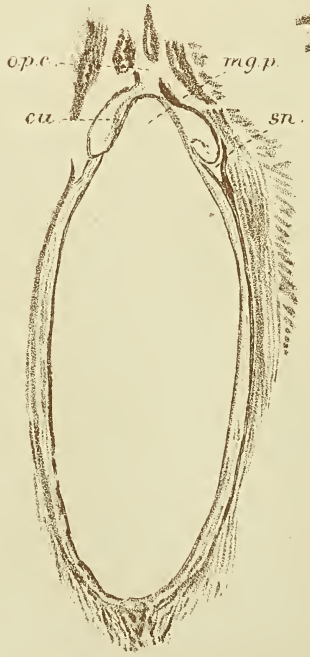
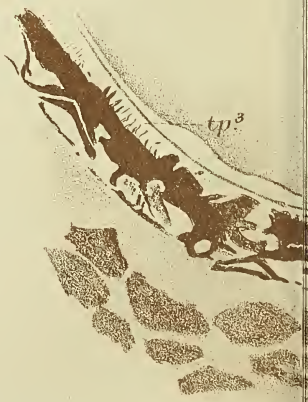
OLIVER — PHYSOSTOMA.











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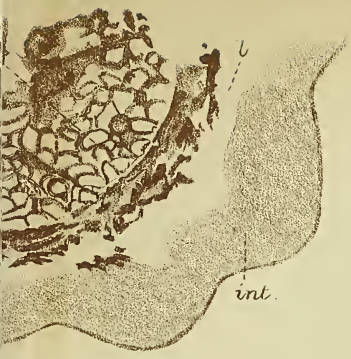

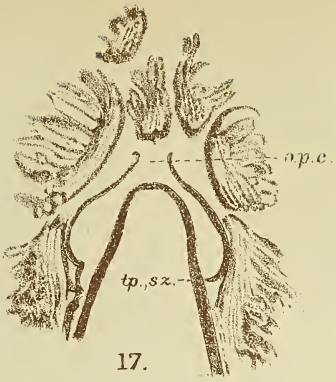

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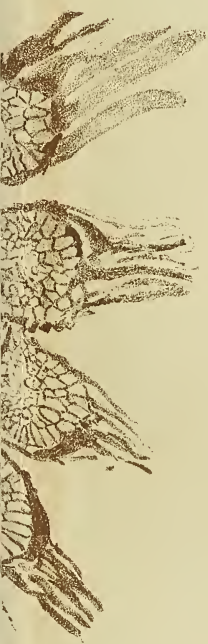

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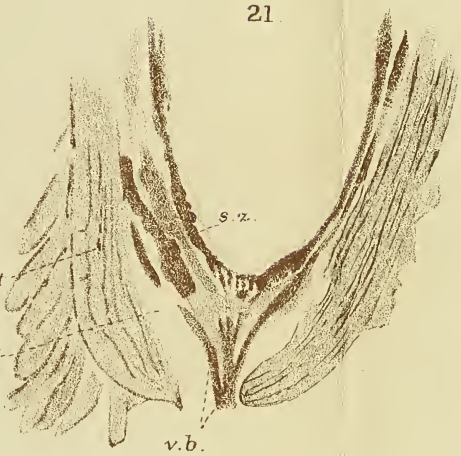

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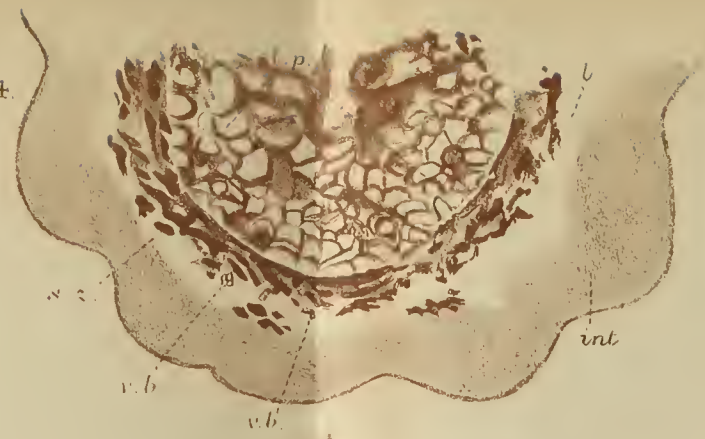

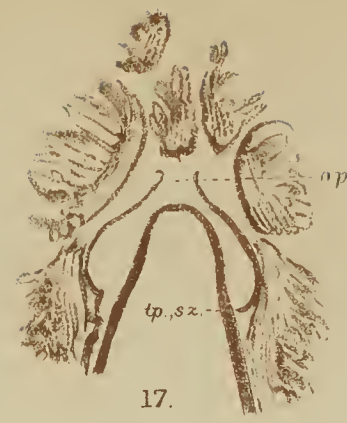

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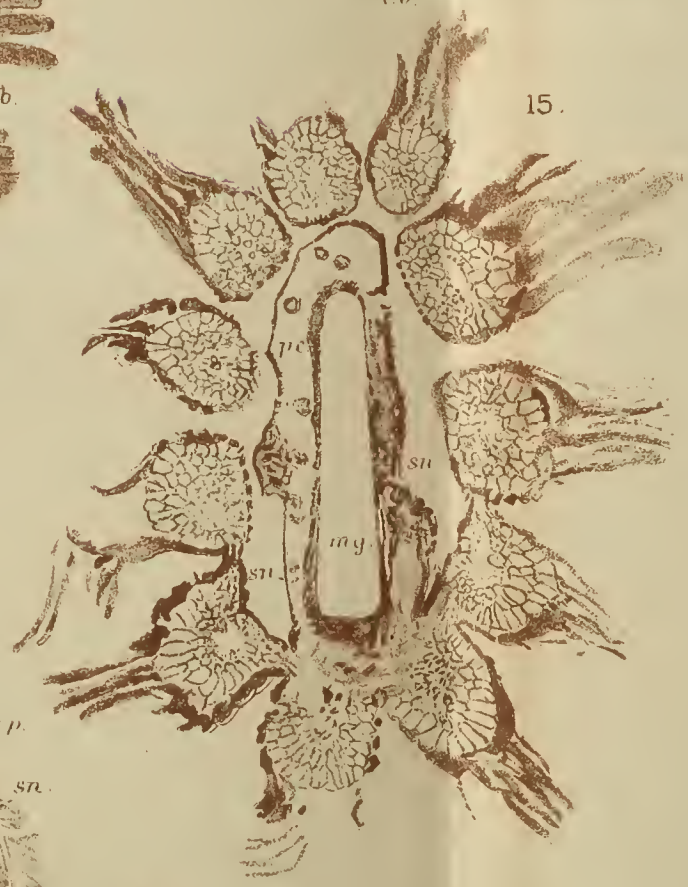

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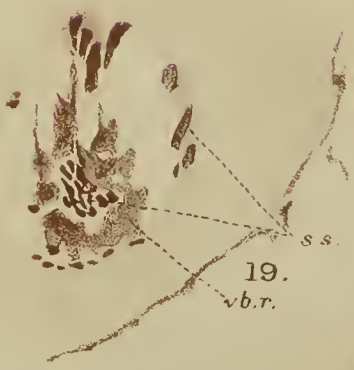

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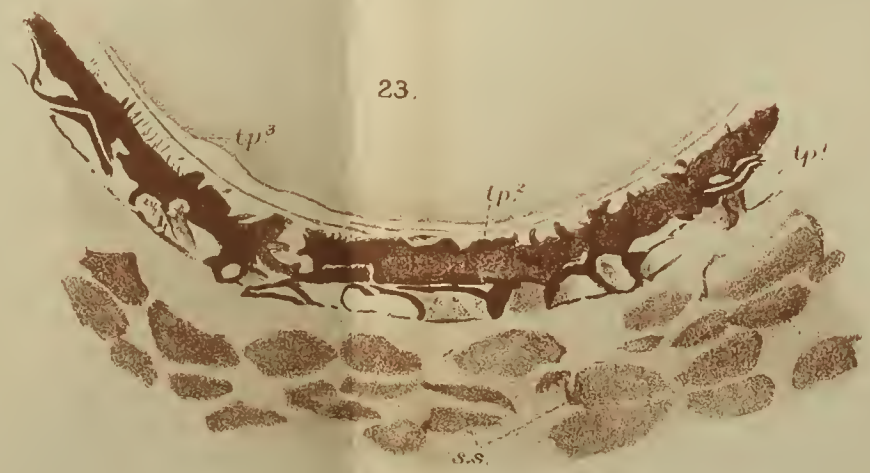

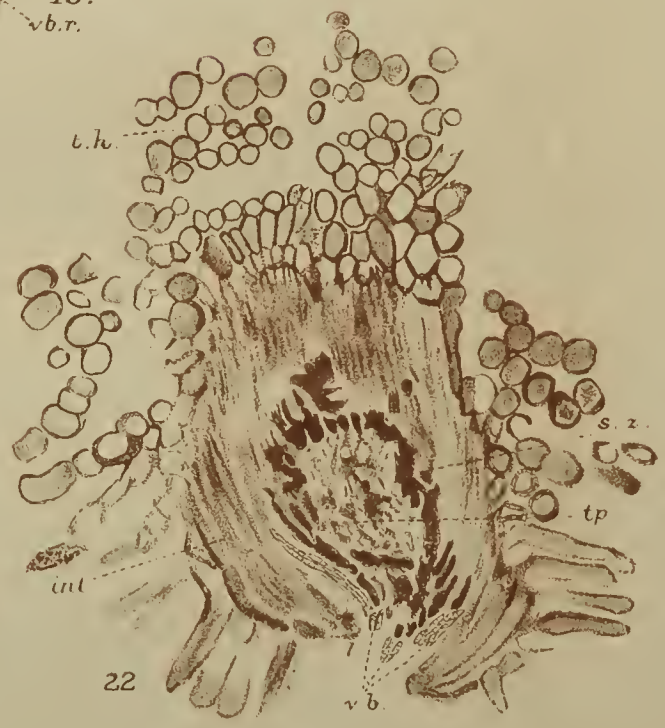

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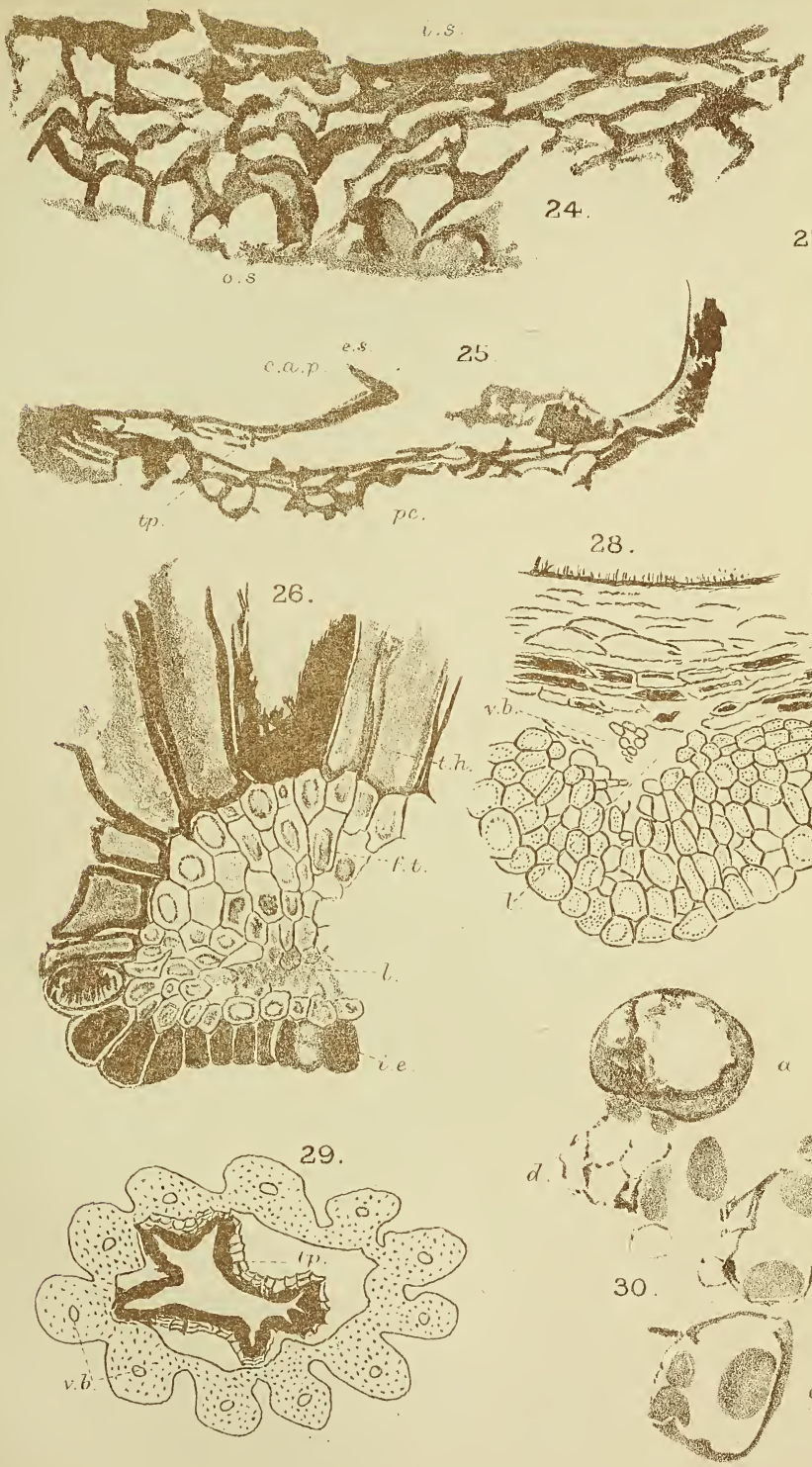
OLIVER — PHYSOSTOMA.



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OLIVER — PHYSOSTOMA.

On the Strength and Development of the Grain of Wheat (Triticum vulgare).

BY

W. E. BRENCHLEY, B.Sc.

Rothamsted Laboratory. (Lawes Agricultural Trust.)

With Plates VIII and IX, and five Figures in the Text.

THIS investigation on the wheat grain was originally started in 1906 with the idea of approaching the question of the 'strength' of wheat from a biological standpoint. This term 'strength' is one that is by no means easy to define exactly. As is well known, the flours obtained from different species of wheat grown in different localities vary very much in the value they have for bread-making. One flour will produce what the baker calls a 'well-piled' loaf, of good shape, size, and texture; another may work up into an equally large loaf from the same amount of flour, but of a very bad shape, while yet a third may yield a loaf bad or indifferent both in shape and size, and close and heavy in texture. These differences are all said to be due to the varying 'strength' of the wheat flour. Authorities differ very much in the way they define this term (1, 2), but perhaps the best working definition to take at the present time, when the matter is so much debated, is that given by Biffen (3), that 'strength is the capacity of the wheat to produce a large, well-piled loaf'.

Of recent years a great deal of work has been done on the consideration of this point, all more or less from the chemical side, the latest contribution being that of Wood (4), who maintains that there are at least two factors concerned with strength, i. e. the ratio of soluble salts to total proteid, governing the shape of the loaf, and also the sugars and nitrogen-free extract of the flour, controlling the size of the loaf.

Since strength had also been correlated with the date of cutting and the character of the season, an attempt was begun at Rothamsted in the latter part of 1906 to find out whether this varying strength is in any way associated with cytological differences in the wheat grain during the process of ripening, and also whether the diverse manuring of soil in the same locality or the variety of wheat play any part in causing such differences, if they exist.

[Annals of Botany, Vol. XXIII. No. LXXXIX. January, 1909.]

Material was examined which had been collected during the summer of 1904, killed and fixed in acetic alcohol. This consisted of samples of a typical English wheat, Square Head's Master, taken from two plots in the Broadbalk Field, Rothamsted, one of which has received no manure whatever since 1843, while the other has yearly a very heavy dressing of nitrogenous manure in the form of ammonium salts ; the other samples were Red Fife, a typical strong Canadian wheat, and a Hungarian wheat, very strong when grown in Hungary, both grown at Rothamsted. The grains were collected at irregular intervals between July 21 and August 30, note being made of the dates of cutting and carting the corn.

When the examination of the material was begun, a great deal of difficulty was experienced in securing the permeation of the grains by paraffin wax, preparatory to sectioning with the microtome. The pericarp of the caryopsis is very tough, and offers a decided obstacle to penetration by such a heavy liquid as melted paraffin wax. Various clearing media were tried—xylol, bergamot oil, paraffin, and cedar wood oil—but it was ultimately found best to cut the grains in half, transfer into the paraffin wax by way of bergamot oil, and then to let them soak in several changes of wax for one to four weeks previous to embedding, according to the age of the grain. Attempts were made to hasten penetration by placing the material under the action of a suction-pump, the tube being led into the embedding oven for the purpose; but no great measure of success attended the experiment.

After sectioning, numerous trials were made with various stains and double stains. It was found that the best results were obtained by overstaining for several hours (five or more) in very dilute Delafield's haematoxylin, then, after washing with tap-water, dipping the slide for a few seconds into acid alcohol (5 drops HCl. to 100 cc. of 70 per cent. alcohol), finally placing in tap-water till the sections turned blue again. After a light double-staining in orange G, the series were dehydrated, cleared in clove oil and xylol, and mounted up in canada balsam. At a later stage of the investigation, considerable use was made of Heidenhain's iron haematoxylin, which gave excellent differentiation when carefully controlled. With this stain the sections were mordanted with the iron alum, and then allowed to remain in the 0·5 per cent. haematoxylin overnight. It was found easier to control the final differentiation by the ferric alum after a long immersion in haematoxylin.

In addition to the microtome series a good deal of sectioning was done by the aid of a Leitz hand-microtome. The ordinary elder pith proved useless for holding such a large and elastic object as a wheat grain, so after various trials it was found that the pith from lime twigs answered the purpose admirably, as it was firm enough to withstand the considerable amount of pressure put upon it, while it was not difficult to cut. These

sections were carefully kept in series, and were taken at short intervals, each grain furnishing about fifteen sections. Delafield's haematoxylin was used as a single stain in this case, in greater concentration with a shorter immersion.

Three problems presented themselves for consideration with regard to the material so prepared :—

(1) Are any cytological differences, which might affect the strength, to be observed in the developing grains of 'strong' (Red Fife) and 'weak' (Square Head's Master) wheat when grown in the same locality, and therefore subjected to similar climatic conditions?

(2) Are there any cytological differences, indicative of strength, to be found in the developing grains of one variety of wheat (Square Head's Master) when grown in the same field, on similar soil and under the same climatic conditions, but subject to radical differences in manuring?

(3) Is the reputed difference in strength between grains of the same variety cut 'green' and 'dead ripe' due to cytological changes as ripening proceeds?

Considering these three points separately :—

(1) It has been suggested (3) that varying strength is partly or entirely due to different climatic conditions, but bakehouse experiments have shown that this statement is much too general, as, other things being equal, some strong wheats, e.g. Red Fife, remain strong whatever the climate in which they grow, and vice versa. The Red Fife wheat under consideration had been spring sown, and therefore, date for date, was not at such an advanced stage as the autumn-sown Square Head's Master—in fact, while the Broadbalk wheat was cut on August 5, the Red Fife was left standing till August 15. In comparing the two varieties, due allowance had to be made for this difference in the age of the grain. It was found that the nuclei undergo considerable changes during the progress of ripening of the grain. In the earlier stages they are quite normal and perfect, well formed, and possessing nucleoli. As the grain grows older changes take place, which will be more fully described later under the head of endosperm development, till finally, by the time the grains are matured, the nuclei are very much disorganized in structure, particularly in the deeper-seated cells furthest away from the aleurone layer. The nuclei of the aleurone layer do not undergo this disorganization, and the cell contents seem to remain unchanged when once they are fully organized. The cells of the starchy endosperm which lie nearest to the aleurone layer were found to contain rather more protein than the rest, as was shown by the Xantho-protein reaction. Freshly cut hand-sections were placed in strong nitric acid, and left for a minute or two till they were quite transparent; after washing in water they were transferred to ammonia and left for a few minutes. After a final washing in water, the sections were at once examined under the microscope, when the cells

of the starchy endosperm lying just underneath the aleurone layer were found to be stained more deeply yellow than the rest, thus indicating the presence of a larger quantity of protein. The manner and extent of infilling of starch were noted, with other details of cell contents and structure, but both the types showed similar phenomena. Altogether it seems that the various points in the history of development are quite parallel in both the strong and weak wheats, and, after due consideration, the conclusion was arrived at that no cytological differences exist under these circumstances which can in any way affect the strength of the grain —at least, no differences that can be observed by the methods used. The information gained as to the developmental history of the wheat grain will be treated fully in the latter part of this paper.

(2) As previously stated, Square Head's Master is an example of a typically weak English wheat, even under the most favourable circumstances, but largely grown because of its good yielding properties.

The crop from the unmanured Plot 3 yields flour of a practically normal strength for the variety (5), which in 1902 was awarded a 'baker's mark' of 42 for its bread-making capacity. The flour from Plot 10, however, with its very heavy nitrogenous manuring, behaves most abnormally in the bake-house. Although the grain looks strong, and contains a large percentage of nitrogen, as is shown by analyses, yet the flour proves to be excessively weak when tested just after harvesting, only receiving a nominal baker's mark of 1, indicating that it is hardly possible to make bread from it at all. A curious and unaccountable change occurs as time goes on, as flour from the same wheat, when ground and tested again by baking about nine months later, proves to be quite as strong as the ordinary wheat from the Rothamsted Fields (6), but this improvement with age only occurs in the wheat from this one particular plot.

Again, an examination of numerous preparations carried on in the same way as in the elucidation of the first problem failed to reveal any cytological differences in the structure of the two sets of grains—though the material obtained from Plot 10 seemed to be a trifle less advanced than that from Plot 3, date for date, as the heavy nitrogenous manuring has a tendency to prolong the growing period, and so to slightly delay the progress of maturation.

(3) Examination of grains of the same variety cut 'green' and 'dead ripe' showed certain cytological differences between them. When the grain begins to ripen, changing colour from green to brown, the nuclei in the amyliferous cells of the endosperm are perfect and complete, but as ripening proceeds these nuclei become much disorganized, owing to the pressure exerted by the increasing amount of starch in the cells, and also to the desiccation which goes on as maturation proceeds. With this exception the cytological structure does not seem to undergo any change, and it is hardly

probable that this disorganization of nuclei has any bearing on the question of strength. As a matter of fact, baking tests do not bear out the idea that grain cut green is stronger than that cut dead ripe, the latter more frequently proving stronger than the former.

To a large extent all these observations were repeated later with material collected in 1907 from the same plots and of the same varieties, with results which corroborated those previously obtained.

To sum up, it seems that so far only negative results have been obtained, and that no cytological distinctions, indicative of strength, present themselves between wheat grains of varying strengths, whether of the same or of different varieties.

Although the original investigation yielded very little result, the examination of the 1904 material seemed to indicate that it would be desirable to pursue the inquiry along different lines, and to study the development of the grain from the time of pollination up to maturity, so this work was undertaken during the summer of 1907.

DEVELOPMENT OF THE WHEAT GRAIN.

(*a*) Preparation of Material.

For the purpose of studying the development it was essential that the grains should be comparable as to age. As is well known, the flowering ear of wheat is an inflorescence composed of numerous flowers of different ages, which therefore 'come into flower' and are pollinated in succession, and not all on the same day. The varieties chosen for investigation in the first place were autumn-sown Square Head's Master from Broadbalk, Plots 3 and 10, and spring-sown Red Fife, from an adjoining field. The first flowers to open and protrude their anthers are the ones in the middle of the groups of flowering glumes situated about four or five down from the tip of the ear.

The plots were watched as the time for flowering drew near, and directly it was seen that the first anthers were hanging out, which always begins on the leading shoots, a staff of workers went through the field and loosely knotted a piece of red wool immediately below each ear which had stamens projecting from one flower. Any ears in which two or three flowers were open in a vertical row were rejected, as these were the forerunners of the general flowering, and were probably a day older than the chosen ears. Thus all the marked ears which were afterwards cut in succession, may be considered to have been of the same age, or with a variation of a few hours only. Two days after this collecting began, and was continued daily at approximately the same hour right up to the time of harvesting, seven weeks later. One grain only was taken from each marked ear, and the one selected was that which had been first pollinated;

this was easily known by its position and also by the fact that it was usually a trifle larger and better developed than those above and below. To ensure that a second grain was never selected from the same ear the ends of the marking wool were clipped off close to the knot. The material thus collected was fixed in the field, half in Flemming's weak solution and half in acetic alcohol. It was found that immersion in Flemming's solution for any length of time had a decided tendency to blacken the grain; indeed, in the younger stages, this discoloration penetrated right through the tissues. Attempts were made to shorten the time of immersion, even reducing it to half an hour, but while the blackening still occurred to some extent, fixation was imperfect. Much better results were obtained by killing and fixing in acetic alcohol for twenty minutes, washing out well in two or three changes of spirit, and preserving in a mixture of one-third glycerine, two-thirds spirit. As with the 1904 material, the grains were embedded through bergamot oil and cut in microtome series. Various stains were used, including Flemming's triple stain, brasilin, Ehrlich's haematoxylin (also with O. G. or eosin), but the best results were obtained with Heidenhain's and Delafield's haematoxylin (either with or without O. G.).

Material was collected from all the three plots mentioned, but as the sequence of events was parallel in all, attention will be chiefly confined to that obtained from Plot 3, Broadbalk, (Square Head's Master).

(*b*) Early Stages of Development.

The ovule of wheat is anatropous, curved on its funicle, so that the micropyle is brought to face inwards towards the stalk of the ear. For the sake of convenience the micropylar end will be consistently referred to as the tip of the grain.

Assuming that pollination in the wheat occurs on the same day that the flower opens, the earliest stage in the material at hand is that taken two days after pollination, though one grain of this date seems a trifle less advanced than the rest. In this case the ovule is cut rather obliquely through the micropyle, and shows a mass of nucellar tissue still present, bounded on the periphery by a single layer of very regularly arranged square-shaped cells, with well-developed nuclei, which eventually merges itself in the tissues of the placenta to which the ovule is attached. Outside this layer are one or two more rows of cells which seem to have broken away from the surrounding pericarp in the process of fixing and preparing. Within the nucellus the boundary line of the embryo-sac can usually be made out, though in places it is disintegrating or destroyed. At the tip of the grain, just where the position of the micropyle is indicated, is a group of two or three nuclei. One of these is a synergid, distinguished by its dense appearance, and there is some indication of the second synergid.

Below this is the ovum, and in the immediate neighbourhood is a crescent-shaped structure, presumably the tip of the pollen-tube, which shows a few well-marked, darkly-staining bodies. Embedded in the nucleus of the ovum, just below the tip of the pollen tube, is an irregular-shaped mass distinguished in the section by its darker colour, which is probably the generative nucleus that has not yet quite lost its entity. The ovum with its nucleus and cytoplasm is bounded by a delicate membrane, a further indication that fertilization has occurred. As Cannon (7) also points out, Koernicke (8) does not mention a division of the generative nucleus in the pollen grain of wheat, and this would be an apparent exception to the statement by Strassburger ('84) that the division of the generative nucleus is a constant character of the grasses. If this were really the case, then it would preclude all possibility of double fertilization occurring in *Triticum*, though it has been demonstrated by Guignard (9) in *Zea mais*, which possesses two generative nuclei. Golinski (10), on the contrary, figures two generative nuclei in the pollen grain of wheat, and describes them as being elongated and curved and embedded in a special protoplasm, stating that they are not unlike the Antherozoids of Ferns and Characeae in appearance.

In the ovule under consideration dense cytoplasm appears on the other side of the embryo-sac, in which are embedded two large nuclei, the result of the first division of the definitive nucleus, if we may assume that the polar nuclei have already fused. According to Golinski, the two polar nuclei are at first in close contact with the ovum: before fertilization, however, they pass down into the vicinity of the antipodal cells, after which the ovum is ready to be fertilized. Apparently the polar nuclei fuse to form the definitive nucleus just at the time of fertilization, so it is probable that the two nuclei in question are really the two first endosperm nuclei and not the unfused polar nuclei. The fusion and subsequent division evidently take place very rapidly. Guignard (9) found in maize that the fusion of polar nuclei and the incorporation of the generative nucleus with them takes place in such a short space of time that the phenomenon can very rarely be observed.

The antipodal cells at this stage are still well in evidence, but show traces of incipient disorganization. In *Triticum*, as in many of the Gramineae, the antipodals are numerous, forming a regular tissue in the embryo-sac adjacent to the placenta. Koernicke (8) indicates the presence of thirty-six antipodals in this case, while Westermaier (11) gives a full account of the literature relating to these cells in the grasses.

The indications are that fertilization normally occurs between one and two days after pollination, as most of the grains taken on the second day after are rather more advanced than the one described. In these, part of the cytoplasm is massed at the micropylar end about the ovum, and the rest, while still remaining in connexion, is gathered round the periphery of

the embryo-sac, surrounding a central vacuole. Embedded in the lining plasm are, not two, but several nuclei, which are evidently the result of the division of the definitive nucleus, and hence are the early endosperm nuclei. These are quite free, no trace of cell walls appearing. Some series, notably those stained with Ehrlich's haematoxylin and O. G., show the arrangement of nucellar and pericarp tissues very clearly. On the outer side of the regularly arranged boundary of the nucellus is a double layer, forming the inner integument of the ovule. Beyond this are two more rows of cells, thin-walled, and appearing rather crushed in places, which have sometimes remained in contact with the inner integument, sometimes with the inner layer of the pericarp in those preparations in which the ovule has shrunk away from the pericarp. This layer evidently represents the outer integument of the seed, which gradually disappears in the course of development. The pericarp shows on its inner side one or two rows of regularly arranged cells with dense protoplasmic contents, beyond which occurs the larger-celled tissue containing much starch. The outer boundary is an epidermis of uniform cells, with much less starch, if any.

By the next day, the *third* from pollination, evident progress has been made. The fertilized ovum has entered on a temporary resting-stage, and no change has yet occurred in it, while the synergidae have practically disappeared. The central vacuole has enlarged, and the nuclei embedded in the protoplasmic lining have greatly increased in number. These embryonic endosperm nuclei are multiplying at the expense of nutriment obtained from the nucellar tissue, the innermost cells of which have been emptied of their contents and are disorganizing, except at the tip of the grain, where they are being reserved for the future use of the embryo. The cells of the limiting layer of the nucellus have resumed their activity—many of the nuclei are in process of division, and some of the cells are narrow, only about half the normal width, showing that division has occurred, but the daughter-cells have not yet grown to their proper size.

On the *fifth* day after pollination the first indication of division occurs in the fertilized egg cell. At this stage the future embryo lies at the tip of the grain in a little *cul-de-sac* formed by the nucellar tissue persisting in this region. In attendance on it is a dense mass of cytoplasm which is full of nuclei, while the protoplasmic lining of the embryo-sac contains very numerous nuclei characterized by the large number of nucleoli they possess ; as many as eight have been counted in some cases (cf. Cannon on *Avena fatua*, where the nuclei have from three to eight nucleoli). From this point onwards the development of the endosperm and embryo will be considered separately. The limiting layer or epidermis of the nucellus behaves somewhat differently from the rest of the tissue. As previously stated, about three days after pollination the cells of which it is constituted renew their activity, nuclear division beginning again and continuing at a rapid rate, so

that the growth of the tissue keeps pace with the expansion of the embryo-sac for some time. At this stage the appearances indicate that this layer may persist in the ripe seed in the form of the aleurone layer, but later sections do not bear this out. The epidermis continues apparently unaltered for some days after the rest of the thin-walled nucellus has been absorbed by the developing endosperm. About a fortnight after pollination, however the epidermal cells lose their contents, just about the same time that the aleurone layer comes into evidence, and the cells get crushed, though the outer and inner walls thicken up and persist in the ripe seed (13).

(*c*) Endosperm.

1. *Development.* It has already been shown that the endosperm arises in the first place by free cell formation, i.e. a large number of nuclei are derived from the definitive nucleus by repeated division, and are embedded in the protoplasm lining the wall of the embryo-sac, no cell walls being formed. During this period the protoplasm seems to be more dense in the immediate neighbourhood of the disorganizing antipodal cells, possibly because, as Koernicke (8) suggests, the developing endosperm is drawing very largely on the substance of the antipodal tissue for its own nutrition. The surrounding nucellar tissue, with the exception of its epidermis or limiting layer, is gradually absorbed, except in the immediate neighbourhood of the fertilized ovum, in which position there is also a large accumulation of protoplasm with many embedded nuclei.

About seven or eight days after pollination a change occurs and the formation of cell walls begins. These start at the periphery of the endosperm and develop inwards very rapidly, till within about two days the endosperm is changed from a mere nucleated protoplasmic layer surrounding a large central vacuole to a definite tissue extending through practically the whole of the embryo-sac. The central vacuole of the embryo-sac fills up first with endosperm tissue at the micropylar end (7), though some sections seem to indicate that the cytoplasm immediately surrounding the embryo does not develop its cell walls till a little later. At first the mass of cytoplasm completely surrounds the young embryo except just at the tip, but as the latter develops it gradually absorbs the endosperm on the outer side away from the furrow, and also the nucellar tissue in the same region, so that it is displaced from its central position to a somewhat lateral one on the outer side of the endosperm. Eventually walls are developed all the way through, but the cells formed from the cytoplasm in the neighbourhood of the embryo retain their dense protoplasmic contents for a long time after the main mass of endosperm tissue begins to appear very poor in protoplasm. Westermaier (11) describes a similar phenomenon as occurring in *Zea mais*.

Quite early in development the endosperm cells on the ventral side

opposite the furrow, are marked off from the rest by their smaller size and denser protoplasmic contents. Eventually a peripheral layer of such smaller cells is marked off, appearing on the dorsal side about a fortnight after pollination. This external layer develops into the aleurone layer, which is highly specialized in the cereals, and consists of small regularly-arranged cells with dense protoplasmic contents and well-marked nuclei, which persist unaltered right up to maturity. No starch grains are present, but instead there is a great accumulation of protein matters which are probably of different chemical composition from those in the rest of the endosperm. The cells in the bulk of the tissue, on the other hand, are large, varying much in size and shape. The protoplasmic contents are reduced to a minimum in the ripe grain, being drawn out into a very attenuated reticulum, and the nuclei undergo definite disorganization, as will be seen later. The protein matters are chiefly to be seen in the cells lying just below the aleurone layer, but the most marked feature of the cell contents is the enormous amount of starch deposited as grains of different sizes.

Certain microscopical tests were carried out to ascertain the behaviour of the aleurone layer during the manufacture of flour, two separate sets of wheat flour, specially milled by Mr. A. E. Humphries, from Coxes Lock Mills, Weybridge, being used for the purpose.

In the modern process of roller milling, the wheat grains are gradually reduced by being passed through successive pairs of revolving rollers (14), so that eventually the whole of the central part of the grain is cleared away from the coverings, which form the bran. After each crushing or rolling the finest particles are sifted out and form the various grades of flour, and samples of these, including the bran, were sent to me for investigation. The flours were subjected to close microscopic scrutiny, with and without staining in various ways, in order to find out whether the aleurone cells were ever present in the flour itself. Very seldom indeed could any of these cells, even isolated ones, be observed in the flour of any grade—all the samples could be considered as practically pure in this respect. The reason was obvious when an examination of the bran was undertaken. In the initial processes of cracking or 'breaking' the grain, the aleurone layer had split simultaneously with the fruit-coats, and remained firmly adherent to them; in the subsequent milling the whole of the starchy endosperm tissue was torn away, and ultimately converted into flour of different grades, while the aleurone layer remained behind in small sheets, so to speak, and formed part of the bran. This is probably due to the fact that the junction between the thin-walled cells of the starchy endosperm and the very resistant and thick-walled aleurone cells offers the line of least resistance to the pressure applied during the process of milling.

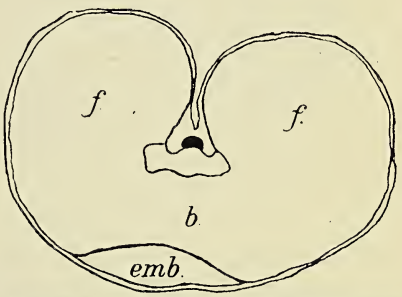
2. *Infiltration of Starch.* The deposition of starch in the endosperm

cells was studied on 1907 material from Plot 10, Broadbalk, by means of series of hand-sections stained with iodine. It was afterwards found that grains from Plot 3 reached a similar stage of development about a day earlier, which is probably due to the fact that the heavy nitrogenous manuring of Plot 10 slightly retarded development.

In all the younger stages there is a dense accumulation of starch in the pericarp, which gradually decreases in quantity, being utilized for respiration and possibly for the nourishment of the growing seed. Traces of this pericarp starch linger for a long time at the micropylar end, especially in the neighbourhood of the furrow.

By the *ninth* day after pollination the endosperm is still incomplete, cell-walls only extending part of the way inwards from the periphery, while as yet there is no vestige of starch.

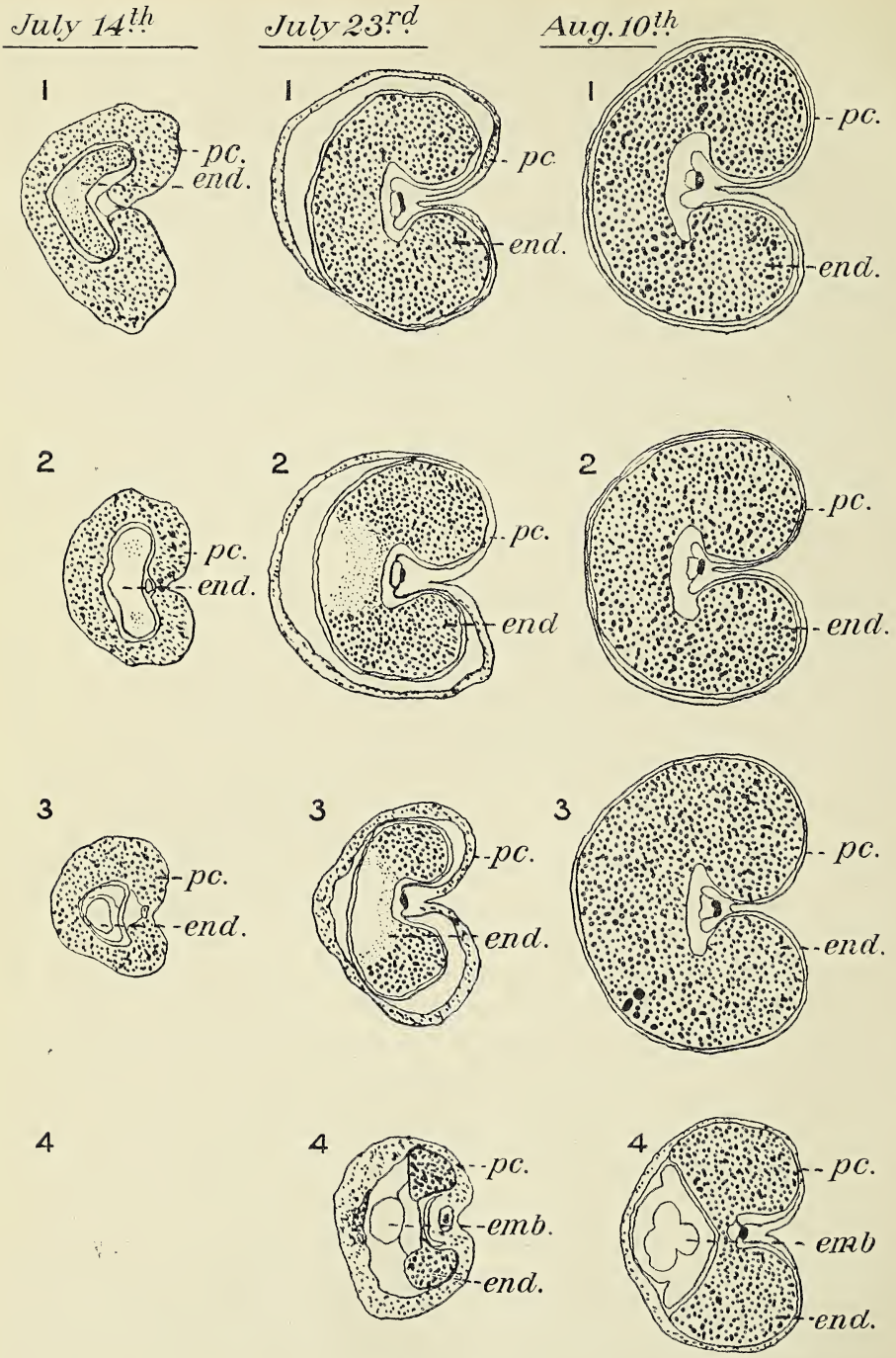
Rapid progress is now made, for by the next day endosperm structure is nearly complete, except just in the middle of the tissue. Towards the lower end of the grain, one or two cells in the middle of the 'flanks' show minute starch grains in the closest connexion with the nuclei. Lower down rather more cells show this, but the starch all through remains very small and is only in association with the nuclei, never scattered throughout the cells.



TEXT-FIG. 1. Diagram to illustrate position of 'flanks' and 'bridge' in wheat endosperm. *f.* flank; *b.* bridge; *emb.* embryo.

By the *eleventh* day from pollination the endosperm is completely formed and some starch is seen in the flanks well up towards the tip. The quantity in the flanks increases gradually as one goes down the grain, and though chiefly associated with the nuclei, the starch eventually begins to get scattered in the cells. At first there is no starch at all in the portion of the endosperm connecting the two flanks across the furrow, which we may call the 'bridge'. Quite late in the series a little starch puts in its appearance across the bridge, but only in the cells on the side nearest to the furrow—none at all occurring on the outer side, except quite at the base of the grain.

The next day, the *twelfth* from pollination, gives one of the most representative stages in the infilling of the starch in the endosperm. The embryo itself is still very small and little developed, and in its neighbourhood the endosperm, though quite complete, shows no sign of starch. A good deal of protein matter seems to be present, as a deep yellow coloration is obtained with iodine, both here and in most other series where the reaction is not masked by the deep staining of starch. Proceeding downwards



TEXT-FIG. 2. Diagrammatic representation of the infilling of starch into the wheat endosperm at different stages of growth, showing the distribution on July 14, July 23, and August 10—12, 21, and 39 days after pollination. (1) Trans. sect. cut near base of grain, (2) higher up, (3) just below embryo, (4) at level of embryo. *emb.* embryo; *end.* endosperm; *pc.* pericarp, with starch in many cases. Dark shading in endosperm indicates dense starch, light shading scattered starch, and no shading shows its total absence. × 9.

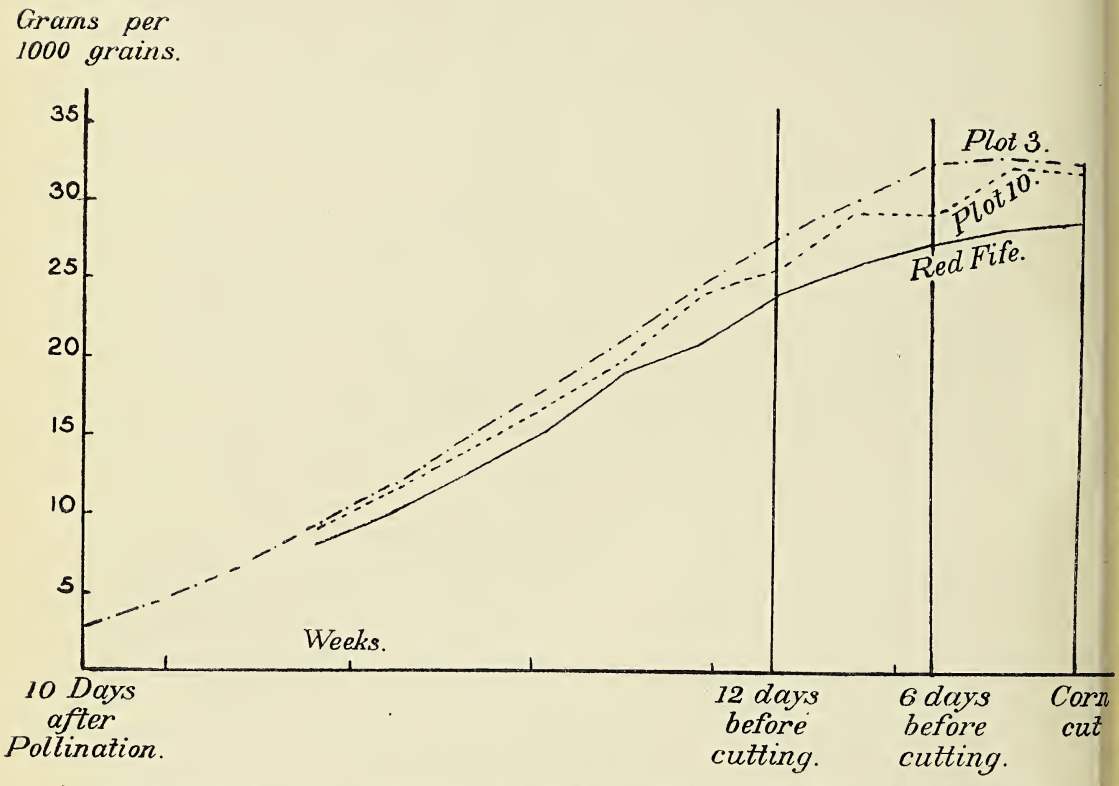
some cells in the flanks begin to show small starch grains, congregated almost entirely round the nuclei, which are large and well developed, in full activity. Further away from the embryo the starch in the flanks increases in amount, while a certain quantity gradually fills in across the bridge, but is at first confined to the side next the furrow, only spreading outwards lower down the series. At the extreme end of the grain a fair amount of starch is present, though right up to the end there is less towards the outer side of the bridge than in the flanks, or on that side of the bridge next the furrow.

During the next three or four days the infilling of reserve material makes rapid strides. Starch is now found in the flanks right up to the level of the embryo, and at this stage it also occurs across the bridge, next to the furrow. The carbohydrate rapidly extends outwards, till a short distance down the whole endosperm is filled in.

From this time onwards no radical change takes place. The starch appears to get more dense and gradually fills up the cells on the outer side of the bridge adjacent to the embryo. Five weeks after pollination the infilling may be regarded as practically complete, judging by microscopical tests, though there are usually one or two rows of much deformed cells on the outer side destitute of starch all the way through, which have been crushed back by the developing embryo. It is just at this time that the grain begins to change its colour from green to brown, after which it takes about another fortnight to become properly ripe.

When one comes to consider the manner in which the reserve starch is packed into the cells, there are certain points which are somewhat contrary to one's expectations. The wheat ovule is anatropous, and therefore a conducting strand runs up the raphe alongside the seed, at the base of the furrow, and so one would naturally expect that insoluble carbohydrate would first be laid down in the cells at the extreme limit of the grain, as actually happens. The flanks of the endosperm, where the first vestiges of starch occur, continue to keep the lead, as, at any level, it is always the cells in this position which first reveal starch grains. Then, instead of the soluble carbohydrate being carried across the width of the endosperm, and being finally deposited in the first place at the *outer* edge of the grain, thus leaving a clear passage behind it, the cells which first deposit their starch are those nearest to the furrow, and therefore to the conducting strand. This makes the percolation of the soluble reserves much more difficult, and, judging by the appearance presented by the various series of sections, it seems probable that the infilling does not only take place laterally, but also in an obliquely downward direction. This implies that soluble reserves are brought to a certain point in the conducting strand—thence some probably pass into the endosperm obliquely downwards, filling up the cells at a lower level which have been blocked

by the deposition of starch in the cells next the furrow at their own level. Part of the remainder of the reserve material so brought passes laterally outwards, and is deposited in the flanks of the endosperm, and eventually the cells next the furrow are filled up, so placing that particular portion of the conducting strand out of action, or rather crippling its activity to a very great extent. It is most probable that a certain amount of percolation can take place through the starch-filled cells next to the



TEXT-FIG. 3. Curve illustrating the influx of starch into the grain during the course of development.

furrow, as these cells are blocked to the very end of the seed some little while before all the outer cells are filled with starch, but these latter cells fill up very slowly indeed, showing how difficult the percolation must be.

During the year 1907 a good deal of progressive quantitative work, as yet unpublished, was carried out on the wheat grain in the course of development. The 'green and dry weights of 1,000 grains' were ascertained every three days, and formed the basis to which all other calculations of quantitative analyses were ultimately reduced. Determinations were made of the actual amounts of ash, nitrogen, and phosphoric acid in the grain at

three-day intervals, from about the tenth day after pollination till the time of harvesting.

From these determinations it was possible to make an approximately accurate calculation of the quantity of carbohydrate per 1,000 grains—the results of which have been expressed as a curve for each of the three plots. It is readily seen that, at least in two cases, the curve is very smooth and very straight for a period corresponding to about six weeks from the time of pollination, while it flattens off considerably during the last week. This indicates that the infilling of starch goes on very steadily and regularly for about six weeks, the rate of inflow hardly fluctuating at all. Then, shortly before the grain is what the farmer would call 'ripe', this influx practically ceases.

A parallel series of analyses showed that during the whole period of starch deposition, protein matters are entering the endosperm at the same time, but the stains used to place the starch in evidence for microscopic work tended to mask the increasing amounts of nitrogenous reserve material.

3. *Disorganization of Nuclei.* The various changes in the endosperm nuclei in the later stages of development were studied on the 1904 material chiefly by means of series of hand-sections stained with Delafield's haematoxylin.

As we have seen, starch has filled into practically all the endosperm cells a fortnight before the grain can be regarded as ripe enough to cut, but the curves illustrating the inflow of carbohydrates show that starch continues to be crammed into the cells for some while longer. Just about the time that all the endosperm cells have received some quota of their starch, when the grain is just beginning to turn from green to brown, changes occur in the nuclei of the cells. Up to this period these have remained perfect and complete, rather large in proportion to the size of the cells, possessing good nucleoli and taking stains well. Those nuclei in the neighbourhood of the embryo are the first to exhibit change, in fact disorganization seems to proceed gradually from the tip downwards, in exactly the opposite direction to that in which the starch is deposited. First of all the nuclei in the middle of the flanks seem to lose their nucleoli and become very dense in structure, staining more deeply than those near the aleurone layer, which still retain their nucleoli. This change continues downwards and outwards as time goes on, though some of the nuclei in the sub-aleuronic cells round the furrow retain their nucleoli till quite a late stage in maturation. While this is going on a further alteration occurs—those nuclei which first become dense apparently lose their solidity, and exhibit a network structure in section, at first appearing as very coarse networks, with thick walls and relatively small cavities, later as fine reticula with large cavities. Investigation showed that this is due to the pressure

of the surrounding carbohydrates. At first the dense nuclei become somewhat deformed, as the neighbouring starch grains begin to press upon them, making dents in the nuclear matter. As the pressure from without increases the substance of the nucleus is squeezed out between the starch grains, eventually surrounding and enclosing them. The method of procedure might be illustrated by taking small balls of putty and pushing peas into them. A section across such a ball would resemble the 'network' nuclei of the wheat grain—if only a few peas were present, the putty would appear as a thick-walled network, while if the peas were in contact the matrix of putty would be well spread out into quite a fine reticulum, filling up the interspaces between the intruding bodies.

This later stage in nuclear disorganization follows the same course as the initial stage and spreads outwards and downwards, though many of the nuclei in the sub-aleuronic layer never get so far, simply losing their nucleoli and becoming dense, but not being subjected to the intrusion of starch grains. The nuclei which are furthest advanced in disorganization, showing an exceedingly attenuated reticulum, do not stain at all well, and need careful observation in some cases if they are not to be overlooked.

This disorganization is by no means completed by the time the corn is cut, but continues its progress while it is standing in 'stook' previous to carrying. The Square Head's Master from Plot 3, Broadbalk, was cut on August 5, 1904, and carted on August 12. On the former date nuclear networks had spread as far downwards as the base of the embryo, below which solid nuclei still persisted. Seven days later, when carted, networks could be found right through the grain from top to bottom. Generally speaking, at this late stage in maturation the endosperm cells at the tip of the grain contained nuclei in only the very finest network stage; towards the lower end of the kernel, the networks occurred chiefly in the middle of the flanks and were coarser, while at this level the nuclei in the sub-aleuronic layers still remained in most cases dense and solid. These latter changes are evidently due to desiccation, which the analyses show to be a marked feature of the last stages of ripening, especially after the corn is cut, and not to the further influx of reserve food material. Two other varieties of wheat were examined with similar results, but in no case was such disorganization seen in any cells of the aleurone layer.

There is just a possibility that these facts may have some bearing on the problem of the vitality of the endosperm tissue, corroborating from the cytological standpoint some of the recent work on the subject. Miss Bruschi (15), fully admitting the vitality of the aleurone layer, maintains that only the immediately sub-aleuronic cells can be regarded as in any sense living, all the rest of the tissue being quite dead. As we have seen, the nuclear networks in the mass of endosperm tissue only stain very

slightly in the later stages, while most of the sub-aleuronic cells retain dense nuclei to the end. This may indicate that the nuclei which stain so slightly are to all intents and purposes dead, while the others still retain at least some degree of vital energy; but such a suggestion can only be brought forward with the utmost reserve, because at the time that the nuclear networks are forming the wheat grains are undergoing considerable desiccation, and so the pressure on the tissues is probably increased by shrinkage. It may easily happen that the desiccation also affects the nuclei, and the loss of staining power may be due to mere physical changes partly caused by the withdrawal of water.

A similar phenomenon, due to the squeezing action of starch grains, has been noticed in barley by Brown and Escombe (16), who describe the 'senescence' and disintegration of the nuclei of the endosperm as maturity approaches. According to these authors, deformation of the nucleus begins in the cells underlying the sub-aleuronic layer, and rapidly advances centripetally till nearly the whole of the endosperm is involved, the deformation being followed by complete disintegration of the nucleus. In the later stages of ripening, the cells of the sub-aleuronic layer also become involved, the very last cells to undergo this 'nuclear senescence' being those of this layer in the ventral folds on either side of the furrow. This may be paralleled in the wheat, in which the nuclei in this same position are the last to lose their nucleoli and become dense in structure, but the complete disintegration observed in barley does not occur in *Triticum*, as the fine networks can be made out to the end.

The way in which the disintegration proceeds throughout the length of the grain is not touched on by Brown and Escombe, so it is possible that a more complete examination would reveal some nuclei remaining at the further end of the grain which would compare with the dense sub-aleuronic nuclei of the wheat.

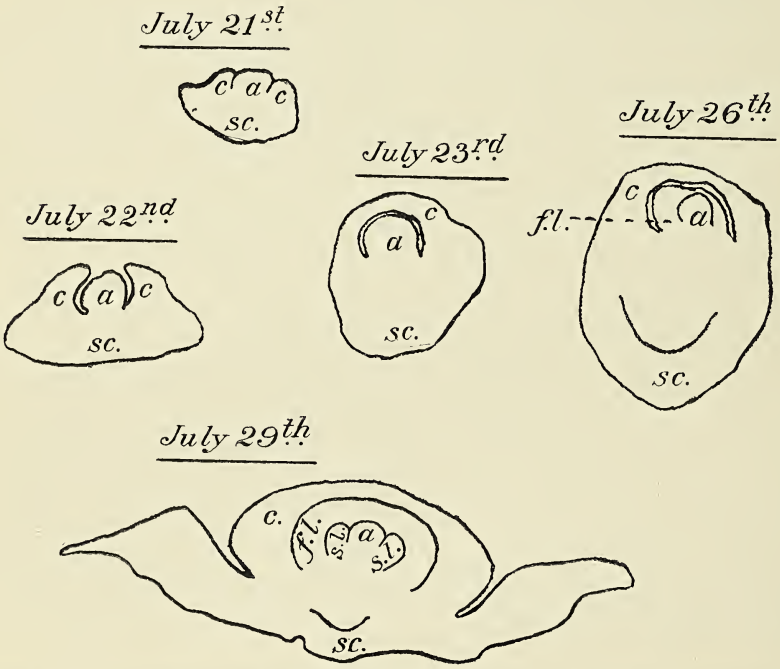
(*d*) Development of the Embyro.

Strasburger (13) gives a very complete account of the structure of the embryo in the ripe seed, but does not enter into the developmental history. The egg cell after fertilization enters on a short period of rest till about the fifth day after pollination, when the first division occurs. Koernicke mentions a three-celled stage as being much in evidence, but unfortunately the material does not show it.

The youngest segmented embryo observed consisted of a mass of four or five cells apparently attached to another cell nearer the micropyle, presumably the suspensor, but the preparations were not sufficiently clear to be certain of this point.

During the next eight or nine days cell-division proceeds actively, and the young embryo develops into a mass of cells, rapidly increasing in size.

At first it is absolutely undifferentiated, but later on the cells at the periphery arrange themselves to form a definite surface layer. About a fortnight after pollination the first traces of differentiation can be made out; towards the tip of the grain the cells in the middle begin to group themselves into a central core, while lower down the initials of the cotyledons appear in the form of two slight projections on the dorsal side of the embryo away from the endosperm. These projections grow towards one another and eventually meet, closing round the inner mass of tissue which forms the rudimentary shoot and shows nuclei in active division; in



TEXT-FIG. 4. Outline sketch showing development of plumule in grains pollinated on July 6, 1907. *c.* cotyledon; *f.l.* 1st leaf; *s.l.* 2nd leaf; *sc.* scutellum; *a.* axis of young shoot. *T.S.* × 62.

about three days' time the edges of the cotyledon not only meet but fuse, so forming a kind of false tube, which, however, gives an indication of its real derivation in the fact that the two edges always remain free quite at the tip.

Meanwhile the central core has become more clearly marked out, and about six days from its first appearance the young radicle begins to separate from the surrounding tissue, which forms the coleorhiza or root-sheath. Just at this time the first traces of the epiblast appear—the ligule which develops on the hypocotyl immediately above the radicle, on the dorsal side of the embryo. A little later the tissues of the radicle are seen

to be arranging themselves into epidermis, cortex, and vascular cylinder. Meanwhile the root-cap has been evolving itself, and is now quite clear.

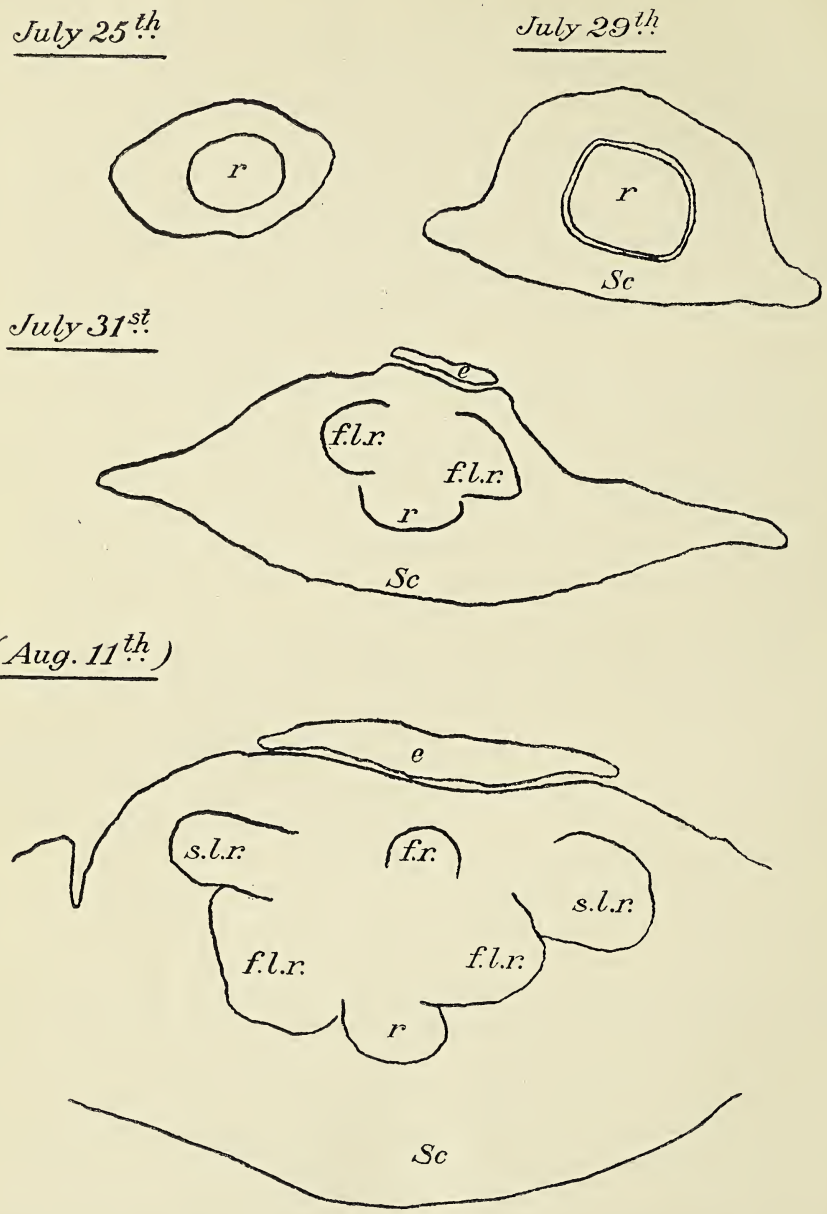
Rather less than four weeks after pollination the first pair of rootlets come into being. These are arranged right and left of the radicle, but rather towards the outer side of the embryo, and arise from the very base of the hypocotyl, just where it joins the young root. About a week later two other rootlets appear above the first pair, with practically the same orientation, but a trifle more dorsal. In the course of another week a fifth rootlet makes its appearance, lying between the others on the outer side, which does not seem to be mentioned by Strasburger. No further root development occurs before the embryo enters upon its resting stage in the ripe seed.

Returning to the developing plumule—about three weeks after pollination the first leaf is marked out, with a divergence of $\frac{1}{2}$ from the cotyledon, with the free tips pointing inwards towards the endosperm. This leaf makes far more growth than any of the subsequent ones, so that eventually its edges are folded over one another at the tip, the lower part forming a tube. Other leaves are soon marked out, so that by the time the grain is ripe three or four are in evidence, all with a divergence of $\frac{1}{2}$.

When the radicle and plumule start differentiating the embryo begins to shape itself. The tissue lying between these rudiments and the endosperm gradually spreads itself out into a shield-like mass forming the scutellum, which develops a well-marked epithelium towards the endosperm, and acts as a sucking organ at the time of germination. This spreads upwards and downwards, as well as laterally, so that at length the young plant appears to be borne on the outer face of the shield.

The initials of the embryonic vascular system are marked out at quite an early stage, when the epiblast begins to develop and at the same level with it. At first a strand of differentiated cells appears in the hypocotyl, which strand turns out into the scutellum, running in it for some way parallel to the plumule, and finally disappearing. As time goes on this vascular strand gets more clearly marked out, and continues its way through the scutellum below the level of the plumule. When first it passes from the hypocotyl the cells are somewhat hexagonal in cross-section, but lower down they appear elongated, with the long axis at right angles to the ventral edge of the scutellum. At length this strand begins to spread out laterally, till eventually a fan-like arrangement of conducting cells is formed, which continues nearly to the extreme end of the scutellum.

Soon after the initials of the vascular strand appear in the hypocotyl branches pass off from it, one into either side of the cotyledon, but these two branches undergo no further division, remaining intact throughout development. In due course vascular strands also pass into the first leaf,



TEXT-FIG. 5. Outline sketch showing development of radicle and rootlets in grains pollinated on July 6, 1907. The last stage was obtained from 1904 material, but would probably correspond in age to grains gathered on August 11, 1907. *r.* radicle; *f.l.r.* first pair of lateral rootlets. *s.l.r.* second pair of lateral rootlets; *f.r.* fifth rootlet; *e.* epiblast; *sc.* scutellum. Radicle is only marked out on July 25, not separated from the surrounding tissue. *T.S.* × 62.

but these divide up till eventually about a dozen are present, and the leaf shows definite ribbing on its inner side. Traces of vascular tissue can also be made out in the second leaf by the time its development is completed.

When the seed germinates the radicle first shows itself, pushing its way through the tissues of the coleorhiza, which remains as a sheath at its base. The two pairs of lateral rootlets appear at intervals, the lowest pair first, and rapidly elongate till they are soon as well marked as the main root. Very much later the solitary fifth rootlet emerges, coming out from between the upper pair of laterals. No tap-root is formed, since the adventitious rootlets develop as strongly as the radicle, and so a fibrous root system is ultimately the result.

Summary.

1. No cytological differences can be observed between wheat grains of different varieties, grown under similar conditions, which produce flour of varying strengths.

2. No cytological differences, indicative of strength, are to be found in the developing grains of one variety of wheat, when grown in the same field, on similar soil, and under the same climatic conditions, but subject to radical differences in manuring.

3. The reputed difference in strength between grains of the same variety, grown under the same conditions, but cut 'green' and 'dead ripe', is not associated with any significant cytological change.

4. Examination of the different grades of flour obtained during the various processes of roller milling show that the cells of the aleurone layer very rarely get into the flour, but remain attached to the bran in small sheets.

5. The endosperm arises in the first place by free-cell-formation, but after about a week wall-formation begins, starting from the periphery of the embryo-sac and proceeding inwards, while the central vacuole fills up first at the micropylar end. The peripheral layer is marked off about a fortnight after pollination, and develops into the aleurone layer.

6. Deposition of starch grains begins in the middle of the flanks of the endosperm at the lower end of the grain, and proceeds upwards and outwards. In the bridge the cells nearest the furrow are the first to fill up, the deposition proceeding from this point towards the dorsal side of the grains. Reserve nitrogenous material enters at the same time as the starch.

7. During the process of maturation, disorganization of endosperm nuclei takes place, caused by the increasing pressure of the surrounding food substances, which is largely due to the desiccation going on at this stage. The nuclei lose their nucleoli, become deformed, and finally

appear as very fine networks spread out between the intruding starch grains.

8. The development of the embryo is normal. A fifth lateral rootlet is formed in addition to the two pairs of laterals usually described, lying between them on the dorsal side, which does not appear in germination until quite late, after the other rootlets and the plumule are well developed.

In conclusion, I have to express my thanks to Mr. A. D. Hall, at whose suggestion the work was undertaken, for the valuable advice he has given me all through the progress of the investigation.

November, 1908.

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EXPLANATION OF FIGURES IN PLATES VIII AND IX.

Illustrating Miss Brenchley's Paper on the Grain of Wheat.

Abbreviations used: *o.* ovum; *sy.* synergid; *g.n.* generative nucleus; *e.n.* endosperm nuclei; *e.s.* embryo sac; *p.t.* pollen tube; *d.* deeply-staining bodies in pollen tube; *ep. nuc.* limiting layer or nuclear epidermis; *ov. integ.* ovular integuments; *pl.* placenta; *pc.* pericarp; *nuc.* nucellus; *end.* endosperm; *st. end.* starchy endosperm; *a.l.* aleurone layer; *st.* starch grains; *sc.* ordinary cells of scutellum; *v.s.* conducting strand in scutellum; *m.* micropylar end of *e.s.*; *pm.* protoplasm of *e.s.*; *emb.* embryo; *f.* furrow of grain; *r.* radicle; *f.l.r.* first pair of lateral rootlets; *s.l.r.* second pair of rootlets; *f.r.* fifth rootlet; *c.* cotyledon; *f.l.* first leaf; *s.l.* second leaf; *w.* germinating wheat grain; *T.S.* transverse section; *L.S.* longitudinal section.

PLATE VIII. Figs. 1–16.

Fig. 1. Rather oblique section through embryo-sac about two days after pollination. Enclosed in the ovum is a dark mass which probably is the male generative nucleus which has not yet lost its individuality. Two nuclei are present at the other side of the sac, the result of the first division of the definitive nucleus. × 270.

Fig. 2. The adjacent section to Fig. 1, showing the tip of the pollen tube overlying the ovum. × 260.

Fig. 3. Part of *T.S.* of *e.s.* three days after pollination. The cells of the nucellar epidermis have renewed their activity and the nuclei are in process of division. × 270.

Fig. 4. Part of *L.S.* through *e.s.* six days after pollination, showing the numerous endosperm nuclei embedded in the protoplasmic lining which surrounds the central vacuole. × 170.

Fig. 5. *L.S.* through *e.s.* seven or eight days after pollination. Cell wall formation is proceeding inwards from the periphery of the endosperm, but the cells are not complete in the middle. × 170.

Fig. 6. Part of *T.S.* of grain eight days after pollination. Remains of the thin-walled nucellus still occur between the nucellar epidermis and the endosperm. × 270.

Fig. 7. *T.S.* ten days after pollination. Thin-walled nucellus has entirely disappeared. × 270.

Fig. 8. *T.S.* fourteen days after pollination. The nucellar epidermis is losing its cell contents, and the outer layer of the endosperm is marked out to form the future aleurone layer. × 270.

Fig. 9. *T.S.* nineteen days after pollination. Aleurone layer clearly marked out. × 270.

Fig. 10. *T.S.* of mature grain, showing endosperm fully developed. × 270.

Fig. 11. *T.S.* Perfect endosperm nuclei, with nucleoli. × 320.

Fig. 12. *T.S.* Endosperm nuclei rather deformed by pressure; no nucleoli. × 320.

Fig. 13. *T.S.* Endosperm nuclei in 'coarse network' stage. × 320.

Fig. 14. *T.S.* Endosperm nuclei in 'fine network' stage. × 320.

Fig. 15. *T.S.* of scutellum one week before the corn was cut, showing the conducting strand just after it has turned out from the hypocotyl—cells roughly hexagonal in *T.S.* Cell contents omitted. × 180.

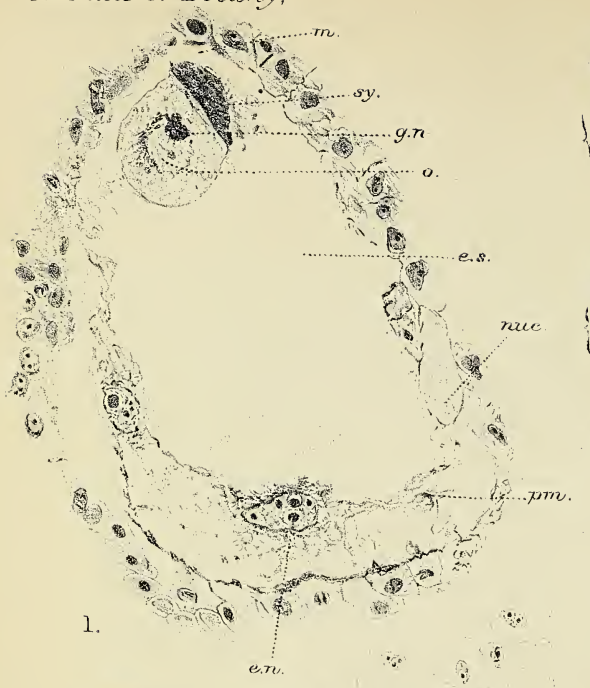
Fig. 16. Same embryo as Fig. 15, showing conducting strand at a point lower down the grain, where the cells are elongated in *T.S.* On one side the strand is just beginning to spread out laterally. Cell contents omitted. × 180.

PLATE IX. Figs. 17–23.

Fig. 17. Same embryo as Fig. 15, but lower down than Fig. 16, where the conducting strand has spread out in a fan-like manner. Cell contents omitted. × 180.

Figs. 18–23. Six successive stages in germination, showing the order of development of the rootlets. About natural size.

N.B.—In Figs. 4, 5, 6, 7 the endosperm has somewhat shrunk away from the surrounding tissues in the course of preparation.



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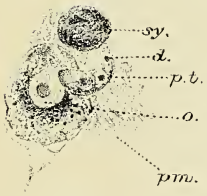

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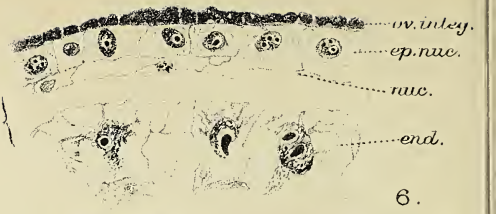

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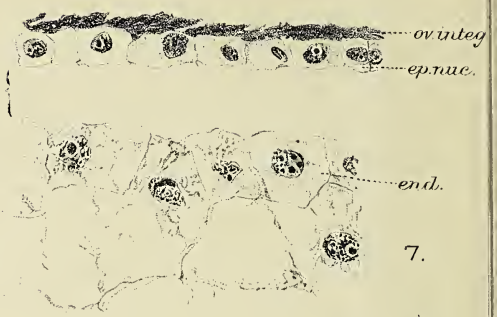

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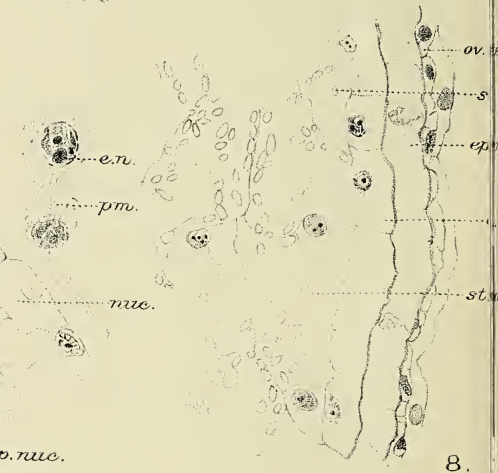

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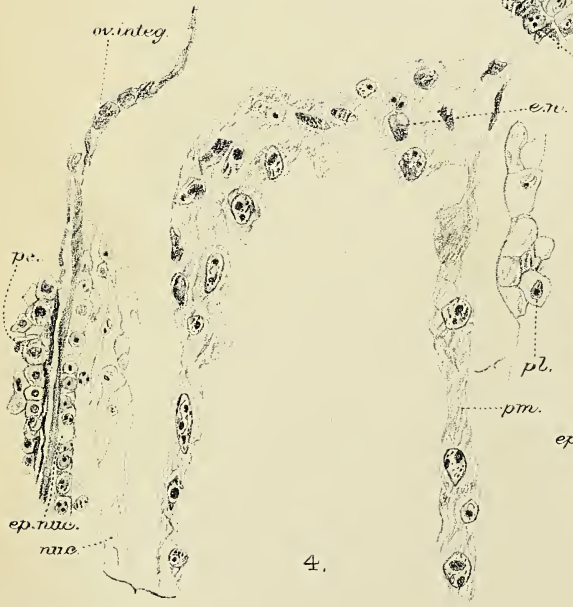

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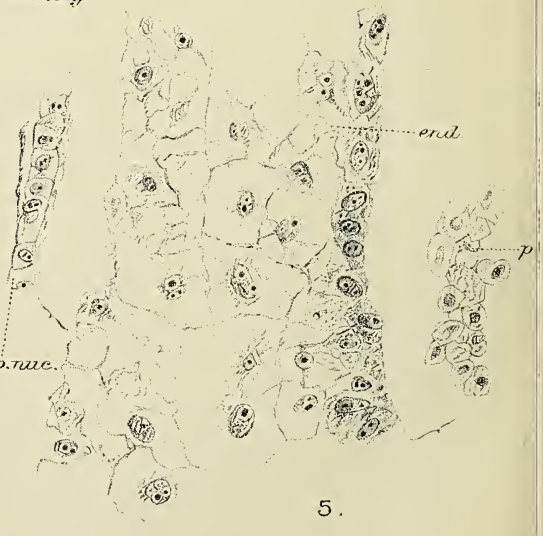

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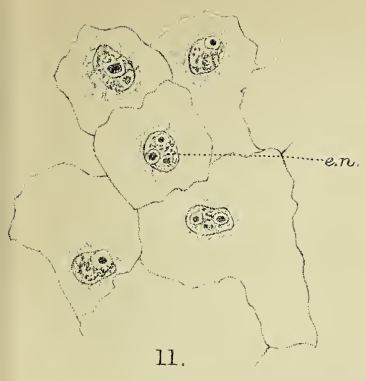
BRENCHLEY — GRAIN OF WHEAT.



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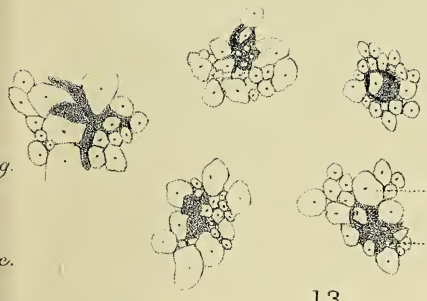

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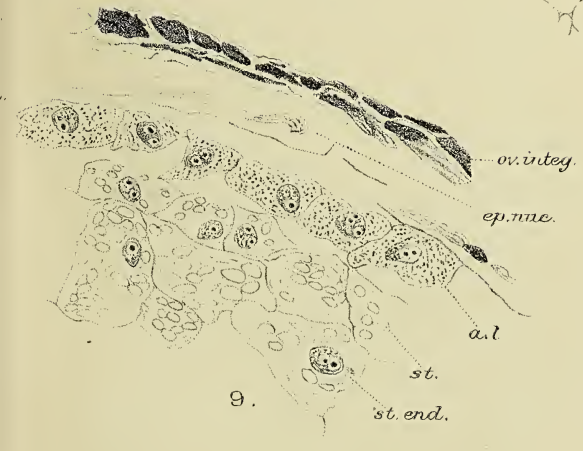

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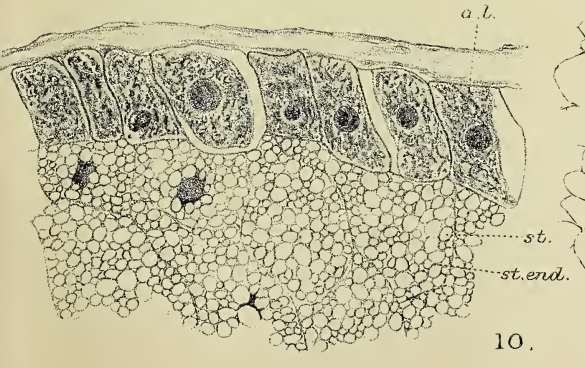

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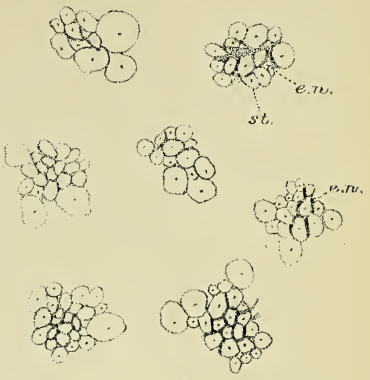

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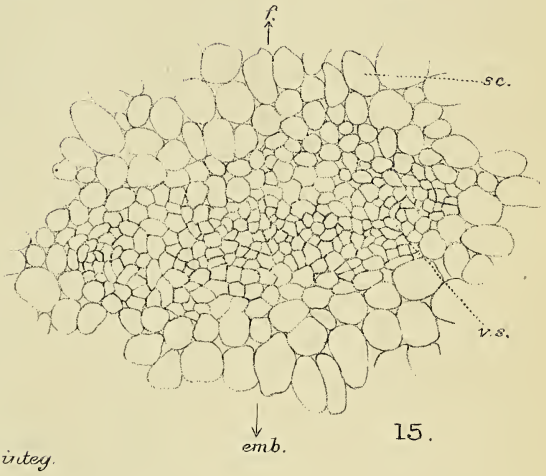

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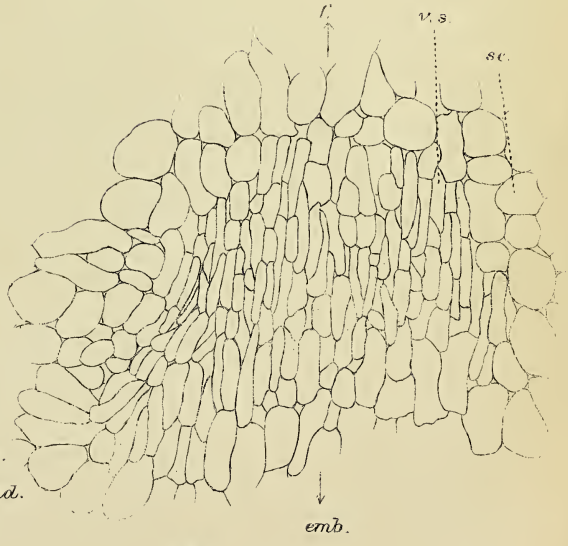

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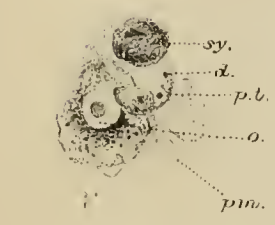

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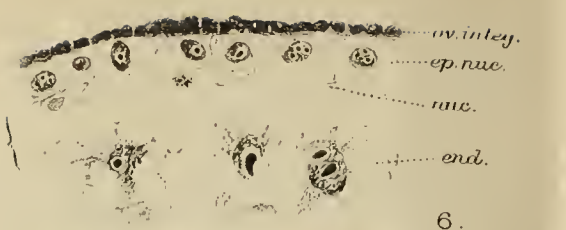

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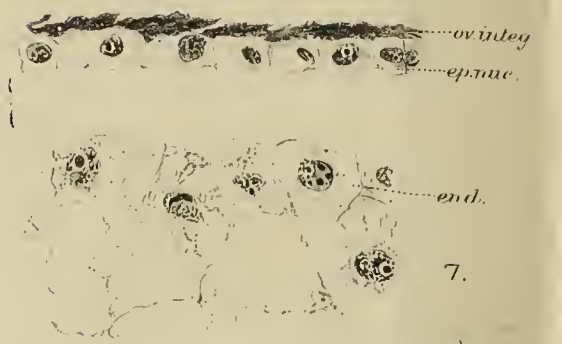

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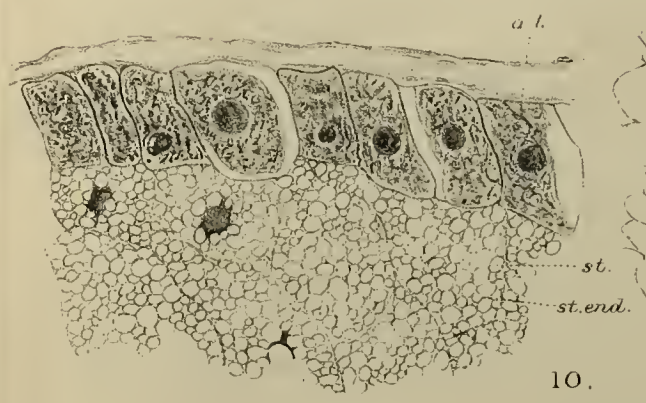

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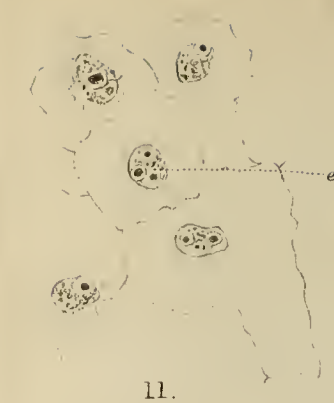

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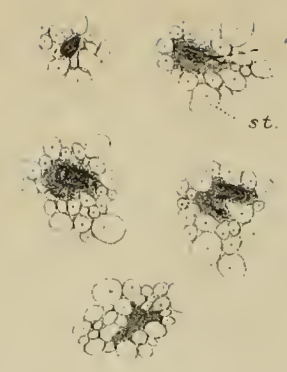

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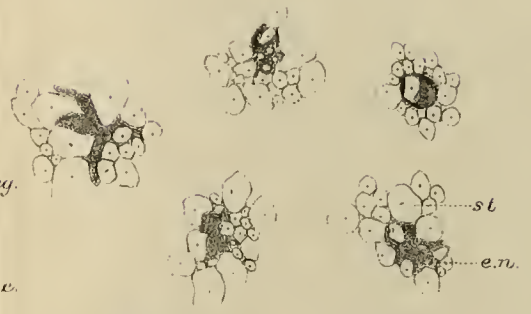

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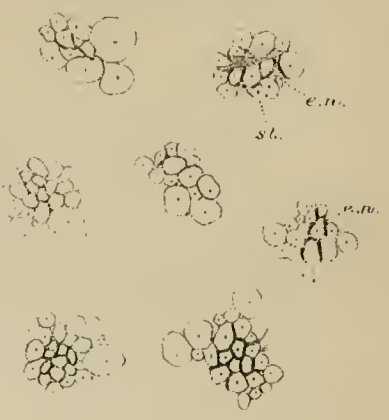

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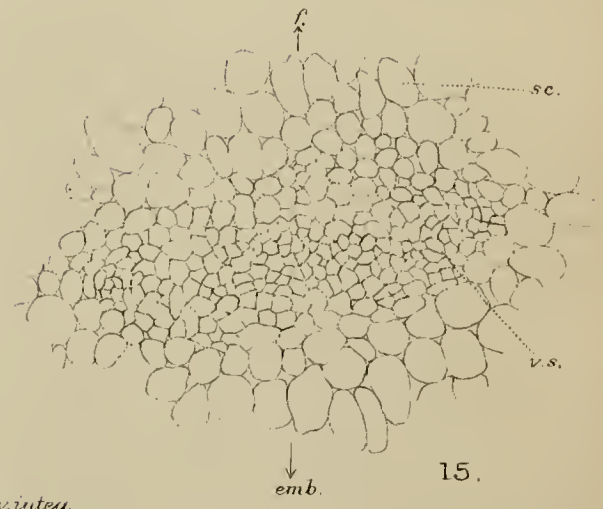

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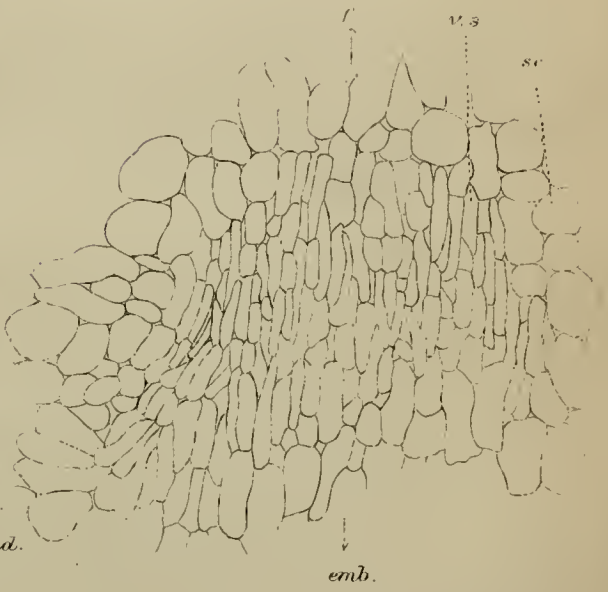

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BRENCHLEY — GRAIN OF WHEAT.

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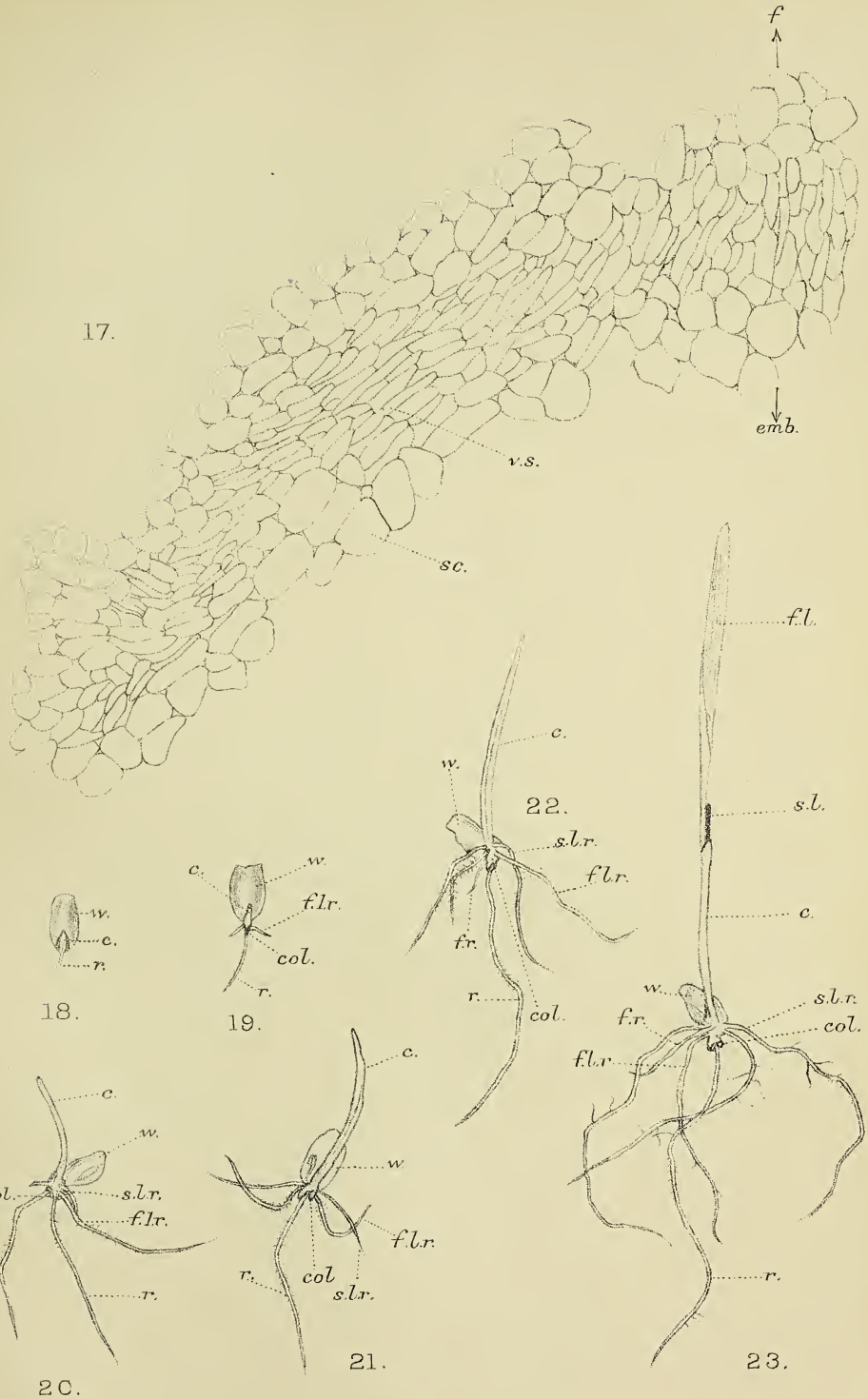

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BRENCHLEY — GRAIN OF WHEAT.

On Spore Formation and Nuclear Division in Mnium hornum.

BY

MALCOLM WILSON, B.Sc., A.R.C.S.

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With Plates X and XI.

AT the present time the Meiotic Phase has been described in almost all the larger groups of plants, but the Muscineae form a notable exception to this statement. Although the reduction divisions have been frequently described in the Liverworts no complete account has yet been given of the corresponding process in the Mosses. This, no doubt, is partly due to the small size of the nuclei found in this group and also to the difficulty experienced in finding sufficiently numerous nuclear divisions during the development of the spores. A short description of the Meiosis in *Mnium hornum* has already been given (24), and it is the purpose of the present paper to complete this account.

Several investigators have already noted nuclear divisions in various mosses. Ikeno (18, p. 219) has found that eight chromosomes appear at the division of the nuclei in the antheridial cells of *Atrichum angustatum* and *Pogonatum rhopalophorum*. Holferty (16) has incidentally figured several nuclear divisions in the cells of the developing archegonia of *Mnium cuspidatum*, but on so small a scale that the chromosomes cannot be counted; no description of these divisions is given. Gayet (13, p. 232), in his work on the development of the archegonium in the Muscineae, described four chromosomes both in the spermatozoid and in the fertilized ovum of *Fissidens incurvus*, but it is very doubtful whether the bodies discovered were really chromosomes. Beer (3, p. 166) found four chromosomes in the dividing spore-mother-cells of *Funaria hygrometrica*, but stated later (4, p. 278, footnote) that the number in this plant and in several other mosses, including *Mnium hornum*, was much greater. He also refers to the compound nucleoli in the spermatogenic cells of *Atrichum undulatum*. Dr. Arens (1) has recently published an account of spermatogenesis in *Polytrichum juniperinum* and *Mnium*

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hornum, but only gives a complete account of the first species. In this the nuclei of the cells of the young antheridium contain a very large, extremely deeply staining nucleolus: a few small grains of chromatin are embedded in the extra-nucleolar material, which otherwise exhibits no structure. He therefore concludes that the nucleolus contains the whole of the chromatic material. Eight chromosomes appeared at the metaphase of division. No centrosomes were found until the final division of the spermatogenic cells. *Mnium hornum* was found to be almost similar in structure, and in this not more than eight chromosomes appear during division. In a summary of this work, published later in the Botanisches Centralblatt, the number of chromosomes is corrected by the writer's authority, and is given as six (2, p. 611, footnote).

The most complete description of nuclear division in the Muscineae yet given is that of Drs. J. and W. van Leeuwen-Reijnvaan (20 and 21). They have examined four species of *Polytrichum* (*P. piliferum*, *juniperinum*, *formosum* and *commune*), but were unable to find the reduction divisions. A few divisions in the cells of the sporogenous layer are described, while more numerous divisions were found in the epidermis and parenchymatous tissue of the sporogonium. The chromosomes are described as very small and rod-like; twelve appear during the division, and this number was found in all the species examined. No centrosomes were seen during the process. During spermatogenesis numerous dividing cells were found in the young antheridium. Immediately before division a small body is cut off from the nucleolus; this passes into the cytoplasm, elongates, and then divides, giving rise to two centrospheres, which are later found at the poles of the spindle. Six chromosomes appear, and these show considerable differences in size; two are small, two intermediate, and two large, and thus three distinct pairs can be distinguished. The authors therefore conclude, by analogy with Liverworts and Ferns, that a reduction in number of chromosomes has taken place on spore formation. At the last division of the spermatogenic cells the two chromosomes of each pair fuse laterally, and at the metaphase the three masses thus produced divide longitudinally, and three entire chromosomes pass to each pole of the spindle. In this way a second reduction in number of chromosomes is brought about. A similar second reduction is considered to take place during the development of the egg cell. At fertilization the ovum fuses with two spermatozoids and with the ventral canal cell, and in this way the original number of chromosomes is regained in the fertilized egg.

Methods.

Flemming's Strong and Weak Mixtures, acetic alcohol (absolute alcohol two parts, glacial acetic acid one part), and Flemming's Strong Mixture diluted with an equal quantity of water, were used as fixing

reagents. The acetic alcohol was allowed to act from 15–20 minutes, and the material was then washed in several changes of absolute alcohol. When Flemming's Mixtures were used, the air present in the tissues was extracted by means of an air-pump. They were allowed to act from 18–24 hours, after which the material was washed in running water for 24 hours, and then placed in a 10 per cent. solution of glycerine in large watch glasses. These were kept in a warm place, so that after about 24 hours the whole of the water had evaporated, leaving the pure glycerine. The material was then transferred directly to methylated spirit and ultimately to absolute alcohol. Much better results were obtained by this method than by dehydration by using gradually increasing strengths of alcohol.

The sporogonia were preserved from the beginning of January until about the end of April, when spore formation was completed. Clumps of the plants were dug up in the afternoon and taken into the laboratory or to a greenhouse, and the sporogonia were preserved during the next and following days at hours varying from 6 a.m. to 8 p.m. In the early part of the year the soil in which the plants were growing was often frozen when collected, and the higher temperature of the laboratory probably caused increased cell division. Material was also preserved in the field. The younger capsules were preserved entire, but when further developed the hypophysis and operculum were cut off immediately before fixing. In some cases the capsular wall was also removed, thus laying bare the sporogenous cells, but it was found that the preservation of the cells was not improved by the treatment. Transverse sections of the sporogonium varying from $3\,\mu$–$6\,\mu$ in thickness were generally used, but longitudinal sections were also cut. These were stained either with Flemming's Triple Stain (safranin-gentian violet-orange G) or with Heidenhain's Iron Haematoxylin.

Premeiotic Development.

Fertilization in *Mnium hornum* usually takes place about the beginning of May. The development of the sporogonium is slow, and in the following January the archesporium becomes differentiated. At this stage the sporogonium consists of a long seta and capsule, but the latter is yet only little developed, and can only be distinguished with difficulty from the stalk. In a transverse section of the capsule taken at this stage the single-layered archesporium is very obvious. Its cells are filled with dense finely alveolar protoplasm, and contain a large centrally placed nucleus (Fig. 1, Pl. X). No vacuoles are present and the protoplasm at this and at all later stages completely fills the cell; with a high magnification alveoli can be distinguished in it, but with moderate magnification the appearance is granular, and usually, in well-fixed cells, very regular in texture. In a considerable number of cases the protoplasm towards the centre of the cell appears to be less dense,

and in the preparations forms a lightly-stained irregularly-shaped area around the nucleus, sharply limited from the remaining protoplasm (Fig. 2). At first sight, this suggests improper fixation, but examination of the other parts of the cell leads to the conclusion that this represents a real structural difference. The nucleus is large in proportion to the cell, and contains one large deeply-staining nucleolus. The remainder of the nuclear cavity is filled with a very fine homogeneous network, somewhat resembling the cytoplasm in structure; chromatin is very scanty or almost absent in the resting nucleus, a few granules sometimes occurring scattered in the intersections of the alveolar walls.

A nucleus of the type can be considered to be characteristic of the Muscineae. It has been described by Beer (4) in *Atrichum undulatum*, by Arens (1) in the spermatogenic cells of *Polytrichum juniperinum* and *Mnium hornum*, and by Drs. Leeuwen-Reijnvaan (20 and 21) in several species of *Polytrichum*. In the case of *Atrichum undulatum*, Beer has also noted the presence of compound nucleoli, and he has described similar nucleoli in the nuclei of the vegetative cells of *Ricciaglauca* (4). These, he states, 'consist of a lightly coloured matrix, in which are embedded a number of grains of chromatin.' Such nucleoli have been described by other observers, and they have been met with in the resting nuclei, both of the archesporial cells and of the spore-mother-cells of *Mnium*, but it is open to question whether this appearance is due to a real structure or is an effect brought about by the fixing reagent. These nucleoli were found much more frequently in preparations fixed with acetic alcohol than in those which had been treated with Flemming's Mixture. Fischer (11) has emphasized the fact that the former reagent causes the precipitation of some of the proteids of the cell in a soluble form, and it is possible that this appearance is due to partial solution of the nucleolus at the time of fixing or during subsequent treatment.

The archesporial cells rapidly increase in size, and division soon takes place. Material preserved at various hours of the day was examined, but no definite relation could be found between the time of preservation and the progress of division. On the whole, divisions were found to be most frequent during the morning, but this, no doubt, was partly due to the rise of temperature; various stages could generally be found in the same section. The first dividing wall is generally periclinal, and this is soon followed by two anticlinal divisions at right angles, so that each of the original cells becomes divided into octants. In some cases no further divisions occur before meiosis, but in others each octant may again divide by a periclinal wall; in a few cases this is followed by yet another division resulting in the formation of a wall transverse to the axis of the sporogonium. Thus, groups of 8, 16, or 32 cells may be produced from each original cell of the archesporium. Each group retains the outline of the original cell, and its limits can be distinguished up to the time of spore formation. Nuclear

division, although simultaneous in the cells of each group, does not take place at the same time in the different groups. The process in all these divisions is identical, and the following description applies to any premeiotic mitosis. Divisions of the vegetative cells of the sporogonium have been examined, but differ in no respect from those of the archesporial cells.

In some of the nuclei the extremely homogeneous structure of the nuclear reticulum is replaced by a more granular appearance; the alveoli are larger and not so regular, and small granules of darkly-staining material make their appearance at the intersections of the network. Nuclei in this condition are constantly found in preparations fixed with acetic alcohol and the above-described homogeneous structure is only obtained when Flemming's Mixture has been used. At this stage the superiority of the latter is clearly demonstrated. But even in material thus fixed some nuclei still exhibit the more granular appearance, and this condition, although frequently due to imperfect fixation, is in some cases the first sign of approaching mitosis. A little later a clear space appears in the middle of the nucleus (Fig. 3). This space is to be distinguished from the light area surrounding the nucleolus mentioned by Strasburger (23, p. 10), and considered by him to be due to contraction at the time of fixing. Such an area is often seen in the nuclei of the resting cells (Fig. 1). The chromatin increases in amount and broad band-like areas appear in the more peripheral parts of the nucleus (Fig. 4). As the result of further contraction, these become gradually narrower and more elongated (Fig. 5), and ultimately give rise to the spireme (Fig. 6). During this time the nucleolus has been losing its staining capacity, and frequently shows lighter and darker areas within it. At the differentiation of the spireme it has completely disappeared. At this stage only a few small irregular masses of deeply-staining material can be seen, and these are in close connexion with the chromatic thread.

Comparison of these stages with those described by Farmer and Shove (9) in divisions of the cells of the root of *Tradescantia* reveal a close similarity, particularly with regard to the formation of the band-like chromatin masses. (Compare Figs. 4 and 5 with Figs. 3 and 4 of above work.)

It could not be definitely ascertained whether the spireme consists of a continuous thread. In several cases it appears that free ends are present (Fig. 6), and that these are not due to removal of part of the nucleus in sectioning. Although careful search has been made, no traces of longitudinal fission have been discovered in the thread at this or at a later stage. The nuclear membrane, present in the earlier prophases, now completely disappears, and the spireme lies free in the protoplasm. Free ends can now be seen with certainty in the thread, and portions of it are arranged about a central plate, although, at this time, no sign of the spindle can be discovered (Fig. 7). Partition of the thread is not yet complete, and loops are found between the free ends; the number of chromosomes cannot yet be ascer-

tained. At the next stage the chromosomes have become arranged on the equatorial plate (Fig. 8), but even now the spindle is not strongly marked, although the individual fibres can be distinguished; the latter converge towards the poles, but no signs of centrosomes or of polar radiations were seen. The number of chromosomes can now be ascertained, and in all cases twelve are found to be present. They take the form of somewhat elongated sharply-bent rods with the hook-like arm lying at the equatorial plate, and, as far as can be judged, they are all of equal size. The number is most clearly shown in polar views (Fig. 9), where each chromosome is generally seen as a dumbbell-shaped structure, consisting of two clearly marked circular portions, united by a less distinct band. This appearance is given by the two arms of the chromosome in optical transverse section, while the connecting portion is dimly seen at a slightly different level. Up to this time twelve is the only number of chromosomes recorded in cytological examinations of the diploid generation in the Muscineae (Leeuwen-Reijnvaan, 20 and 21), and this agrees with the number found in the present investigation.

Longitudinal splitting of the chromosomes takes place on the equatorial plate, and the resulting halves retreat to the poles forming the usual diaster (Figs. 10 and 11). The retreating chromosomes are of the characteristic V-shape, and a polar view at this stage allows the number to be easily counted. Each chromosome is seen as two small dots, since the two arms of the V are seen in optical transverse section. On reaching the poles the chromosomes lose their sharp outline and become fused together, forming an irregularly-shaped mass of chromatin. This gradually loses its staining capacity, and, at the same time, several rounded nucleoli appear. The nuclear membrane becomes evident in each of the daughter-nuclei, which at first possess a flattened surface on the side towards the centre of the spindle (Fig. 12). Later on the nucleoli decrease in number, and ultimately one is produced by general fusion. The nucleus takes on a rounded form, finally becoming spherical or ellipsoidal (Fig. 13); during the telophase numerous darkly-staining masses of material are found in the cytoplasm.

During the anaphase the spindle broadens out towards the poles and becomes very obvious, the new cell wall is laid down at the equatorial plate, and can first be distinguished as a fine granular deposit, extending across the spindle (Fig. 11). At the telophase the wall has become more obvious, and extends completely across the cell.

The Meiotic Divisions.

After the final divisions in the archesporial tissue have taken place the cells pass into a resting condition which continues for some time—as far as could be judged, from seven to ten days. Growth takes place and the cells increase considerably in size; after a time, contraction of the protoplasm

sets in, and each protoplast separates slightly from the wall and lies free in the cavity. As a general rule, nuclear division takes place simultaneously in the whole of the spore-mother-cells of the sporogonium, but a few exceptional cases have been discovered. In these, although the majority of the nuclei were in the same state, some few were found in the immediately preceding or following stages; in all cases, cells of the same group divide simultaneously.

The nuclei resemble those already described in the premeiotic resting cells, but in the spore-mother-cells the nuclear reticulum is, on the whole, coarser, and the chromatin is slightly more abundant (Fig. 14). In most of the nuclei, in addition to the nucleolus, a small deeply-staining rounded body is present. This varies in position, being sometimes situated in contact with the nucleolus, but generally about equidistant between the latter and the nuclear membrane (Figs. 14 and 17). A careful examination leads to the conclusion that this body arises from the nucleolus by a process of budding (Fig. 15), gradually increases in size, and is ultimately set free (Fig. 16). This structure is constantly found in preparations which have been fixed with Flemming's solution, but it is absent from those previously treated with acetic alcohol. This fact confirms the view previously stated (p. 144), that by the use of the latter reagent some of the nuclear material is actually lost.

In the spermatogenesis of *Marchantia polymorpha*, Ikeno (19) has described the formation of a similar body; here, after its constriction from the nucleolus, it passes out through the nuclear membrane into the cytoplasm, and there, by division, gives rise to the two centrosomes. An examination of the spermatogenic cells of *Mnium hornum* reveals the presence of a similar structure with a like origin, but its ultimate fate has not yet been determined. In the spore-mother-cells of *Mnium*, the body in question certainly does not pass into the cytoplasm, and it can still be distinguished within the nucleus during the earlier stages of division. Gates (12, p. 6) has figured a similar body in the heterotype division of *Oenothera rubrinervis*. He states that a large nucleolus and several smaller ones are present in the resting sporogenous cells, and that, as division approaches, fusion takes place until generally one large nucleolus and one smaller one remain; these persist during synapsis and diakinesis. Miss Nichols (22) has described a budding of the nucleolus in the pollen-mother-cells of *Sarracenia*, but this is interpreted as a fusion by Gates (12, p. 6, footnote). Cardiff (7) has figured a similar budding in the pollen-mother-cell of *Acer platanoides*. In the case of *Mnium*, there can be no question of fusion, since only one of these bodies is present.

The first sign of division is seen in nuclei which exhibit a coarser reticulum and more abundant chromatin (Fig. 17). The remarks made during the description of the early prophase of the premeiotic division (see

p. 144) will equally apply to this stage. In some nuclei exhibiting this structure, the cutting off of the above-mentioned body from the nucleolus is taking place (Fig. 16), and, if this is to be regarded as a criterion of the resting state, the coarser reticulum in these cases must be the result of imperfect fixation. The enlargement of the meshes of the reticulum and the increase in the amount of chromatin go on simultaneously; short thread-like filaments of the latter substance are gradually differentiated (Fig. 17), until finally a distinct deeply-staining network results (Fig. 18). The latter generally extends over the whole nuclear cavity, and consists of a thin chromatic thread with regular structure, but interrupted at intervals by small irregular masses of chromatic material. Whether at this stage the spireme forms one continuous thread, could not be determined on account of the numerous intersections which take place. This thin spireme is evidently similar to the 'filaments minces' of Grégoire, and comparison of Fig. 18 with Fig. 2 of his work on *Lilium Martagon* (14) shows considerable agreement, although, in the case of *Mnium*, no such distinct areas of aggregation ('plages') of the network are present.

Contraction now begins in the network as a whole, and finally the spireme becomes aggregated to a dense tangled mass which often occupies a position towards one side of the nucleus (Fig. 21), but may in some cases be centrally disposed (Fig. 19). The exact mode of formation of this first contraction figure is somewhat difficult to determine; it seems that the meshes of the network decrease in size, and that during the whole of the process the spireme retains its tangled arrangement. Figs. 20 *a* and 20 *b* were drawn from two successive sections of the same nucleus in which the contraction had not proceeded very far, and in these, no parallel arrangement of the thread, such as described by Grégoire (14) and by Bergs (5) could be discovered; where the individual filaments could be distinguished they were found to be irregularly entangled. In other nuclei at a similar stage, portions of the spireme could be found which had not yet become contracted into the general mass (Fig. 19), and a casual investigation might lead to the conclusion that in these cases a lateral approximation of separate threads was in progress. But careful examination with a high magnification proved that these appearances were really due to looping of the thread, and not to approximation of distinct filaments. Contraction continues until the whole of the thread is collected into a densely tangled mass (Fig. 21), generally in close proximity to the nucleolus. At this stage the small rounded body derived from the latter during the resting period is still found within the nuclear cavity; its position varies, but it is often found at some distance, both from the nucleolus and from the contracted threadwork.

Numerous preparations were obtained in which the nuclei were in the condition of the first synapsis; but, on the other hand, few were found showing the uncontracted network which immediately precedes it, and

intermediate stages were not very abundant. Judging from this the synapsis is of considerable duration, but the preceding stages are rapidly passed through. During the period of the greatest contraction the nucleus was found close to the periphery of the cell (Fig. 21), and, as this was of constant occurrence in different preparations, it may be concluded that the position is characteristic of the synaptic stage.

The first indication of the emergence of the spireme from the contraction is given by the appearance of a number of thick indefinitely tangled threads within the deeply-staining mass (Fig. 22). This can be at once distinguished from the somewhat similar stages immediately preceding the contraction on account of the thickness of the emerging thread characteristic of this stage. At this time the nucleus also begins to pass back to the centre of the cell. During synapsis there is a considerable increase in the amount of chromatin, and the spireme on its reappearance is at least twice as thick as the threadwork which formed the original reticulum. The spireme gradually becomes more loosely coiled and spreads out, and is soon found distributed throughout the nuclear cavity (Fig. 23, Pl. XI). This second network obviously consists of a shorter length of thread than the presynaptic reticulum ; it appears that besides the increase in amount of chromatin an actual shortening of the thread goes on during the contracted state. Irregular masses of deeply-staining material are found in the course of the thread, but the body previously cut off from the nucleolus has now disappeared ; the nucleolus itself still shows no diminution in staining capacity. Although careful examination of the thread was made at this period, no definite evidence either of fusion or approximation was obtained.

The second contraction now supervenes (Fig. 24); the spireme tends to become collected around the nucleolus, but the aggregation is not nearly so complete as at the first synapsis. Loops are found radiating outwards from the central mass, and in some of these longitudinal fission could undoubtedly be seen at this stage (see loop in upper part of Fig. 24). The loops, although for the most part consisting of undivided filaments, in some cases exhibit the splitting at frequent short intervals along their course. At the next stage the individual chromosomes can be recognized. As far as can be discovered these are not produced by the direct transverse division of the spireme, but rather by the aggregation of the chromatic material to certain lengths of the thread which are subsequently drawn asunder. The intervening portions of the spireme become thinner and take up the stain much less deeply. On the other hand, the lengths destined to form the chromosomes increase in thickness and staining capacity. The previously mentioned masses of chromatic substance which are situated at intervals in the spireme appear to give up material to the chromosomes (Fig. 25) ; as the latter become more distinct the masses of chromatic substance gradually disappear. The whole process strongly suggests the transference of the material from

them to the chromosomes. At this stage the longitudinal fission was again seen in the individual chromosomes (Fig. 25). These become arranged near the periphery of the nucleus, but the final stages of diakinesis were not observed.

Up to this time no sign of spindle formation is to be seen, but a little later the fibres become evident in the cytoplasm. These are directed towards the two bluntly-pointed poles; as in the premeiotic divisions no polar radiations or centrosomes were found. The nuclear membrane has now disappeared and the chromosomes lie free near the centre of the cell (Fig. 26). The nucleolus is found near one of the poles of the spindle, and only takes up the cytoplasmic stain. This position was found to be characteristic of the stage. Humphrey (17) in an early paper figured large bodies of similar appearance during the metaphase of the reduction division of *Osmunda* and *Allium*, and it is possible that in these cases also the appearance may be explained by the persistence of the nucleolus. At this stage the chromosomes are of rather irregular shape, and it is difficult to correctly ascertain the number present; some are hook-like, others U-shaped, and yet others bent in two directions and elongated. The impression that more than six are present is given, and this may perhaps be explained by the division of one or more of the bivalent chromosomes into the constituent parts.

A little later the last trace of the nuceolus is lost, and the spindle becomes more marked. The chromosomes take on their characteristic shapes and become arranged on the equatorial plate (Fig. 27). The number can now be easily counted, and six were found to be constantly present. The commonest form is the ring, but X- and U- figures are frequently met with. Transverse division now takes place in each chromosome, and this can be particularly well seen in the ring forms. These are attached to the spindle by a fibre on each of the opposite sides, and the first sign of division is the appearance of a break in each in the plane of the equatorial plate. This may take place on one side of the ring while the other is still intact (Fig. 28). Finally, two semi-circular portions are set free, but these almost at once become V-shaped, the spindle fibres being attached at the apex on the V (Fig. 29). The impression given that the fibres exert a pulling strain is very strong. As the chromosomes separate the V form becomes elongated, and soon changes to a Y-shaped figure with the long arm of the Y directed towards the pole (Fig. 29; the pair of chromosomes towards the centre of the spindle). The short arms of the Y remain thick, and an aggregation of chromatin is found at the distal end of the long arm, while the central portion of the latter becomes thin and drawn out. The U-shaped forms divide so that the parallel arms are separated, and the latter at first remain short and thick. As the anaphase continues the chromosomes become thinner and more elongated, but on reaching the poles they again contract and thicken (Fig. 30). A polar view at this stage shows the six

chromosomes each as a dumbbell-shaped structure. As before described, this appearance is produced by the two arms of the V seen in optical transverse section. No evidence for longitudinal fission in the chromosomes was discovered during their passage to the poles. At this stage one chromosome is often found lagging behind the rest near each pole of the spindle (Fig. 30). This, no doubt, is caused by the retarded division of one of the bivalent structures.

No subsequent stages of the heterotype division were discovered, and the reconstitution of the daughter-nuclei must proceed very rapidly. It could not be determined whether these latter regain the completely resting condition, but, judging from analogy with other forms and from the short period allowed for these changes, this seems to be improbable. The telophase of the homotype division was found, and in this six chromosomes are easily distinguishable at each pole (Fig. 31). The divisions of each of the two nuclei resulting from the heterotype mitosis take place in planes at right angles, and in the stage figured one division is seen from the side, while a polar view is obtained of the corresponding process in the other cell. A definite cell wall separates the two divisions, and although no such structure was found in the telophase of the heterotype division, its formation must take place before reconstitution of the daughter-nuclei. After the homotype mitosis walls are formed across the spindles of the two final divisions. These join up with the wall already present, and four daughter-cells are thus produced (Fig. 32). Spindle fibres can still be seen between the pairs of resting nuclei formed by the last mitosis. These nuclei possess a small lightly-staining nucleolus and a rather coarse reticulum; at this stage numerous deeply-staining granules are found in the cytoplasm, particularly in the neighbourhood of the nuclei.

Considerable growth takes place on the differentiation of the spores, and the spore-sac becomes completely filled with the developing tetrads. Very soon the spores become closely packed, and, on account of movement of the cells due to pressure, the original arrangement is soon lost. At this stage the spores only possess one coat; the nucleus is fairly large and possesses a distinct nucleolus, but the latter is not so large and evident as in the case of other cells of the plant. No details of the formation of the various walls during spore formation are discussed here, and this will form the subject of a separate communication.

General Discussion.

At the present time the descriptions of the course of events during the early prophase of the somatic divisions given by various investigators show considerable agreement. The accounts of Strasburger (23), Farmer and Shove (9), and Grégoire (15) agree in the essential facts. The importance of fixation in the elucidation of the early stages cannot be

over estimated. In the above description of the premeiotic prophases it was suggested that the increase of granularity in the nuclear reticulum was frequently a result of improper fixation due to the use of acetic alcohol. It is probable that in many cases the similar appearances obtained at this stage by other investigators are partly due to use of this or of other fixing reagents. Flemming's Mixtures gave much better results at this stage, and this was especially the case when the dehydration was performed by the glycerine method (see p. 143). Too rapid changes in the concentration of the liquids used both at this and at later stages are quite sufficient to produce an apparently early prophase. These difficulties can only be avoided by the use of great care during the transference of the material from water to alcohol and vice versa, and these remarks will also apply in a less degree to the change from alcohol to xylol, cedarwood oil, or chloroform.

The use of acetic alcohol produces preparations which are characterized by a ragged appearance, and possibly this is due to solution of a portion of the material composing the cell. As above noted, this reagent precipitates some of the proteids present in the plant in a form soluble in water, and these would be lost during the subsequent staining operations. Although well fixed preparations of the chromosomes are obtained by the use of acetic alcohol, this mixture is not a reliable fixing reagent, especially for the earlier stages of division. Farmer (10, p. 490) has already noted that at the reduction divisions the spore-mother-cells of the Hepaticae can only be fixed with great difficulty. This is especially the case in the Muscineae. Methods which resulted in perfect fixation of the premeiotic stages, gave very inferior results at the reduction divisions, and at this period acetic alcohol is valuable on account of its penetrating power.

A large amount of discussion has taken place with regard to the formation of the bivalent chromosomes at the meiosis. It is generally admitted that at the heterotype division the equivalents of somatic chromosomes are separated, but there is considerable divergence of opinion concerning the details of formation of these bodies.

Farmer and Moore (8) find that a longitudinal fission of the spireme takes place during or shortly subsequent to the first contraction; this fission temporarily closes during the second contraction. Approximation of the thread into more or less parallel lines now goes on, whether by looping or otherwise. At emergence from the second contraction traces of the original longitudinal fission can be found in the spireme, and these persist in the individual chromosomes. The latter are bivalent, and consist of two somatic chromosomes fused end to end. At the metaphase transverse division goes on, and during the anaphase the original longitudinal fission may again be seen in the chromosomes.

Grégoire (14) considers that lateral approximation of the thin spireme takes place at or immediately before the first contraction. The parallel

arrangement of the spireme can be seen after emergence, and close approximation of the two threads goes on before the second contraction, resulting in the formation of the thick spireme. Later on this gives rise to the chromosomes by transverse divisions. The latter consist of two somatic chromosomes in close lateral approximation. At the metaphase the approximated somatic chromosomes are separated. The difference between the two theories lies essentially in the formation of the split spireme; in the first this is considered to be due to longitudinal fission of an original single thread, in the second to the lateral approximation of originally separate filaments.

In the case of *Mnium* no evidence for lateral approximation was discovered during or previous to the first contraction. Contraction goes on in the network as a whole, and there is no rearrangement of the thread into parallel lengths. Sections of the contracted mass likewise gave no evidence for approximation. No signs of longitudinal fission were found in the emerging thread, but traces of this were discovered at the time of second contraction, and also in the differentiating chromosomes, and no doubt is entertained as to its presence at this stage. The small size of the nuclei in the plant under examination makes satisfactory elucidation of the events of chromosome formation difficult, but the evidence obtained confirms the conclusions arrived at by Farmer and Moore.

The above investigation into the nuclear phenomena of *Mnium hornum* has proved that both in the somatic and in the reduction divisions the normal course of events is followed; the first division of the spore-mother-cells is of the characteristic type. The chromosome numbers found in the Muscineae up to the present are comparatively small. The only recorded number for the diploid generation is twelve, and in the haploid generation eight, given by Ikeno for *Atrichum angustatum* and *Pogonatum rhopolophorum*, is the only variant from the six found in *Mnium hornum* and various species of *Polytrichum*. It may be taken that twelve is the characteristic number for several individuals of the Muscineae. The latter agree with the Hepaticae in the possession of a comparatively small number of chromosomes.

The absence of centrospheres during the reduction division is a noteworthy fact. These structures have been described in a considerable number of representatives of the Hepaticae during the meiosis, and their absence in *Mnium* may perhaps be correlated with the greater specialization for the land habitat found in the Muscineae.

In their examination of *Polytrichum*, Drs. Leeuwen-Reijnvaan were unable to obtain the reduction division, but since twelve chromosomes were found during vegetative divisions in the sporogonium, and six during division in the antheridium, they concluded that reduction took place as usual on spore formation. This, which was already justified by analogy

with the Hepaticae and Ferns, is further strengthened by the description of the process in *Mnium hornum*. Their further surprising statements, that a second reduction takes place on the last division of the spermatogenic cells, and that at fertilization the egg fuses with two spermatozoids, and with the ventral canal cell, will need abundant confirmation before they can be accepted.

This is especially the case, as Dr. Arens, who has recently investigated the spermatogenesis of both *Polytrichum juniperinum* and *Mnium hornum*, finds that in each case the development is normal. At present no statements can be made with regard to the spermatogenesis and fertilization of *Mnium hornum*, but the investigation of this plant is being continued, and it is hoped that results will be forthcoming within a short period.

Summary.

1. Fertilization takes place in *Mnium hornum* during May; the single-celled archesporium can be recognized early in the following January. Spore formation and chromosome reduction are completed about the middle of April.

2. The resting nucleus, both in vegetative and reproductive cells, is characterized by the presence of a very large deeply-staining nucleolus; the nuclear reticulum is very fine, and contains little or no chromatin.

3. Each original cell of the archesporium gives rise to 8, 16, or 32 spore-mother-cells. On the approach of somatic division the nucleus becomes more granular, and broad band-like masses of chromatin are differentiated; the nucleolus gradually loses its staining power and disappears. By contraction of the chromatin masses the spireme is formed, and in this no sign of longitudinal fission could be discovered.

4. The spireme divides transversely into twelve chromosomes of approximately equal size, and these become arranged on the equatorial plate of the spindle. No centrosomes or polar radiations are present.

5. The chromosomes split longitudinally and the halves retreat towards the poles. The telophase follows the usual course; several nucleoli are at first formed, but later these fuse into one.

6. The wall dividing the daughter-cells is laid down as usual at the equatorial plate.

7. There is a period of rest after the formation of the spore-mother-cells; the resting nucleus resembles that of the premeiotic cells, but contains slightly more chromatin. While in the resting state a body is budded off from the nucleolus, and this persists until after the first synapsis, and then disappears.

8. The spireme is gradually differentiated from the nuclear reticulum, forms a definite network, and then gives rise to the first contraction figure. In this there is no lateral approximation of individual filaments.

9. On emergence the spireme has become shorter and thicker; a network is formed, and soon the second contraction supervenes; this is characterized by the looped arrangement of the spireme, which now exhibits traces of longitudinal fission.

10. The chromosomes are formed by aggregation of the chromatin to certain portions of the thread, and in these longitudinal fission can still be seen. They are separated by transverse division of the thread. The nucleolus passes into the cytoplasm and soon disappears, while the chromosomes remain in the centre of the cell.

11. No centrospheres are found at the poles of the spindle. The chromosomes, six in number, become arranged on the equatorial plate, and show the characteristic O and X forms. They divide transversely and pass to the poles.

12. The homotype division results in the formation of four nuclei, which are later found in the spores.

The investigation was commenced in the Botanical Department of the University of Glasgow, and I am greatly indebted to Professor Bower, F.R.S., for the facilities which he afforded me. In conclusion, I wish especially to express my thanks to Professor Farmer, F.R.S., for his constant help and valuable advice during the progress of the research.

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EXPLANATIONS OF FIGURES IN PLATES X AND XI.

Illustrating Mr. Wilson's Paper on *Mnium hornum*.

Figs. 1–13 illustrate somatic divisions, Figs. 14–22 the reduction divisions.

(All the figures were drawn with the camera lucida under 2 mm. Hom. Imm. Zeiss with Comp. Oc. 12 or 18, giving magnification of 1500 and 2250 respectively.)

Fig. 1. Cell with resting nucleus from single-celled archesporium. × 1500.
Fig. 2. Cell with resting nucleus showing differentiation in cytoplasm. × 1500.
Fig. 3. Nucleus in early prophase. × 1500.
Fig. 4. Band-like chromatin areas differentiated. × 1500.
Fig. 5. Further contraction of chromatin areas. × 1500.
Fig. 6. Spireme. × 1500.
Fig. 7. Division of spireme to form chromosomes. × 1500.
Fig. 8. Chromosomes on equatorial plate. × 1500.
Fig. 9. Polar view of metaphase. × 1500.
Figs. 10 and 11. Diasters. × 1500.
Fig. 12. Telophase showing formation of nucleoli and new cell wall. × 1500.
Fig. 13. Almost resting daughter-nuclei. × 1500.
Fig. 14. Resting nucleus from spore-mother-cell. × 1500.
Fig. 15. Resting nucleus showing budding of nucleolus. × 1500.
Fig. 16. Nucleus with more granular reticulum showing constriction of body from nucleolus. × 1500.
Fig. 17. Spore-mother-cell with nucleus in very early prophase. × 1500.

Fig. 18. Spore-mother-cell with nucleus showing the thin spireme. × 1500.

Fig. 19. Nucleus in early stage of first contraction. × 2250.

Fig. 20, *a* and *b*. Two successive sections of a nucleus immediately before the first contraction. × 2250.

Fig. 21. Cell with nucleus showing first contraction figure. × 1500.

Fig. 22. Nucleus with spireme emerging from the first contraction. × 2250.

Fig. 23. Nucleus showing the thick spireme. × 2250.

Fig. 24. Nucleus showing spireme in second contraction, with radiating loops, in some of which longitudinal fission can be seen. × 2250.

Fig. 25. Nucleus in early stage of diakinesis with differentiating chromosomes. × 2250.

Fig. 26. Stage immediately before chromosomes pass on to the equatorial plate showing the faintly-staining nucleolus. × 1500.

Fig. 27. Chromosomes on the equatorial plate; seen obliquely. × 1500.

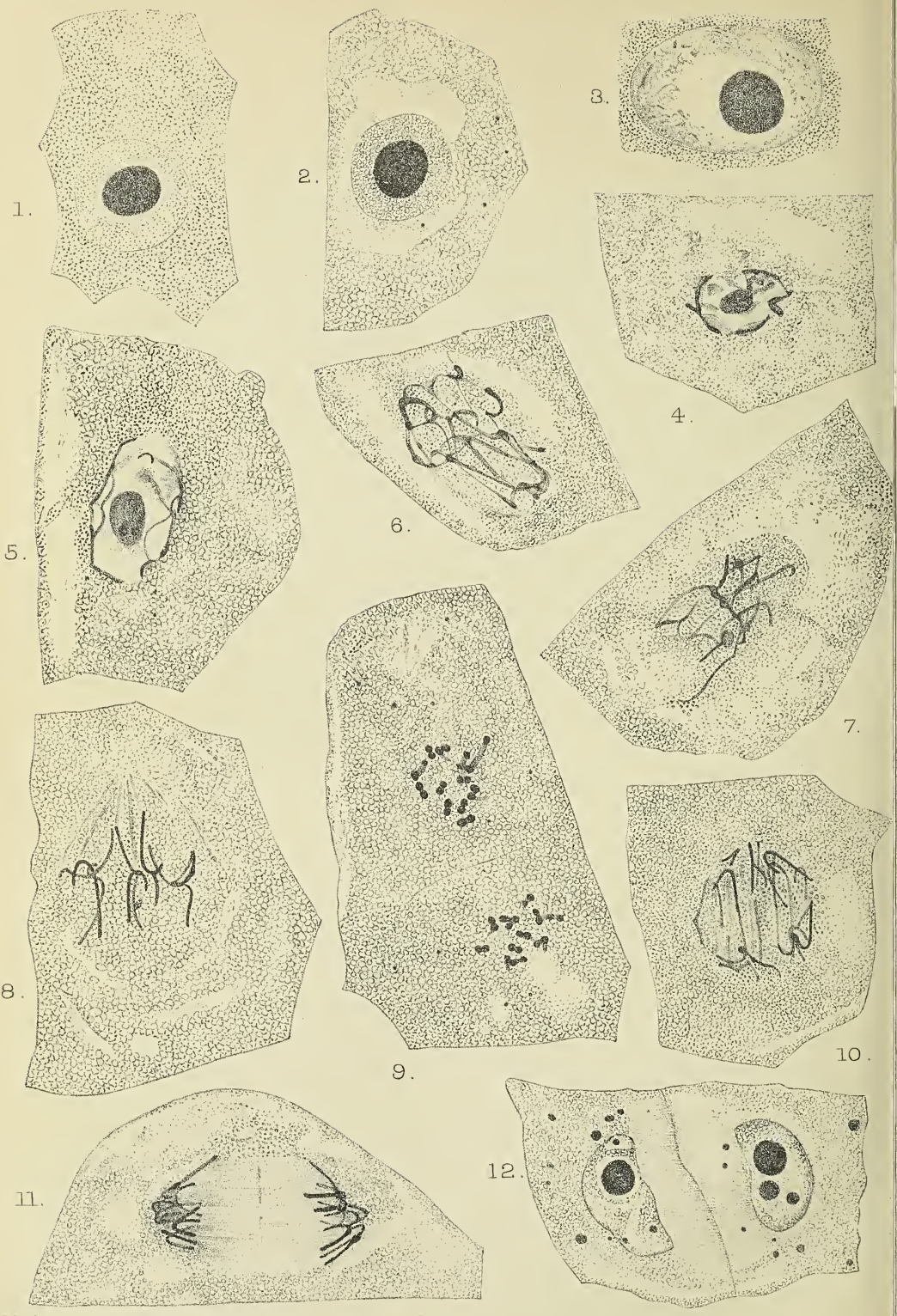
Fig. 28. Chromosomes dividing transversely on the spindle; drawn at one focus only. × 1500.

Fig. 29. Anaphase showing chromosomes just after division. × 1500.

Fig. 30. Telophase. × 1500.

Fig. 31. Telophase of homotype division. × 1500.

Fig. 32. Resting nuclei after homotype division. × 1500.



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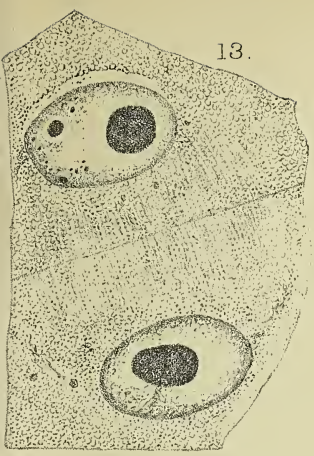
WILSON — MNIUM HORNUM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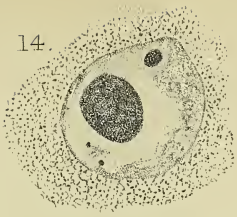
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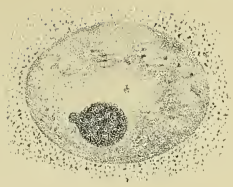
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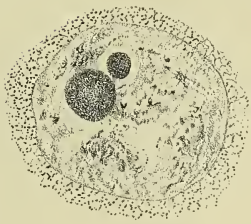
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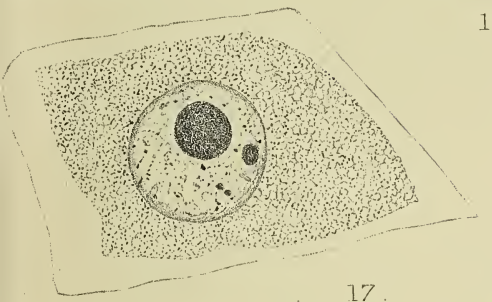
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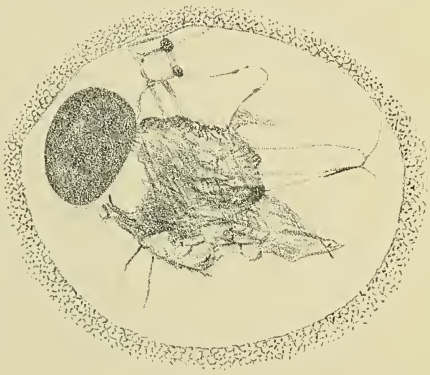
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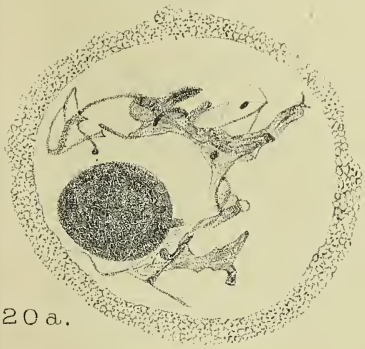
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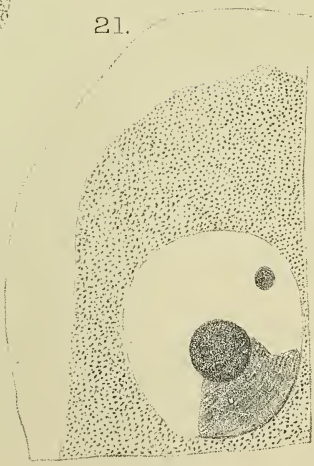
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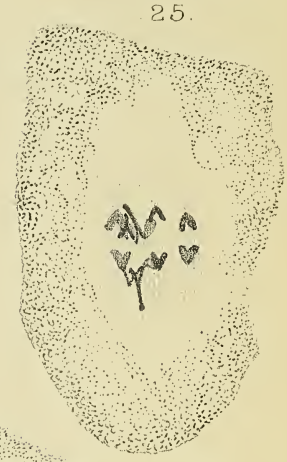

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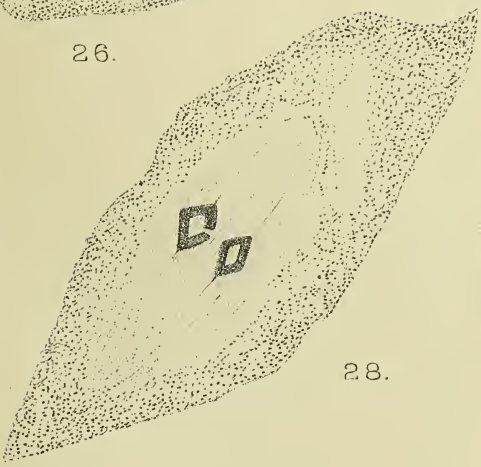

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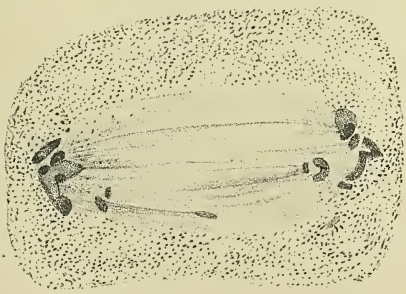

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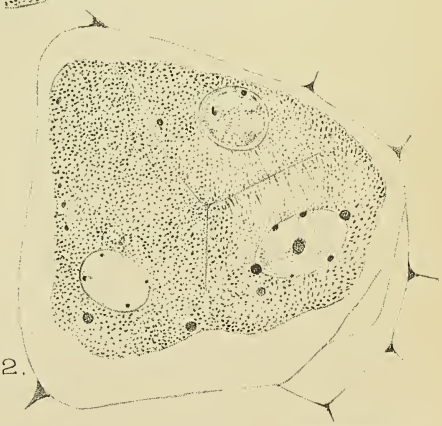

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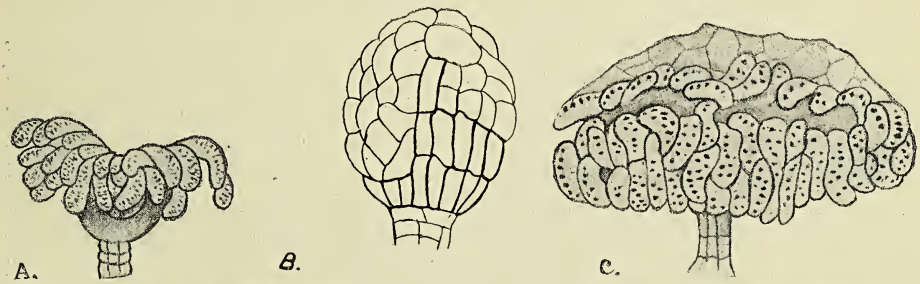
NOTE.

DISCHARGE OF ANTHEROZOIDS IN FOSSOMBRONIA AND HAPLOMITRIUM HOOKERI.—Campbell,[1] in describing the discharge of antherozoids in *Porella Bolanderi* Aust., states that the cells of the wall absorb water with great avidity, and finally the upper part bursts open by a number of irregular lobes, which curl back so strongly that many of the marginal cells become completely detached. According to Humphrey,[2] the method is similar in *Fossombronia longiseta* Aust. In 1903, Cavers [3] pointed out in connexion with the explosive ejection of antherozoids in *Fegatella* Radd., that the mechanism of discharge was partly associated with the mucilaginous nature of the antheridial wall. This seems to be the case also in certain species of *Fossombronia* [4] recently described by the writer, and in *Haplomitrium Hookeri*. In both cases the discharge of antherozoids is preceded by a progressive series of changes in the wall of the antheridium.

In *Fossombronia*, the antheridia arise singly between the base of the leaf and the stem, and are generally shut in by a little basal leaf-flap. When young they are pale green, and the contents can be seen through the wall. As the organ ripens, the chlorophyll granules of the wall-cells become orange coloured, and appear to become mucilaginous. The change in the chlorophyll corpuscles is progressively downward



A, B. *Fossombronia*. C. *Haplomitrium Hookeri*.

from the top of the antheridium. The corpuscles, in their final stage, are situated in the protoplasm close to the convex wall of the cell. They are of irregular shape, and are arranged in roughly parallel lines of granules or dentate rods, whose ends point toward the colourless concave side of the cell.

The wall of the antheridium shows differentiation into two forms of cells. Those standing up from the base of the antheridium are three or four times longer than broad, the others are polygonal and of irregular outline. If the latter cells were removed, the former would make with the stalk a wineglass-looking object. As the antheridium develops the wall-cells become more convex in outline, so that it has a warty appearance, and finally, on the point of dehiscence, it is difficult to convince oneself that the antheridium is not encased in tightly-fitting filaments. Dehiscence

[1] Mosses and Ferns, 1st ed., p. 101.
[2] Annals of Botany, xx, 1906, p. 94.
[3] Annals of Botany, xvii, p. 274.
[4] 'Observations on *Fossombronia* from Devonshire.'

began with the rise of one or two of the apical wall-cells. Then the remaining cells drew back with little jerks, presenting the appearance of the loosening of a claw of filaments. At the same time a stream of antherozoids appeared, which was helped out of the antheridium by the pushes given by individual cells jumping-off, and by filaments of wall-cells rising and drawing back. The appearance was very striking, and the discharge occupied about two minutes. The individual cells of the wall were turgid sausage-shaped bodies. After the rejection of the antherozoids, what remained of the cell-filaments could be seen attached to the rim of lower cells which acted as a kind of stalk.

The antheridia of *Haplomitrium Hookeri* are oval bodies, and are much larger than the more or less spherical ones of *Fossombronia*. When ripe, these are of an orange colour, due to a similar change in the chlorophyll corpuscles of the wall, which are roundish in outline. The wall of the antheridium lacks the differentiation seen in *Fossombronia*. The cells are five- or six-angled, and are arranged in a diamond pattern. As the antheridium ripens first, the apical cells, and later the lower cells, bulge outward, giving it a warty appearance, which is not so marked towards the base. In nature the discharge of antherozoids is sudden and rapid. In attempting to examine the process, dehiscence is often premature, and the discharge less rapid. In one case the contents passed out in a cloud, which became several times longer than the antheridium during a couple of sudden movements which resulted in the complete collapse of the spirally-arranged, turgid wall-cells. These remained attached to the basal portion of the antheridium.

In another case where the process was retarded, an orifice was formed at the apex, owing to the uprising of a few turgid cells. Previous to this the radial walls of the apical cells appeared thick and discoloured, due to the arrangement of a yellowish-brown mucilaginous matter about their walls. The free cells, bounded by a thin, clear membrane, soon lost their turgidity, and the orange or orange-brown coloured matter diffused from the changed chlorophyll corpuscles into the protoplasm. After the formation of an orifice, more and more cells rose, but did not float off, and the antherozoids oozed out. The wall of the antheridium bulged in places, and it could be seen that the membrane of the sausage-shaped cells was free from the limiting membrane (cuticle) of the wall.

To sum up, the following processes take place during ripening of the antheridium :—

1. Change in character of the chlorophyll corpuscles of the wall from above downwards.

2. Local degeneration of the middle lamella in the wall of the antheridium. Cell filaments are formed in *Fossombronia*. Special cells rise in *Haplomitrium*.

3. The upper wall-cells become free from the limiting membrane of the wall.

When these processes have operated for a certain time—that is to say, when the antheridia are ripe—dehiscence takes place, and, since the wall-cells are normally in a turgid state, the mere access of water would be sufficient to start dehiscence. The disposition and behaviour of the wall-cells in each case provide a simple and efficient mechanism for the discharge of antherozoids.

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A PRELIMINARY NOTE ON THE EMBRYO-SAC OF PROTEA LEPIDOCARPON. R.Br.—The Proteaceae form one of the outstanding features of the flora of Cape Colony. As very little is known of the structure and development of the embryo-sac in the Order, an investigation was commenced on *Protea Lepidocarpon*, one of the commonest and best known of the Cape species.

It is a xerophytic shrub often attaining a height of eight feet. It usually exhibits a subsocial tendency. Each terminal bud gives rise to a capitulum. Several sugar-birds and a number of insects visit the flowers in search of the honey which they contain.[1]

A preliminary study of *Protea grandiflora* was made in 1906 by Mr. E. P. Phillips, B.A.; the results agree in the main with those obtained for other species of *Protea* which have been examined.

The young ovule arises laterally near the top of the ovary, but turns through an angle of 90° and soon becomes pendulous. There are two integuments, which arise in basipetal succession. While they are making their appearance the sporogenous tissue is also becoming differentiated. A small group of large cells situated below the hypodermal layer includes one which becomes the megaspore mother-cell. By this time a very definite meristematic tissue has arisen at the base of the nucellus. This tissue remains active until about the time of fertilization, and owing to its activity the ovule becomes about 5 mm. long. The megaspore mother-cell increases in size, and divides in the usual manner to a row of four cells. The reduced number of chromosomes is twelve. The four megaspores are readily distinguished from the surrounding cells.

The basal cell of the row rapidly increases in size, while the other three become crushed. The single surviving megaspore increases in length very rapidly, and on division gives the typical eight-nucleate embryo-sac. The usual polar arrangement of the nuclei is very early established. Meanwhile the cells of the nucellus immediately surrounding the embryo-sac become densely packed with starch. The growth of the sac keeps pace with the elongating nucellus, and at the eight-nucleate stage the former is extremely long and narrow, except at the poles, where it is somewhat expanded. The inner integument becomes very long and several cells thick, the outer remaining only two cells thick.

The cells of the inner integument surrounding the micropyle are very large and glandular, and they dovetail more or less into one another. They are also very closely applied to the cells of the tip of the nucellus, which are also somewhat glandular in appearance.

When the embryo-sac has reached its adult form it has forced its way through the tip of the nucellus, and its upper end forms a short wide tube projecting into the micropylar tube of the sac. The antipodal nuclei disintegrate about the time of fertilization.

Owing to the difficulty of obtaining satisfactory preparations of the very large embryo-sac, neither fertilization nor the fusion of the polar nuclei has yet been seen. The first recognizable stage of the embryo was a small group of cells, with definite

[1] Scott Elliott, Ornithophilous Flowers in South Africa. Annals of Botany, vol. iv, p. 274.

cell-walls, situated in the micropylar prolongation of the sac. The embryo has no suspensor. In this respect and in its globular form it closely resembles the young embryo of *Penaea*.[1] The endosperm is copious. In many of the South African representatives of the Proteaceae only a small percentage of the flowers set seed. In *Protea Lepidocarpon* only about 30 per cent. of the flowers in the capitulum are fertile, while in some of the other species the percentage is lower. Mr. Phillips found that of the 1260 flowers on five capitula from different plants of *Protea grandiflora*, Thunb., 1067 contained aborted ovaries. A careful search for fertile seeds of this species has not yet met with any success, though seedling plants are occasionally established. In *Protea mellifera*, Thunb., it appears that less than 50 per cent. of the ovaries become aborted.

Irregular protuberances of the nucellus—the origin of which calls for further investigation—penetrate the tissue of the integuments. These are presumably haustorial in function. The nucellar tissue disappears very soon after the appearance of the embryo.

The tetragonal pollen-grains show some peculiarities. The two divisions of the pollen mother-cell are rapid. Here also the reduced number of chromosomes is twelve. After the first division of the mother-cell a thick transitory wall appears between the nuclei. The microspores are in tetrads by the time the megaspore mother-cell is dividing.

These investigations were carried out in the Botanical Laboratory of the South African College, Cape Town, at the suggestion and under the supervision of Professor H. H. W. Pearson. The work was done during the early part of 1908, before the Winter Session at Edinburgh commenced.

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[1] Stephens, E. L. A Preliminary Note on the Embryo-sac of certain Penaeaceae. Annals of Botany, vol. xxii, p. 329.

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The Gametophytes and Embryo of Pseudotsuga Douglasii.

BY

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Lecturer in Botany, the University of Glasgow.

With Plates XII, XIII, and XIV.

DURING an examination of the gametophytic structure of various genera of the Abietineae certain features in regard to *Pseudotsuga Douglasii* were brought to light which proved to be unusual and interesting. On account of the accumulating evidence in support of the view that the Abietineae constitute a very ancient group of the Coniferales, and especially as *Pseudotsuga* is one of the few genera which have not been investigated, it has been thought advisable to record these unusual features in connexion with the gametophytes of this genus.

The material was collected in Santa Clara County, California. The investigation was, for a considerable part, carried on at the Jodrell Laboratory, Royal Gardens, Kew, but was finished in the Laboratory of the University of Glasgow. For facilities afforded at the Jodrell Laboratory I am much indebted to Lieut.-Col. Prain, F.R.S., and also to Mr. L. A. Boodle, F.L S., for many kindnesses.

THE MALE GAMETOPHYTE.

Pollination takes place early in April or the latter part of May, and lasts for two or three weeks. The first material for the study of the microspores was collected on April 2. At this date many of the sporangia at the base of the cones had discharged their microspores, but the sporangia in the middle and upper regions of the cones were quite full of pollen. There appeared to be no difference in the state of development, however, between the microspores within the sporangia at this time, and those which had been discharged. The spores were practically globular in form, and showed a distinct and well-developed exine. Unlike *Cedrus*, *Pinus*, and many other Abietineae, there were no wings or bladder-like swellings to give buoyancy to the spore. A cross-section of a microspore at the time of pollination is

represented in Pl. XII, Fig. 1. Within the spore two degenerating prothallial cells, lying close to the spore-wall (p', p'') were readily recognized. One of these appears as a mere film of deeply-staining nuclear substance, closely applied to the inner surface of the spore wall, while the other is represented by a small irregular mass of chromatin situated close to its neighbour but in a line towards the interior of the spore. The presence of these prothallial cells in the pollen grains, in which there are no bladder-like swellings of the exine to afford buoyancy, does not give support to the view (Robertson, '04) that a correlation exists between the survival of the prothallial cells and the winged character of the pollen.

In addition to the two prothallial cells, there are two large free nuclei within the microspore. One of these is centrally situated and is the so-called generative nucleus; the other is the tube nucleus. Both nuclei are surrounded by dense granular cytoplasm, and between the latter and the spore wall are numerous starch grains. As indicated in Fig. 1 all four nuclear structures lie in the same plane; that is in a row one behind the other.

The pollen-receiving device in *Pseudotsuga* is quite peculiar and unlike anything yet described for Gymnosperms. For some little time after pollination, the nucellus presents the form of a small protuberance with a perfectly rounded apex. The integument extends for a considerable distance beyond the nucellus. At a point immediately above the apex of the nucellus the integument bends inward in such a fashion as to partly close or narrow the micropylar canal, and then sharply bends outward again. This results in the formation of a distinct stricture midway between the apex of the nucellus and the mouth of the mieropyle. As a result of this peculiar curvature of the integument, the micropylar canal is not a straight passage of uniform width, but consists of two chambers, one immediately above the apex of the nucellus, and the other near the mouth of the mycropyle. The relative position and form of these two chambers are shown in Fig. 3, as well as the narrowness of the passage between them. The fact is also made clear in this figure that the curvature of the integument is much greater on the side nearest the ovuliferous scale.

In addition to this narrowing in the middle region of the micropyle, the integument is still further modified. It will be seen in Fig. 3 that the extremity of the integument which forms the mouth of the micropyle is folded inward. On the inner surface of this infolding extremity numerous fine hair-like processes are present. A close examination of these processes made it clear that they were not cellular in structure, but were merely outgrowths from the external walls of the epidermal cells. They serve very effectively, however, as a stigmatic surface, for during pollination the extremity of the integument is directed outward instead of inward.

It will be remembered that in nearly all of the Coniferales the pollen

grains find a lodgement on the apex of the nucellus, and upon germination directly penetrate the nucellar tissue. This is certainly not the case with *Pseudotsuga*. Of the numerous preparations examined I was unable to find a single case of a pollen grain that had reached the nucellus. They were invariably—and sometimes in great number—found in the upper chamber of the micropyle and frequently entangled in the hair-like processes at the mouth. They are evidently caught on the stigmatic surface when the mouth is extended, and when the extremity turns or folds inwards as shown in Fig. 3 the pollen grains find themselves enclosed in the upper chamber. It will be seen in Figs. 3 and 4 that the stigmatic processes are now all pointing backwards towards the interior of the micropyle. It is in this upper chamber of the micropyle that the pollen grains commence their germination. In Figs. 3, 4, and 10 some characteristic positions of germinating grains are shown.

It will also be observed from these figures that after pollination—as a result of continued growth—the whole micropylar region of the integument bends or curves towards the ovuliferous scale. This is evidently due to the fact that growth of the integument on the side nearest the ovuliferous scale is less rapid than that on the opposite side. This irregular growth continues until—as indicated in Figs. 4 and 10—the more distal part of the micropyle lies at right angles to the long axis of the ovule.

The pollen grains remain in the upper chamber of the micropyle for a week or ten days before germination begins. This is marked first by a rupture of the exine and a slight protrusion of the pollen-tube. It was observed that the rupture of the exine and the protrusion of the pollen-tube always take place at the side of the pollen-grain directly opposite to that occupied by the two degenerated prothallial cells—that is, on the side nearest the tube nucleus. In Fig. 3 one may see the position and germinated condition of the pollen grains as they appeared on April 28.

The division of the generative nucleus evidently takes place about the time the exine is ruptured, and just before the appearance of the pollen-tube; for although the actual dividing stages were not found, the immediate results of this division were frequently observed. As indicated in Fig. 2 the division of the generative nucleus results in the organization of two distinct cells, one of which is considerably larger than the other. The cytoplasm of these two cells is much more dense and granular than the cytoplasm of the pollen-tube, and each is completely surrounded by a thin but sharply defined cell-membrane. The smaller of the two is the so-called stalk-cell, while the larger one is the body-cell. The tube nucleus descends with the tip of the growing tube, but the stalk- and body-cells remain in their original positions for some considerable time. This may be seen in Fig. 2.

After the conditions shown in Fig 2 the growth of the pollen-tube is quite rapid. As the tube elongates, both the body- and stalk-cells move forward

and descend slowly in the wake of the tube nucleus. In their movement down the tube both these structures enlarge to three or four times their original size. But the body-cell—which during these stages was always found in advance of the stalk-cell—increases in size at a much greater rate than the latter, so that before they have advanced very far into the tube the one is more than twice the size of the other. These conditions are indicated in Fig. 5.

Owing to the large number of pollen-tubes that were found growing in the upper chamber of the micropyle, and also owing to the fact that there was no tissue in the way to offer resistance to their growth, the tubes elongated towards the nucellus in a more or less irregular fashion. These circumstances made it impossible to obtain a section showing the full length of any one tube. On comparing the series of sections from which Fig. 4 was drawn it was evident that the tubes folded about each other in such a fashion as to form a complex tangle as they elongated. Fragments of at least five different tubes are shown in Fig. 4. Fig. 6 represents a portion of a section of a young tube with the body-cell descending towards the tip. It will be observed from this figure that the nucleus of the body-cell has enlarged enormously. The entire cell becomes very much elongated with the nucleus in advance of the drawn-out cytoplasm. At this time the membrane enveloping the body-cell becomes exceedingly thin, and in many cases could only be distinguished by careful focusing. Its location, however, could always be made out as the line separating the coarse granular cytoplasm, characteristic of the body-cell, from the more delicate and less dense cytoplasm of the tube.

In Fig. 7 is represented the condition of the body-cell at a time when the tip of the tube has traversed about half the length of the micropylar canal. As it was possible to obtain only short segments of the pollen-tubes in longitudinal section I was unable to determine the relative positions of the stalk- and body-cells at this time. There was some evidence, however, that the stalk-cell was now in advance of the body-cell as happens in *Pinus* (Ferguson, '04), but with so many segments of different tubes in the micropyle one could not be certain that one was following the same tube in serial sections.

The actual division of the body-cell was not observed, but after a study of the chromatin and the cytoplasm immediately surrounding the nucleus, it seemed quite certain that this division takes place before the tip of the pollen-tube reaches the nucellus. In Fig. 7 the nucleus is represented very much enlarged—its diameter being nearly equal to the width of the pollen-tube. A large, deeply-staining nucleolus was always present, and the chromatin at this time was in the so-called reticulum stage. This reticulum seemed to be made up of very delicate threads which crossed and anastomosed with one another in such a fashion as to give the appearance of

a network. Suspended in this network—especially at the angles where the threads crossed one another—numerous irregularly-shaped granules were observed. Although these granules were undoubtedly chromatin, and although they stained more deeply than the thinner portions of the threads which constitute the reticulum, it was quite impossible to determine how much of the thread consisted of chromatin, and how much of linin. Indeed, as far as one could judge from its affinity for stain, the entire reticulum seemed to be made up of chromatin threads—staining less deeply in the thinner places and more deeply at the points where the threads intersected one another.

Accompanying the enlargement of the nucleus and the organization of the loose chromatic reticulum as shown in Fig. 7, the approach of nuclear division was also indicated by changes in the cytoplasm. Immediately surrounding the nucleus, the cytoplasm becomes densely granular until a more or less definite zone is differentiated—this being the first step towards the organization of the achromatic spindle. Fig. 8 shows a slightly older stage where the dense zone of cytoplasm becomes still further differentiated into definite but very fine spindle fibrils. It will be observed that these fibrils at first appear only on one side of the nucleus—the remaining surface being quite free of them. The details of this spindle in *Pinus* have been carefully worked out by Miss Ferguson ('04), and here this same peculiarity of the early stages of spindle-formation has been observed.

From a study of Fig. 8 it will be seen that during the differentiation of the spindle fibrils the nucleus has lost its reticular structure. The chromatin threads no longer form a network, but become much thicker, more granular, and stain much more deeply than in the stage represented in Fig. 7. The reticulum has given rise to the spireme.

While the mature spindle of the dividing body-cell was not found, sufficient observations on the early stages of spindle-formation were made to convince me that no structures which could be interpreted as blepharoplasts were present during the organization of the sperm nuclei. This, however, is what one would naturally expect, for in the Abietineae as well as in all other Coniferales the last vestige of the motility of the male gamete disappeared with the specialization of the pollen-tube as a means of conveying the sperm nuclei into the egg. The next stage observed in *Pseudotsuga* was that at the time of fertilization. The two sperm nuclei, accompanied by the tube- and stalk-nuclei, were found just inside the archegonium. As in *Pinus* (Ferguson, '04, Blackman, '98, Coulter and Chamberlain, '01), *Abies* (Miyake, '03), *Tsuga* (Murrill, '00) and *Picea* (Miyake, '03), these nuclei are of unequal size—the functional one being quite twice the size of its neighbour. These are represented in Fig. 36.[1]

[1] The meaning and causes of this inequality of the sperm nuclei have already been fully discussed in this journal (Lawson, Ann. Bot., vol. xxi, No. LXXXII, 1907, pp. 292–3).

Compared with *Pinus*, *Torreya*, *Cephalotaxus*, and certain other Conifers, the time required for the complete development of the male gametophyte is very short. The sperm nuclei are organized, and fertilization takes place within sixty days after pollination.

The number and history of the nuclear structures which constitute the male sexual generation in *Pseudotsuga* are in all essentials similar to those of *Pinus* and *Picea*, the only two genera of the Abietineae in which a full account has been recorded.

The Female Gametophyte.

The collection of material was commenced too late to allow of a study of the development of the megaspores. In the ovules taken March 30 the megaspore was not only fully organized but had already germinated—its nucleus having undergone repeated free division. The earliest stage found is represented in Fig. 9. It may be seen from this figure that the functional megaspore is very much enlarged, and at the micropylar end are at least two flattened structures which I interpret as the remains of disintegrating functionless spores. These remains were so far disintegrated that it was impossible to distinguish a membrane between them. They appeared to be two fragmented nuclei. They were, however, separated from the functional spore by a definite membrane. It was impossible to say whether a fourth spore had been formed. The position of the three observed, however, makes it clear that, whether three or four spores are originally formed, they are arranged in an axial row.

The interior of the functional megaspore consisted mainly of cell-sap contained within two or three large vacuoles. In proportion to its size comparatively little cytoplasm was present, and this was found mainly at the periphery and surrounding the free nuclei. Three of the free nuclei are to be seen in Fig. 9. The spore wall could be made out as a very thin delicate membrane, apparently of a plasmatic nature.

Completely enveloping the growing megaspore, and in close contact with the spore membrane, there is a layer of large sporogenous-like cells. These large cells form practically a single layer, although at places, as indicated in Fig. 9, they may appear two cells deep. They are at least three or four times the size of the ordinary surrounding cells of the nucellus, and as their cytoplasm is very highly granular, and their nuclei large and deeply staining, the entire layer has the characteristic appearance of sporogenous tissue.

From the fact that these cells are so closely associated with the young growing prothallium, and from the fact that they are so fully charged with nutritive substances, one can only conclude that they are nutritive in function. They constitute an early stage in the formation of the so-called 'spongy tissue' or 'tapetum' which has been frequently described for *Pinus* and other

Gymnosperms. The nature and function of these cells has been discussed at some length by Miss Ferguson (1903–4). In her paper on *Pinus* this writer rejects the idea that these cells are sporogenous on the ground that 'the divisions in this tissue are according to the typic method, and present the number of chromosomes characteristic of the sporophyte'. It should be remembered, however, that the haploid phase does not appear until the division of the mother-cell, and that there may be more than one generation of diploid archesporial cells. In his work on *Stangeria*, Lang ('00) describes these cells as a 'sporogenous group', the outermost of which 'form a more definite tapetal layer'. Arnoldi ('01) also describes in *Cunninghamia* an 'archesporial tissue' surrounding the young embryo sac.

From what we know of the development and nature of the 'spongy tissue' in the Gymnosperm ovule in the various types studied, there are good reasons for believing that it is not only tapetal in function but is also archesporial in origin. My belief in this regard has been very much strengthened after a study of the development and behaviour of this tissue in *Pseudotsuga*. All of the facts point to the conclusion that it consists of archesporial cells which do not reach or go beyond the mother-cell stage.

Although in *Pseudotsuga* the tapetum at an early stage consists of a single layer of cells closely packed together, it eventually becomes several layers thick. It increases with the growth of the young prothallium. This increase and growth are, however, not very rapid. In Fig. 10 the prothallium is represented in the parietal multinucleate condition surrounded by several layers of tapetal cells. It will be observed that the latter are no longer closely packed together, but are arranged quite loosely with numerous intercellular spaces. It was also observed at this time that the megaspore wall appeared as a sharp well-defined membrane of measurable thickness.

From the time of the free nuclear stage represented in Fig. 9 to the multinucleate parietal condition represented in Fig. 10 four weeks have elapsed. The development is slow but not interrupted by a resting period as is the case with *Pinus* (Ferguson, '04). The parietal condition is represented in Fig. 11. This is some little time before the primary prothallial cells are formed. It will be seen that there is a very large central vacuole which keeps the cytoplasm closely pressed against the megaspore membrane. As illustrated in Fig. 12 the cytoplasm consists of a thin film no wider than the diameter of one of the free nuclei which are distributed at regular intervals in it. The regular distribution of the nuclei is probably due to the fact that they have undergone their last free division and are preparing for the mitosis which will result in the formation of walls between them. A stage immediately following this division is represented in Fig. 13. It will be observed that there is now a cell-wall separating each nucleus from its neighbour, and that the cells thus formed are open on the inside and freely exposed to the sap of the central vacuole.

These are the first or primary cells of the prothallium which were first described by Mlle. Sokolowa ('90) as alveoli, and which have since been found in many other Gymnosperms. The nuclei of the primary cells were nearly always found occupying a position at the surface of the cytoplasm nearest the central vacuole. In Fig. 15 is represented a group of these cells as seen in a section taken at right angles to the dividing walls. It was observed that these walls were nearly always six in number for each cell, and were arranged about the respective nuclei in such a fashion as to give the appearance of a regular symmetrical mosaic.

Rapid development immediately follows the organization of the primary prothallial cells. These structures elongate in an inward direction, and encroach upon the central vacuole in the manner already described for other Conifers (Sokolowa, '90; Arnoldi, '01; Coker, '04; Ferguson, '05; Lawson, '04 and '07). This is represented in Fig. 16. The later stages in the formation of the permanent prothallial tissue were not found, but it was observed that free nuclear division may proceed in the primary cells for some time before cross-walls are formed. Some cross-walls were, however, found at quite an early period. The multinucleate condition of the primary prothallial cells may be seen in Fig. 16.

During these stages in the development of the prothallium the megaspore membrane becomes decidedly thicker and much more conspicuous. Its structure in the mature condition is very similar to that described for *Pinus* (Thomson, '05). The fibrillae of the exosporium, however, seemed to be longer, finer, and more regularly arranged than that of *Pinus*. From measurements taken it was found that the membrane varied in thickness—being thicker at the base of the prothallium than along the sides, its approximate average thickness being 4·5 μ (Fig. 14). At a plane almost level with the base of the archegonia the membrane thins out rather abruptly, and from this region to the very apex of the prothallium no trace of the membrane could be detected. A section of the prothallium showing this is represented in Fig. 17. It will be seen from this that the archegonial region extends beyond the limits of the spore. In its distribution the megaspore membrane suggests that of *Larix* (Thomson, '05), but is quite unlike that of *Tsuga*, where, according to my own observations, the membrane completely surrounds the archegonial region.

The Archegonia.

The young archegonia make their appearance during the second and third week of May. The earliest stage observed was that where the primary neck-cell had already been cut off from the central cell, and the latter had enlarged to several times the size of the neighbouring sterile prothallial cells. From the position of these two cells it was quite evident that the archegonial initials originate as superficial cells at the apex of the prothallium, as in

Pinus, *Picea*, *Abies*, and other Conifers. The primary neck-cell shows little, or no further increase in size but very soon divides by an anticlinal wall. The two cells thus formed may undergo division by periclinal walls to form two tiers of cells in the neck. In many archegonia, however, especially in the later stages of development, but a single tier of cells could be found. This confirms the observations of other writers (Murrill, '00; Coulter and Chamberlain, '01; Coker, '02; Ferguson, '04), that the variation in the number of neck-cells is quite common among the Coniferales.

The central cell increases in size quite rapidly and its elongation is directed towards the centre of the prothallium. It is at first vacuolate, with a large nucleus and granular cytoplasm. Later, the small vacuoles become so numerous that the cytoplasm takes on a frothy appearance quite like that of certain other Abietineae (Miyake,' 03) and *Cephalotaxus* (Lawson, '07).

At a very early stage in the development of the central cell a single layer of jacket cells becomes organized. This nourishing sheath appeared in all respects similar to those already fully described for other Abietineae. It is, however, a significant fact that the original cells of the jacket in many of the Abietineae make their appearance simultaneously with the archegonial initials. In certain cases it is only their relative position which allows us to distinguish between the primary jacket cells and the archegonial initials at these very early stages.

Stages in the development of the central cell are shown in Figs. 18, 19, 20, 21. The nucleus, although very large, remains in the vicinity of the neck. The sterile tissue at the apex of the prothallium continues to grow forward, leaving a free passage to the neck of each archegonium. This passage or archegonial chamber is, however, not very deep—resembling more closely the conditions found in *Pinus*, *Picea*, and *Larix* than those described for *Tsuga* (Murrill, '00).

The largest number of archegonia found in one prothallium was six. The number seems to vary from four to six. Four, however, was the commonest number met with. They are situated quite closely together (Figs. 27 and 28), there being very little sterile tissue between them. Although the necks are separated from one another by a considerable amount of tissue, they may come in contact with one another in the middle region where they are widest. This condition may be seen in Fig. 28. This figure also illustrates the fact that the archegonia are much longer in proportion to their width than is the case in *Pinus*, *Picea*, *Larix*, or *Tsuga*. The form they present in cross-section is shown in Fig. 27.

The archegonium reaches nearly its mature size before the central nucleus—which retains its position near the neck-cells—shows any further activity. But from the material collected upon June 3 many evidences of nuclear activity were found in the central cell. Transition-stages from the reticulum to the spireme, and from the spireme to the definite chromosomes,

were frequently met with. While these changes in the form of the chromatin were in progress, it was observed that the cytoplasm immediately surrounding the nucleus was also undergoing a change of structure. From the series of stages found it was evident that on the anterior and posterior (in regard to the long axis of the archegonium) surfaces of the nucleus, the cytoplasm becomes differentiated into delicate kinoplasmic threads. These threads or fibrils are the first indications of the achromatic spindles. At the two places where they appear the nuclear wall breaks down and the fibrils extend into the nuclear cavity, and there come in contact with the chromosomes. The details of the further organization of the ventral canal spindle have been so fully described for *Picea* (Miyake, '03) and *Pinus* (Ferguson, '05), and agree so closely with my own observations on *Pseudotsuga*, that a further description is unnecessary.

In *Pseudotsuga*—as in all other Abietineae where the development of the egg has been observed—a definite membrane is formed which separates the ventral canal cell from the egg. The position of this membrane is shown in Figs. 23, 24, 25, and 26. It will also be observed from these figures that the nucleus of the ventral canal cell immediately shows signs of disintegration, while the nucleus of the egg descends towards the centre of the archegonium and becomes enormously enlarged. From the drawn-out appearance of the cytoplasm between the egg-nucleus and the ventral canal cell it would appear that the descent of the enlarged nucleus is quite rapid. A very characteristic appearance of the archegonium and the condition of the cytoplasm is shown in Fig. 26. The membrane of the ventral canal cell persists up to the time of fertilization. The phylogenetic importance of this membrane has already been discussed in a previous memoir (Lawson, '07). Its presence in the Abietineae and its entire absence in the Cupressineae, Taxodineae, and Taxineae is certainly significant.

In Fig. 28 are represented two mature archegonia ready for fertilization.

Fertilization.

It will be remembered that the pollen-tubes have attained a great length before they reach the nucellus. In this regard *Pseudotsuga* is quite unlike any other of the Abietineae in which the stages of fertilization have been studied. It is the rule among the Abietineae that the pollen is deposited directly upon the apex of the nucellus and the tissue of the latter is penetrated immediately by the growing tubes. It is remarkable that there should be this difference in the behaviour of the pollen-tubes of forms that are so closely related. It nevertheless demonstrates how plastic a structure the pollen-tube really is, and how unreliable is the evidence it affords for phylogenetic purposes. It will be remembered how unusual are the growth and distribution of the pollen-tubes in *Sequoia* (Lawson, '04). Here also there is a very early growth, and their distribution, although irregular, is

established before the prothallium is formed. The conditions in *Pseudotsuga*, however, suggest those in the Araucarineae (Thomson, '05 ; Lopriore, '05), but unlike the latter there are no additional free nuclei in the tubes. The development of the pollen-tubes in a position so far removed from the nucellus is no doubt a specialization to meet the peculiar pollen-receiving device.

Correlated with the position of the pollen-tubes in *Pseudotsuga* there is an early disintegration of the tissue of the apex of the nucellus. The cells in this region of the nucellus separate from one another in places, and there appears to be a general breaking-down or dissolving of the tissues in advance of the descending pollen-tubes. These latter structures, therefore, find no obstructions in their path. There is no firm tissue to penetrate before reaching the archegonial chambers. Indeed, at the time of fertilization the apex of the nucellus is completely broken down, and we find an appearance very unlike that of *Pinus* and the majority of other Coniferales where the nucellus persists for some time after fertilization.

As in other Abietineae the archegonia are so arranged that the necks are separated from one another by sterile prothallial tissue and each archegonium has its own archegonial chamber. By this arrangement the tip of the pollen-tube enters an archegonial chamber and its entire contents are discharged into the egg. It is therefore possible for one pollen-tube to fertilize but a single archegonium. It was noticed that the depth of the archegonial chambers was not as great as that in *Tsuga*, and the number of neck-cells is also less (Murrill, '00). In fact there is a considerable difference in the appearance of the archegonial group of the two genera. This seems to be in harmony with Jeffrey's ('05) conclusions that *Pseudotsuga* is more closely related to *Larix*, *Picea*, and *Pinus* than it is to *Abies*, *Pseudolarix*, *Cedrus*, and *Tsuga*.

By the time the egg nucleus reaches its central position, after the organization of the central canal cell, it becomes very much enlarged. This enlargement is indicated in Figs. 29, 30, and 32. During this period the egg cytoplasm loses its 'frothy' or vacuolate appearance, and becomes very coarsely granular by the presence of numerous so-called ' proteid vacuoles '.

As the egg-nucleus approaches its mature size, and prepares for fertilization it takes on a very extraordinary appearance due to the presence of several dense masses of cytoplasm which seem to project at intervals into the interior of the nucleus. As a matter of fact these masses do not project into the nucleus but are contained in small indentations or pockets which give the nuclear membrane a very irregular outline in the manner shown in Fig. 31. These masses of dense cytoplasm proved to be quite interesting and important because it was found that one or more of them take, eventually, an active part in the formation of the fibrils of the spindle immediately following fertilization. This has thrown considerable doubt on the statement repeatedly made (Blackman, '98, Murrill, '00, Ferguson, '05, and others)

that the fibrils of the first cleavage spindle are differentiated out of nuclear substance. The following observations I think will show that these fibrils are of cytoplasmic origin. These cytoplasmic masses first appear very soon after the ventral canal cell is organized, and while the egg-nucleus is comparatively small. The cytoplasm surrounding the young egg-nucleus becomes more dense, forming a more or less definite zone, much as in the early spindle-formation stages in the Angiosperms. This zone, however, owing to the very rapid growth and distension of the nucleus, becomes interrupted at intervals. As a result of this continued rapid growth we find, instead of a zone, numerous patches or masses of cytoplasm which become quite separated from one another, and which stain more deeply than the surrounding cytoplasm. These dense masses—which soon show a finely fibrous structure—being in contact with the nuclear membrane, offer a resistance to the growth of the nucleus. The osmotic pressure within—which no doubt causes the enlargement of the nucleus—forces the membrane between these dense masses, and almost completely envelops them. The result is that the contour of the nucleus becomes interrupted by several infolding pockets, each of which contains a dense mass of cytoplasm which is distinctly fibrous in structure. On account of their distribution they could be studied to much better advantage in cross- than in longitudinal-sections. A characteristic appearance of a cross-section of a nucleus at this time is shown in Fig. 31.

Very similar pockets of cytoplasm have been noted and figured in *Pinus* by Ferguson ('05) and in *Tsuga* by Murrill ('00), but neither of these writers mentions the fact, although it is clearly demonstrated in their figures, that a considerable amount of cytoplasm is carried into the egg-nucleus at the time of the fusion of the sex nuclei. Indeed, both of these writers describe the spindle fibrils of the first mitosis of the pro-embryo as originating from transformed nuclear substance. The chromatic contents of the egg-nucleus at maturity are quite like that of *Pinus* which has been described by Blackman ('98) and Ferguson ('05), as is shown in Fig. 30. Immediately before fusion the chromatin becomes collected near the centre of the nucleus in the manner indicated in Fig. 32. Although the actual first contact of the male nucleus with the egg-nucleus was not observed, the stages immediately following were found in several preparations. One of these stages is shown in Fig. 33. It became evident from this figure that there is no resting period of the fusion-nucleus, for the first segmentation spindle is already in process of formation. Although the chromosomes could be observed quite easily, it was impossible to distinguish the male group from the female. As shown in Fig. 33, the first spindle of the pro-embryo is formed within the area bounded by the membrane of the egg-nucleus. There seems little doubt that its fibrils are formed out of the dense masses of cytoplasm carried in by the male nucleus, and not out of nuclear substance.

The Embryo.

With the organization of the first segmentation spindle, the membrane of the fusion-nucleus becomes very indefinite, and gradually fades out completely. As indicated in Figs. 34 and 35, the axis of the spindle comes to lie nearly at right angles to the long axis of the archegonium. During the period of the disorganization of the nuclear membrane an interesting observation was made in connexion with the numerous nucleoli-like bodies which are so abundant in the mature egg-nucleus, and from which the chromatin proper becomes segregated. As shown in Fig. 35, these granules, which stain like chromatin, collect at one side of the first spindle, and numerous kinoplasmic threads develop among them as if a second spindle were in process of formation. These threads show all the characteristics of regular spindle fibrils, especially those found in the early stages of spindle-formation. Whether this second pseudo-spindle ever develops farther I am unable to say, but it is certainly a point of cytological interest that these discarded chromatin-like granules should become associated with kinoplasmic threads as the chromosomes do in the regular spindle. The behaviour of these granules would suggest a close relationship with chromatin.

The result of the division of the fusion-nucleus is indicated in Fig. 37. The daughter-nuclei remains in the upper part of the egg until the second mitosis takes place. The four free nuclei of the pro-embryo now pass to the base of the archegonium and are found in the positions represented in Figs. 38 and 39. These early stages in the development of the embryo, as well as the later ones, are essentially as in *Pinus*. The free nuclei enlarge considerably as they descend towards the base of the egg and become enveloped by dense granules of cytoplasm. The archegonium thus becomes differentiated into two regions—a basal nutritive region containing the pro-embryo, and an upper clearer region which is not so rich in food-granules. This differentiation is, however, not so clearly marked as in the Cupressineae or in *Cephalotaxus* (Lawson, '07). The four free nuclei now at the base of the archegonium undergo further division. The latter, however, result in the formation of walls between the nuclei as shown in Fig. 40. Eventually there are three tiers of cells and a tier of free nuclei. The uppermost of these tiers becomes the 'rosette', the middle tier becomes the suspensors, and the end tier the embryo proper.

By the rapid elongation of the suspensor cells the embryo is carried deep into the tissue of the prothallium. This is shown in Fig. 41. Associated with the elongation of the suspensors a peculiar differentiation of the contents of the base of the archegonium was observed. The cytoplasm in this region becomes replaced by a homogeneous mucilaginous substance which apparently acts as a resisting plug to the growing suspensors.

Coker ('02) reports a similar structure in connexion with the suspensors in *Podocarpus*. In the latter case, however, Coker describes this plug as cellulose. The suspensor plug in *Pseudotsuga* failed to give the cellulose reactions. It is more probably mucilaginous in composition. The position and evident function of this plug is brought out in Fig. 42.

Summary and Conclusions.

The microspore at the time of pollination is globular in form and differs in appearance from that of the majority of other Abietineae in the entire absence of bladder-like appendages.

The mature microspore contains four cells. Two of these are represented by the fragmented remains of two vestigial prothallial cells, and the other two represent the tube and generative cells respectively.

Owing to the peculiar form of the micropyle, which has a stigmatic surface at the mouth, the pollen grains fail to reach the apex of the nucellus, but are caught at the mouth of the micropyle and here germinate.

This pollen-receiving device and the formation of pollen-tubes so far removed from the nucellus is unlike anything yet reported for the Abietineae, and is evidently a novelty as far as the Gymnosperms are concerned.

With the first appearance of the pollen-tube the generative nucleus divides, and as a result of this division two distinct cells are organized, one of which is considerably larger than the other. These are the body- and stalk-cells respectively.

The pollen-tubes grow down the micropylar canal and attain a considerable length before the nucellus is reached.

The tissue of the apex of the nucellus disintegrates in advance of the approaching pollen-tubes, so that the latter structures find little or no obstruction in their path towards the archegonial chambers.

The division of the body-cell results in the formation of two male nuclei of unequal size.

The entire nuclear contents of a pollen-tube are discharged into one archegonium.

There are probably three megaspores resulting from a single mother-cell. Two of these are abortive and one functional.

Upon the enlargement of the functional megaspore free nuclear division takes place, and this is followed by the formation of a large central vacuole.

Completely enveloping the growing megaspore there is a single layer of large sporogenous-like cells which are closely packed together. This layer of cells, although single at first, soon becomes several layers thick, and eventually becomes quite loose and sponge-like—with numerous intercellular spaces—as the young prothallium increases in size. This tissue is regarded as sporogenous in origin and tapetal in function.

The megaspore membrane makes its appearance at a very early period,

and although quite thin at first it increases in thickness with the growth of the prothallium, and eventually becomes very conspicuous. In the mature stages it surrounds the prothallium except in the region of the archegonia. In this region it is entirely absent, and in this regard differs quite markedly from *Tsuga*.

With the increase in the size of the central vacuole, and the consequent formation of the parietal layer of cytoplasm, free nuclear division continues for some time.

The parietal layer now increases in thickness, and the primary prothallial cells are formed in the ordinary way.

These latter structures elongate in an inward direction, and gradually close the central vacuole. Free nuclear division now takes place within the primary prothallial cells, before cross-walls are formed to organize permanent prothallial tissue.

The archegonia originate as superficial cells at the apex of the prothallium.

They are generally four in number, and each is enveloped by a single layer of nourishing jacket-cells. There are generally two tiers, but frequently a single tier of neck-cells.

The archegonia are separated from one another—especially in the region of the necks—by several layers of sterile prothallial cells, and each is provided with a separate archegonial chamber.

A distinct ventral canal-cell is formed as a result of the division of the central cell.

The membrane of the ventral canal-cell persists up to the time of fertilization.

The fusion of the sex nuclei takes place in the middle of the archegonium. The female is many times the size of the male.

The first segmentation-spindle is formed within the area bounded by the membrane of the fusion-nucleus. It is, however, of cytoplasmic origin. One or more dense masses of cytoplasm are carried into the egg-nucleus by the sperm-nucleus.

The first division is very soon followed by a second, and the four free nuclei thus formed pass to the base of the archegonium.

After the division that follows, cell-walls are formed separating the nuclei.

Eventually the pro-embryo consists of three tiers of cells and one tier of free nuclei. The lowermost of these becomes the embryo proper. The middle one becomes the suspensor, and the next one the rosette.

As the suspensors elongate, the cytoplasm in the base of the archegonium becomes replaced by a mass of mucilaginous substance which acts as a plug to prevent the suspensors from growing forward.

The account here given of the gametophytes of *Pseudotsuga* makes it

clear that this genus is not closely related to *Tsuga*. And considering the state of development of the various vestigial and semi-vestigial structures present, the view that the Abietineae are the most ancient group of the Coniferales is very much strengthened.

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EXPLANATION OF FIGURES IN PLATES XII, XIII, AND XIV.

Illustrating Dr. Lawson's paper on *Pseudotsuga Douglasii.*

All figures were drawn with the aid of the Camera Lucida.

Fig. 1. A cross-section of a microspore before pollination, showing the generative nucleus, the tube-nucleus, and two degenerating prothallial nuclei. April 2.

Fig. 2. A longitudinal section of a young pollen-tube, showing the body-cell (*B*), the stalk-cell (*S*), and two degenerating prothallial cells (P′, P″). April 28.

Fig. 3. A longitudinal section of an ovule some time after pollination, showing the upper expanded region of the micropyle with a 'stigmatic' surface, and in which two germinating microspores are seen. April 28.

Fig. 4. The same at a later stage, showing numerous pollen-tubes growing from the distal or expanded end of the micropyle towards the nucellus. May 23.

Fig. 5. A pollen-tube soon after the division of the generative nucleus, showing the stalk- and body-nuclei and the tube-nucleus. May 3.

Fig. 6. A pollen-tube at a later stage, showing the body-nucleus advancing towards the tip of the tube. May 23.

Fig. 7. The body-cell as it lies near the tip of the pollen-tube. May 23.

Fig. 8. The same at a later stage. The body-nucleus is preparing for division. May 23.

Fig. 9. A longitudinal section through the sporogenous region of a megasporangium, showing one large functional megaspore which has already germinated, and two very small abortive megaspores. A distinct layer of large cells, the tapetum, surrounds the megaspores. March 30.

Fig. 10. A longitudinal section through an ovule, showing the functional megaspore and the tapetum considerably enlarged. The young prothallium within the megaspore consists of a thin parietal layer of cytoplasm in which numerous free nuclei are distributed, and a large central vacuole. A distinct megaspore-membrane is already quite visible at this stage. April 28.

Fig. 11. The young prothallium shown in Fig. 10 more highly magnified. April 28.

Fig. 12. A small portion of the parietal layer of cytoplasm, showing the relative size of the free nuclei. April 28.

Fig. 13. A portion of a young prothallium at a later stage, showing the formation of the first or primary prothallial cells. These primary cells have no walls on the inner side exposed to the central vacuole. The megaspore membrane shows a considerable increase in thickness at this stage. April 16.

Fig. 14. A portion of the periphery of a mature prothallium, showing the structure of the megaspore membrane at this stage. May 23.

Fig. 15. A small portion of a section of a very young prothallium, showing the formation of the primary prothallial cells. The section is taken parallel to the inner or open surfaces of the primary cells. April 16.

Fig. 16. A longitudinal section through the lower or sterile end of a young prothallium, showing the primary prothallial cells very much elongated, and growing inward and encroaching upon the space occupied by the central vacuole. April 16.

Fig. 17. A section through a portion of the archegonial end of a mature prothallium, showing the gradual tapering off of the megaspore membrane in the region of the archegonia, and the entire

absence of the membrane at, and for a considerable distance back of, the apex of the prothallium. June 3.

Fig. 18. A longitudinal section of a young archegonium, showing the central cell, two neck-cells, and a single layer of jacket-cells. May 17.

Fig. 19. The same at a later stage, showing the frothy appearance of the cytoplasm of the central cell. May 20.

Fig. 20. The same at a still later stage, showing the central nucleus in the region of the neck and preparing for division. May 23.

Fig. 21. The same at a still later stage, showing the position of the central nucleus at the time of spindle-formation. June 3.

Fig. 22. The nucleus of the central cell undergoing division. June 3.

Fig. 23. A later stage of the same. The central-canal nucleus is completely separated from the egg-cell by a distinct cell membrane. June 3.

Fig. 24. A later stage of the same, showing the degeneration of the ventral canal nucleus and a great increase in the size of the egg-nucleus. June 3.

Fig. 25. The same at a still later stage. The ventral canal nucleus has become fragmented, and the egg-nucleus has continued to increase in size, and has begun to move back towards the centre of the archegonium. The spindle fibrils stretching between the two cells are still visible. June 3.

Fig. 26. The egg-nucleus has reached an enormous size, and occupies a position near the central region of the archegonium. Its movement from its original position near the ventral canal cell is clearly indicated by the structure and drawn-out appearance of the cytoplasm in the forward half of the egg. June 3.

Fig. 27. A cross-section near the apex of a mature prothallium, showing four archegonia. Each archegonium is enveloped by a complete single layer of jacket-cells, and each is separated from its neighbour by sterile tissue. May 10.

Fig. 28. A longitudinal section through the apex of a mature prothallium, showing the appearance of the archegonia just before fertilization. June 3.

Fig. 29. A longitudinal section of the egg-nucleus at a stage soon after the organization of the ventral canal cell. The egg-nucleus has attained a considerable size. Very close to the nucleus membrane there is an accumulation of several masses of very dense cytoplasm. June 3.

Fig. 30. A later stage of the same, showing the egg-nucleus more than twice the size of that shown in Fig. 29. The dense masses of cytoplasm are larger and more numerous. June 3.

Fig. 31. The same, showing how much more numerous the dense masses of cytoplasm appear in cross-section. By the distending of the nuclear membrane outward between the dense masses of cytoplasm, the latter resemble small pockets projecting towards the interior of the nucleus. June 3.

Fig. 32. A longitudinal section of the egg-nucleus showing the segregation of the chromatin from the other nuclear substances. June 3.

Fig. 33. The same at a later stage, showing a rupture in the nuclear membrane, caused very probably by the entrance of the male nucleus. The first sporophyte spindle is being organized within the membrane of the egg-nucleus. June 3.

Fig. 34. A longitudinal section of an archegonium, showing the position of the first spindle after fertilization. The nuclear membrane has completely disappeared. June 9.

Fig. 35. A more highly magnified view of the spindle shown in Fig. 34. The chromatin-like granules discarded from the egg-cell become associated with kinoplasmic threads as if a second spindle were in process of formation.

Fig. 36. A longitudinal section of the upper half of an archegonium, showing the vacuolated appearance of the cytoplasm caused by the discharge of the contents of the pollen-tubes into the egg. Two distinct male nuclei of unequal size are to be seen accompanied by a much smaller nucleus which is probably the tube-nucleus. June 3.

Fig. 37. A longitudinal section of an archegonium, showing the first two free nuclei of the pro-embryo. June 3.

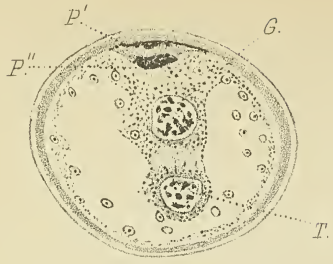
Fig. 38. Four free nuclei of the pro-embryo have passed to the base of the archegonium.

Fig. 39. Another view of the same.

Fig. 40. A later stage of the pro-embryo, showing the formation of walls between the nuclei.

Fig. 41. A view of the embryo proper with suspensors.

Fig. 42. Another view of the same, showing the suspensor plug.



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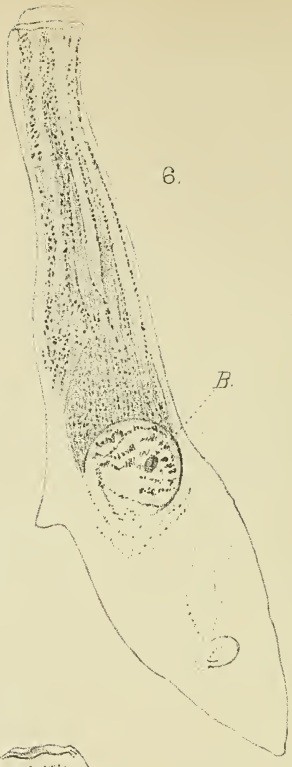

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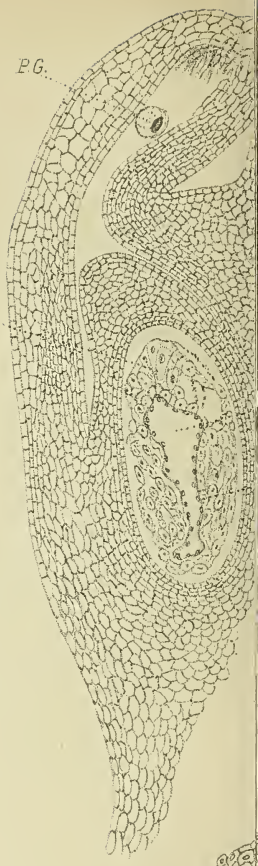

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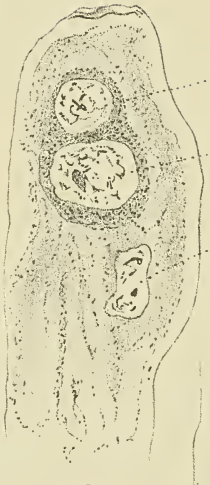

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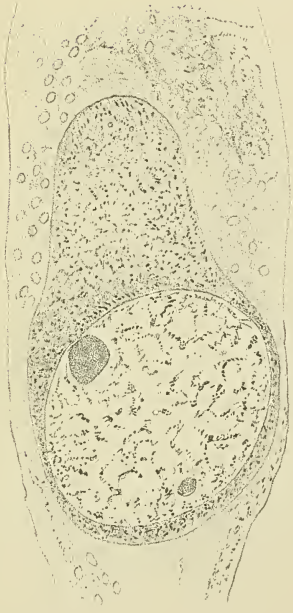

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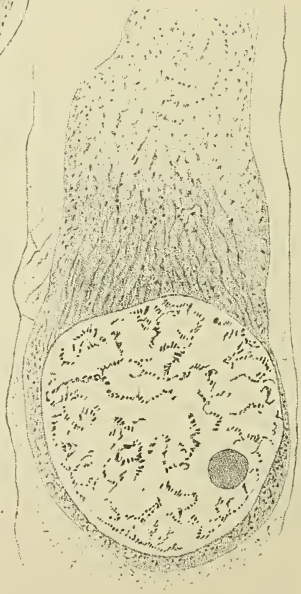

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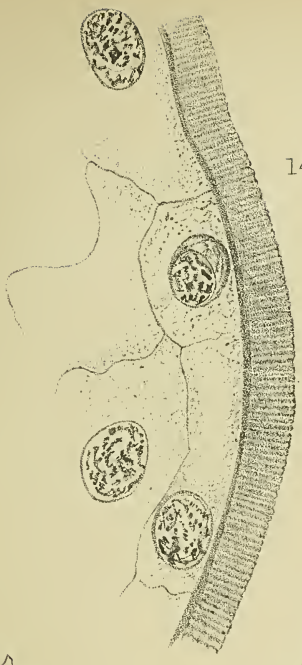

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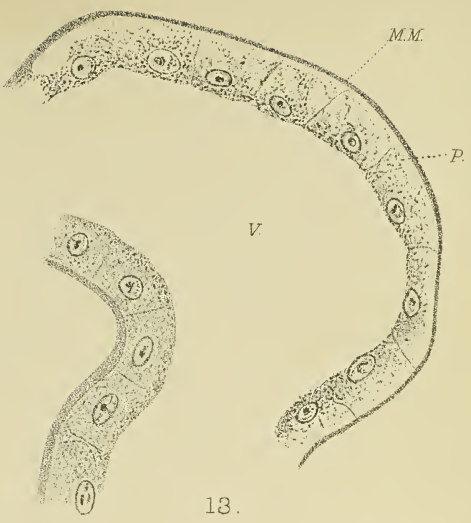

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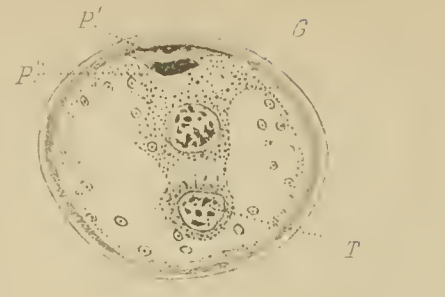

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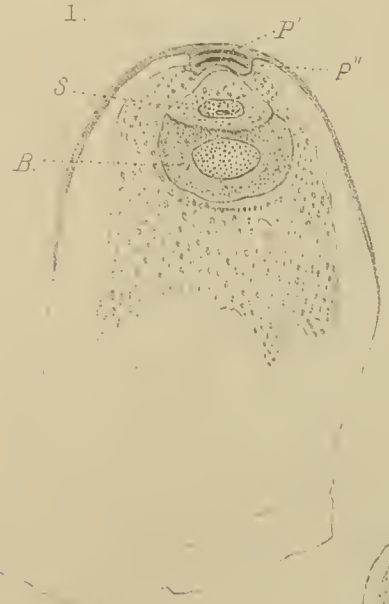

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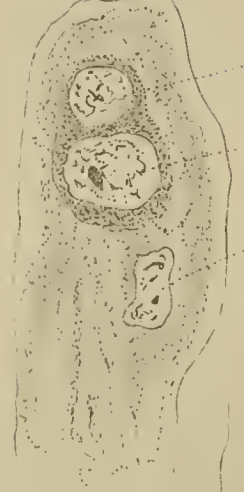

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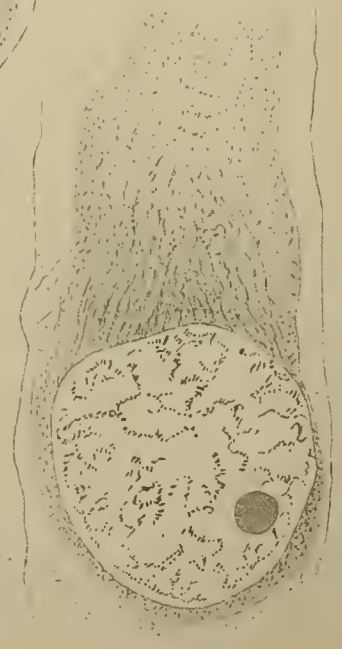
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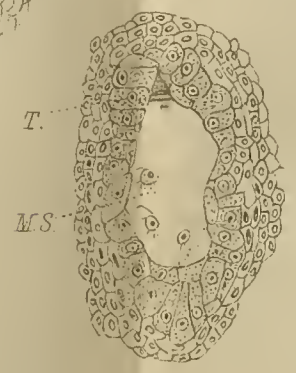

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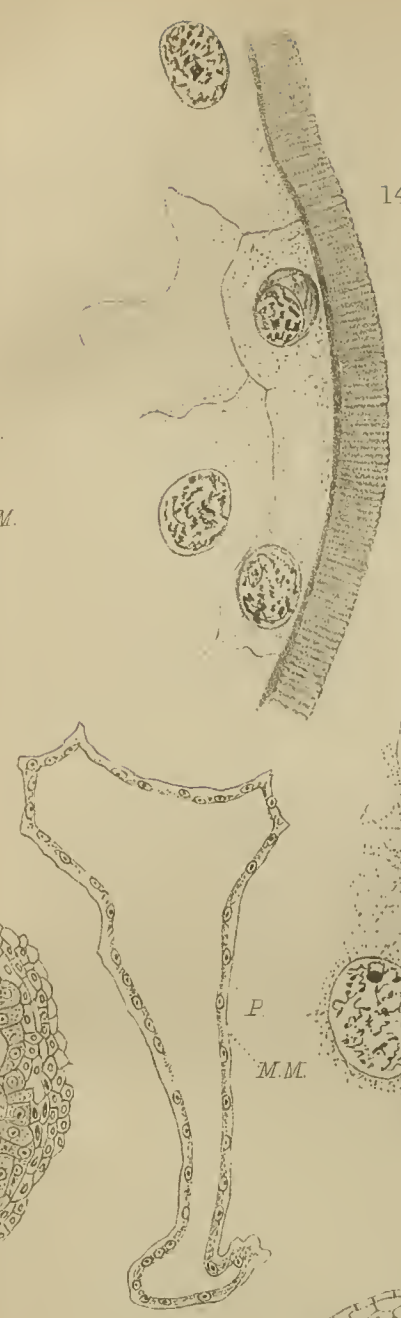

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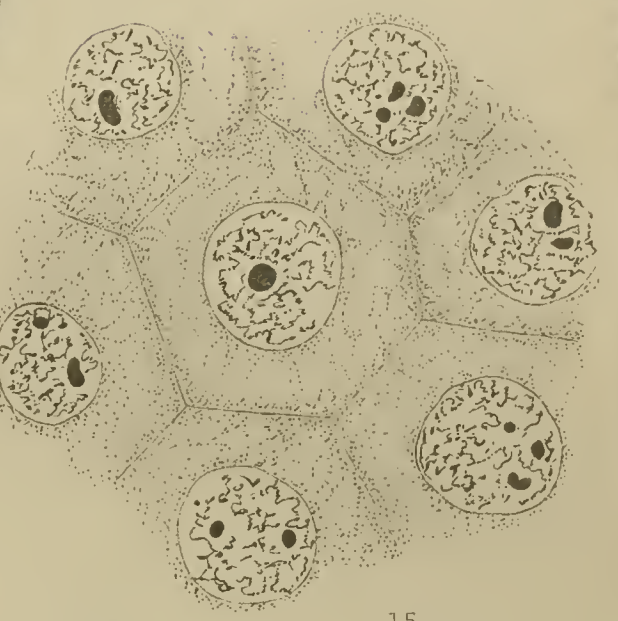
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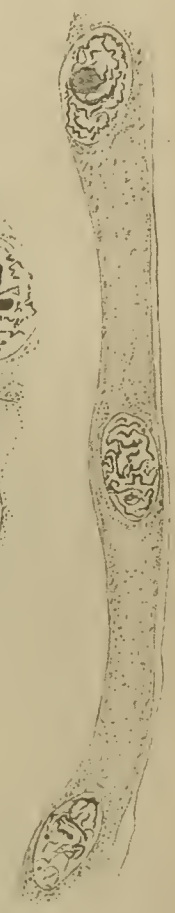
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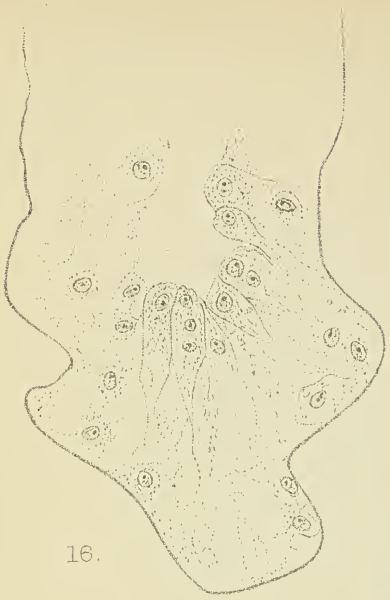

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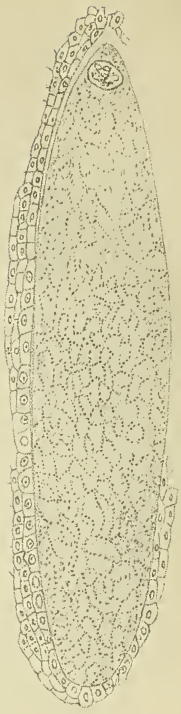

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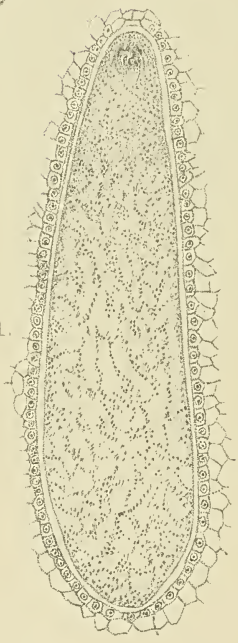

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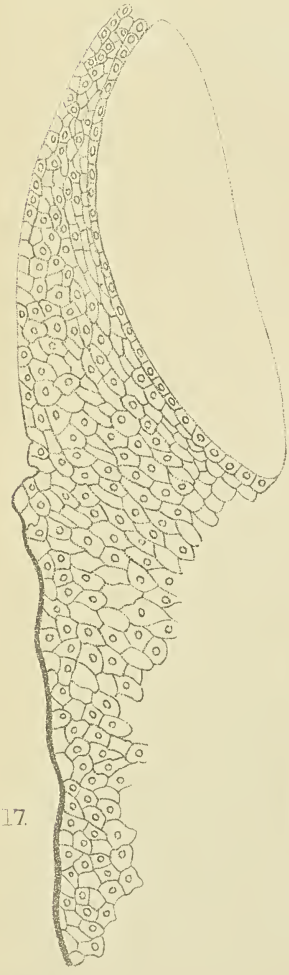

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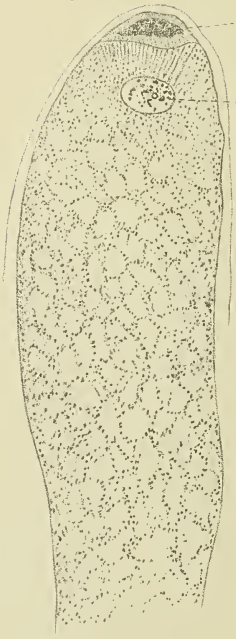

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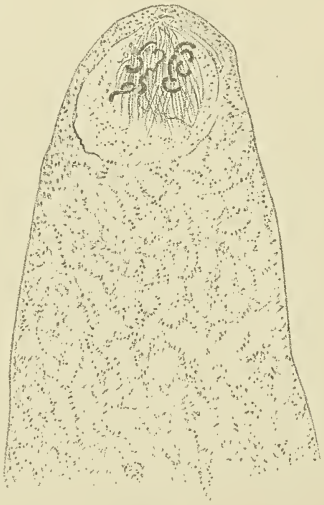

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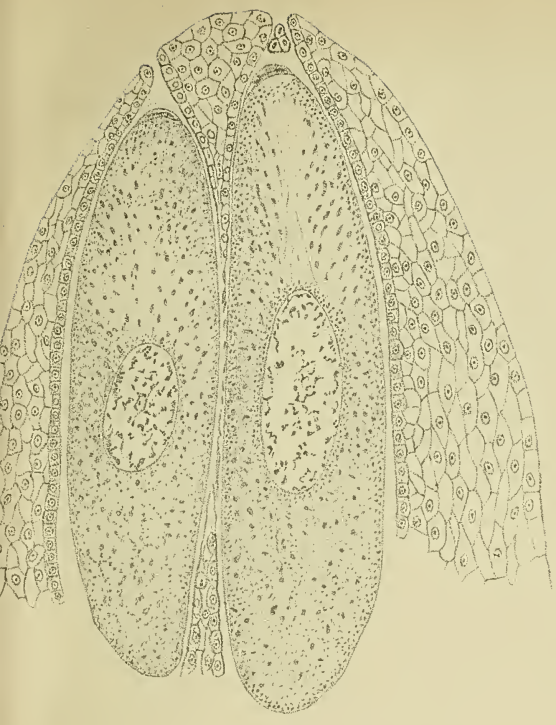

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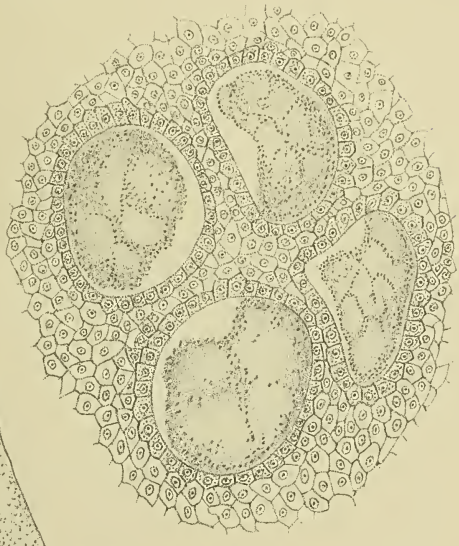

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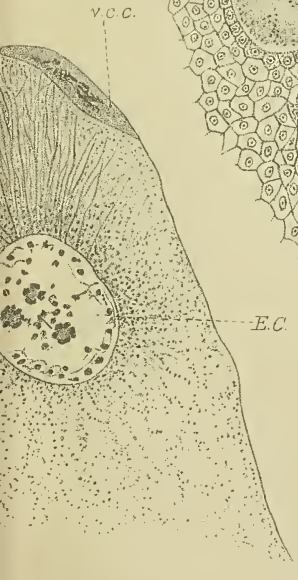

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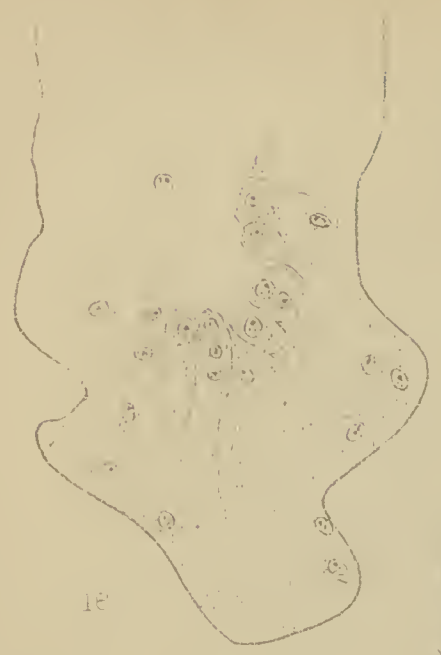

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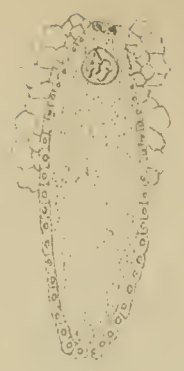

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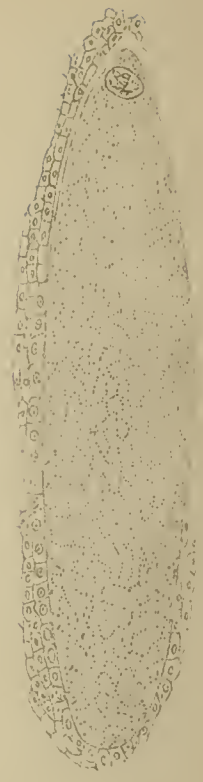

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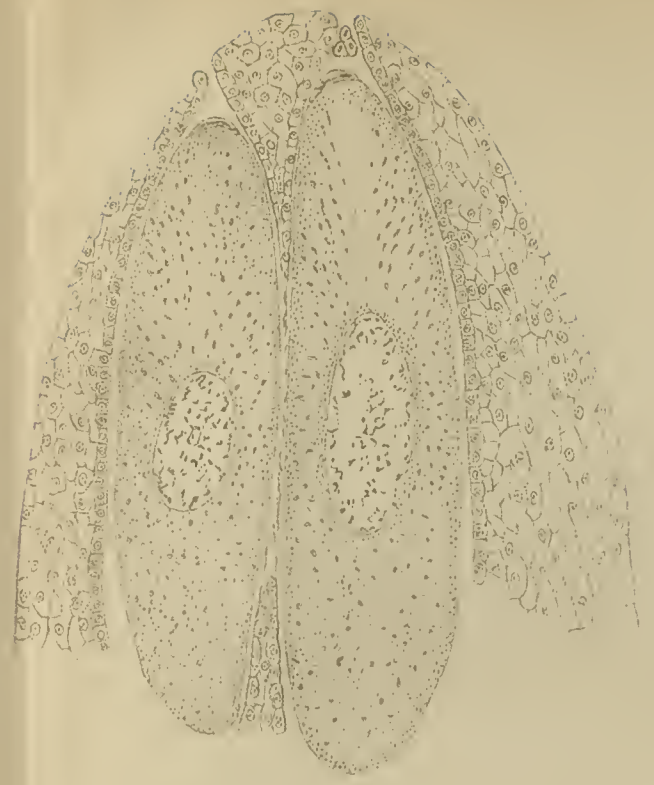

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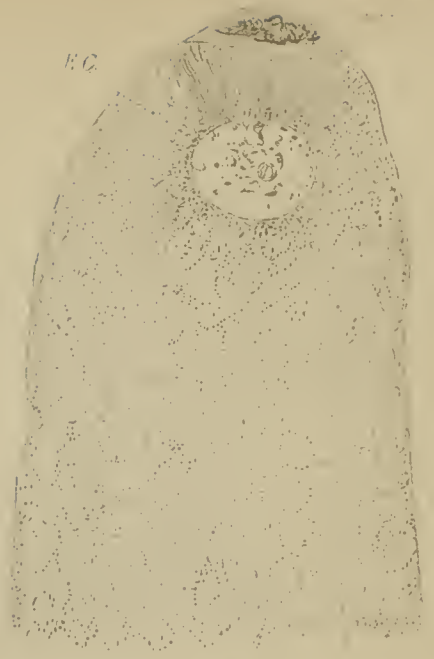

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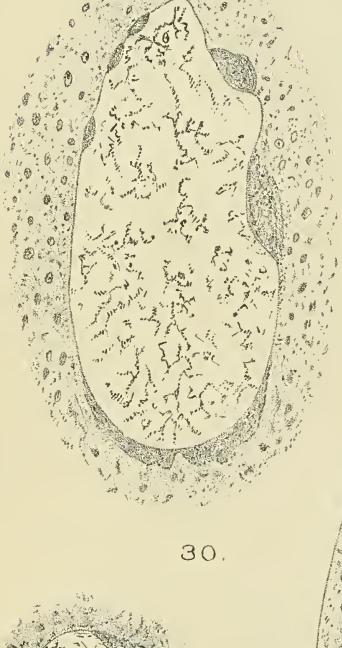

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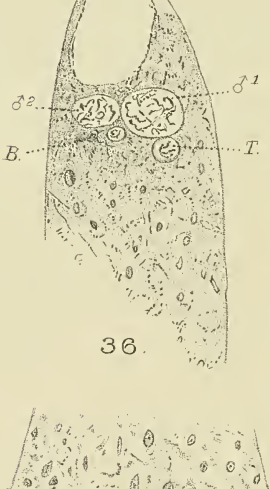

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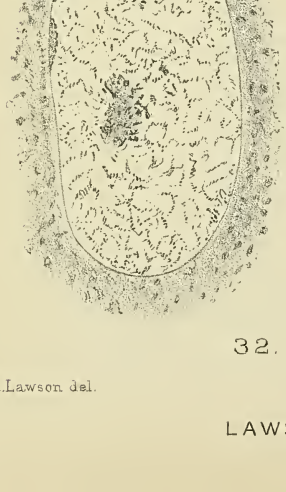

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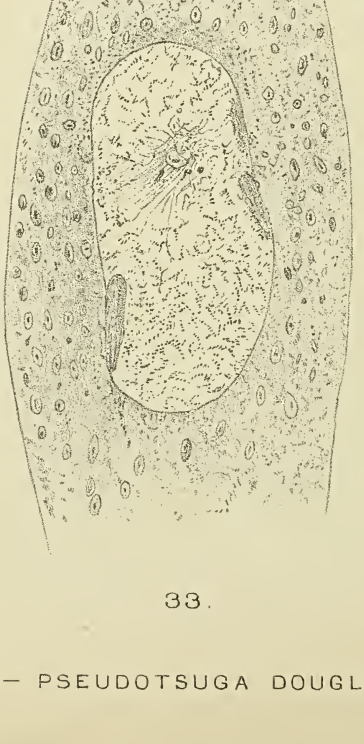

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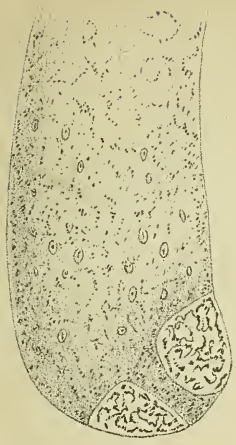

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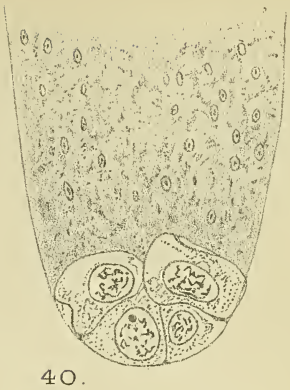

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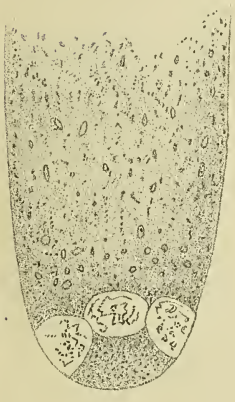

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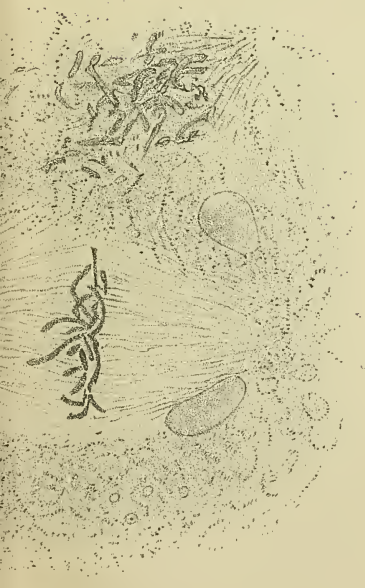

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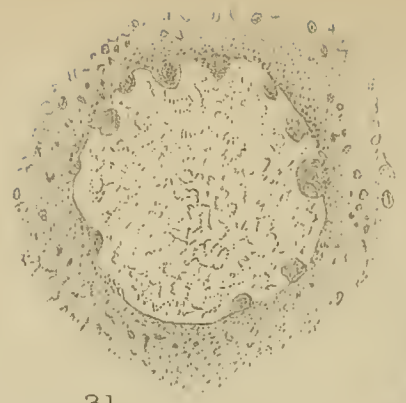

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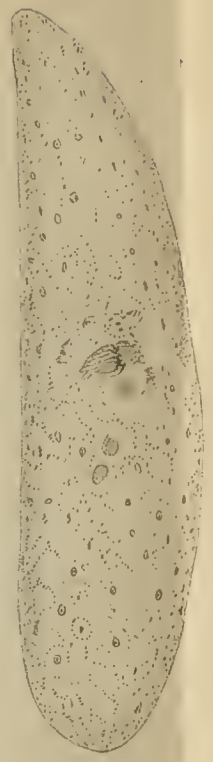

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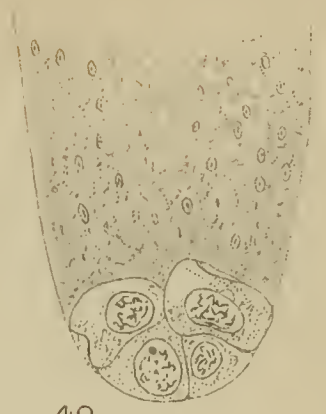

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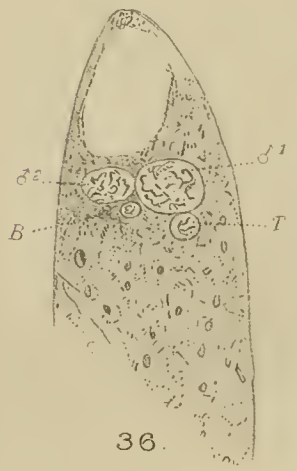

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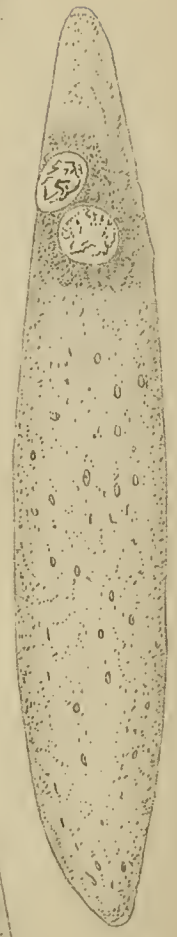

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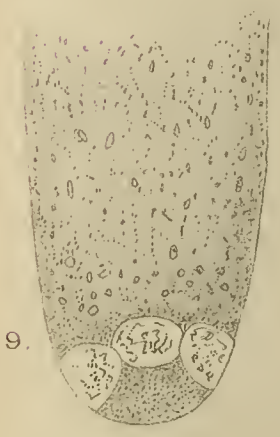

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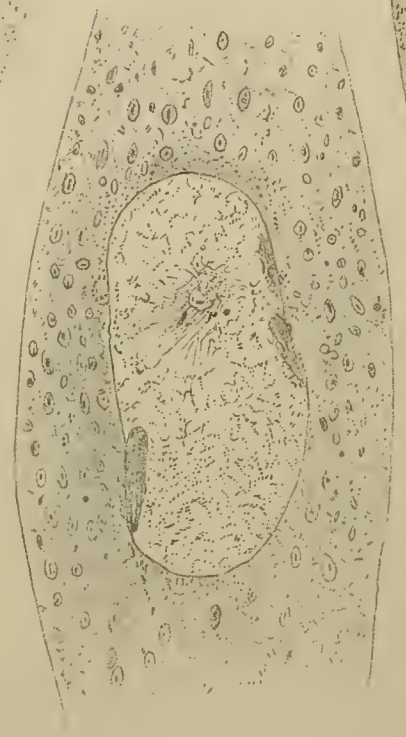

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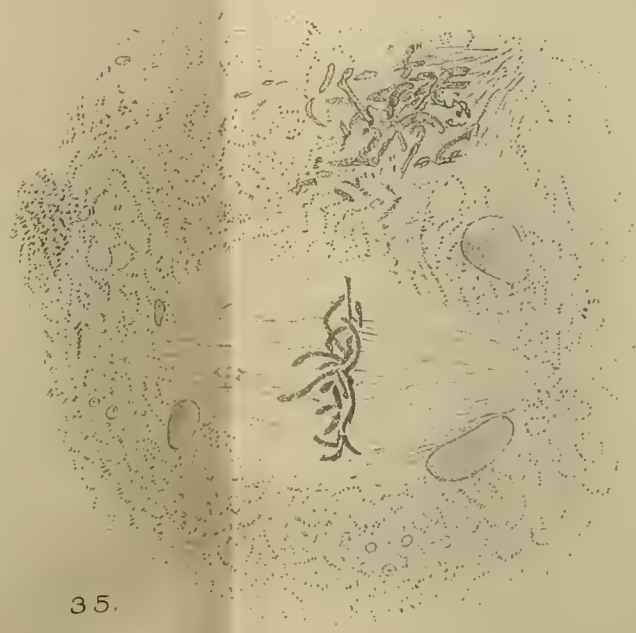

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The Action of Poisons upon Chlamydomonas and other vegetable Cells.

BY

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1. *The Toxic Action of Various Isomers upon Vegetable Cells.*

THE toxic action of dilute solutions of the isomers of various benzene derivatives upon the roots of young lupine plants has been studied by Hunkel and True,[1] and also the effect of a small number of these isomers upon *Spirogyra*. The toxic activity in these cases does not depend upon the hydrogen ions, for the dissociation in each case is shown to be too slight to have any very appreciable effect. Hunkel and True determined the minimal concentration of the isomers required to kill practically all the young lupine roots immersed in them within forty-eight hours; these concentrations, expressed in gramme molecular weights per litre, are shown for a few cases in the second column of Table I. From a comparison of these concentrations it is seen that '*the toxic power of the para-derivative is in most cases, but not invariably, much greater than that of the ortho- or meta-derivative*'. This conclusion was previously arrived at for bacteria by Carnelli and Frue,[2] who experimented with gelatine plates containing various concentrations of the isomers, which were exposed to the air of a dusty room in order to inoculate them.

In the third column of Table I the order of toxic strength of the poisons is given; these were obtained, for the cresols by Fränkel and for the phthalic acids by myself, by adding a drop of a culture of the bacteria to a tube of dilute poison, and inoculating tubes of suitable media from this at regular intervals.

I have investigated the effects of these isomers upon *Chlamydomonas*, and determined the minimal concentrations of the various isomers required to cause cessation of movement in a culture of actively motile *Chlamydomonas multifilis* in ten minutes. These concentrations are given in the first column of Table I. It was found possible to estimate the concentrations required

[1] Hunkel and True: Botanisches Centralblatt, B. 76, pp. 231, 289, 361.
[2] Carnelli and Frue: Journal Chemical Society, vol. 57, p. 636.

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to within 10–15 per cent., a much greater degree of accuracy than was possible with lupines. In some cases, as with resorcin, the difference in the effects caused by a $\frac{1}{6}$ per cent. and a $\frac{1}{7}$ per cent. solution was well marked; in other cases a similar difference in the concentration of the poison caused a much less marked effect.

The Table shows both the very similar reactivity of widely different vegetable cells towards the same poison—a somewhat striking phenomenon—and also a fairly constant relation between the chemical constitution of a poison and its toxic strength, since the para-derivative is in most cases the strongest poison of the three isomers. A close relation between the cessation of the power of movement of the Chlamydomonadine cell and its death-point is indicated.

TABLE I.

Poison.	*Minimal Conct. in Gm. Molecular Wts. per Litre.*		
	Chlamydomonas.	*Lupinus Albus.*[1]	*Bacteria.*
ortho dihydroxy phenol	0·0091	0·00125	
meta dihydroxy phenol	0·0167	0·005	
para dihydroxy phenol	0·000182	0·000062	A powerful disinfectant [1]
ortho Cresol	0·00316	0·00125	1[2]
meta Cresol		0·00125	3
para Cresol	0·00159	0·00062	2
ortho Phthalic acid	0·0018		1[3]
meta Phthalic acid	0·00072		2
para Phthalic acid	0·000012		3

2. *The Reaction Velocity of Hydrochloric acid as a Poison.*

A small quantity of a culture of a large and actively-moving species of *Chlamydomonas* was introduced into a cylindrical glass vessel with a thin glass bottom. The cells swam about rapidly within a short distance from the bottom of the vessel, and were viewed with an inverted microscope in a strong red light from above. After introduction of the poison the cells were seen to fall one by one and rest on the bottom. The number thus brought to rest within the field of the microscope was counted at intervals of five minutes.

If the cessation of movement of the cells in this case was a measure of

[1] Hunkel and True: Botanisches Centralblatt, B. 76, pp. 231, 289, 361.

[2] C. Fränkel: Zeitsch. für Hygiene, 1889. The numbers give the order of increasing toxic strength.

[3] Order of toxic strength with Bacterium subtilis obtained in the same manner as in Fränkel's experiment.

the time of their death, the counts gave a measure of the number of deaths which had taken place after the various intervals of time, and consequently of the number surviving after these intervals. That this is the case seems most probable from the results obtained. If n_1 = number of cells surviving after an interval of time t_1, and n_2 the number surviving after t_2, then the equation

$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = \text{a constant}$$

is found to be approximately obeyed, within the limits of experimental

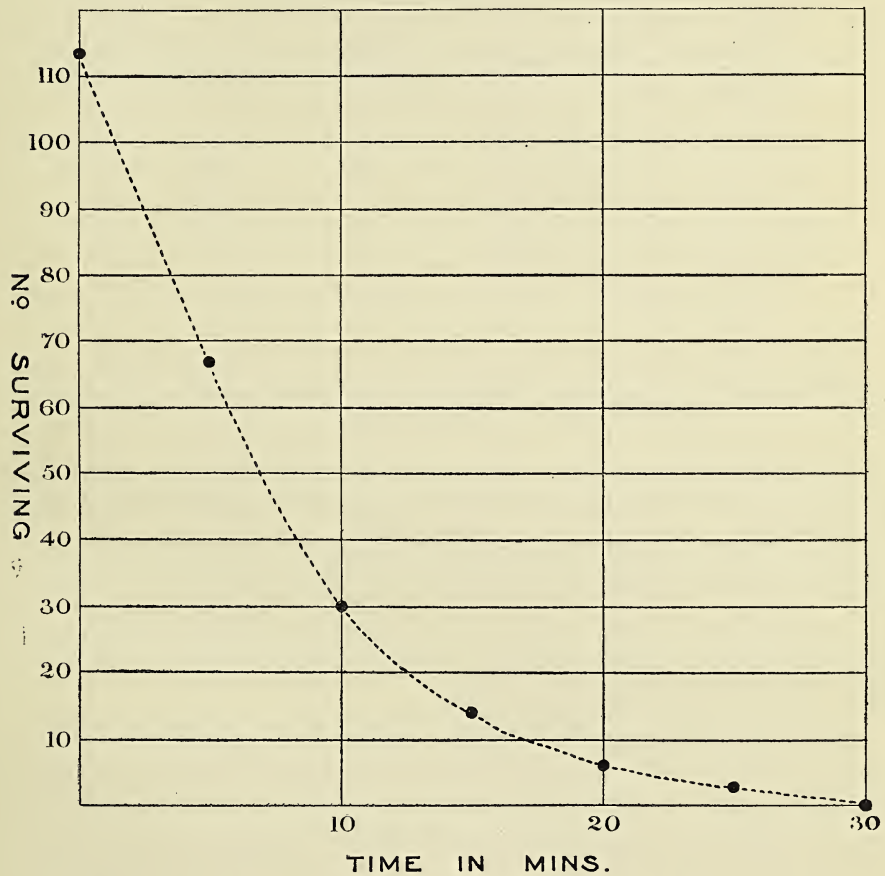

Fig. 1. Curve showing relation between number surviving and time (actual).

error, as seen in the fourth column of Table II. Owing to the difficulty of obtaining suitable material, I was unable to repeat the two experiments, the results of which are shown, or to obtain larger and more satisfactory numbers.

The above equation is the same as that for a unimolecular chemical reaction, if n_1 is the active mass after time t_1, and n_2 is the active mass after time t_2. A bimolecular chemical reaction in which one active mass is in great excess approximates in velocity to a unimolecular reaction. The same equation as the above was arrived at by Madsen and Nyman, and independently by Miss Chick [1] in 1907, for the rate of disinfection of bacteria.

Results of a similar nature have been obtained by Dr. F. F. Blackman [2] and Miss N. Darwin for the death-rate with higher plants.

TABLE II.

Strength of hydrochloric acid = ·009 per cent.

Time in mins.	*Number fallen in two experiments.*	*Number surviving*	$\frac{1}{t_2-t_1} \log \frac{n_1}{n_2} = k.$
0	2 + 1 = 3	113	
5	19 + 28 = 49	67	(0·045)
10	34 + 52 = 86	30	0·070
15	39 + 63 = 102	14	0·066
20	45 + 65 = 110	6	0·074
25	49 + 65 = 113	3	0·060
30	51 + 65 = 116	0	
35	51 + 65 = 116	0	

Miss Chick points out the analogy between the death-rate of bacteria and the rate of decomposition of molecules of a chemical compound; as, for instance, those of cane-sugar undergoing hydrolysis in a weak acid solution. The same analogy applies to the relations between the death-rate and the concentration of the poison, and between the end point of a bimolecular reaction and the concentration of the active substance in excess. An explanation has been given for the rate of a chemical reaction to the effect that 'at a particular time only a proportion of the molecules are temporarily in such a state as to permit of the combination'. The same explanation seems to apply to the reaction between a poison and cells of bacteria and *Chlamydomonas*.

The death of the cells might be expected to occur after a fixed proportion of one or more substances in the protoplasm had decomposed, but, although the rate of decomposition of such a substance or substances would follow a logarithmic curve obeying the above equation, the time for death to take place would depend upon the quantity of such substances in the individual cells, and upon the proportion necessary to be decomposed before death took place. Why the cells of bacteria and *Chlamydomonas* behave in this way, like the molecules of a chemical compound, has yet to be explained.

[1] Harriette Chick: Journal of Hygiene, Jan. 1908.
[2] British Association, Sept. 1908.

3. *Variation of the death-rate with the Concentration of the Poison.*

To small quantities of a culture of very numerous actively-moving cells of *Chlamydomonas* about 1 cc. of Resorcin solutions of different strengths

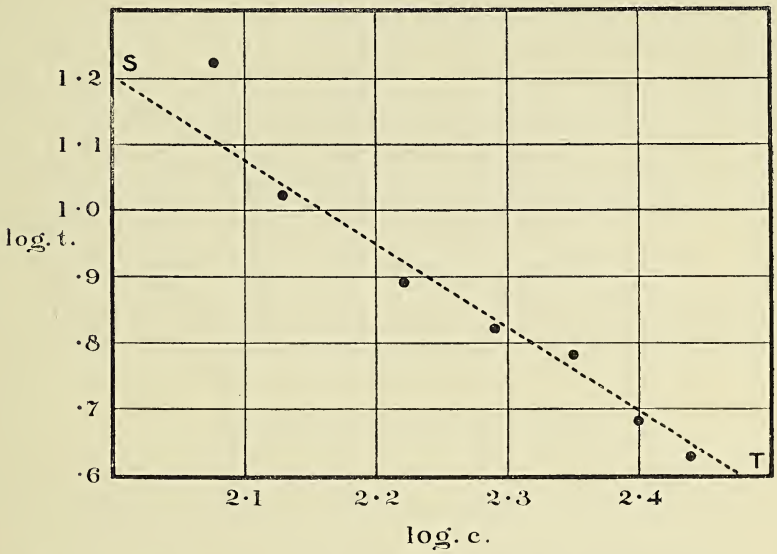

FIG. 2. Diagram showing direct proportion between logarithm of concentration of poison and logarithm of time for poisoning to reach completion.

were added and the times recorded for all, except 3–5 cells, to cease moving under the field of the microscope. The concentrations of the poison and the times required for cessation of movement are given in the first two columns of Table III, and in the third column the average times required for movement to cease.

On plotting the logarithms of the times and of the concentrations the points are seen to lie upon a straight line; that is, the logarithms show a direct proportion. In the case of the point corresponding to the least concentration, the discrepancy is accounted for by the difficulty in determining the exact time-value, as may be seen from the varying values given in the table.

Since the logarithms of time (t) and of concentration (c) are directly proportional

$$\log t + k \log c = \text{a constant.}$$

This is found to be the case, taking $k = 1{\cdot}21$, as shown by the constant values in the last column.

In a chemical reaction where one molecule of one compound reacts with k molecules of another compound which is in great excess, this equation is

obeyed when the concentration (c) is made to vary, t being the time for the reaction to reach completion in each case.

In the disinfection of bacteria the same equation is obeyed, the value of k being dependent upon the nature of the poison and the bacterium.[1]

TABLE III.

C = concentration of Resorcin in parts per 100,000.	*Times in minutes.*	*t or average time.*	*log c.*	*log t.*	*log t + 1·21 log c.*
102	Several hours—indefinite				
121	16, 18, 17, 16, 18	17	2·0828	1·23	3·75
136	10, 11, 10, 11, 11, 10, 10	10·5	2·13	1·02	3·60
165	8, 8, 8, 8, 7·5, 8, 7·5, 8	7·8	2·22	0·89	3·58
195	6·5, 7, 7, 6·5, 6·7, 6	6·6	2·29	0·82	3·59
224	6, 6, 6, 6, 6	6·0	2·35	0·78	3·60
254	4·5, 5, 5, 5, 4·5, 5, 5	4·8	2·40	0·68	3·58
278	4·5, 4, 4, 4·5, 4·5, 4, 4, 4·5	4·25	2·44	0·63	3·58

4. *Action of a mixture of Poisons.*

The following concentrations of poisons were each found just to cause cessation of movement in a culture of *Chlamydomonas multifilis* in ten minutes :—

Hydrochloric acid	0·009 per cent.
Pyrocatechin	0·1 per cent.
Resorcin	0·18 per cent.

A mixture of 0·009 per cent. HCl and 0·18 per cent. Resorcin caused cessation of movement before ten minutes, and so did a mixture of 0·009 per cent. HCl and 0·10 per cent. Pyrocatechin, also a mixture of 0·18 per cent. Resorcin and 0·10 per cent. Pyrocatechin. Hence no one of these poisons retards the action of another to any considerable extent.

Further experiments showed the striking fact that a mixture of the poisons, each slightly weaker, viz. :—

0·007 per cent. HCl
0·08 per cent. Pyrocatechin
and 0·14 per cent. Resorcin

does not cause cessation of movement in ten minutes, although each of the three poisons exceeds in strength three-quarters of the concentration which, by itself, will cause movement to cease.

Here it is evident that *the action of each poison is specific*, and, from the great difference in toxic activity of isomeric modifications of the same compound, the specific action is seen to vary greatly for slight changes in chemical constitution. A mixture of strong acids behaves as if its toxic

[1] Watson : Journal of Hygiene, Oct. 1908.

activity was proportional to the number of free hydrogen ions present, unless, of course, any one of the acids has a further specific toxic action dependent upon the negative ion. (Hunkel and True.)

It seems probable, in the above instance, that the velocity of the reaction due to the mixture of poisons is equal to the sum of the velocities of each specific reaction. When a number of chemical reactions are simultaneously taking place in any system, each proceeds independently of the others, and the total change is the sum of all the independent reactions.

Owing to the difficulty in procuring suitable cultures of *Chlamydomonas* I have been unable to make conclusive experiments with other mixtures of poisons, but hope to extend the above results next summer. In the meanwhile these results can only be regarded as preliminary.

Finally I have to express my thanks to Miss Chick for several valuable suggestions, and to Dr. F. F. Blackman for his kind help throughout.

On the Seedling Structure of Gymnosperms. II.

BY

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With Plate XV and eleven Figures in the Text.

ABIETINEAE (*continued*).

MANY investigators have recorded various observations on the seedlings of this group of the Gymnosperms. Lestiboudois [1] drew attention to the fact that the number of cotyledons is often double the number of the bundles of the root, e.g. *Pinus Pinea*, &c.; further, he pointed out that this is not at all constant even in the same species; for, while some of the vascular strands of the root may give rise only to one cotyledonary trace, others may give rise to more than one.

Duchartre [2] described the occurrence of polycotyledony in the Abietineae and certain Cupressineae, and remarked upon the rarity of the phenomenon in *Juniperus*, *Thuja*, and *Cupressus*. He also observed the inconstancy of the number of seed-leaves in these polycotyledonous plants, and the lack of a direct relation between the number of bundles coming in from the cotyledons.

Van Tieghem [3] and Strasburger [4] cite examples of species of *Pinus* and other Gymnosperms which show this same variation both as regards the number of seed-leaves and the relations of their bundles to the primary root-structure.

De Bary [5] gave a brief general description of the bundle system of the seedlings, with a few details of the transition phenomena. Dangeard [6]

[1] Lestiboudois: Phyllotaxis anatomique (Ann. Sci. Nat., Bot., sér. 3, t. x, 1848).

[2] Duchartre: Mémoire sur les embryons qui ont été décrits comme polycotylés, Id. 1848.

[3] Van Tieghem: Sur la symétrie de structure des plantes vasculaires (Ann. Sci. Nat., Bot., sér. 3, t. xiii, 1870–1).

[4] Strasburger: Die Coniferen und die Gnetaceen (Jena, 1872).

[5] De Bary: Comparative Anatomy of the Phanerogams and Ferns (Oxford, 1884).

[6] Dangeard: La mode d'union de la tige et de la racine chez les Gymnospermes. Compt. Rend., Feb. 1890.

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states that if there are two cotyledons, the root is diarch; if three seed-leaves are present, a triarch root-structure results; but, if the number of cotyledons be more numerous, agreement between their number and the number of poles in the root no longer obtains.

Dangeard was the first to work out in any detail the relationships between the cotyledonary strands and the root-structure; his results are best expressed by the following quotation: 'Chaque faisceau de la racine, soit libérien, soit ligneux, s'insère sur deux traces cotylédonaires, ce qui peut être indiqué par le rapport $\frac{2n}{n}$'. He then mentions some exceptions:—

'1. A ce que l'une des traces cotylédonaires se divise en deux pour donner insertion à un faisceau de la racine, les autres traces cotylédonaires conservent la disposition ordinaire: ce fait correspond aux rapports $\frac{5}{3}$, $\frac{7}{4}$, $\frac{9}{5}$, $\frac{11}{6}$, $\frac{13}{7}$.

2. A ce que l'une des traces cotylédonaires se réunit à une autre sans servir à l'insertion: ce cas correspond aux rapports $\frac{7}{3}$, $\frac{9}{4}$, $\frac{11}{5}$, $\frac{13}{6}$, $\frac{15}{7}$'.

It may here be remarked that these relations, according to our experience, do not occur so regularly as the above indicates. Further, that on our hypothesis Dangeard's first case $\left(\frac{2n}{n}\right)$ corresponds to all the seed-leaves being half-cotyledons; his second case corresponds to one seed-leaf being a whole cotyledon, and the rest half-cotyledons; while his third case corresponds to one seed-leaf being a subsidiary cotyledon and the rest half-cotyledons.

In a later paper Dangeard[1] describes many histological features in the seedlings of some Gymnosperms, and also transition-phenomena which will be referred to when necessary.[2]

Masters[3] described the morphology of the seedlings of many species, and showed how variable is the number of seed-leaves in one and the same genus.

Tsuga.

Tsuga canadensis, Carr. The normal number of cotyledons is apparently three, Masters[4] gives 3–5 as the range in number, and Dangeard[5]

[1] Dangeard: Recherches sur les plantules des Conifères (Le Botaniste, sér. 3, 1892).

[2] We did not discover the existence of this paper until after this present communication had been written, and it is for this reason that mention was not made of Dangeard's work in our Part I (Annals, 1908). He briefly describes and illustrates the transition-phenomena in *Cupressus funebris* Endl., *C. Corneyana* Hort., *C. Lindleyi* Klotsch, *Actinostrobus pyramidalis* Miq., *Thuja orientalis* L., and *Taxus baccata*, Tourn. We are in agreement with him in essentials; but as regards details we have not found the bifurcation of the cotyledonary strand and rotation of the protoxylem quite so strongly marked in *Thuia orientalis* and *Taxus baccata* as he indicates.

[3] Masters: Comparative Morphology, Anatomy and Life-history of the Coniferae (Journ. Linn. Soc., Lond., Bot., xxvii, 1891). Notes on the Genera of Taxaceae and Coniferae. Id. xxx, 1895.

[4] Masters: 1895, loc. cit.

[5] Dangeard: Le Botaniste, iii, 1892.

states that three are generally present. Their structure resembles many of the Cupressineae; the amount of transfusion tissue is very small, and there may be made out, towards the bases of the seed-leaves, a few xylem elements situated in a centripetal position. Resin ducts are absent.

The seed-leaves form a short but fairly well-marked cotyledonary tube, and in this region the bundle of each becomes somewhat tangentially elongated, an arrangement which is retained during the inward passage. After the central region of the hypocotyl has been reached, each trace splits into two halves, the bifurcation generally appearing first in the phloem. Each half-bundle then rotates around the protoxylem which acts, as it were, as the pivot. The wood is thus exposed, and the protoxylem takes up its exarch position, in some instances very gradually. The opposing groups of phloem elements effect a junction so that a triarch root-structure obtains. These changes agree with the brief description given by Dangeard [1].

Tsuga diversifolia, Mast., as far as can be ascertained from seedlings having much secondary thickening, does not differ in any essential feature from *T. canadensis*.

Abies.

The structure of the cotyledons calls for but little comment; mention need only be made of three features. In *Abies sibirica*, *A. Veitchii*, *A. balsamea*, *A. amabilis*, and *A. magnifica* var. *Shastensis*, each seed-leaf has two resin ducts situated in each corner towards the dorsal surface; transfusion tracheides also are present in varying amounts; and, finally, a cap of broad, elongated, thin-walled elements abuts directly on to the dorsal surface of the phloem.

Abies pectinata appears to be somewhat abnormal, for the seed-leaves have no resin ducts, and transfusion tissue has not been observed.

In all the species examined each cotyledon had a single bundle which retained its endarch character until the central region of the hypocotyl had been reached.

Abies sibirica has three or four cotyledons. The examination of a specimen with the former number showed the transition to be identical with what obtains in *Tsuga canadensis*, and resulted in the formation of a triarch root-structure. A seedling (Series B) which had four cotyledons behaved somewhat differently, and requires some description. All the seed-leaves were quite free for the greater part of their length, but towards the basal region two of them were fused together, so that a transverse section at this level had the appearance illustrated in the first figure of Diagram 1. The four bundles of the seed-leaves retained their identity within the hypocotyledonary axis for some time, and were surrounded by a practically closed ring formed by the fusion of the fibrous elements of the four phloem masses.

[1] Dangeard, loc. cit.

The bundles *a*, *b*, and *c* underwent bifurcation of their vascular elements, and rotation of the protoxylems as described for *Tsuga canadensis* and illustrated in Diagram 1. The strand *d*, however, performed differently. In the upper part of the hypocotyl, *d* converged towards *a* (Diagram 1, Fig. 2), and at the same time a rearrangement of the xylem elements began which ultimately resulted in the protoxylem occupying an exarch position: the phloem of *d* showed no signs of bifurcation but fused with the adjoining portion of the bast of *a* (Diagram 1, Figs. 3 and 4). At lower levels of the hypocotyl the protoxylem of *d* gradually made its way through the inter-

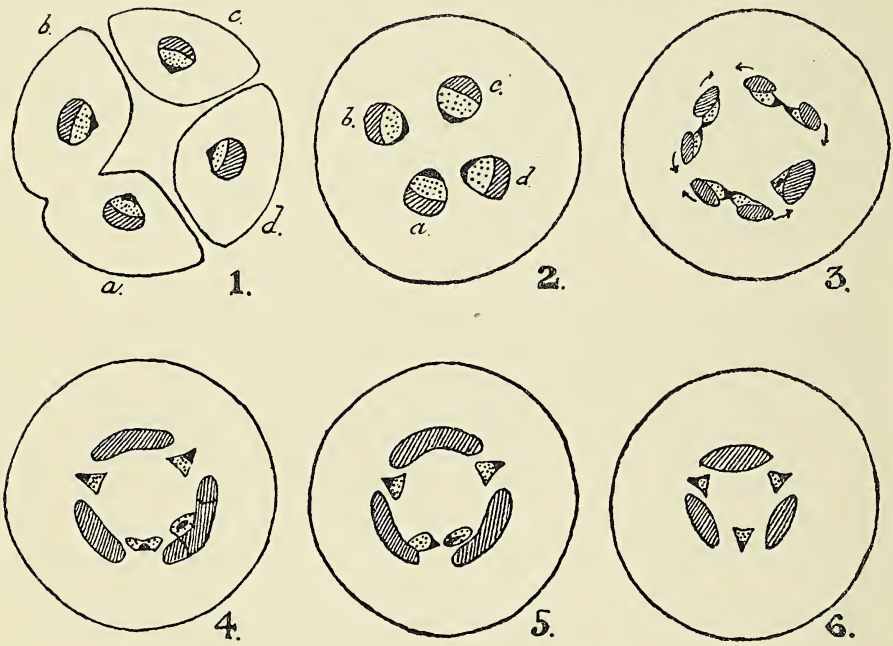


DIAGRAM 1. *Abies sibirica.* In this, and in all the following text-figures, the protoxylem is indicated by black areas, the metaxylem by dots, and the phloem by shading.

vening metaxylem elements, and effected a junction with the same tissue of *a* (Diagram 1, Figs. 4–6). A triarch root-structure was thus attained.

Different views may be held regarding the features described for this particular individual: the seedling may represent a late stage in the splitting of one seed-leaf to form two cotyledons, but a comparison with what obtains in *Cupressus torulosa* [1] shows important and obvious features of difference. The bundles *a* and *d* of *Abies sibirica* do not converge, and by a comparatively simple sequence of events give rise to one pole of the root as in the case of *Cupressus torulosa*; in the seedling of *Abies sibirica*, the phloem of *a* bifurcates and one portion accompanies the bast of *d* to effect a junction

[1] Part I, Annals of Botany, 1908, p. 700, Diagram 4.

with half the phloem of *c* ; again the protoxylems of *a* and *d* retain their identity longer than would be expected if these two strands represented the bundles of half-cotyledons ; further, from what obtains in the similar seedling of *Cupressus torulosa*, and in other plants to be described hereafter, the bundles *a* and *b* would be expected to form one pole of the root, since these are the traces of the two cotyledons which fuse together ; finally, the protoxylem of *d* becomes exarch, and, at a lower level, makes its way through the metaxylem to join with the protoxylem of *a*. These characteristics are not those associated with the strands of either half-cotyledons or subsidiary cotyledons ; for these reasons this particular seedling may be looked upon as an example showing a tetrarch root-structure becoming triarch in a very short space. The fusion of the seed-leaves *a* and *b* is the beginning of the formation of a cotyledonary tube which is not at all uncommon in these polycotyledonous forms.

Abies Veitchii. Of this species, only one seedling, having three cotyledons, was available. The transition-phenomena leading to the formation of the root-structure, although similar to what occurs in *A. sibirica* (Series A), is not nearly so well marked as in the last-named plant. The redistribution of the xylem elements in order to bring the protoxylem into the exarch position is of a very indefinite nature, there being in two bundles no separation of the xylem masses into two halves followed by a rotation (cf. *Juniperus*[1]) ; the xylem of one bundle did, however, partly split in the manner described for *Actinostrobus*,[2] but the V very soon closed up again.

Abies balsamea, Mill. All the plants of this species had four cotyledons, each with one vascular bundle.

The transition resembles *A. sibirica* in all essentials, but the rotation of the protoxylem is not so well marked in all cases, a character which recalls that obtaining in *Callitris rhomboidea*.[3]

Series A. Two of the cotyledonary bundles underwent bifurcation and rotation to form two poles of the triarch root ; the two remaining seed-leaf-traces together formed the third pole.

Series B. All the four vascular bundles derived from the seed-leaves showed bifurcation and rotation of their vascular elements, a tetrarch root-structure resulting. The exarch position of the protoxylem, however, was brought about partly by the rotatory movement and partly by an indefinite rearrangement of the xylem-elements.

Series C proved more interesting. Of the four cotyledonary bundles, three exhibited bifurcation and rotation of the xylem as in the other plants of this species ; in a very short space, however, half the xylem of one of these strands, which, for convenience may be termed *c*, fused with the xylem of the adjoining fourth seed-leaf-trace (*d*), and the combination of the two gave rise to one pole of the triarch root-structure. The fourth bundle was

[1] Part I, Annals, 1908, p. 696. [2] Part I, Id. p. 703. [3] Part I, Id. p. 706.

clearly that of a half-cotyledon, and the bundle *c*, although it first behaved as the strand of a whole cotyledon, finally performed as that of a half-cotyledon.

Abies firma, Sieb. and Zucc. One seedling only was available for study. It had four cotyledons, and the transition-phenomena closely resembled those described for *A. balsamea*. The rotation of the xylem was more strongly marked in *A. firma* than in *A. balsamea*.

Abies sachaliensis, as far as our inadequate material indicates, is essentially similar to *A. firma*.

Abies pectinata, DC. The usual number of cotyledons is eight, which

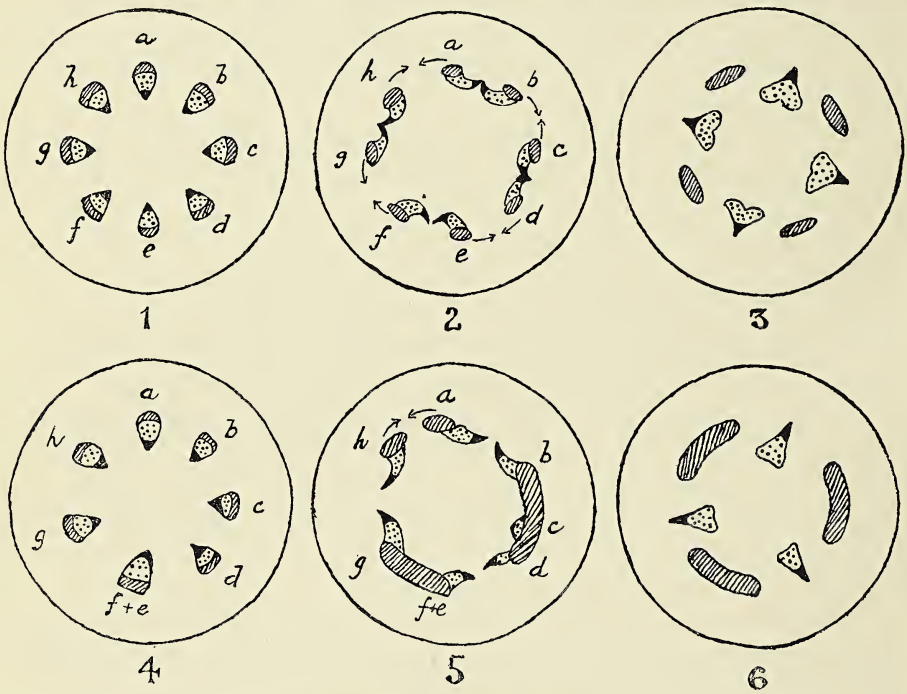


DIAGRAM 2. *Abies pectinata.*

in some cases form a short tube. The root is triarch in structure, which arrangement is arrived at in two different ways.

In the first case, the eight seed-leaf-traces form a well-marked zone in the upper part of the hypocotyl ; rotation then takes place, the protoxylems of two adjacent bundles are thus brought into contact and gradually assume the exarch position, concurrently the eight phloem masses fuse in pairs. The inward rotation of the metaxylem still continues until the fusion of each pair is completed, and hence a tetrarch root-structure results. At a lower level a triarch arrangement supervenes by the obliteration of one xylem group and the fusion of the two lateral phloem masses (Diagram 2, Figs. 1, 2 and 3).

In other instances, a different course is pursued. In the upper part of the axis two of the cotyledonary traces fuse together, and, at a slightly lower level, two others effect a junction, so that six bundles result which by rotation of the xylem and fusion of the phloem, as in the case first described, form a triarch root. (Diagram 2, Figs. 1, 4, 5 and 6.)

The first seedling of the plant under consideration clearly represents an instance of four cotyledons having split to form eight; while in the second case, six of the seed-leaves represent half-cotyledons, and the two which play no prominent part in the transition are to be considered subsidiary seed-leaves.

Abies amabilis, Forb. The one available seedling of this plant had eight seed-leaves which fused to form a short cotyledonary tube.

The transition-phenomena took place in a similar manner to those in *A. sibirica*; the bundle of one cotyledon played no part in the process, and thus is to be placed in the category of subsidiary cotyledons. Of the remaining bundles, each formed a pole of the root, which organ was 7-arch in the higher region, but was reduced to 6-arch, and finally 5-arch.

Abies magnifica var. *Shastensis*. Here, again, only one seed germinated, giving rise to a seedling with nine cotyledons. The transition resembled both *A. sibirica* and *A. pectinata*. The bundles of six of the seed-leaves underwent bifurcation of the phloem and rearrangement of the xylem to form six poles of the 7-arch root, two of the cotyledons behaved as in *A. pectinata*, and together formed the remaining pole of the root, the remaining cotyledon merely fused with its immediate neighbour and played no further part. Thus of the nine seed-leaves, six represent whole-cotyledons, two are half-cotyledons, and one is subsidiary.

The 7-arch root was reduced to a 6-arch structure by the gradual disappearance of one phloem mass, and the fusion of the two protoxylems originally separated by the obliterated bast.

Picea.

The cotyledons do not call for much comment. In transverse section they are triangular in shape; the mesophyll is very loose with a few secretory cells; the transfusion tracheides are not very numerous, and are more abundant in *P. ajanensis* and *P. morinda* than in *P. nigra*; resin ducts are absent; and fibrous elements occur on the dorsal side of the phloem, they are not, however, so numerous as in *Abies*. Each seed-leaf has one endarch vascular strand which shows no vascular rearrangement until entry into the hypocotyl has been gained. The cotyledons, at their basal regions, may fuse together laterally to form a more or less well-defined cotyledonary tube; *P. ajanensis* has the tube fairly well defined, *P. morinda* less so, and in *P. nigra* there is no lateral union of the seed-leaves.

Picea ajanensis. The seed-leaves vary in number from six to nine, for the genus Masters [1] states that the number of cotyledons is five to fifteen.

Series A. Six cotyledons. The endarch collateral bundles move towards the centre of the axis, and are arranged in three pairs (Diagram 3, Fig. 1, *a. b.*, *c. d.*, and *e. f.*), alternating with the three plumular traces derived from the foliage leaves from the first whorl (*p.* 1, *p.* 2, *p.* 3). The centripetal displacement continues so that the individuals of each pair of cotyledonary strands become contiguous; finally a complete vascular ring is formed. At a lower level the protoxylems of each pair of bundles rotate towards each other, the metaxylem, in two cases out of three, still being in continuity, and the phloem still forming a practically uninterrupted ring (Diagram 3, Fig. 2). This rotation of the xylem before the phloem and metaxylem has split is somewhat unusual, although it does sometimes occur in *Tsuga canadensis*. Then the original pairs of seed-leaf-traces separate so as to leave the protoxylem exposed and, at the same time, the fusion

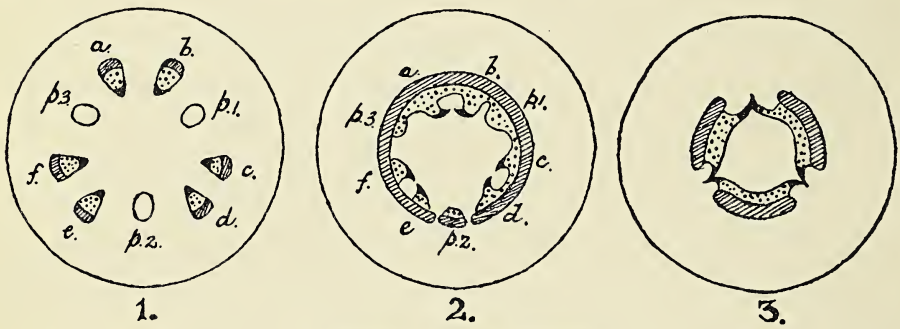


DIAGRAM 3. *Picea ajanensis.*

of the plumular bundles with the strands of the seed-leaves becomes so intimate that it is no longer possible to delimit their boundaries. There thus results three phloem masses with much metaxylem on their inner sides alternating with three groups of protoxylem elements (Diagram 3, Fig. 3). The completion of the rearrangement results in a triarch root-structure. From these facts it is clear that each seed-leaf represents a half-cotyledon.

Series B. Seven cotyledons. The transition was effected as in Series A, but instead of there being three pairs of cotyledon-traces, there were two pairs and a group of three. The root as before was triarch. The behaviour of the seed-leaf-strands showed that one cotyledon was subsidiary and the rest were half-cotyledons.

Series C. This seedling was very much younger than the rest and had eight seed-leaves, of which two were subsidiary and six were half-cotyledons. A triarch root was formed.

[1] Masters, loc. cit.

Series D. Nine cotyledons. The changes resulting in root-structure were the same as described for the previous individual, the vascular ring was, however, so close as to render the absolute identification of the separate bundles almost impossible. A triarch root resulted; and of the cotyledons, three may be considered subsidiary and the rest, half-cotyledons. The transition took place in a manner identical to that followed by *Abies pectinata* (see Diagram 2, Figs. 1, 4, 5, and 6).

Picea nigra, Link. The number of seed-leaves is six or seven, and the rearrangement of their vascular tissues takes place as in *P. ajanensis* and requires no further detailed description. Attention, however, may be drawn to one seedling (Series C). It has been seen that one seedling of *Abies sibirica* provided an example of a tetrarch root-structure becoming triarch in a very short space; this particular seedling of *P. nigra* illustrates the same change in a more marked degree. The plant had seven seed-leaves, the bundles of which behaved in a normal fashion; two gave rise to one pole of the root, and two, together with a subsidiary strand, gave rise to another, the remaining two commenced to rotate so that it was naturally expected that a triarch root would result. This, however, did not occur; the last pair of bundles mentioned, which commenced redistribution of their vascular elements, stopped the rearrangement so that a diarch root resulted. These changes recall the similar ones which occur in some species of *Mirabilis*.

Picea morinda, Link. The seed-leaves vary from eight to ten, and the transition-phenomena are the same as in *P. ajanensis*, a triarch root being formed.

Picea alba, Ait, is described by Dangeard [1]. The number of seed-leaves varies from five to seven, the mesophyll is homogeneous and resin ducts are absent. In the case of a seedling with six cotyledons, the transition resembles in all essentials that described above for *P. ajanensis*.

Cedrus.

The cotyledons are numerous, varying in number from nine to eleven, which is in agreement with the number found by Masters [2]. Their structure calls for but little comment; each has a single vascular bundle which is endarch throughout the whole length of the member. Transfusion tracheides in our material were absent; older cotyledons might exhibit them, for a certain number of immature elements occurred in places where they might be supposed to be formed. Fibrous elements occur on the dorsal side of the soft bast, and secretory cells also are abundant. Resin ducts are present in the seed-leaves of *C. Deodara*; but are absent in the cotyledons of *C. atlantica*. When present, there are two resin-canals in each leaf, one being situated in each of the dorsal corners.

[1] Dangeard: Le Botaniste, iii. 1892. [2] loc. cit.

Towards their bases the seed-leaves fuse together to form a particularly well-marked tube, on the inner side of which foliage leaves are inserted, which indicates that, in the seedling, the apex of the stem is depressed.

Cedrus atlantica.

Series A. The seed-leaf-traces enter the hypocotyl and gradually converge towards the centre, forming a closed ring of vascular tissue with the protoxylem groups in an endarch position. The transition in this species, and also in *C. Deodara*, is, in essentials, similar to that in *Abies pectinata* and species of *Picea*. It is, however, slower than in many of the preceding and foregoing plants. Eight of the bundles form four pairs, and the protoxylems of the vascular strands of each pair rotate towards one another and outwards, so that ultimately a tetrarch root-structure results, the phloem being interrupted opposite the exarch protoxylem masses.

The ninth bundle was much slower in its movements than the rest; ultimately it converged towards, and fused with one of the strands lateral to it, and its protoxylem also showed indications of an outward rotation. The fate of these particular elements was extremely hard to trace, for the adjoining bundles had already formed a pole of the root and their metaxylems were very compact. However, the appearances warrant the assumption that the protoxylem of this ninth bundle fused with the same tissue of the lateral strand mentioned above, thus this particular strand played the part of the bundle of a subsidiary cotyledon; but, at the same time it showed characters usually associated with the trace of a half-cotyledon.

Series B. This seedling had ten cotyledons, the bundles of which behaved in the same manner as the majority in Series A; six bundles paired to form the poles of the triarch root, and the remaining four played no important part in the transition, and are therefore to be classed as subsidiary.

One other individual (Series C) was also examined, but the vascular ring formed by the eleven cotyledonary bundles and the intervening plumular traces was so compact as to make it impossible to follow the changes with any degree of satisfaction. It is probable that of the eleven cotyledons, eight were half-cotyledons and three subsidiary; the resulting root-structure was tetrarch.

Cedrus Deodara. The number of seed-leaves is eleven, and the transition follows seemingly precisely that obtaining in *C. atlantica*. We add the slight qualification because the cotyledonary bundles are so numerous, and, in the hypocotyl, are situated so closely together that it is impossible to identify them with absolute certainty.

In the seedlings examined, the resulting root-structure was pentarch, which in one case became reduced to tetrarch.

Attention also may be drawn to another feature. In Series A there were eleven seed-leaves and the same number of leaves in the first foliage whorl of the plumule. Of these foliage leaves one was much larger than the rest, with a more massive vascular bundle which showed a greater vascular differentiation. Further, it fused on to the cotyledonary tube before any of its fellows, and its bundle took up a position slightly more external than the other strands; it also retained its vascular differentiation for a longer period than the other bundles of the first foliage whorl. The probable significance of this will be dealt with below (see *Pinus Pinea*, Series C).

Pinus.

The cotyledons vary in number from three to about twelve, and their structure does not differ, excepting in a few features, in certain species, from the foregoing plants. Resin ducts are generally present, the only two species in which they have not been observed being *P. halepensis* and *P. Coulteri*. In *P. Pinea*, *P. Gerardiana*, and *P. canariensis* each cotyledon has two resin ducts situated in the dorsal corners of the leaf; in *P. australis*, *P. insignis*, *P. contorta*, *P. contorta* var. *Murrayana*, *P. montana* var. *gallica*, and *P. sylvestris* each seed-leaf has one resin duct situated immediately on the dorsal side of the vascular bundle or else in between the two halves of the divided trace (see Diagram 4, Fig. 1). The general rule is for each cotyledon to have a single vascular strand; there are, however, exceptions, thus in *P. montana* var. *gallica* and *P. contorta* var. *Murrayana*, some of the seed-leaves have two separate vascular strands.

The cotyledonary bundles may show no rearrangement at all in the seed-leaf, as in *P. Pinea*, *P. Thunbergii*, *P. Gerardiana*, and *P. halepensis*; or they may undergo bifurcation of the phloem and rotation of the xylem before entry into the hypocotyledonary axis, as in *P. Coulteri*, *P. canariensis*, *P. australis*, *P. insignis*, *P. contorta*, *P. contorta* var. *Murrayana*, *P. montana* var. *gallica*, and *P. sylvestris*.

The bundles of *P. Pinea* and *P. Gerardiana*, very occasionally show one or two metaxylem elements on the ventral side of the protoxylem, the strands thus being very slightly mesarch. Fibrous elements on the dorsal side of the soft bast are generally present; and, finally, the majority of the species examined exhibit in varying degrees a lateral fusion of the cotyledons towards their bases; the best cotyledonary tubes were seen in *P. Pinea* and *P. canariensis*, in the other species the tube was either very short or incomplete.

Pinus Pinea. Excellent figures representing different stages in the germination of this plant are given in Sachs's Text-book.[1]

[1] Sachs: Text-book, Oxford, 1882, p. 508.

Series A and B. Eleven cotyledons were present each with one massive endarch collateral bundle, which became tangentially elongated as the cotyledonary node was approached.

The transition took place in the manner described for *Cedrus atlantica* and other plants; the seed-leaf bundles rotated in pairs giving rise to a pentarch root-structure, the remaining cotyledonary bundle took no part in the vascular rearrangement, and the seed-leaf to which it belonged thus is to be classed as a subsidiary member, while the rest are half-cotyledons. This agrees with the outline given by Strasburger [1], who mentions that the cotyledons vary from 11–4, and also with more extensive description by Dangeard [2] who gives many figures to illustrate the course of the bundles, and enters fully into histological details.

Series C. The number of seed-leaves was nine, eight of which formed a well-marked cotyledonary tube. The ninth also fitted into the tube, but was clearly out of place. In the hypocotyl the vascular strand derived from this ninth seed-leaf was situated within the zone formed by the other cotyledonary bundles, and was orientated obliquely. Gradually, it passed outwards, rotating on its own axis through an angle of about 90°, and fused with the nearest cotyledonary trace. From its behaviour and position we conclude that this ninth seed-leaf is in reality not a cotyledon at all, but a foliage leaf out of place.

This point, taken in conjunction with the peculiarity described in *Cedrus Deodara* (Series A), is of some interest as it shows a second way in which the number of cotyledons may have been increased.

We look upon the case of *Cedrus Deodara* as an earlier stage in the formation of a seed-leaf from a foliage member of the first plumular whorl. It may also be remarked that Tansley and Thomas, in the discussion on seedlings at the British Association Meeting at York, expressed their opinion that in some cases the number of cotyledons may be increased by the displacement of leaves of the first foliage whorl.

Pinus Thunbergii, Parl., closely resembles *P. Pinea.*

Pinus Gerardiana, Wall. The only seedling available had ten seed-leaves [3], the majority of the vascular strands of which behaved like those of *P. Pinea.* Some description, however, is necessary on account of the part played by one of the cotyledonary bundles. All the bundles entered the axis as collateral structures and formed, with the traces derived from the plumule, a close vascular cylinder. One seed-leaf-strand then bifurcated and rotated to bring its protoxylem into the exarch position. A resin duct very soon was formed opposite this protoxylem. The rest of the cotyledonary traces behaved as in *P. Pinea*, one being subsidiary and the rest

[1] Strasburger, loc. cit. [2] Dangeard, loc. cit.

[3] Masters (loc. cit.) gives 3–8.

rotated in pairs so that a pentarch root resulted. During these changes resin canals developed opposite the four remaining protoxylem groups in exactly the same way as in *P. Pinea.*

Pinus halepensis, Mill., closely resembles *P. Gerardiana*; there are, however, more whole-cotyledons in *P. halepensis*, and, further, the bifurcation and rotation of the bundles of these whole-cotyledons may take place at a higher level.

Pinus Coulteri, D. Don.

Series A. A well-marked tube was formed by the thirteen cotyledons [1], the bundles of eleven of which were endarch and collateral throughout; the remaining two showed bifurcation of the phloem and rotation of the xylem in different degrees, before an entry into the axis had been made, a feature not observed in any of the foregoing plants. Within the hypocotyl the phloem of another bundle quickly divided and, at the same time, the protoxylem commenced to take up its exarch position. Thus, there were three bundles which underwent bifurcation of the phloem, and a certain amount of rotation of the xylem: generally, these three bundles would form three poles of the root; but, in this particular case, two only of these particular strands behaved as would have been expected, the third, in the transition region, behaved as a half-cotyledon and with an adjoining bundle formed one pole of the root. The other strands acted as the bundles of either half- or subsidiary-cotyledons.

Series B. A similar phenomenon occurred in this seedling. There were eleven cotyledons forming a tube as before; and the bundle of one seed-leaf showed rotation of the xylem and bifurcation of the phloem while still contained within the cotyledon. Within the hypocotyl four other strands showed a similar rearrangement; of these one exhibited the redistribution of vascular elements very fully, but the change came to nothing, the bundle being doomed to play the insignificant rôle of a subsidiary cotyledon.

The theoretical importance of these facts will be dealt with, when like occurrences have been described in other plants.

Pinus canariensis, C. Sm. One plant only was available for examination, which differed in no important feature from *P. Coulteri.* One bundle only showed signs of the rearrangement of the vascular elements while the strand was still contained within the seed-leaf, and no trace showed the curious change especially mentioned as occurring in *P. Coulteri.* There were nine seed-leaves [2], of which two were whole-cotyledons, three were subsidiary-, and four were half-cotyledons. The root was tetrarch.

[1] According to Masters (loc. cit.), 10–14 cotyledons occur.

[2] According to Masters (loc. cit.), 6–8 cotyledons.

Pinus australis.

Series A. Nine cotyledons were present,[1] the bundles of all of which showed division of the bast and rotation of the xylem for the greater part of their length. Thus, at about the level of the cotyledonary node, there was in each seed-leaf a resin duct occupying a central position towards the upper surface; immediately ventral to this canal the protoxylem was situated, while on each side of the resin tube were the two groups of phloem bounded by metaxylem (Diagram 4, Fig. 1). The appearance of these strands within the axis would warrant the assumption that a 9-arch root would result, for the contiguous half-bundles have only to fuse to bring about the typical root-structure. But on tracing the bundles downwards,

DIAGRAM 4. *Pinus australis.*

it was found that a 7-arch root obtained, a result brought about by the obliteration of the half-portions of four bundles, and the fusion of the neighbouring protoxylems. This is illustrated in the accompanying Diagram 4, Figs. 2 and 3, where *p.* indicates the phloem; *mx.* represents the metaxylem, and *px.* stands for the protoxylem of the bundles of the eighth or ninth cotyledon. It will be seen that the vascular tissue of cotyledon 8 has two groups of phloem, two masses of metaxylem, and one collection of protoxylem elements situated on the inner side of a resin duct (*r.d.*). The same applies to seed-leaf 9. On tracing these structures downwards, the metaxylems pass more towards the centre of the axis, leaving the protoxylems fully exposed, any small amount of metaxylem, which would other-

[1] According to Masters (loc. cit.), 7–10 cotyledons.

wise be in the way, dies out. The neighbouring phloem masses (*p.* 8 and *p.* 9) become obliterated, the protoxylems of 8 and 9 approach, ultimately fuse, and, finally, the two resin ducts also effect a junction. The same series of changes takes place between the bundles of the sixth and seventh cotyledons. The rest of the strands behave normally; their protoxylems attain the exarch position before their entry into the axis, and it only remains for the contiguous masses of phloem to fuse in pairs; thus, there is formed a 7-arch root.

Series B. This seedling behaved in an exactly similar manner as regards the details of the transition, excepting for the fact that only two cotyledon-traces fused to form one pole of the root. The number of seed-leaves was nine and the root 8-arch.

Pinus insignis, Dougl.

Series A. The five cotyledons[1] each had the bifurcated bundle as described for *P. australis*; the top of the hypocotyl was thus occupied by ten groups of phloem elements bounded internally by metaxylem. Between each pair of bast bundles a resin duct was situated, on the inner side of which the protoxylem occurred. As the centripetal displacement of these strands took place, the metaxylem rotated further inwards, and the phloem masses fused in pairs, so that a pentarch root-structure quickly obtained.

Series B. Of the eight seed-leaves, all exhibited the divided bundle with the exception of one, the strand of which retained its undivided nature throughout its whole course. Within the axis, the same phenomena occurred as have been described for *P. australis.* Thus terming the cotyledonary traces *a*, *b*, *c* . . . *h*; *a* played a subsidiary part in the formation of the root-structure; *b* formed one pole of the root; *c* and *d* together formed one pole in the same manner as the bundles 6 and 7, and 8 and 9 of *P. australis* (Diagram 4); *e*, although its protoxylem contributed to form one xylem ray of the root, must be considered subsidiary; *f* formed one pole; and, finally, *g* and *h* showed an unequal bifurcation of the vascular elements, and behaved in an exactly similar manner to *c* and *d*.

Pinus contorta, Dougl., resembles *P. insignis* pretty closely, although the number of cotyledons is fewer. One plant (Series C) exhibited a very unequal bifurcation of one of the seed-leaf bundles; the smaller portion eventually died out, and the remaining part, together with the adjoining non-bifurcated strand, formed one pole of the root.

Pinus contorta, var. *Murrayana.*

The number of cotyledons varies from three to five. Of 25 seedlings examined 2 had three cotyledons (8 per cent.), 18 had four (72 per cent.), and 5 had five seed-leaves (20 per cent.). The transition in many

[1] According to Masters (loc. cit.), 6–9.

(Series A–F) took place in exactly the same way as in Series A of *P. insignis*, although the bifurcation of the phloem and rotation of the xylem occurred generally at a lower level in the cotyledons. The other seedlings showed certain variations which may be briefly alluded to.

Series G. Of the four seed-leaves, three behaved in the same manner as in the preceding individuals (Series A–F); but the fourth seed-leaf showed no rearrangement of the vascular elements until entry into the hypocotyl had been made: then, the phloem divided into two portions which passed one on each side away from the xylem, and ultimately fused with the adjoining phloem masses derived from the other strands. The metaxylem similarly divided and moved to each side, leaving the protoxylem exposed; there was no definite rotation of the protoxylem, the bundle followed in the fashion usually associated with *Juniperus*.

Series H. Here also, there were four seed-leaves, the bundles of three of which underwent the redistribution of the vascular tissues while still contained within the cotyledon. The strand of the fourth seed-leaf showed no such rearrangement to begin with, but at a slightly lower level signs of rotation of the xylem were seen, the movement, however, came to nothing, and the bundle finally behaved as a subsidiary structure.

Series I. This seedling, in its essential features, recalled the changes described above for *P. australis* and *P. insignis* (Series B). The bundles of two of the cotyledons showed the divided phloem and exarch position of the protoxylem before the cotyledonary node was reached; the vascular strands of the remaining two seed-leaves exhibited a similar rearrangement at a lower level within the hypocotyledonary axis. But the division of these two bundles was very unequal; the two smaller neighbouring portions practically died out, and the two remaining parts together formed one pole of the triarch root (Figs. 1 and 2, Pl. XV). The two seed-leaves from which these bundles were derived are thus to be looked upon as half-cotyledons.

Series J. The four seed-leaves of this seedling were of unequal size, one (Diagram 5, Fig. 1 *a*) was larger than the rest, *b* was slightly smaller than *a* but larger than *c* and *d*, which were equal in size. The distal region of *a* showed two small bundles, the phloem masses of which fused at a lower level and, later, the xylem elements of each became joined by the formation of new metaxylem in the intervening space (Diagram 5, Fig. 2). At a much lower level this bundle underwent the usual bifurcation, and so also did the strand of the neighbouring seed-leaf *b*. In each case a resin duct occurred between the two separated portions of the bast. The traces of *c* and *d* showed no such division; but, just above the cotyledonary node, they rotated slightly in such a manner that their protoxylems pointed towards one another. Further, *c* had no resin duct, this structure was, however, present in *d* and occurred on that side of the bundle towards *e*

(Diagram 5, Fig. 3). These two bundles, within the hypocotyl, quickly approached one another, and, as they did so, the rotation became more and more marked; finally they together formed one pole of the triarch root, the other two being produced by the bundles of *a* and *b* (Diagram 5, Figs. 4 and 5). From this it is clear that this particular seedling is of some interest;

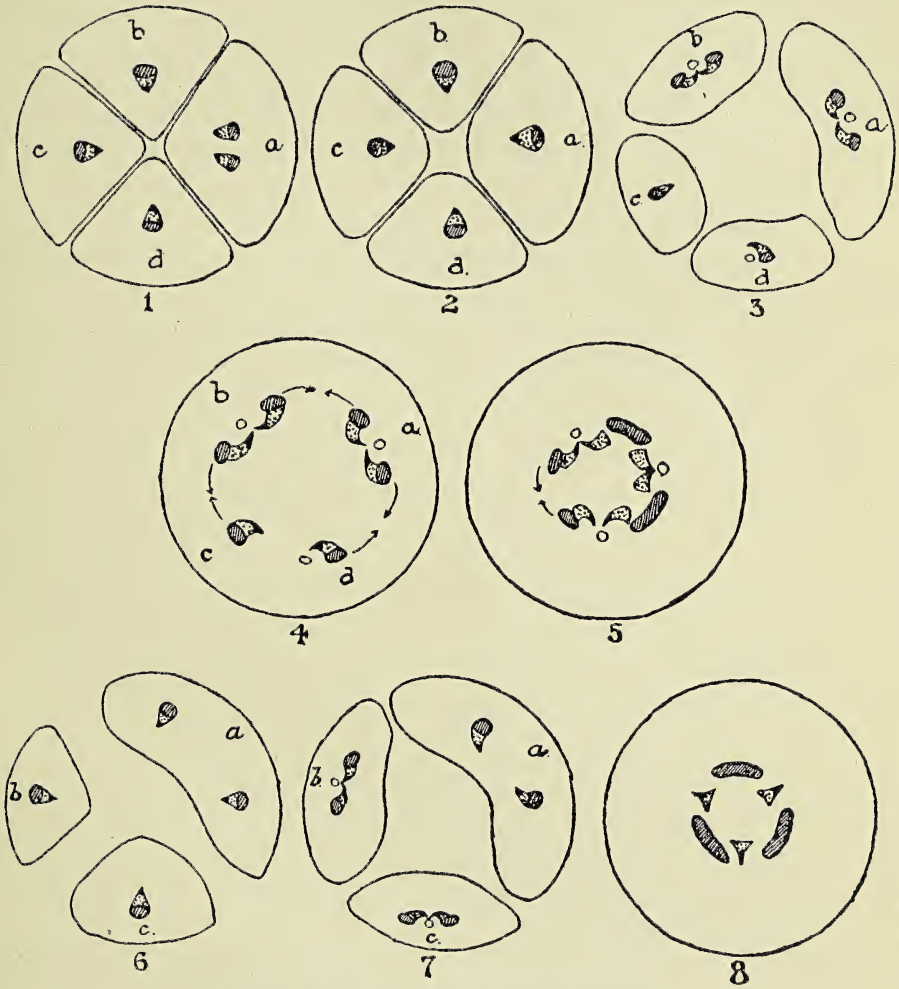


DIAGRAM 5. *Pinus contorta*, var. *Murrayana*.

the division of the bundle of *a*, in its apical region, may be looked upon as the initial step towards the splitting of this cotyledon to form two seed-leaves; the consummation of which is clearly indicated in the cotyledons *c* and *d*.

Series K showed an advance upon the last seedling. There were three seed-leaves, one of which was about twice as large as the others.

This cotyledon showed no external signs of a division, but the examination of the sections showed that there were, throughout its whole length, two distinct bundles, well separated one from the other, the structure strongly recalling the condition which obtains in *Ephedra* (Diagram 5, Fig. 6, *a*). The two strands gradually approached one another and entered the axis as entirely distinct endarch structures: no bifurcation took place, but the xylem of each rotated towards the other and outwards; thus the exarch position of the protoxylem was assumed. The phloem of each strand fused with the adjoining bast derived from one of the other seed-leaves *b* and *c*, the bundles of which showed the essential rearrangement of the vascular tissues while still contained within the seed-leaves (Diagram 5, Figs. 7 and 8).

Pinus montana, var. *gallica*.

The majority of the seedlings examined had four cotyledons, one only possessed three.[1]

Series A. The transition in this plant followed, on the whole, a normal course. It is only necessary to draw attention to two features. In one

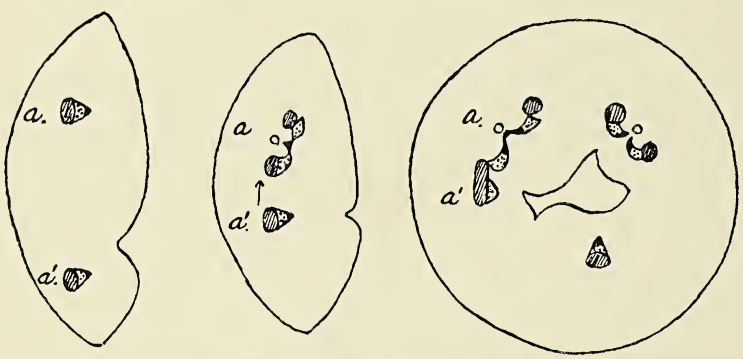


DIAGRAM 6. *Pinus montana*, var. *gallica*.

seed-leaf, the bifurcation of the phloem and rotation of the xylem took place, not within the seed-leaf, but during the inward passage towards the centre of the axis. Another cotyledon-trace showed this rearrangement of the vascular tissues within the seed-leaf, but the division of the bundle was unequal (Fig. 3, Pl. XV).

Series B. Two of the three cotyledons showed signs of splitting at their apices. Each of these seed-leaves had two vascular strands well separated one from the other. The behaviour of the bundles of one of these cotyledons was exactly the same as those of the seed-leaf *a* of *Pinus contorta*, var. *Murrayana*, Series K (Diagram 5, Figs. 6–8). The changes exhibited by the pair of bundles of the other cotyledon are illustrated in Diagram 6, from which it will be seen that the two strands (*a* and *a'*) approach one another and, at the same time, *a* undergoes bifurcation of the

[1] Masters (loc. cit.), stated that the species has five cotyledons.

phloem and rotation of the xylem, a rearrangement of tissues which was not shown by *a'*. Very quickly a junction between the phloem of *a'* and half the bast of *a* was effected, and in this condition the vascular strands entered the axis. A pole of the root-structure was entirely formed by the elements derived from the strand *a*, the other bundle *a'* played no part. This means that one bundle (*a*) is the equivalent of the trace of a whole cotyledon, while the other (*a'*) represents the bundle of a subsidiary seed-leaf.

It only remains to be remarked, that the bundle of the third cotyledon first showed the characteristic redistribution of the vascular tissues within the hypocotyl, not in the cotyledon. This seedling thus shows three cotyledons on the way to become five.

Series C. This seedling, in many respects, resembled the foregoing individual. There were four seed-leaves, the bundles of two of which behaved normally ; one as that of a whole-cotyledon, and the other as that of a subsidiary seed-leaf, although the xylem of this last strand retained an abnormal and isolated position beside one of the poles of the diarch root. The two remaining cotyledons fused together at some little distance above the cotyledonary node ; so that in transverse section the appearance of this single structure resembled the seed-leaf illustrated in Diagram 6. Of these two strands one bifurcated very unequally ; that part of the bundle situated nearest the other strand being very small (see Fig. 3, Pl. XV). The subsequent events resembled those described for Series B sufficiently closely to render any further description unnecessary.

Series D showed similar phenomena, as regards the essential features.

Pinus sylvestris, L.

The following table shows the variation in the number of the cotyledons ; it will be observed that the number varies from 3 to 8, which agrees with the observations of Masters [1] ; and that seedlings with six seed-leaves are the commonest. Dangeard [2] gives 6–9 as the range in the number of the cotyledons, and enters somewhat fully into the structural details.

No. of cotyledons.	*No. of seedlings.*	%
3	2	0·21
4	28	2·97
5	171	18·11
6	500	52·97
7	209	22·14
8	34	3·60
	Total 944	

The majority of the seedlings examined showed transition-phenomena of exactly the same nature as in many of the foregoing plants, especially

[1] loc. cit. [2] Le Botaniste, iii, 1892.

Pinus contorta var. *Murrayana* and *P. montana* var. *gallica*, and agrees with the short description given by Gerard,[1] so that the table at the end of this paper, showing the relationships between the cotyledons and the root-structure, will indicate sufficiently well the vascular rearrangements observed. The following three seedlings require a more detailed description.

Series G. Six seed-leaves were present, of which two were subsidiary, two showed the bifurcation and rotation of the bundle very clearly and one underwent a similar but very unequal division. The smaller portion of this unequally bifurcated bundle died out, and the remaining part eventually

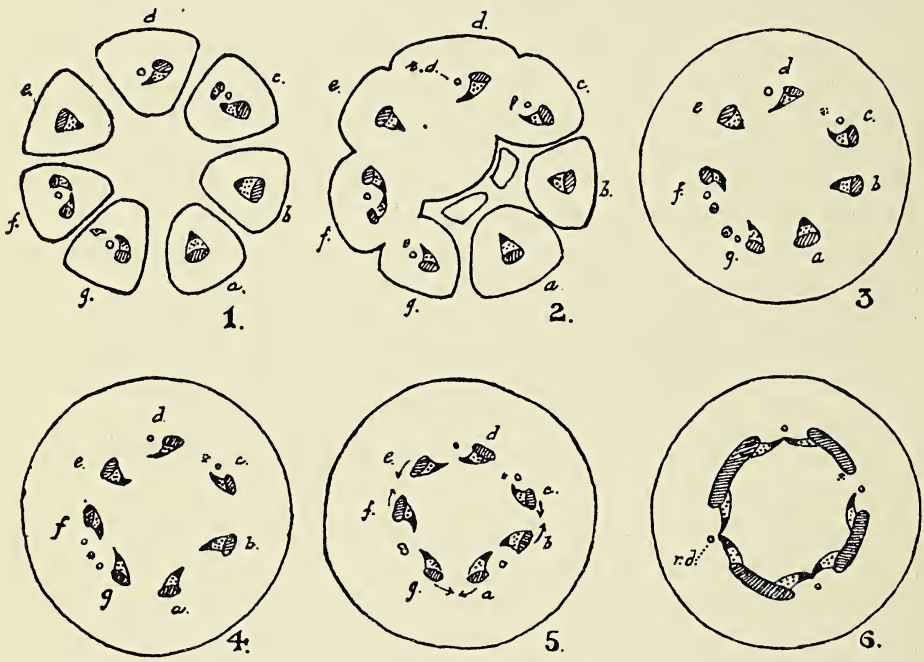


DIAGRAM 7. *Pinus sylvestris.*

combined with the strand of the adjoining half-cotyledon and formed one pole of the root. This is similar to what occurs in some seedlings of *P. contorta* and *P. insignis*.

Series H. The behaviour of the seed-leaf-traces of this seedling was of a complex character, and is illustrated in the accompanying Diagram 7.

The bundles of the seed-leaves showed various vascular rearrangements; it will be observed (Diagram 7, Fig. 1) that the bundles *a*, *b*, and *e* are endarch; *d* has its protoxylem turned towards *e*, and there is a resin duct immediately lateral to it; *c*, *f*, and *g* show the bundles to be bifurcated, but very unequally, and, in the case of *g*, the bifurcation only affects the xylem.

[1] Gerard: Recherches sur le passage de la racine à la tige (Ann. Sci. Nat., Bot., xi, 1881).

Tracing these strands downwards *a*, *b*, *d*, and *e* act as the bundles of half-cotyledons. The trace *d* may be placed on a plane slightly higher than *a*, *b*, and *e*, since it possesses the dignity of a resin duct. As regards the other bundles, *g* soon loses its smaller group of xylem elements and combines with *f* to form a pole of the root, the intervening half-bundle of *f* having died out (Diagram 7, Figs. 2–5). The two seed-leaves to which they belong are therefore half-cotyledons. The bundle *c* was of a somewhat undecided character, the phloem of the smaller portion of the bifurcated bundle speedily disappeared (Diagram 7, Fig. 2), and, at a lower level, the corresponding xylem-mass followed suit (Diagram 7, Fig. 4). From this it would be supposed that it would ultimately act as the strand of a half-cotyledon, but at a still lower level (Diagram 7, Fig. 5) the missing xylem reappears and the whole bundle forms one pole of the tetrarch root.

Series M had seven cotyledons, but it is only necessary to draw attention to the bundle of one of them. This strand showed the bifurcation, but the half-bundle of one side soon disappeared; it would therefore be imagined that its performance would be that of the trace of a half-cotyledon, but this was not the case. The whole of the remaining phloem passed over and fused with the similar tissue of the adjoining bundle, and a rearrangement of the xylem took place so that the protoxylem became exarch in position. This is something like what obtained in bundle *c* of series H of this same plant.

Pinus maritima. The structure of the seedling of this plant has been worked out by Chauveaud,[1] whose account shows that this species resembles *P. sylvestris*, *P. contorta*, var. *Murrayana*, &c., very closely as regards the features under consideration.

He points out that there is no constant relation between the number of cotyledons and the root-structure; for instance, a plant with five seed-leaves may have a pentarch root, while a seedling with seven cotyledons may have a tetrarch root-structure; generally, the seed-leaves are more numerous than the bundles of the root. Further, he recognizes different kinds of cotyledons; the primitive ones (corresponding to our whole-cotyledons), the bundles of which show a vascular rearrangement, viz. the bifurcation of the vascular tissue and the assumption of the exarch position by the protoxylem, as they are traced from the apex downwards; and the non-primitive seed-leaves (corresponding to our subsidiary-cotyledons), in the bundles of which no such rearrangement obtains, they being collateral throughout. They are further characterized by the absence of a resin canal.

Dangeard [2] also briefly alludes to this species, and gives figures of the seedlings and the course of the bundles.

[1] Chauveaud: Passage de la disposition primitive à la disposition secondaire dans les cotylédons du Pin maritime. (Extr. Bull. Mus. Hist. Nat., Paris, 1902.)

[2] Dangeard: Le Botaniste, iii, 1892.

Pinus laricio, Poir, is also described by Dangeard.[1] The cotyledons vary from eight to ten, and the vascular rearrangements show the same variations as we have found in other species of the genus.

Larix.

The structure of the seed-leaves requires but a short description. Resin ducts have not been observed in *L. occidentalis*; they are present in *L. leptolepis* and *L. europaea*, in which plants each cotyledon has two canals, situated one in each dorsal corner of the leaf. Each seed-leaf has a single vascular strand; there may be a little variation in this respect, for one plant showed two separate bundles in one cotyledon similar to what has been described above for *Pinus montana*, var. *gallica* and *P. contorta*, var. *Murrayana*. The vascular bundle is endarch and collateral throughout the whole length of the member, and transfusion tracheides have not been observed. Finally, the cotyledons tend to fuse laterally at their bases to form more or less complete tubes.

Larix occidentalis, Nutt. The only seedling available had six cotyledons, each of which had a single endarch collateral bundle. The vascular rearrangements all took place in the hypocotyledonary axis and resembled those described for *Pinus Gerardiana* and *P. halepensis* very closely. Thus of the six seed-leaves, the behaviour of the bundles showed one to be a whole-cotyledon, four to be half-cotyledons, and the remaining one subsidiary.

Larix leptolepis does not differ in any essential feature from *L. occidentalis*.

Larix europaea, DC.

Series A and B. Six seed-leaves were present, the bundles of which showed the same rearrangements as have been described for *Abies pectinata* and other plants; in brief, two pairs of bundles rotated so as to form the two poles of the diarch root, and the remaining bundles merely fused on to the others, taking no part in the transition. Dangeard[2] states that the transition resembles *Picea alba*.

Series C. In this seedling there were seven cotyledons, the bundles of which behaved somewhat differently from any of the foregoing plants. The first figure of Diagram 8 shows the seed-leaf-traces *a*, *b* . . . *g* arranged in a zone in the upper part of the hypocotyl. On tracing the strands downwards, *b* and *c* approach one another and each fuses with the vascular tissue on either side, their protoxylems rotate towards each other and outwards and form a pole of the triarch root, the strand *b* contributes most of the protoxylem. These strands are therefore derived from half-cotyledons. The bundle *a* very soon bifurcates, one half passing over and fusing

[1] Dangeard: Le Botaniste, iii, 1892. [2] loc. cit.

with *g* and the other portion joining with a small plumular trace (*pl. t*). The two compound bundles thus formed (Diagram 8, Fig. 3, $g + \frac{1}{2}a$, and *pl. t* + $\frac{1}{2}a$) behave as half-cotyledons and form one pole of the root. The fusion between the half portion of *a* and *g* is very intimate, which makes the allocation of values rather difficult. It is simpler to consider *a* as the strand of a whole-cotyledon, and *g* as that of a subsidiary seed-leaf. The bundles *d*, *e*, and *f* all converge ; *e* then bifurcates, one-half joining up with *d* and the other part fusing with *f* (Diagram 8, Fig. 2). The protoxylem of *e* is left *in situ* (Diagram 8, Fig. 3) for a time, but at a lower level it

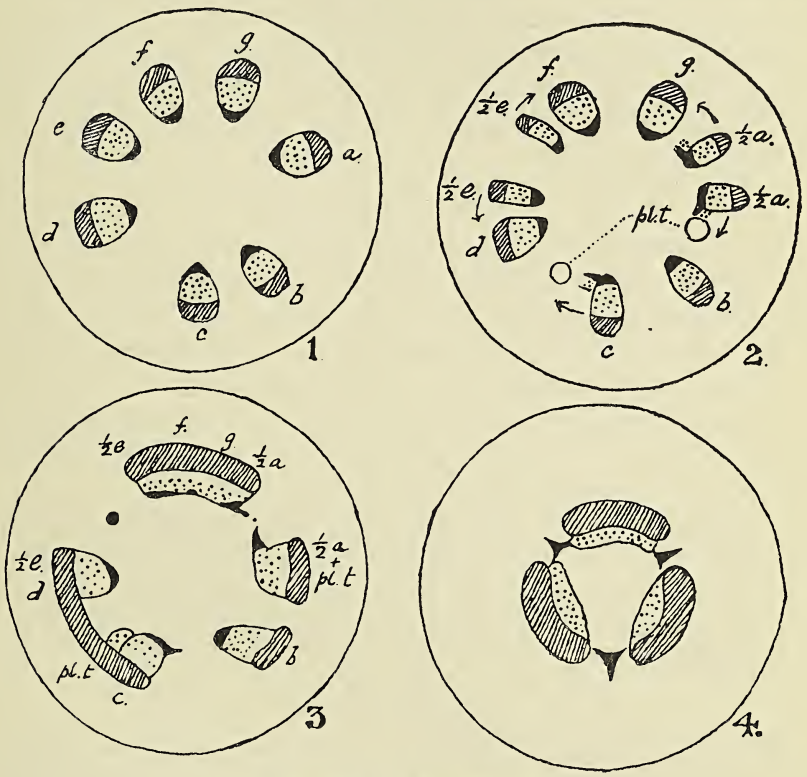


DIAGRAM 8. *Larix europaea.*

passes over to one side. Here, again, the fusion of the two parts of *e* with the strands *d* and *f* is very complete, and the combined protoxylems of the compound bundles, $d + \frac{1}{2}e$, and $f + \frac{1}{2}e$, rotate to form the remaining pole of the root (Diagram 8, Fig. 4).

In all the foregoing plants no difficulty has been encountered in determining whether any particular strand is the bundle of a whole-cotyledon, or of a half-cotyledon, or of a subsidiary seed-leaf; in the case of a whole-cotyledon the bifurcation and rotation of the two xylem masses is generally very strongly marked, but in this particular seedling the strands *a* and *e*

fuse up with adjoining structures before any rotation begins so that it is possible to give values other than those assigned; for instance *d*, *e*, and *f* might conceivably be the strands of a cotyledon which had split into three parts, so that the existing structures are really the third portions of a cotyledon.

Pseudolarix.

Pseudolarix Kaempferi, Gord. The number of cotyledons is four or five, which resemble in their structure those of *Larix leptolepsis* and *L. europaea*. No cotyledonary tube is formed, and no tendency towards the basal fusion of the seed-leaves has been observed.

The transition takes place entirely within the hypocotyledonary axis, and it resembles very closely that already described for *Tsuga canadensis*, *Abies sibirica*, and other plants; further description, therefore, is uncalled for.

ARAUCARIEAE.

The seedlings of the Araucarieae naturally fall into two groups; those of the section Eutacta characterized by a slender hypocotyl and two to four epigeal cotyledons, e. g. *A. Cunninghamii*, *A. Cookii* and *A. excelsa*; and those of the section Colymbea distinguished by the possession of a more or less tuberous hypocotyl and two hypogeal seed-leaves, e. g. *A. brasiliensis*, *A. imbricata*, and *A. Bidwillii*.[1] The number of the cotyledons appears to be fairly constant; Masters[2] gives two to four, and Richard[3] states that two is the normal number for *A. imbricata*.

Exceptions, however, occur; thus, according to Richard,[3] one or three seed-leaves may occur in *A. imbricata*. Strasburger[4] mentions that three or four may be present in the same plant, while Dangeard[5] states that three may be present. We ourselves have observed similar abnormalities in *A. Cunninghamii*.

Araucaria.

The seeds of several species of this genus, and also of *Agathis*, were planted, but two species only germinated, *Araucaria Cunninghamii* and *A. brasiliensis*.

A. Cunninghamii, Forbes. The external appearance of a normal seedling of this plant is illustrated in Fig. 4 *a*, Plate XV. The cotyledons are epigeal and, apparently, four in number, arranged in two well-marked

[1] See Seward and Ford: The Araucarieae, recent and extinct (Phil. Trans. Roy. Soc. Lond., B. 247, 1906).

[2] Masters: Notes on the Genera of Taxaceae and Coniferae (Journ. Linn. Soc. Lond., Bot., xxx, 1895).

[3] Richard: Commentatio botanica de Coniferis et Cycadeis (Stuttgart, 1826).

[4] Strasburger: Die Coniferen und die Gnetaceen (Jena, 1872).

[5] Dangeard, loc. cit.

pairs. A more careful examination shows that the members of each pair fuse together immediately above the cotyledonary node, thus having the appearance of a two-pronged fork with a short handle, which suggests that each pair has been derived by the longitudinal fission of a single structure. Fig. 4 *b*, Plate XV, represents an abnormal seedling with seemingly three cotyledons; really, one with two slender prongs and one single member.

Structure. The epidermis is covered with a thick cuticle, and so also is the outer surface of the guard-cells of the stomates, which are sunken well

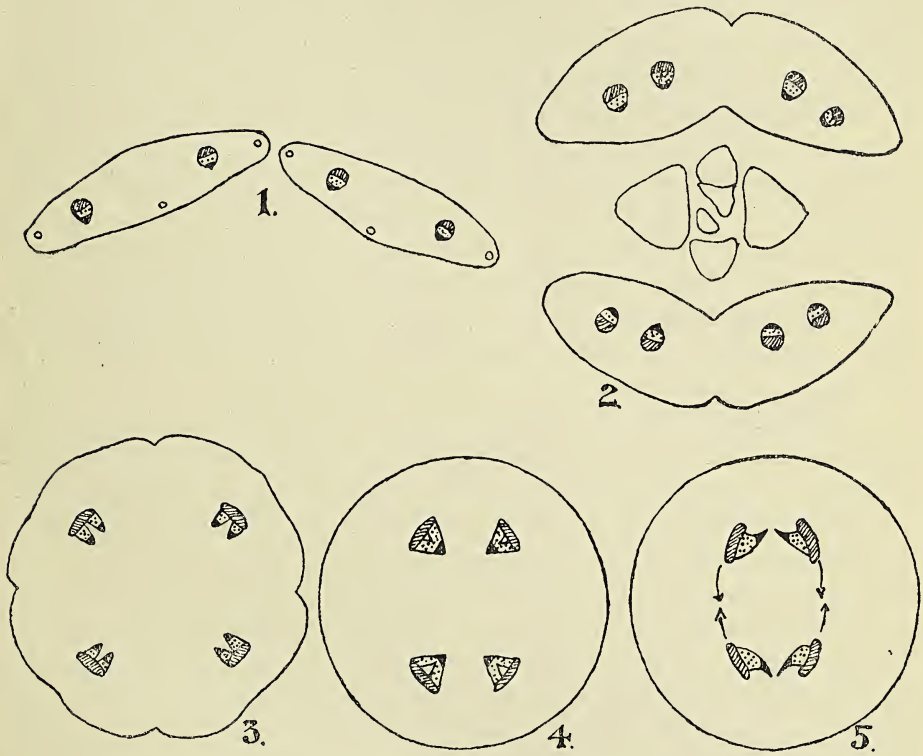


DIAGRAM 9. *Araucaria Cunninghamii.*

below the general level of the epidermis; secretory cells are abundant and each prong of a cotyledon has three resin ducts in the upper region, situated one at each end and the third just beneath the hypodermis in the middle line of the ventral side (Diagram 9, Fig. 1). In the lower regions the resin ducts become more numerous, some being situated in the mesophyll. The vascular bundles are endarch and collateral throughout the whole length of the cotyledon and, at the extreme tip, consist only of tracheae. Transfusion elements are fairly common, more especially in the apical region.

Transition.

Series A and B. Each fork of the bifurcated cotyledons contains two vascular strands, well separated one from the other (Diagram 9, Fig. 1); in the basal region of each cotyledon, below the fork, there are obviously four bundles arranged in two pairs (Diagram 9, Fig. 2). No union of bundles takes place in the cotyledons themselves, so that eight vascular strands arranged in four pairs enter the hypocotyl. The individuals of each pair of bundles, derived from the four prongs of two-forked cotyledons, continue to approach one another and ultimately fuse; the union of the two phloem-groups takes place first, then the junction of the protoxylems is effected. The two groups of metaxylem elements remain separated by parenchymatous elements; thus the wood has a V-like appearance (Diagram 9, Figs. 3 and 4). The four bundles thus produced gradually rotate and become situated in a position roughly at right angles to the plane of insertion of the cotyledons. The actual transition takes place very slowly; the two bundles derived from one cotyledon approach one another, and their protoxylems rotate towards the exarch position. Concurrently, each xylem-mass becomes more compact (Diagram 9, Fig. 5), and the corresponding portions ultimately fuse together; finally, the opposing phloems effect a junction so that a diarch root results.

Series C. This seedling differed from the rest of this species inasmuch as there were but three apparent cotyledons. The structure showed that really there were two seed-leaves, one undivided and the other one forked.

The rearrangements of the vascular strands of this specimen differed from those of the previous Series, and are illustrated in Diagram 10. The first figure shows that one prong of the bifurcated cotyledon had one bundle *a*, and the other portion two, *b* and *c*. The other cotyledon had five bundles instead of four. Eight bundles entered the hypocotyl as in the foregoing Series, but their arrangement was different (cf. Diagram 9, Fig. 3, and Diagram 10, Fig. 3). In the upper region of the axis the traces *b* and *c* rapidly approached one another and fused (Diagram 10, Fig. 3), and, at a lower level, this compound bundle fused on to the strand *a* (Diagram 10, Fig. 4). Thus there were six bundles, five in one group derived from the undivided seed-leaf, and one derived from the forked cotyledon. The five strands fused together (Diagram 10, Fig. 5) and then separated out as two single structures (Diagram 10, Fig. 6), which behaved in the same way as in the previous Series and formed one pole of the diarch root. The single strand $a+b+c$ underwent a lateral displacement, and its protoxylem commenced its rotation towards the exarch position; then, in a corresponding position on the other side of the hypocotyl, a new group of phloem elements appeared which gradually became more abundant, and were followed by the appearance of xylem (Diagram 10, Figs. 7 and 8). A new bundle was

thus organized which balanced the original single structure, and the two together formed the other pole of the diarch root.

The facts of the transition corroborate the view, based on the external morphology, that there are really only two cotyledons in this species ; and as the resemblance between the seedling of this species and those of

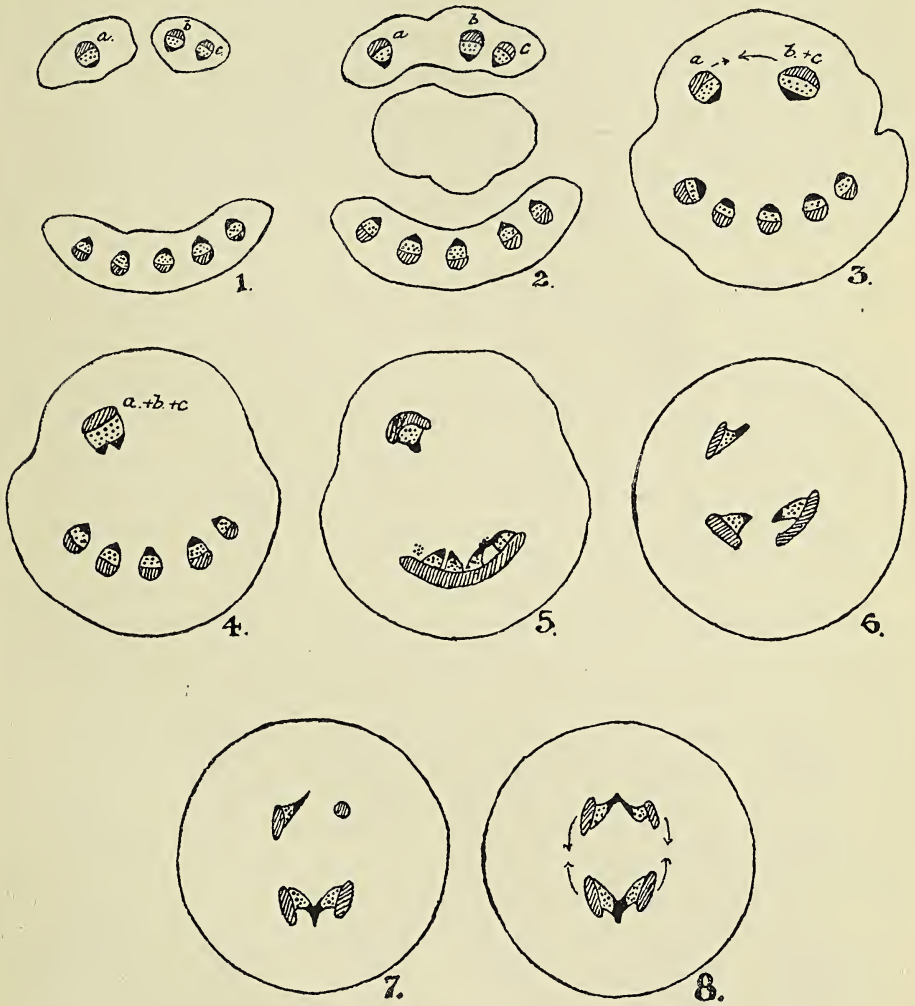


DIAGRAM 10. *Araucaria Cunninghamii.*

A. excelsa and *A. Cookii*, as indicated by the published illustrations,[1] is extremely close, the description of the species of the section Eutacta appears to require some emendation. Rather they should be described as having typically two cotyledons, each of which is forked. At the same time

[1] See Seward and Ford, loc. cit.

this feature may prove to be very obscure in some species, for the fission only requires to be extended a very little further to completely divide each seed-leaf into two.

Araucaria brasiliensis.

The seedling of this species differs from that of *A. Cunninghamia* in the following more or less important features :—it is hypogeal ; the cotyledons are two in number, unbranched and rather massive ; the hypocotyl is relatively much thicker. The internal structure also differs ; each cotyledon has more vascular strands, five, six, or seven, instead of four as in *A. Cunninghamii* ; the resin ducts are more numerous and are not restricted to the peripheral regions. The presence of stomata and transfusion tracheides are points of similarity to *A. Cunninghamii*, and so also is the character of the bundles which are endarch and collateral throughout the whole length of the seed-leaf.

Transition.

Series A. Although in the more distal parts of the cotyledons the vascular strands may number more than six, six enter the axis, the reduction being effected by the fusion of certain of them. In the hypocotyl the most laterally placed bundles fuse with their neighbours so that there are eight traces, arranged in two groups of four (Diagram 11, Figs. 1–5). At a lower level each group of four bundles fuse in pairs, although, in the seedling now being described, this was masked to some extent by the presence of well-developed plumular traces.

In the upper region of the hypocotyl the plumular traces form an uninterrupted vascular cylinder enclosing a mass of parenchymatous tissue (Diagram 11, Fig. 3) ; at a lower level this vascular ring breaks up into four parts, two of which are situated in a plane at right angles to that of the cotyledons and the others are placed one in each gap (Diagram 11, Fig. 4). The disposition of the bundles obtaining at the commencement of the transition is illustrated in Diagram 11, Fig. 4, from which it may be seen that the cotyledon-traces are arranged in two main groups (*c* and *c* 1), each group being further divided by an intervening plumular strand (*p* and *p* 1) ; there are thus four pairs of seed-leaf-traces, the individuals of one pair of which have joined together. The space between the two large groups of four is bridged by the elongated masses of epicotyledonary vascular tissue. Tracing these structures downwards, the cotyledonary traces fuse in pairs, move inwards, and join on to the large masses of plumular vascular tissue (*e* and *e* 1). A comparison of Figs. 5, 6, and 7 in Diagram 11 shows that the details of this movement are not precisely the same on each side of the axis. The cotyledon-traces *c*, on the left side, fuse with the intervening plumular strand *p*, and forming a broad band of vascular tissue which, however, soon separates into two parts, one half joining with *e* and the other portion with *e* 1. The strands, on the right side, *c* 1, join up with *e* and *e* 1

without any previous fusion and subsequent splitting. These changes lead to the formation of two bands of vascular tissue, each of which immediately divides into two parts. Avoiding subsidiary features for the time being, these

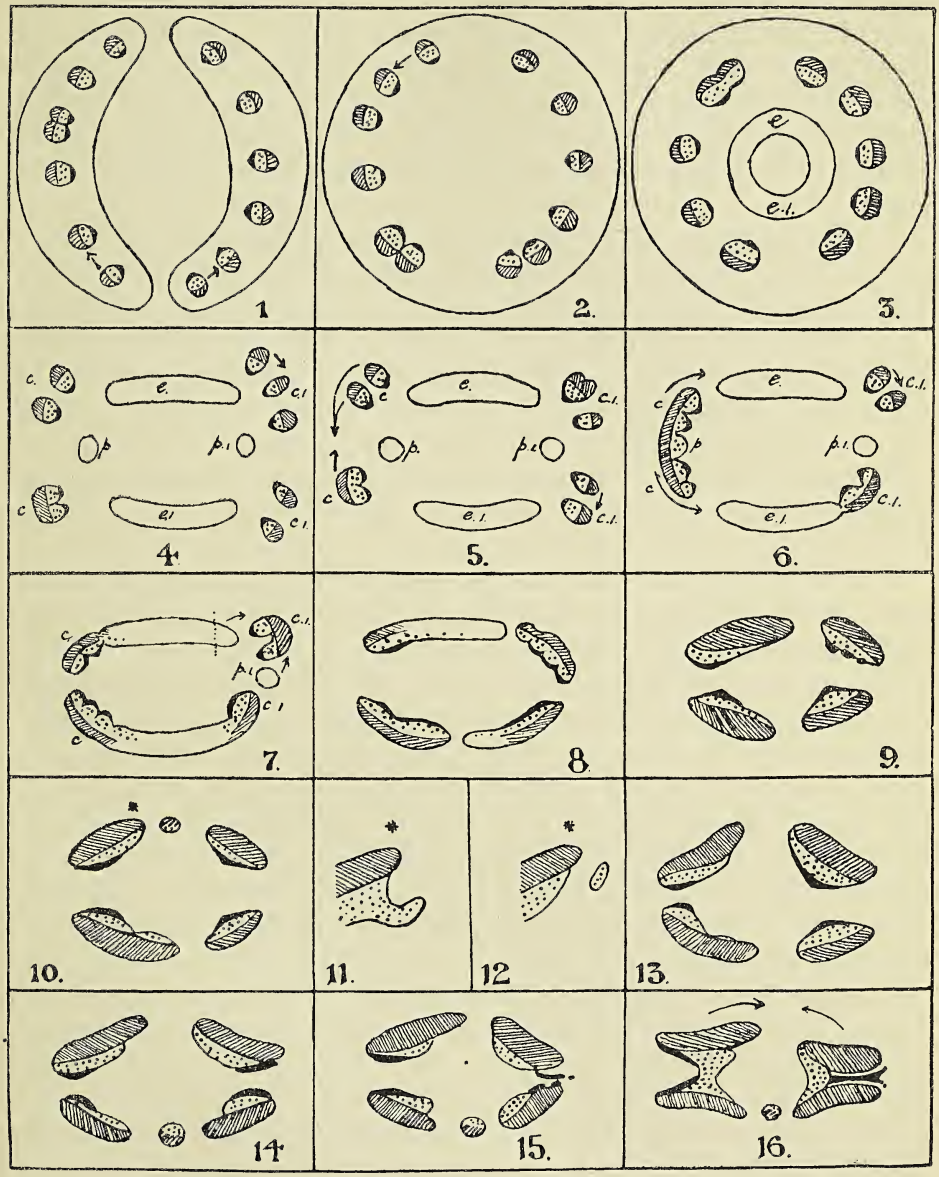


DIAGRAM II. *Araucaria brasiliensis.*

four strands each have an endarch protoxylem, a point to which Masters[1] has drawn attention, which gradually becomes concentrated towards the outer parts until its position is fully exarch (Diagram II, Figs. 8–10 and

[1] Masters, 1891, loc. cit.

13–15). At the same time the opposing bundles move towards the cotyledonary plane until their metaxylems are contiguous (Diagram 11, Fig. 16); at a lower level the corresponding groups of phloem elements join together; thus a diarch root-structure obtains. A comparison of the critical stages shows that the transition phenomena of *A. brasiliensis* are essentially like those of *A. Cunninghamii*, although, in the former plant, they appear more complicated owing to the greater number of cotyledonary vascular bundles, and the large amount of plumular vascular tissue. This similarity may be seen by a comparison of figures 5, 6, 13–16 in Diagram 11 with Figs. 2–5 of Diagram 10.

The resemblance is the more marked when reference is made to Strasburger's [1] brief description of the transition-phenomena in this same species (*A. brasiliensis*), which account describes what we have found to obtain in *A. Cunninghamii*, with the exception that in the latter plant there are four bundles for each cotyledon, and in *A. brasiliensis* there are eight. We ourselves found fewer vascular strands, but it is likely that eight is the typical number for each seed-leaf of the hypogeal forms, for Seward and Ford [2] give this number for the cotyledons of *A. imbricata*; on the other hand Dangeard [3] states that seven strands occur in the seed-leaves of this species (*A. imbricata*).

To return to the minor features in the transition-region of *A. brasiliensis* (Series A); there is a tendency for some of the vascular strands to detach portions which gradually become smaller and die away at lower levels; in other words, the large bundles give off smaller branches which end blindly (Diagram 11, Figs. 10 and 14). At a much lower level a branch, consisting only of xylem, and very much smaller than those mentioned above, was given off and appeared in transverse section as a finger-like process. This strand underwent an exarch displacement, and followed a sinuous course downwards, being, at different levels, alternately attached to and detached from the main bundle from which it took its origin. Ultimately, it died away (Diagram 11, Figs. 11 and 12, in which the portions illustrated are of the strand marked with an * in Fig. 10).

Series B. This seedling was in the stage represented in Figure 5, Plate XV. Secondary thickening was well advanced, and each cotyledon had five vascular bundles at its base. Some of these strands, in the cortical region of the uppermost part of the hypocotyl, fused together so that two groups of four bundles resulted. The transition took place in the manner described above for Series A.

The seedling anatomy of *A. imbricata* has been described by Dangeard [4] and Seward and Ford.[5] Dangeard states that at the base of the cotyledons the bundles unite tangentially to form two large elongated strands, which in the hypocotyl divide into two. Thus there are four large bundles which

[1] loc. cit. [2] loc. cit. [3] loc. cit. [4] loc. cit. [5] loc. cit.

form the diarch root-structure in the manner already indicated. The account given by Seward and Ford for this same plant does not agree with that given by Dangeard in respect to the union of the cotyledonary bundles at the base of the seed-leaves. It appears, however, that there may be some variation as regards this particular feature, for we have described a tangential union of the seed-leaf-traces in *A. Cunninghamii* (Series C), and on one side of the seedling in *A. brasiliensis* (Series A). With this exception there is no material difference, and a comparison of the descriptions and corresponding illustrations warrants the conclusion that *A. imbricata* does not differ in any material feature from *A. brasiliensis*.

If the rearrangements of the vascular strands in these seedlings of *Araucaria* be traced in the opposite direction, from the root upwards, they may be summarized thus:—each pole of the diarch root gives off two bundles, which, during their passage to the cotyledons, first rotate to bring the protoxylems into the endarch position and then dichotomize, once in the case of *A. Cunninghamii*, and ideally twice in the case of *A. brasiliensis*, and some other hypogeal forms, so that the former has four bundles in each seed-leaf and the latter eight, subject to reduction in some instances.

If reference be made to *Podocarpus*,[1] it will be seen that there is a marked resemblance in the actual transition between that plant and *Araucaria*; in each case the upper regions of the hypocotyl are occupied by four cotyledonary bundles, two on each side, which rotate and give rise to a diarch root-structure, a fact which is clearly brought out in the relative figures. The only difference is that each seed-leaf in *Podocarpus* has two bundles, while in *Araucaria* there are typically either four or eight.

Conclusions.

The chief immediate conclusion arrived at by the study of the seedling-structure of the Taxaceae and Araucariaceae is that the polycotyledonous condition, in the majority of cases, has been attained by the longitudinal division of the pre-existing single members which were in all probability two in number.

Before reviewing our evidence upon which this conclusion is based, some mention must be made of earlier work, which directly bears upon the subject.

The botanists of the earlier part of the nineteenth century were divided in their opinions regarding the nature of polycotyledony. Adanson and Jussieu both believed that the multiplicity of seed-leaves was more apparent than real, and that it had arisen by the division of two cotyledons, which opinion was not shared by Salisbury and Richard.

Duchartre[2], on the evidence summarized above (p. 189), shared the belief of the two former investigators.

[1] Part I, loc. cit., p. 694.

[2] loc. cit.

Coming to more recent times, Sachs[1] made the following statement: 'The Taxineae and most Cupressineae and Araucarieae have two opposite cotyledons, although in some Cupressineae there are from three to nine, and in some Araucarieae whorls of four cotyledons; while among the Abietineae there are rarely so few as two, more often four, or even as many as fifteen. To refer this larger number of cotyledons to the division of two opposite ones, as Duchartre proposes, is entirely opposed to the other processes of leaf-formation in these plants, especially to the common occurrence of whorls consisting of several leaves on the growing axis of seedlings.' This disbelief was seemingly shared by Masters,[2] who, after mentioning the opinion of Adanson and his followers, remarked: 'If, however, the vascular bundles be traced from the caulicle [hypocotyl], it will be seen that the vascular cylinder breaks up not first into two divisions, which subsequently branch, but into a variable number not always in direct relation to the number of cotyledons.'

Dangeard[3] recognizes that polycotyledony may have arisen either by the intercalation of members of the first foliage whorl between the existing cotyledons, or by the splitting of the original seed-leaves. He examines both hypotheses, and, discarding the former, he says, 'Nous pensons que l'augmentation du nombre des cotylédons chez les Gymnospermes provient de la division de deux larges cotylédons; cette transformation s'est effectuée, dans la série des temps géologiques, sur un type voisin des *Araucaria*: mais ces deux cotylédons ont perdu actuellement toute individualité, elle s'est fragmentée pour ainsi dire en nouvelles individualités bien caractérisées.'

Clearly botanical opinion is divided, and it must be owned that the proof advanced in support of the contention of Adanson and Jussieu leaves much to be desired.

We may now pass on to the examination of our own evidence.

The Taxeae, Podocarpeae, and many of the Cupressineae, are characterized by the possession of two cotyledons; each of them, with the exception of those of the Podocarpeae, has a single vascular bundle which undergoes a bifurcation and a more or less well-marked rotation of the xylem elements to produce one pole of a diarch primary root. The Abietineae, with the exception of the Araucarieae, are tri- to polycotyledonous; each seed-leaf again has a single vascular strand, with certain exceptions which will be considered below, but the primary root may be diarch, triarch, tetrarch, and so on, the number of poles bearing no obvious relation to the number of cotyledons,[4] a feature which has struck more than one observer.[5] The

[1] Sachs: Textbook of Botany, 2nd ed. (Oxford, 1882, p. 507).
[2] 1891, loc. cit.
[3] Dangeard: Plantules des Conifères (Le Botaniste, sér. 3, 1892, p. 196).
[4] See the table at the end of this paper.
[5] Van Tieghem, Chauveaud, &c., op. cit.

behaviour of the bundles of the seed-leaves of these polycotyledonous forms must therefore vary in the same plant. The statement of our observations on the behaviour of the cotyledonary traces, contained in this present communication and in the first part of this research (Annals, '08), very strongly brings out the fact that the seed-leaves, as judged by the behaviour of their bundles in the transition-region, naturally fall into three categories, viz. (*a*) whole cotyledons, characterized by the bundle of each forming one pole of the root; (*b*) half-cotyledons, which are recognized by the bundles of two of them being required to form one pole of the root-structure; and (*c*) subsidiary cotyledons, the strands of which have no influence on the number of bundles in the root-structure.

Attention has already been drawn (p. 209) to the conclusions arrived at by Chauveaud in this connexion.

Any or all of these classes of seed-leaves may be represented in one seedling.

The external morphology is not infrequently useful in this present connexion; attention already has been drawn to the occurrence of seed-leaves partially split in the longitudinal direction, e. g. *Cupressus torulosa*, *Abies sibirica*, *Pinus montana*, var. *gallica*, and *Araucaria Cunninghamii*; and also to some cotyledons of a larger growth than their fellows, e. g. *Pinus contorta*, var. *Murrayana*. Again, seedlings have sometimes been seen in which the seed-leaves were obviously grouped together, a fact already recorded by Duchartre,[1] who stated that this grouping is more evident in the embryo than in the seedling. Finally, Mrs. Tansley[2] has described the cotyledons of *Torreya* as showing a marked tendency to lobing, and other observers have recorded the same thing for the *Cycads* and for *Ginkgo*.[3]

There is thus an abundance of macroscopic evidence partly to justify the above conclusion; but, at the same time, if this hypothesis be correct, the examination of a large number of plants should provide some transitional cotyledons, in which the bundles should be more or less divided and accompanied by an actual division of the seed-leaf itself to a degree more or less corresponding. Such examples are not wanting.

Pinus contorta, var. *Murrayana* (Series K), had three entire seed-leaves, one very much larger than the rest, and having two vascular strands entirely separate throughout the whole length of the member. These two bundles rotated towards one another within the axis and together formed one pole of the root. This is a case of one whole-cotyledon on the way to the formation of two half-cotyledons (Diagram 5).

Pinus montana, var. *gallica* (Series B). Two of the three seed-leaves were split longitudinally at their apices, and each of them had two vascular

[1] loc. cit. [2] Chick: New Phytologist, ii, 1903.

[3] See Part I, Annals, 1908, p. 693.

strands entirely separate one from the other. The bundles of one of these partly-divided cotyledons behaved in exactly the same manner as obtained in the similar case of *P. contorta*, var. *Murrayana* (Series K), mentioned above. The bundles of the other bifurcated cotyledon behaved differently ; one took no part in the transition and was therefore subsidiary, while the other underwent the equal division and rotation associated with the strands of a whole-cotyledon. Thus we have an example of the formation of two half-cotyledons from a whole-cotyledon, and also the formation of a subsidiary seed-leaf from a whole-cotyledon.

Another plant (Series C) of this same species provides a similar instance of the formation of a subsidiary cotyledon from a pre-existing single structure; in this case, however, there is a further advance, for the cotyledon in question was split more deeply. *Araucaria Cunninghamii* in which the two cotyledons are deeply bifid, may also be cited.

These examples, taken in conjunction with the other facts mentioned above, furnish a proof as complete as may be expected, but our hypothesis, on first thoughts, apparently does not lead us any great distance; for we can, so far, only derive a seedling with, say, ten half-cotyledons from a form which had five whole-seed-leaves, which is some way removed from the simpler condition of two cotyledons obtaining in *Taxus*, &c.

The examination, however, of the foregoing plants has provided several instances which show that this difficulty is more apparent than real. Many seedlings exhibited cotyledons, the bundles of which behaved differently from what they should if they had been of an absolutely rigid nature. In other words, it does not follow that because a seed-leaf is a subsidiary cotyledon, or a half-cotyledon, that therefore it will always remain as such; a subsidiary seed-leaf may, in the course of events, be promoted, as it were, to the rank of a half-cotyledon ; while a half-seed-leaf may be raised to the dignity of a whole-cotyledon.

The following examples have a bearing on this particular point :—

Cedrus atlantica (Series A). One bundle commenced to rotate in the manner usually associated with that of a half-cotyledon, but the movement came to nothing, and the strand in question finally behaved as that of a subsidiary seed-leaf.

Pinus Coulteri (Series A and B). A bundle from a seed-leaf bifurcated and commenced to rotate exactly like the strand of a whole-cotyledon, but finally it acted like a trace of a half-cotyledon.

Pinus australis, *P. insignis*, and *Abies balsamea* showed the same thing in a more marked degree.

Pinus contorta, *P. contorta*, var. *Murrayana*, *P. insignis*, and *P. sylvestris*, provided many examples of the bundles of cotyledons dividing and rotating within the seed-leaves in the manner they should if they belonged to whole-cotyledons. The bifurcation was sometimes very unequal (see

Diagram 7, and Fig. 3, Pl. XV), and the smaller portion often died away so that the strands finally performed as those of half-cotyledons; in other cases the smaller portion of the divided bundles persisted, so that notwithstanding the unequal division the strand behaved right through as that of a whole cotyledon.

Thus we have instances of cotyledonary bundles with intermediate characteristics, and these we consider examples illustrating the promotion of seed-leaves from a lower rank to a higher. There is thus no difficulty in the conception of the origin of a polycotyledonous from a dicotyledonous seedling by the process of splitting and promotion.

At the same time, we do not desire to maintain that this is the invariable rule: tricotyledonous seedlings may be due to nothing more than the formation of three seed-leaf primordia instead of the normal number, such as obtains in many seedlings of Dicotyledons, e. g. *Anemone* sp. and *Salicornia herbacea*; further, we have drawn attention to the fact that some subsidiary seed-leaves may be due to the displacement of leaves from the first foliage whorl to the cotyledonary node, as in *Cedrus Deodara* and *Pinus Pinea*.[1]

It may be mentioned here that Tansley and Thomas [2] have arrived at similar conclusions.

As regards the reasons for this multiplicity of seed-leaves, Masters [3] has stated his opinion that 'It is possible that an increased number of cotyledons might, under certain circumstances, be advantageous by securing a larger surface and a better chance in the competition with the neighbouring herbage'. Avebury [4] has suggested that the reason for the deeply bifid cotyledons in several plants, e. g. *Schizopetalon Walkeri*, and *Opuntia basilaris*, is to enable them to be withdrawn more easily from the seed, and he asks the question 'Is it possible that the multiplicity of the cotyledons in Conifers can be due to the same cause?'

In all cases we have found the mesophyll of the cotyledons to be homogeneous in structure, and the parenchyma never shows the internal shelves or flanges which is so characteristic a feature in the chlorenchyma of the foliage leaves of so many Gymnosperms; thus the available surface in the seed-leaves for the exposure of the chloroplasts is more limited than is often the case in the foliage leaves. We therefore think that it is not altogether improbable that the longitudinal fission of the cotyledons is a means to obtain a greater surface; for these structures persist relatively for a long time, and are thus important contributors to the synthetic food-material of the young plant.

[1] Cf. Dangeard, loc. cit.
[2] Brit. Assoc. York. Sect. K, 1906.
[3] Masters, 1891, loc. cit.
[4] On Seedlings, p. 52.

SUMMARY.

Cotyledons.

1. The number of cotyledons varies: the hypogeal species of *Araucaria* have two, the epigeal also have two, which may be divided so deeply as to form apparently four; *Tsuga* seldom has more than three; the species of the other genera examined may have from three upwards (see table below).

2. Some of the seedlings possess a more or less well-marked cotyledonary tube, e.g. *Pinus Pinea*, *P. canariensis*, *P. contorta*, var. *Murrayana*, *P. montana*, var. *gallica*, *P. sylvestris*, *Abies pectinata*, *A. amabilis*, *Picea ajanensis*, *Cedrus atlantica*, and *C. Deodara*. In the following plants certain of the cotyledons fuse laterally at their bases so that an incomplete tube is produced, *Pinus Pinea*, *P. Gerardiana*, *P. halepensis*, *P. australis*, *P. insignis*, *P. contorta*, var. *Murrayana*, *P. montana*, var. *gallica*, *P. sylvestris*, *Larix*, *Abies sibirica*, and *Picea morinda*.

3. Some seed-leaves exhibit a longitudinal split from the apex towards the base, e.g. *Cupressus torulosa*, *Abies sibirica*, and *Pinus montana*, var. *gallica*. This division is constant and extends almost to the base in *Araucaria Cunninghamii*.

4. Resin ducts are generally present. In the following, each cotyledon has two canals situated one at each dorsal corner, *A. sibirica*, *A. Veitchii*, *A. balsamea*, *A. amabilis*, *A. magnifica*, var. *Shastensis*, *Cedrus Deodara*, *Pinus Pinea*, *P. Gerardiana*, *P. canariensis*, *Larix europaea*, *L. leptolepis*, and *Pseudolarix Kaempferi*. In the following plants, each seed-leaf has one resin duct, situated in the mesophyll immediately dorsal to, or by the side of (in the case of half-cotyledons), the vascular bundle. *Pinus australis*, *P. insignis*, *P. contorta*, *P. contorta*, var. *Murrayana*, *P. montana*, var. *gallica*, and *P. sylvestris*.

The Araucarias have several resin ducts in their cotyledons, while in the following plants no such canals have been observed: *Tsuga*, *Abies pectinata*, *Picea*, *Pinus halepensis*, *P. Coulteri*, and *Larix occidentalis*.

Resin-ducts are absent from subsidiary cotyledons and many half-cotyledons.

5. The mesophyll is, without exception, homogeneous.

6. The seed-leaves of *Araucaria* each contain several bundles (4–8), those of *Tsuga*, *Abies*, *Picea*, *Cedrus*, *Pinus*, and *Larix* each have one vascular bundle.

Exceptions, however, occur; one cotyledon of a seedling of *A. Cunninghamii* had three strands; two vascular bundles occurred in the cotyledons of a few seedlings of *Pinus contorta*, var. *Murrayana*, *P. montana*, var. *gallica*, and *Larix europaea*.

7. Traces of a mesarch structure have been seen in the cotyledonary bundles of occasional plants of *Tsuga canadensis*, *Pinus Pinea*, and *P. Gerardiana*.

8. Transfusion tracheides are generally present, although the amount varies: they have not been observed in *Abies pectinata* and *Cedrus*.

9. Fibrous elements on the dorsal side of the phloem are generally present.

TRANSITION-PHENOMENA.

10. In the following plants the vascular arrangement takes place within the hypocotyl, so that the bundles within the cotyledons are endarch and collateral:—*Tsuga canadensis*, *T. diversifolia*, *Abies sibirica*, *A. Veitchii*, *A. balsamea*, *A. firma*, *A. Sachaliensis*, *A. pectinata*, *A. amabilis*, *A. magnifica*, var. *Shastensis*, *Picea ajanensis*, *P. nigra*, *P. morinda*, *Cedrus atlantica* *C. Deodara*, *Larix occidentalis*, *L. leptolepis*, *L. europaea*, *Pseudolarix Kaempferi*, *Pinus Pinea*, *P. Thunbergii*, *P. Gerardiana*, *P. halepensis*, *Araucaria Cunninghamii*, and *A. brasiliensis*.

In the following plants the bundles of some or all of the seed-leaves may bifurcate and rotate, more or less markedly, in the cotyledon itself:—*Pinus Coulteri*, *P. canariensis*, *P. australis*, *P. insignis*, *P. contorta*, *P. contorta*, var. *Murrayana*, *P. montana*, var. *gallica*, and *P. sylvestris*.

11. The cotyledonary bundles behave differently in the vascular changes in the transition-region; and according to their behaviour the seed-leaves may represent whole-cotyledons, half-cotyledons, or subsidiary cotyledons (see above, Conclusions).

12. When all the seed-leaves are whole-cotyledons, the transition follows Van Tieghem's Type 3.

13. Much variation in the transition occurs when the seed-leaves are mixed, i. e. when various combinations between the three kinds of cotyledons occur in the same species.

14. The polycotyledonous condition has been derived from the dicotyledonous condition, in the vast majority of cases by the splitting of the seed-leaves and by the promotion of cotyledons from a lower to a higher rank. In a few cases, the number of seed-leaves has been increased by the displacement of foliage leaves from the first plumular whorl to the cotyledonary node.

15. The relations between the number and nature of the cotyledons and the root-structure is shown in the following table.

Plant.	*Series.*	*Cotyledons.*				*Root.*
		Total.	*Whole-cots.*	*Half-cots.*	*Sub.-cots.*	
Tsuga canadensis / *T. diversifolia*	all	3	3	—	—	3-arch
Abies sibirica	A	3	3	—	—	3-arch
,,	B	4	4	—	—	4→3-arch
A. Veitchii	—	3	3	—	—	3-arch
A. balsamea	A	4	2	2	—	3-arch
,,	B	4	4	—	—	4-arch
,,	C	4	2	2	—	3-arch
A. firma / *A. sachaliensis*	—	4	4	—	—	4-arch
A. pectinata	A	8	—	8	—	4→3-arch
,,	B	8	—	6	2	3-arch
,,	C	8	—	6	2	3-arch
,,	D	8	—	6	2	3-arch
A. amabilis	—	8	7	—	1	7→6→5-arch
A. magnifica, var. *shastensis*	—	9	6	2	1	7→6-arch
Picea ajanensis	A	6	—	6	—	3-arch
,,	B	7	—	6	1	3-arch
,,	C	8	—	6	2	3-arch
,,	D	9	—	6	3	3-arch
P. nigra	A	6	—	6	—	3-arch
,,	B	6	—	6	—	3-arch
,,	C	7	—	6	1	3→2-arch
Picea morinda	A	8	—	6	2	3-arch
,,	B	10	—	6	4	3-arch
,,	C	10	—	6	4	3-arch
Cedrus atlantica	A	9	—	8	1	4-arch
,,	B	10	—	6	4	3-arch
,,	C	11	—	8	3	4-arch
C. Deodara	—	11	—	10	1	5→4-arch
Pinus Pinea	A	11	—	10	1	5-arch
,,	B	11	—	10	1	5-arch
,,	C	9	—	8	1	4-arch
P. Thunbergii	A	7	—	6	1	3-arch
,,	B	8	—	8	—	4-arch
P. Gerardiana	—	10	1	8	1	5-arch
P. halepensis	A	7	4	—	3	4-arch
,,	B	9	2	4	3	4-arch
P. Coulteri	A	13	2	8	3	6-arch
,,	B	11	4	4	3	6→5-arch
P. canariensis	—	9	2	4	3	4-arch
P. australis	A	9	5	4	—	9→7-arch
,,	B	9	7	2	—	8-arch
P. insignis	A	5	5	—	—	5-arch
,,	B	8	2	4	2	4-arch
P. contorta	A	3	3	—	—	3-arch
,,	B	4	4	—	—	4-arch
,,	C	4	2	2	—	3-arch
P. contorta, var. *Murrayana*	A, K	3	3	—	—	3-arch
,, ,,	B, C, G	4	4	—	—	4-arch
,, ,,	F	4	4	—	—	4→2-arch
,, ,,	H	4	3	—	1	3-arch
,, ,,	I, J	4	2	2	—	3-arch
,, ,,	D, E	5	5	—	—	5-arch
Pinus montana, var. *gallica*	A	4	3	—	1	3-arch
,, ,,	B	3	3	—	3	3-arch
,, ,,	C	4	2	—	2	2-arch
,, ,,	D	4	2	2	—	3-arch
P. sylvestris	A	4	3	—	1	3-arch
,,	B	5	3	—	2	3-arch
,,	C	6	4	—	2	4-arch

Plant.	*Series.*	*Cotyledons.*				*Root.*
		Total.	*Whole-cots.*	*Half-cots.*	*Sub.-cots.*	
P. sylvestris	D	6	3	—	3	3-arch
,,	E	6	2	4	—	4-arch
,,	F	6	2	—	4	2-arch
,,	G	6	2	2	2	3-arch
,,	H	6	1	4	1	3-arch
,,	I	7	4	—	3	4-arch
,,	J	7	2	4	1	4-arch
,,	K	7	1	4	2	3-arch
,,	L	7	1	6	—	4-arch
,,	M	7	3	2	2	4-arch
Larix occidentalis	—	6	1	4	1	3-arch
L. leptolepis	A	5	1	4	—	3-arch
,,	B	6	2	—	4	2-arch
L. europaea	A	6	—	4	2	2-arch
,,	B	6	—	4	2	2-arch
,,	C	7	2	2	3	3-arch
Pseudolarix Kaempferi	A	5	4	—	1	4-arch
,, ,,	B	4	4	—	—	4-arch
Araucaria Cunninghamii } *A. brasiliensis* }	all	2	2	—	—	2-arch

EXPLANATION OF PLATE XV.

Illustrating Mr. T. G. Hill and Miss de Fraine's Paper on the Seedling Structure of Gymnosperms. II.

Abbreviations :—*mx.* metaxylem ; *ph.* phloem ; *px.* protoxylem ; *r. d.* resin-duct ; *T. S.* transverse section.

Fig. 1. *Pinus contorta*, var. *Murrayana* (Series I). T. S. through upper region of hypocotyl. × 240.

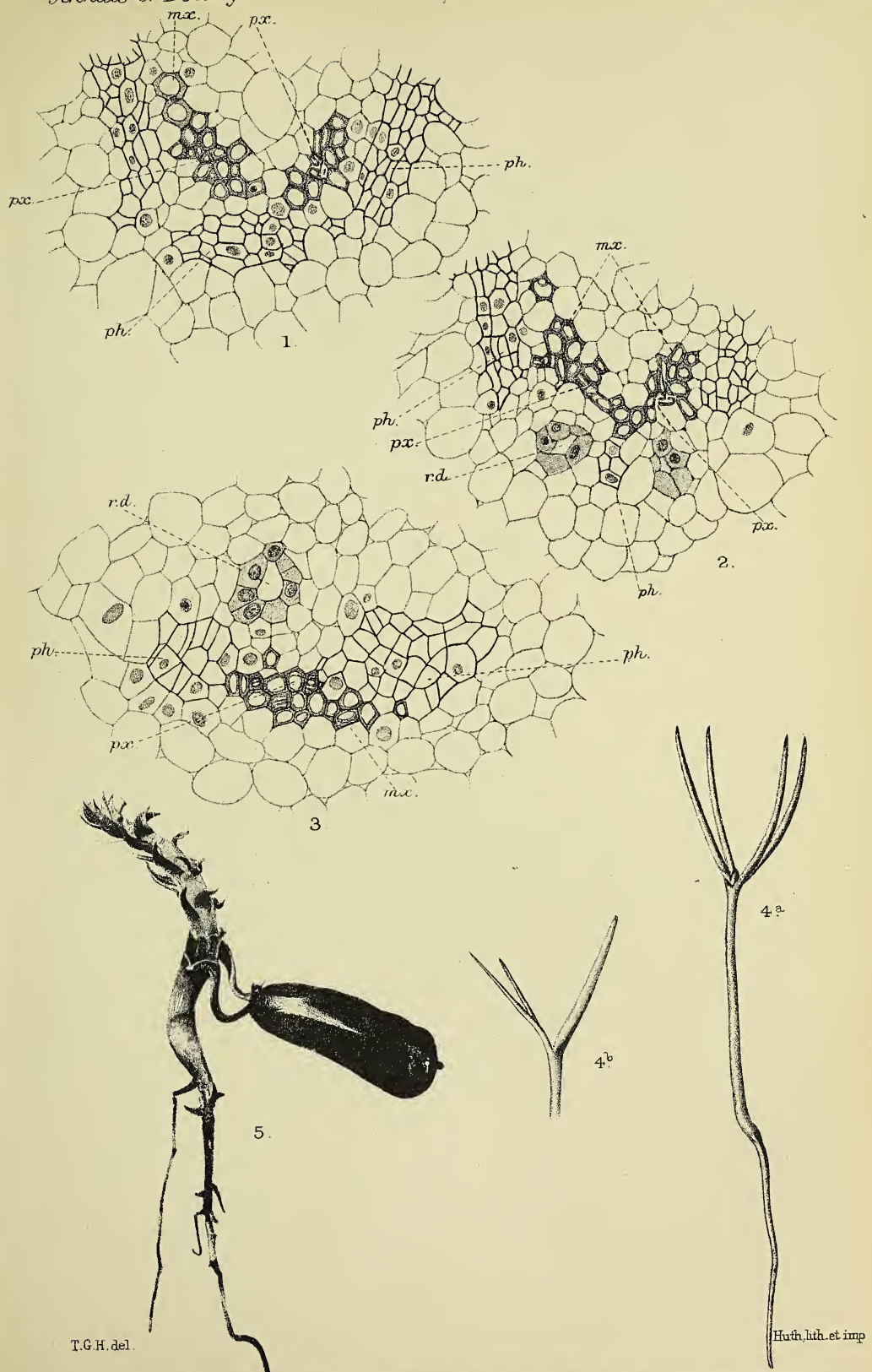
Fig. 2. The same, about 10 μ below. × 240.

Fig. 3. *Pinus montana*, var. *gallica*. T. S. of a cotyledonary bundle, showing unequal bifurcation of the vascular tissues. × 240.

Fig. 4 *a*. *Araucaria Cunninghamii*. A normal seedling. Nat. size.

Fig. 4 *b*. *Araucaria Cunninghamii*. Cotyledons and upper part of hypocotyl of an abnormal seedling. Nat. size.

Fig. 5. *Araucaria brasiliensis*. Photograph of seedling. × about $\frac{3}{5}$.



HILL AND DE FRAINE — SEEDLING STRUCTURE OF GYMNOSPERMS. II.

The Extra-floral Nectaries of the Genus Polygonum.

BY

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With Plate XVI, and six Figures in the Text.

THE present research was undertaken with a view to ascertaining the function of the glands which occur on the underside of the leaf-cushion in various species of Polygonum. The following species have been under observation, viz. :—

Polygonum baldschuanicum, *P. cilinodum*, *P. compactum*, *P. Convolvulus*, *P. cuspidatum*, *P. multiflorum*, *P. sachalinense*, and *P. scandens*.

All the above agree in possessing leaves which are variations upon the cordate-saggitate type and are either herbaceous or annual.

The only woody species is *P. baldschuanicum*, which produces climbing shoots that die back in the winter. *Polygonum cilinodum*, *P. Convolvulus*, and *P. scandens* are also of climbing habit.

The extra-floral nectaries of *P. cuspidatum* have been described by Delpino (5) ; those of *P. baldschuanicum*, *P. Convolvulus*, and *P. multiflorum* by Schwendt (15), and those of *P. sachalinense* by Ono (12). The two latter papers were published during the course of the present research. Detailed descriptions of the above species need not therefore be included.

For convenience, the paper is divided into the following sections, viz. I, General; II, Special; III, Microchemical; IV, Physiological; V, Summary and General Conclusions.

I. General.

The extra-floral nectaries are oval, triangular, or circular depressions. In all except the circular nectary of *P. cilinodum* the depressions are boat-shaped. The nectaries lie in the plane of symmetry of the leaf and are situated upon the underside of the base of the petiole just below the abscission layer which is differentiated at an early stage (Pl. XVI, Fig. 4). *Polygonum cilinodum* is, however, an exception to this rule, for in this species where, though the glands were fully developed, no abscission layer could be recognized. Like most nectaries these stand in close relation

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to the vascular system. The vascular supply of the petiole is therefore of some interest and attains greater significance when viewed in the light of the physiological experiments to be described later on. In general, the petiole of the Polygonums is convex or angled upon its lower surface and concave or grooved upon its upper.

The bundles form a dorsal arc which is closed by a bundle or bundles placed ventrally and which of course have the xylem directed inwards. (The terms dorsal and ventral are applied to the lower and upper surface of the leaf, respectively.)

In the simpler types of petiole the bundles are six in number, so that the ventral and one of the five dorsal bundles lie in the median plane, and

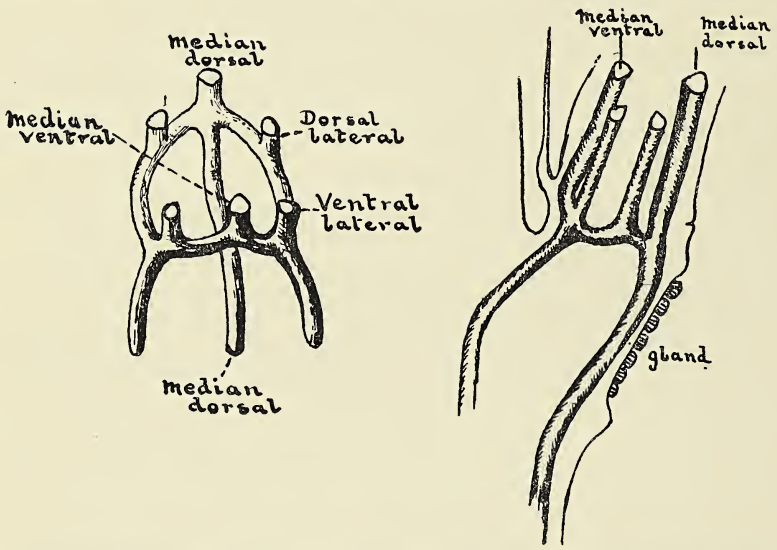

FIG. 1. Vascular supply of petiole in *Polygonum multiflorum*. The bundles passing to the ochrea have been omitted.

the other four are arranged symmetrically with regard to these. (The pair of bundles which occupy the upper angles of the petiole are frequently reduced.)

In the more complex forms the arrangement is the same, but owing to subdivision the bundles are more numerous.

It is opposite the dorsal median bundle that the nectary is situated (Text-fig. 1).

Around the nectary the tissue is somewhat raised, and may even slightly overhang, especially at the upper edge. For convenience of description this will be termed the lip.

The nectaries are fully developed and function before the leaf unfolds: large drops of secretion were observed upon nectaries of *P. cuspidatum* when the lamina was not quite 2 mm. in length.

Winter buds upon their emergence in the spring have actively secreting nectaries upon bud-scales which possess no lamina.

In only one species, viz. *P. Convolvulus* were seedlings obtained; and these showed variations in the occurrence of the nectaries.

In no case were they present upon the cotyledons, but whilst they appear to be normally present on the petiole of the first leaf, occasional specimens had no nectary till the second or third leaf was reached. The depressions forming the nectaries are lined with secretory trichomes which form a closely packed layer over the whole surface. The trichomes or unit structures of the nectary consist of three parts.

The lowest or basal portion consists of large sac-like thin-walled cells, which with their neighbours form an almost continuous layer one cell deep, and for each gland number four or more in a single story (Text-fig. 2, *b*, on left).

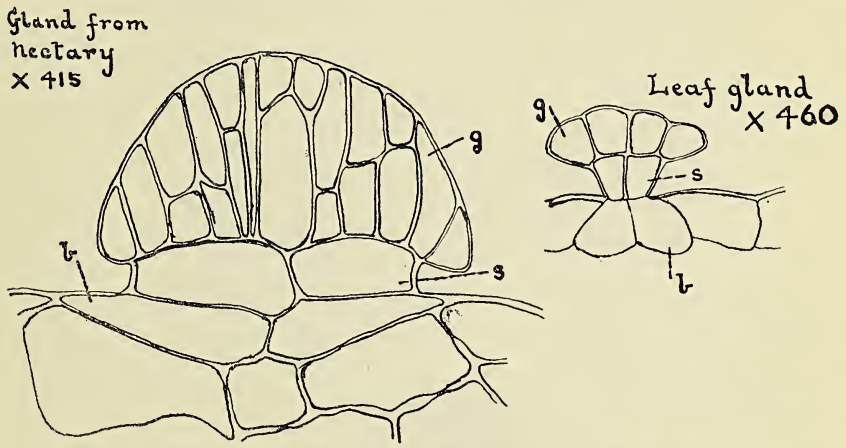

FIG. 2. Nectary- and leaf-glands of *Polygonum compactum*. *g*. gland cells; *s*. stalk cells; *b*. basal cells.

The middle layer is also one cell deep, and consists of flattened cells whose vertical walls are thickened, the horizontal walls—separating them on the one hand from the basal cells, and on the other from the gland cells—remain thin (Text-fig. 2, *s*).

The gland cells forming the third portion are usually not more than one cell deep, but may become subdivided by transverse walls; they form an enlarged head of numerous cells elongated in the vertical direction and with comparatively thin walls (Text-fig. 2, *g*). This type of structure is frequent in secreting trichomes, and also finds its parallel in the hydathodes of Piperaceae, Bignoniaceae, and species of *Artocarpus* as was shown by Haberlandt (8, p. 420).

As regards the cytological details, they are those characteristic for secretory cells in general; the gland cells when young are completely

filled with a dense granular and highly refractive cytoplasm and possess large nuclei. At maturity they become markedly vacuolate. This vacuolation was observed by Gardiner (7) in the gland cells of *Dionaea*, and more recently, Miss Huie has shown that the gland cells of *Drosera* exhibit vacuolation, which is most marked at the period of secretion (9). See also Saunders (13).

The development of the nectaries was studied in *P. cuspidatum*, and agrees with that described by Schwendt (15, p. 250) for *Muehlenbeckia sagittifolia*.

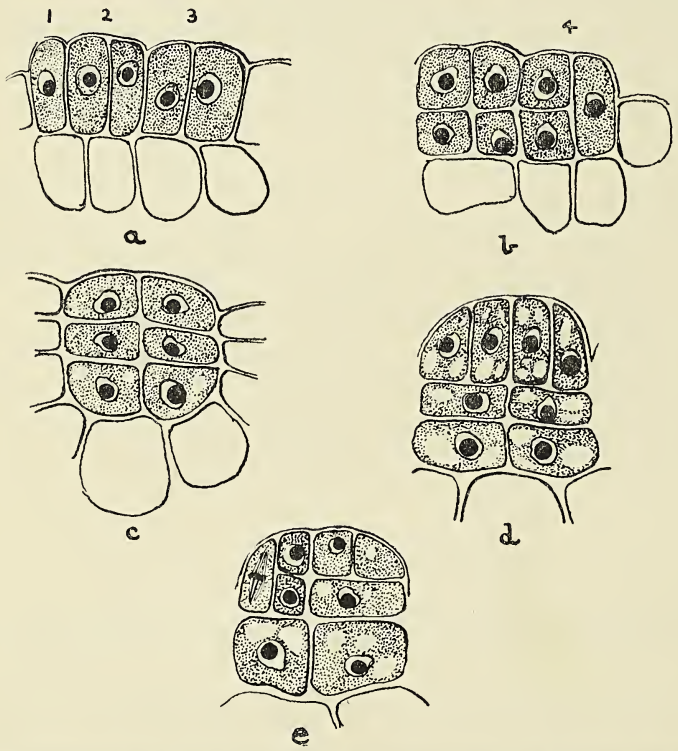

FIG. 3. Development of nectary glands in *Polygonum cuspidatum*, shown in vertical section. The successive stages are seen in *a*, *b*, *c*, *d*. In *e*, the longitudinal division of the glandular portion has taken place before the completion of the last horizontal division. The figures 1–4 denote successive stages in development. × 460.

The development of the trichomes starts from the centre of the nectary and passes to the periphery. It begins with the radial division of the gland mother-cells in two planes at right angles to one another. Division parallel to the surface of the nectary then takes place, and we thus have formed an upper and a lower story, each of four cells. The cells of the upper group again divide horizontally, and we thus have formed the three series representing the basal, stalk, and gland cells. This sequence may, however, be subject to slight variation (Text-fig. 3, *e*).

Further subdivision generally takes place in the glandular portion as this reaches maturity; the walls are usually vertical but sometimes horizontal or inclined. Further subdivision of the stalk and basal cells is not infrequent, but does not take place to the same extent as in the secretory region.

In all the species enumerated, and in others not bearing extra-floral nectaries, isolated glands which secrete gum occur upon the ochrea, petioles, stems, and leaf-blades (Pl. XVI, Fig. 5, *g*).

The structure of these glands is in all essentials identical with that of the nectary-units just described (see Text-fig. 2).

They arise as epidermal papillae, and follow the same course of

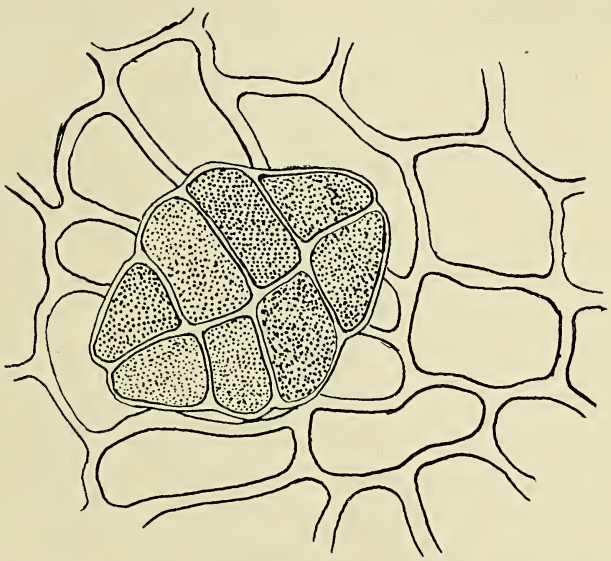

FIG. 4. Gland from ochrea of *Polygonum sachalinense*. × 580.

development as those of the nectary; subdivision is however much less, and practically confined to the secretory portion.

These, like the foregoing, function long before the leaf unrolls, and mostly fall off before the organs reach maturity.

It is interesting to note in this connexion that similar glands secreting a mucilagenous substance and probably functioning as hydathodes have been described by Areschoug (1) on leaves of various species of Mangroves.

II. SPECIAL.

Polygonum cilinodum (Pl. XVI, Fig. 1).

The nectaries in this species are circular in outline with a diameter of about 0·63 mm. They are saucer-shaped depressions surrounded by a well-marked lip, which slightly overhangs the concavity.

The lip consists of slightly thickened cells covered by an epidermis of radially elongated elements and strongly thickened external walls, whilst near the nectary its cells are sometimes divided tangentially. The median dorsal leaf-trace accompanied by a sclerenchyma sheath, which is a continuation of that which surrounds the stele of the stem, passes close beneath the nectary.

Opposite the nectary the sclerenchyma sheath is pierced, and through this opening there passes from the bundle to the nectary a band of small-celled tissue with dense contents, large nuclei, and strongly thickened walls, which latter are deeply pitted (Pl. XVI, Fig. 2, *s p*).

This, which we may term the epithem tissue, broadens out as the nectary is approached, and on reaching the latter forms an almost continuous layer beneath the basal cells. Around this epithem strand is large-

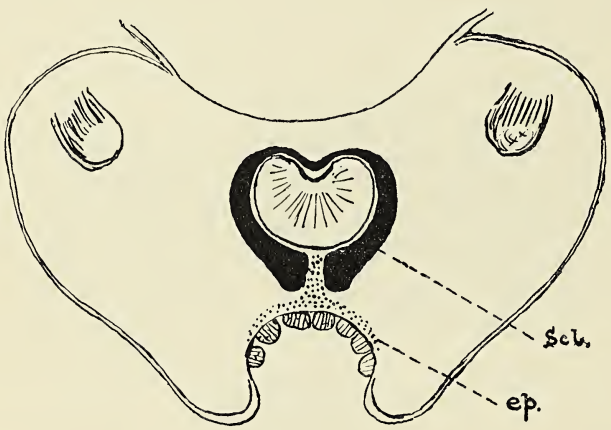

FIG. 5. Diagram of gland of *Polygonum cilinodum*. *Scl.* sclerotic tissue; *ep.* small-celled epithem.

celled parenchyma which in a transverse section appears as two groups to right and left of the small-celled tissue (Pl. XVI, Fig. 2, *l p*).

The secretory trichomes themselves are in this species much more complex than in any of the others examined; this is due to increased division, and applies not only to the secretory but also to the stalk and basal regions.

The glandular head shows both radial and transverse division, the latter may take place in some or all of its cells.

The petiole of this species is rounded in transverse section with a slight concavity above (Pl. XVI, Fig. 9). It contains six bundles, the five forming the dorsal arc are of nearly equal size, whilst the ventral bundle is larger. A noticeable feature in this species is its fringed ochrea. The hairs which arise from the base of the latter are directed downwards and

might perhaps prevent any small insect that had been attracted by the nectar from climbing the stem further.

Sphaerocrystals of calcium oxalate are generally distributed throughout the plant, and are always relatively crowded in the young organs and beneath the nectaries.

The blades, besides possessing mucilage-secreting glands, have cells which appear to contain mucilage scattered at intervals in the epidermis.

Polygonum compactum.

In this species the nectary is an oblong, almost rectangular, shallow, flattened depression. It measures about 1 × 0·75 mm. (Pl. XVI, Fig. 11).

The glandular heads of the trichomes show both radial and tangential subdivision, and the stalk cells also divide. The basal cells seldom appear to do so, and exhibit a tangentially extended appearance.

In this species the sclerotic sheath around the stele of the stem is discontinuous; it only accompanies the median dorsal bundle of the petiole as slight sclerotic strands on either flank, the epithem tissue therefore abuts direct upon the phloem.

It consists of 2–3 layers of cells with dense contents and sclerized walls which are pitted. The lip does not overhang but has a well-developed and strongly thickened epidermis of radially elongated cells.

The petiole is roughly rectangular with rounded corners and with three ridges on the upper surface (Pl. XVI, Fig. 7). Its vascular supply is somewhat more complex than in the previous species. It consists of twelve bundles—two of which, situated in the lateral ridges, are small, and two other small ones occur one on either side of the median dorsal strand. Here again, calcium oxalate crystals are numerous beneath the nectary.

Polygonum sachalinense.

This species, which is by far the largest of those investigated, has a much more complex petiole structure (Pl. XVI, Fig. 6).

In transverse section as many as nineteen or twenty bundles may be present. Associated with this large size and consequent increase of vascular supply we find an augmented system of extra-floral nectaries; a large one about 2 × 1 mm. upon the abaxial face of the leaf-cushion, and from 2–4 paired nectaries of ellipsoid form situated above the main lateral veins of the leaf-base, at the points where they emerge from the stem. All these glands are present on the youngest leaves. The accessory glands measure about 0·25 × 0·75 mm., and except in point of size their structure agrees with the main nectary. The latter is sunk about a millimetre below the surface, and is an almost flat depression (Pl. XVI, Fig. 4). The basal cells are separated from the phloem of the underlying bundles by from 3–4 layers of cells which are strongly sclerized and deeply pitted (Text-fig. 6).

These cells appear to be of two kinds, some show a deeply stained protoplasm, the rest stain but faintly and contain large nuclei. The staining reagents used being Methyl blue and Kleinenberg's hematoxylin and safranin.

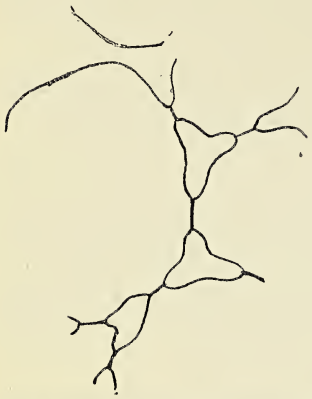

FIG. 6. Pitted epithem of *Polygonum sachalinense*.

Polygonum multiflorum.

The nectary here is triangular in form; only slightly depressed, and with poorly differentiated lips. Immediately beneath the nectary are from 2–3 layers of sclerized and pitted cells, between these and the bundle are parenchymatous cells interspersed with sclerenchymatous groups. The median petiolar bundle has a sclerotic sheath which is perforated opposite the nectary (Pl. XVI, Fig. 8). Calcium oxalate sphaerocrystals occur around the gland and mucilage cells are present in the leaf-epidermis. The petiole is of a rounded pentagonal form; the bundles of the upper angles are somewhat reduced.

Polygonum scandens.

The nectary is slit-like, measuring about 0·8 × 0·75 mm. Immediately beneath are two layers of sclerized and pitted cells, and between these and the bundle 3–4 layers of cells (Pl. XVI, Fig. 3).

The chief interest of this species lies in the fact that it possesses at once the smallest nectary and most reduced petiolar structure of all the species examined. The petiole, which has a deep groove on the upper surface, has six bundles; but two of these, situated in the wings bordering the groove, are extremely reduced (Pl. XVI, Fig. 12). What appear to be wax glands occur on the leaf-blade.

Polygonum baldschuanicum.

The nectary here is not well developed, and the lip is poorly differentiated; the underlying tissue is sclerized and pitted, and between this and the bundle is parenchymatous tissue. The petiole is rounded—pentagonal in outline; it contains six bundles, the upper two being small. An interesting feature of this species, which is a woody climber, is that nectaries are absent from the rosette of leaves at the base of the annual shoots; in some cases it was not till the eighth leaf that the nectary was present.

Polygonum Convolvulus.

This species has a somewhat small elongated nectary of triangular form about 0·9 mm. long by 0·5 mm. as its greatest breadth. The lips are

well developed. Below the glands the tissue is strongly sclerized and pitted. The petiole is pentagonal in outline, the upper side being the longest; the bundles at the upper angles are somewhat smaller than the other four (Pl. XVI, Fig. 10).

Polygonum cuspidatum.

The nectary is oval in form and slightly concave transversely. The tissue beneath is strongly thickened and deeply pitted. The petiole is of oval-rectangular form with prominent ridges on either side of the upper surface. It contains from 8–9 bundles.

III. Microchemical.

In all the investigated species it was found that the tissue below the gland was rich in a substance which gave a blue reaction with hydrochloric and osmic acids. This was especially marked in the stalk cells, whilst the gland cells remained unchanged (Pl. XVI, Fig. 11).

Heating sections with Fehling's solution gave a red precipitate in the gland cells only. But previous heating with H_2SO_4 and neutralization resulted in an instant reduction of the copper, which formed a copious precipitate in the epithem tissue between the nectary and the vascular bundle. Older non-secreting nectaries showed hardly any precipitate in the epithem. Ferric chloride gave a bluish precipitate in the epithem, but in the basal cells this was so marked as to render them almost black. Fehling's solution reduces the secretion itself without any previous treatment with acid.

The above results suggest the presence of a glucoside in the epithem tissue which by its breakdown supplies the glucose secreted from the nectary.

Concentrated sulphuric acid completely dissolves the sections placed in it except the cuticle, the gland cells, and the stalk cells. Sulphuric and iodine causes a brown coloration which is darkest for the stalk cells.

Concentrated caustic potash colours the stalk cells yellow, the tint becoming brighter on heating; the gland cells appear unchanged. Thus it would appear that the gland cells are cuticularized and the radial walls of the stalk cells are strongly suberized.

IV. Physiological.

Most of the following experiments were performed upon plants in pots under glass. The experiments with saturated air were conducted on all species except *P. scandens*. The other experiments were chiefly carried out on *P. cuspidatum* and *P. compactum*.

Washing out young nectaries with water showed that these have the power of repeated excretion of fresh sugar, but after a while this ceases.

If, however, fresh secretion from another nectary be placed upon the glands they function again.

Washing nectaries with 0·1 per cent. corrosive sublimate solution killed the gland cells, and no further secretion took place.

The same result obtained when the nectary was washed and the gland cells carefully scraped off with a scalpel.

The root pressure was determined for an actively secreting plant of *P. cuspidatum*, and was found to be equivalent to 13·1 cm. of mercury. Water injected at a higher pressure only produced an increased secretion or rather bleeding from the lowest nectary of the shoot employed.

Secretion was equally active where the shoots were removed and placed with their cut ends in water.

Placing plants in saturated or nearly saturated air caused marked increase of secretion—this latter appearing as large drops clinging to the nectaries. The control plants showed no such increase. It was found that where the plants which were placed in saturated air possessed few shoots, and therefore few nectaries, the whole of the nectaries showed an increased activity.

But where large plants were used which bore numerous nectaries, only a few showed increased secretion.

One plant which bore fifty-two glands was placed in damp air. When examined after three hours only two nectaries were actively secreting.

The removal of eight leaves was followed after twelve hours by an increase in the number of actively secreting nectaries to six.

The result may have been due to an increase in the saturation, but a plant with a large number of nectaries, of which only three were secreting vigorously, showed an increase to only five after a lapse of sixty hours.

Where in the case of plants bearing numerous shoots one or two of these were enclosed in saturated air, whilst the remainder were in comparatively dry air, no increase of secretion was observed on the nectaries of the enclosed shoots.

Plants, which, in saturated air, had drops of secretion hanging to the nectaries, rapidly lost their drops on removal to dry air. In order to determine whether the loss of the drops was due to evaporation or absorption by the glands, control-drops of water of similar dimensions were placed on the axes and petioles. It was found that the drops of secretion were the first to disappear, notwithstanding their slower rate of evaporation in consequence of the dissolved sugar. We appear then to have glands here which, like those described by Kerner (10), are at once secretory and absorptive.

Experiments in saturated air were also performed upon the gum-secreting leaf-glands, and these too (particularly those of the ochrea) prove to be more active under that condition.

This was most marked in *P. sachalinense* where the nodes of one young shoot were bathed in the gummy secretion which hung down in sticky threads.

With a view to finding if there were any relation between vascular supply and secretory area, the following table was constructed. Where the cross-sectional area of the xylem in the petiole of each species has been estimated, and the area of the corresponding nectary, it will be seen that the value obtained by dividing the one area by the other approaches a constant.

Species.	*Approx. area of nectary* (n).	*Approx. area of xylem in petiole* (x).	*Value* $\frac{n}{x}$
P. baldschuanicum	0·23 sq. mm.	0·031 sq. mm.	7·42
P. cilinodum	0·30 ,, ,,	0·030 ,, ,,	10·00
P. compactum	0·75 ,, ,,	0·076 ,, ,,	9·87
P. Convolvulus	0·22 ,, ,,	0·025 ,, ,,	8·80
P. cuspidatum	0·57 ,, ,,	0·068 ,, ,,	8·51
P. multiflorum	0·26 ,, ,,	0·028 ,, ,,	9·29
P. sachalinense	2·25 ,, ,,	0·247 ,, ,,	9·11
P. scandens	0·16 ,, ,,	0·019 ,, ,,	8·42

V. Summary and General Conclusions.

The petiolar nectaries are in all cases surrounded by a lip which is raised and covered by thickened epidermal cells.

This lip, which projects most at the upper edge (Pl. XVI, Fig. 1, *l*), may serve to protect the gland cells and to prevent the washing out of the nectary by rainwater. It may possibly further serve as a protection for the cells beneath against the plasmolyzing action of the osmotically powerful secretion contained in the nectary.

For it is these thick-walled lip cells with which the secretion is in contact. The tangential division of the epidermis of the lip which was observed in *P. cilinodum* still further supports this view. And in this connexion we may refer to the well-developed lip-structure described by Miss Ewart (6) in *Ipomoea paniculata*, as here, too, the lip exhibits a tangential division which may have the same significance.

A like function may be served by the suberization of the stalk cells, for the secretion has access to these notwithstanding the close packing of the glandular heads.

The high osmotic pressures involved may further account for the sclerization of the epithem cells, as the deep pittings, which are fairly numerous upon their surfaces, allow of sufficient facility for translocation. It is worthy of note that a similar pitted tissue has been observed below the gland cells of *Ipomoea paniculata* (6) which, as we have seen, closely resemble the glands of *Polygonum* in other respects. M. Vuillemin (16) has also called attention to the pitted tissue beneath the glands of the Tamariscineae,

Plumbagineae, and Frankeniaceae, all of which secrete osmotically active substances.

The secretory activity of the nectaries appears to be due to osmotic action, largely independent of root pressure. The thin-walled character of the basal cells may be associated not only with the need for ready permeability, but also with communication of the turgor pressure of the surrounding tissue.

The experiments seem to show that the plant can accommodate itself to local inequalities of transpiration, and that these nectaries are, from the physiological point of view, water-secreting organs whose action is dependent upon osmosis.

To summarize the reasons for this view:

(1) The structure of the glandular trichomes agrees with that of the leaf-glands.

(2) The nectaries function at an early stage in the development of the organs upon which they are borne, and therefore at a period when excess of turgidity would be most injurious.

(3) There is a marked increase of secreted fluid accompanying an increase of humidity.

(4) The nectaries stand in close relation to the vascular bundles, and there is an increase in their number and extent accompanying an increase in complexity of the vascular supply. In those species which have a reduced petiolar vascular supply a reduced or simplified nectary is present.

(5) In no case were glands observed to be visited by ants, either in the exotic species at Kew, or in *P. Convolvulus* in its native haunts. In this latter some dozens of plants in various situations were kept under observation, and in no instance were they found to be visited by insects.

That some physiological purpose was served by extra-floral nectaries was suggested by Schimper (14). That this was, in the case of floral nectaries, of a hydathodal nature was advocated by Burck (4), and the same view in respect to floral nectaries has been put forward by Renner (12) and Schwendt (15, p. 42). The ant protection theory, whilst it is undoubtedly applicable in certain instances, has only been proved for a few. Aufrecht (2) and others have remarked on the absence of insects during their observations. Such protection must be regarded as an altogether secondary adaptation.

An admirable historical summary of the views regarding the function of extra-floral nectaries is given by Niewenhuis-Uxküll (11).

Bonnier (3) showed for floral nectaries that the maximum secretion took place when the soil and air were saturated.

Ono (12), who worked on numerous extra-floral nectaries including those of *P. sachalinense* and *P. cuspidatum*, summarized his results as follows. 'Among different external circumstances, moisture seems to be of the greatest importance' (p. 18).

Burck (4) showed that the floral nectaries in many instances act as hydathodes, causing dehiscence of the anthers even in saturated air.

The frequent occurrence of extra-floral nectaries in tropical plants, where very sudden changes of humidity are often daily phenomena, cannot be without significance.

There does not, therefore, seem any difficulty, so far as known facts are concerned, in deriving all nectaries originally from osmotic hydathodes, subserving a physiological function, which have in certain cases secondarily acquired biological importance.

In conclusion, I should like to take this opportunity of recording my thanks to Professor F. W. Oliver, at whose suggestion and under whose direction the present work was undertaken.

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EXPLANATION OF PLATE XVI.

Illustrating Mr. Salisbury's paper on the Nectaries of *Polygonum*.

Fig. 1. *Polygonum cilinodum.* Median longitudinal section through node. *a.s.* axillary shoot; *l.* lip of nectary; *och.* ochrea; *pet.* petiole; *scl.* sclerenchyma; *t.* trichomes; *v.t.* vascular tissue. × 15.

Fig. 2. *P. cilinodum.* Transverse section through nectary. *s.c.* stalk cells; *scl.* sclerenchyma; *s.p.* small-celled epithem; *l.p.* large-celled parenchyma. × 178.

Fig. 3. *P. scandens.* Transverse section of nectary. *cr.* crystals of calcium oxalate; *l.* lip; *t.* trichomes; *v.b.* vascular bundle. × 35.

Fig. 4. *P. sachalinense.* Longitudinal section of nectary. *a.l.* abscission layer; *ep.* epithem tissue; *t.* trichomes; *v.b.* vascular bundle. × 39.

Fig. 5. *P. scandens.* Transverse section of young leaf. *g.* gum secreting gland. × 35.

Fig. 6. *P. sachalinense.* Transverse section of petiole. × 12.

Fig. 7. *P. compactum.* Transverse section of petiole. × 12.

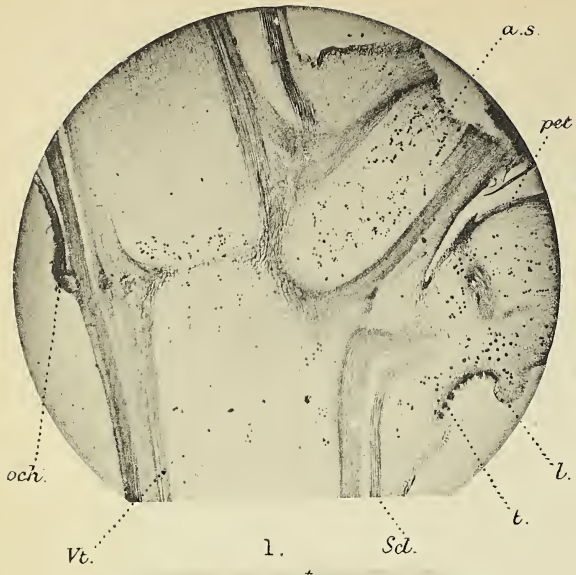
Fig. 8. *P. multiflorum.* Transverse section of nectary at upper end passing through the glandular area twice. *scle.* sclerized cell groups; *t.* trichomes; *v.b.* vascular bundle. × 35.

Fig. 9. *P. cilinodum.* Transverse section of petiole. × 12.

Fig. 10. *P. Convolvulus.* Transverse section of petiole. × 12.

Fig. 11. *P. compactum.* Transverse section of nectary taken after treatment with hydrochloric and osmic acids. *ep.* epithem; *l.* lip; *t.* trichome; *v.b.* vascular bundle. × 27.

Fig. 12. *P. scandens.* Transverse section of petiole. *ch.* ventral channel; *p.b.* phloem bundle. × 31.



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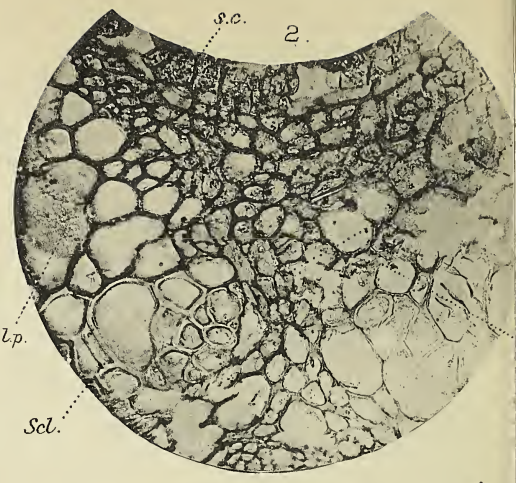

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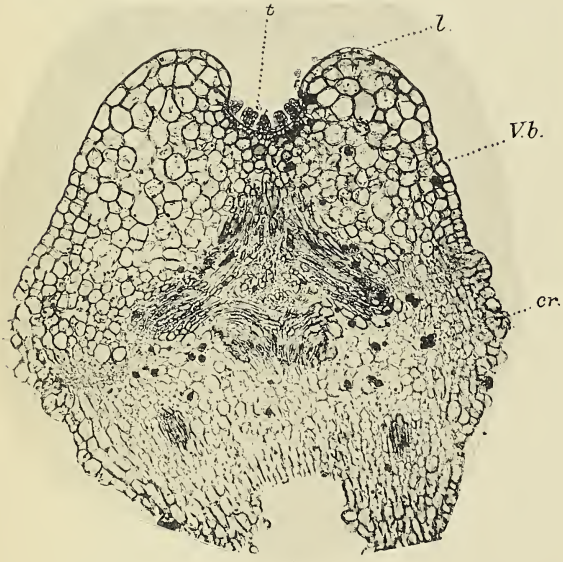

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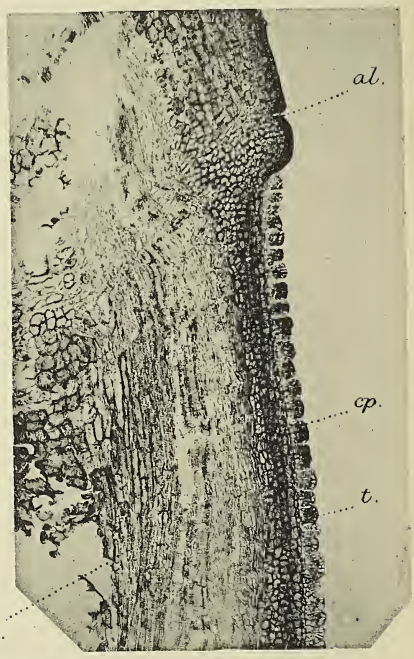

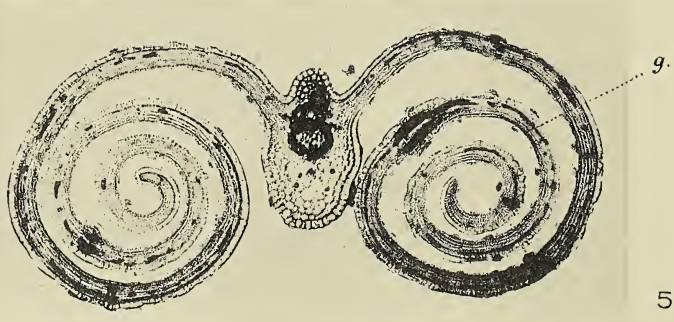

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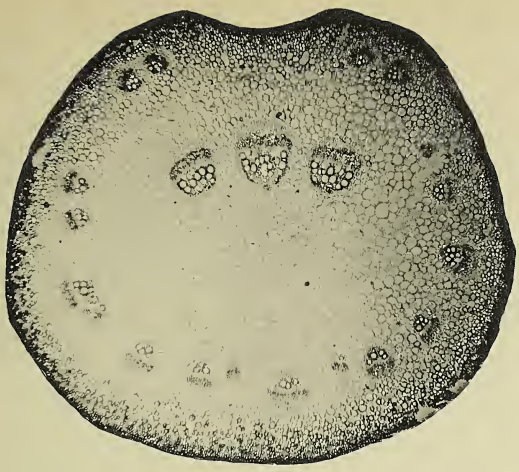

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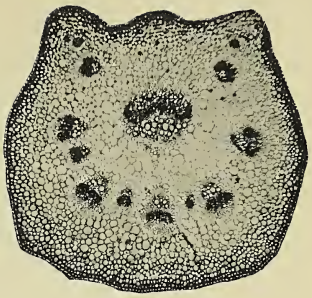

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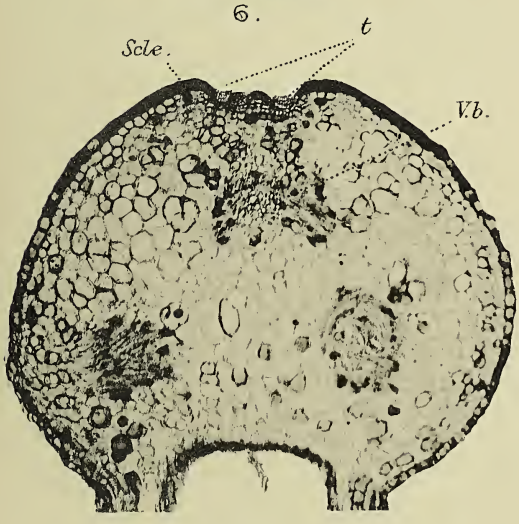

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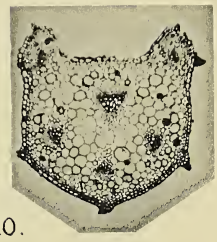

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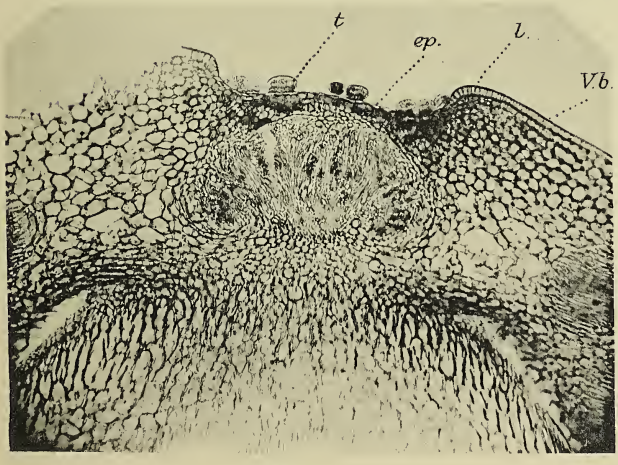

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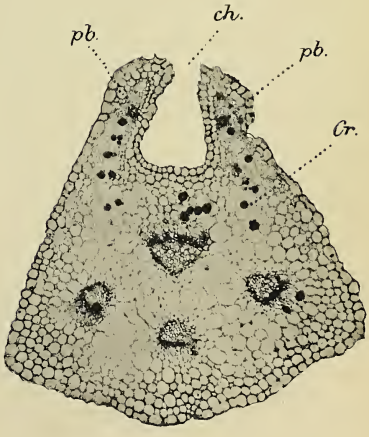

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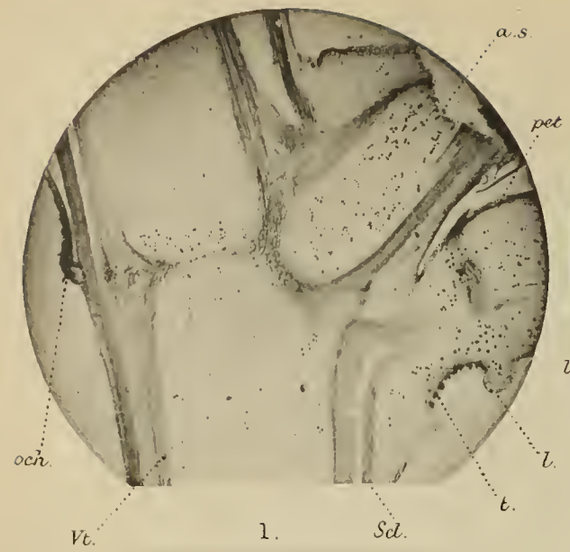

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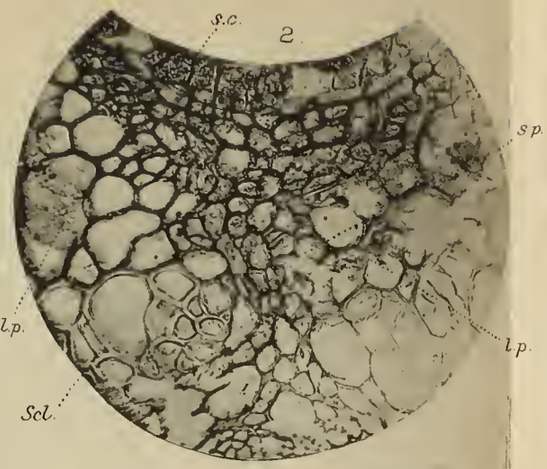

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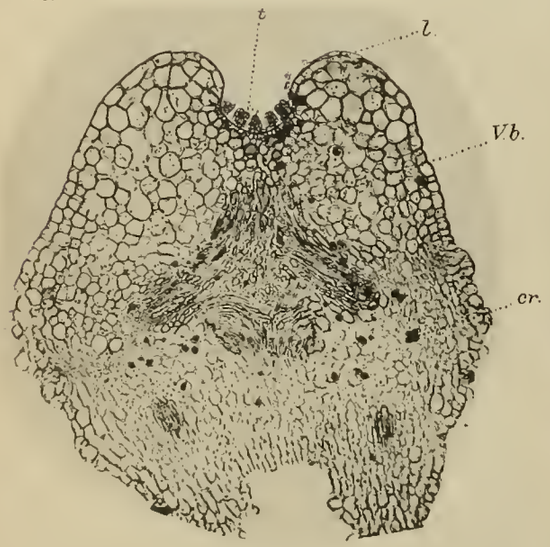

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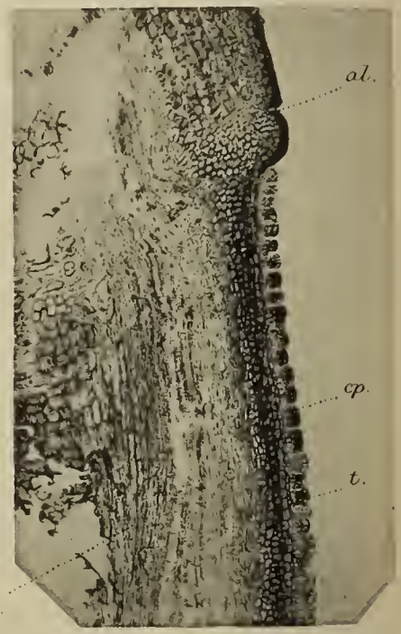

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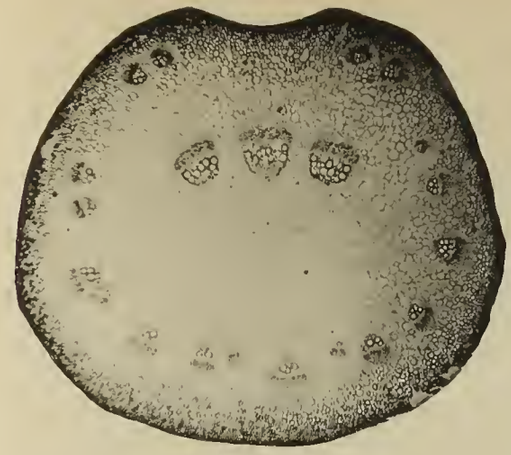
SALISBURY — EXTRA FLORAL NECTARIES OF POLYGONUM.



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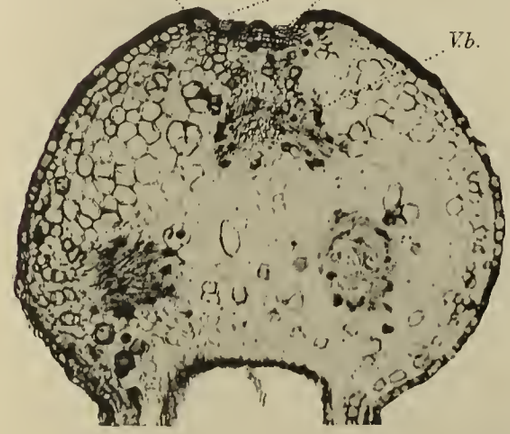

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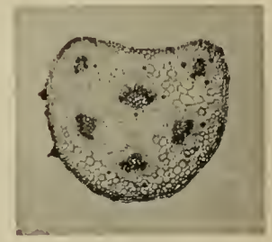

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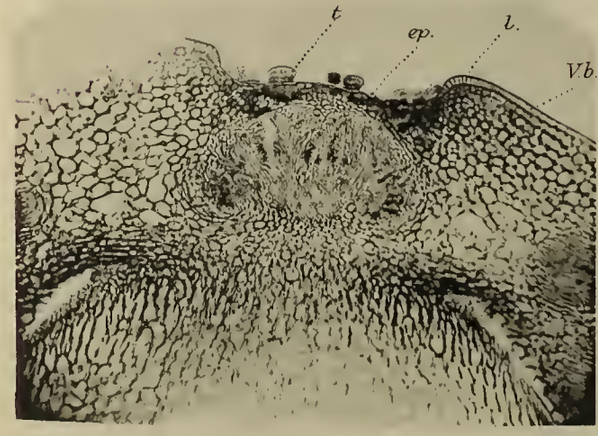

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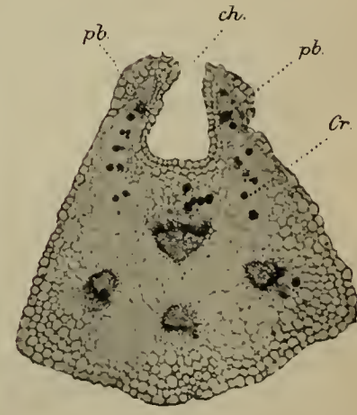

12.

Huth, coll.

The Structure and Affinities of British Tuberaceae.

BY

GEORGE MASSEE, F.L.S.

With Plate XVII.

IT is a somewhat remarkable coincidence, if nothing more, that two of the primary divisions of the Fungi, Ascomycetes and Basidiomycetes respectively, should be represented by subterranean forms possessing many morphological and physiological features in common. Amongst such may be enumerated a comparative absence of differentiation, the large size and marked variability in the number of spores, whether produced on basidia or in asci, and the more or less elaborate ornamentation of the epispore. Hymenogastraceae, the subterranean group belonging to the Basidiomycetes, is characterized by the spores being produced on basidia, whereas in the Tuberaceae, representing the Ascomycetes, the spores are contained in asci. With this marked exception the mode of development and general structure is the same in the two groups.

The sporophore originates as a minute ball from a weft of mycelium, which is generally located some distance underground, and continues to increase in size for some considerable time before differentiation commences. During this period the sporophore consists of a solid mass of densely interwoven hyphae, usually more or less globular in form, although sometimes lobed or irregular. At a later stage the peripheral hyphae become thick-walled and dark-coloured, and form the cortex, which in some species remains smooth and even, in others nodulose, or broken up into pyramidal warts. The ultimate structure of the cortex depends on the relative rate of growth of the peripheral and central portions of the sporophore. If the two portions grow at the same rate and for the same length of time, the cortex remains even. On the other hand, if the cortex ceases to grow and becomes rigid at an early stage of development, before the internal portion ceases to grow, the outward pressure of the latter breaks up the cortex into polygonal warts or pyramids, which become more or less separated from each other at the base, depending on the relative increase in size of the central mass of the sporophore. The warts and spines present on the

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peridium or outer wall of the puff-balls originate in a similar manner, and are not in any instance due to local growth of the cortex. Simultaneous with the differentiation of the cortex, the solid mass of the sporophore becomes pierced by numerous tortuous rifts or cracks, the walls of which gradually recede from each other and bear the asci, which project into the cavity. The general appearance of a section of a mature sporophore may be compared to a section of a nutmeg. From the first the sporophore consists of two distinct systems of hyphae; one, the vegetative hyphae, go to form the peripheral portion of the sporophore, including the cortex, also the walls in the interior of the sporophore, whose free faces bear the hymenium. The second system of hyphae give origin to the asci alone. The components of this system have thinner walls, and are more slender than the vegetative hyphae, with which they grow intermingled until the free surfaces of the internal cavities are reached, where they form a compact layer, the free ends giving origin to a succession of asci. At what point, and under what conditions the two systems of hyphae become differentiated in the minute knob representing the future sporophore, is not known. When the spores are mature, the asci along with the hyphae belonging to the ascigerous system, deliquesce, leaving the framework composed of vegetative hyphae in a more or less moist condition for some time. This state of things is more marked in the descendants of the subterranean forms of the Basidiomycetes, the Gasteromycetes or puff-balls, which at a certain stage of development are completely waterlogged, owing to the liquefaction of the system of spore-producing hyphae. At a later stage the sporophore becomes dry, and contains a dense mass of powdery spores, characteristic of a ripe puff-ball.

When mature, the sporophores of subterranean Fungi emit a powerful odour. From the standpoint of humanity, this odour is sometimes very agreeable, as in some of the edible truffles; in many other species it is very unpleasant, resembling asafoetida, and other indescribable odours. From the standpoint of the fungus, these odours are of the nature of advertisements, indicating their whereabouts to rodents, by whom they are eaten, and the spores, after passing through the alimentary tract, are dispersed.

The Tuberaceae are an ancient group of Fungi, as evidenced by their extreme simplicity of structure. In the most typical genera, as *Tuber*, *Elaphomyces*, &c., the sporophore has a perfectly continuous cortex, and the whole mass continues to increase in size for some time after the mycelium from which it originated, has disappeared. In the genus *Terfezia* the cortex is still continuous, but a basal portion is shadowed in, which remains in continuity with the vegetative mycelium until the spores are mature. Finally, in *Genea*, *Choeromyces*, &c., the mycelium remains adherent to the ascophore, and supplies it with food until the spores are

mature, and, in addition, there is a distinct small opening or perforation in the cortex, shadowing in the structure which is characteristic of the great group of Ascomycetes known as Discomycetes, which originated from the Tuberaceae. So far as our knowledge at present goes, there is no advantage to subterranean Fungi in the possession of an opening in the cortex, but as in many other well-known instances, the presence of a structure of no immediate service to the members in which it originated, becomes a factor of primary importance to a subsequent group. Figures 16 to 19 on the accompanying plate illustrate the sequence of evolution from the typical subterranean condition of the Tuberaceae to the equally typical condition of the above-ground Discomycetes. In *Tuber* (Fig. 16), the cortex is quite continuous, without any opening, hence the hymenium is, as it were, hermetically sealed until the decay of the wall of the peridium or cortex. The hymenium is spread over the free surfaces of sinuous plates which practically fill the peridium. In *Genea* (Fig. 17), the peridium has a small opening, and the hymenial plates or tramal walls do not fill the peridium to the same extent as in *Tuber*. In another species of *Genea* (Fig. 18), the opening of the peridium is still more pronounced, and the projecting tramal plates are almost obliterated. All the above species are truly subterranean, and the dispersion of their spores is effected by rodents. In *Sphaerosoma* the ascophore is only partly buried in the ground, the wall has a small opening, and the projecting tramal plates have disappeared, the hymenium lining the inside wall of the ascophore only. *Sphaerosoma* was until recently placed in the Tuberaceae, but is now considered as belonging to the Discomycetes. It is in reality a transitional genus, connecting the two families named above. Finally the genus *Peziza* (Fig. 19) grows above ground, and in the most primitive types, as the one figured, the ascophore is almost indistinguishable from that of *Sphaerosoma*, except in that it develops above ground, has lost the strong aroma characteristic of the Tuberaceae, and depends on wind for the dispersion of its spores. This combination of characters constitutes the group known as Discomycetes, as contrasted with those of the Tuberaceae, from which it evolved. The modifications necessary to enable the asci to eject their spores into the air, for dispersion by wind, in the Discomycetes, is contemporaneous with the modifications of the ascophore, already enumerated in the Tuberaceae. In typical members of the Tuberaceae, the asci play no part whatever in the distribution of the spores, hence they deliquesce and disappear at an early stage, liberating the spores into the cavities of the ascophore ; furthermore, such asci are usually globose or broadly elliptical, and contain a variable number of spores, and of various sizes, sometimes a single very large spore is present, whereas a neighbouring ascus may contain as many as six, or even eight spores. It is obvious that the nucleus present in such asci is erratic in its division. On the other hand, in those genera of the Tuberaceae

that shadow in the origin of the Discomycetes, through various morphological and physiological changes in the ascophore, the asci also undergo important changes, resulting in the formation of a cylindrical ascus, containing eight spores of equal size, arranged in a single row, as in the genera *Stephensia*, *Genea*, &c. Such an ascus closely resembles those met with in the genus *Peziza*, only lacking the well-known arrangement for suddenly ejecting the mature spores into the air, by the process known as 'puffing'.

In the Tuberaceae, as in all primitive groups of Fungi, when the advantages arising from dispersion by wind had not been realized, the spores are of exceptional size, and usually with a greater or less amount of ornamentation of the epispore. The object of this ornamentation is obscure, possibly its function is protective, while the spores are subjected to various mechanical processes in the stomach of some rodent. The ornamentation may take the form of raised ribs, anastomosing to form a more or less regular network; of coarse warts or tubercles; or of long spines or finger-like processes. The wall of the spore is in all instances relatively very thick, and of a soft or gelatinous consistency, and is in all cases perfectly smooth and even until approaching maturity, when the ornamentation, of whatever nature, is always due to skrinkage of special portions of the wall, and to the bulging out of other portions to form the ribs, warts, &c. If mature spores are treated with a five per cent. solution of potassic hydrate for some time, the ornamentation of the wall disappears, and it becomes smooth and even. In those genera leading up to the Discomycetes, the spores are smaller, and smooth or nearly so.

Geographical Distribution.

Owing to the subterranean habit, members of the Tuberaceae are almost invariably passed over by travellers, and even in Europe the group has not appealed to mycologists generally; some half-dozen, amongst whom may be mentioned, Berkeley and Broome in England, Vittadini in Italy, Tulasne in France, and Hesse in Germany, having furnished the bulk of information we possess at the present day. Harkness has done some valuable pioneer work in the same direction in California, and Rodway has discovered some very interesting species in Tasmania. Duthie has also met with certain edible species in India. On the whole it may be said that the family is cosmopolitan in its distribution.

Numerically the family is quite a small one, we have in Britain 11 genera and 32 species. The total number of known genera is 27, including 140 species.

The following table gives the known distribution of genera and species throughout the world, and clearly indicates that our knowledge of the number of species recorded for a given continent, is in proportion to the

amount of attention paid to the subject, and does not in any sense indicate the actual distribution of either genera or species.

	Europe.	*Asia.*	*Africa.*	*America.*	*Australasia.*
*Amylocarpus	1				
*Balsamia	5			4	
*Choeromyces	6				
Cryptica	1				
Delastria	1				
*Elaphomyces	24				
Genabea	2				1
*Genea	11			2	1
Geopora	2				4
Gyrocratera	1				
*Hydnobolites	4			1	
Hydnocystis	4			1	
*Hydnotrya	3			1	
Leucangium	2				
Lilliputia	1				
Myrmecocystis				2	
*Pachyphloeus	4				
Phaeangium			1		
Picoa			1		
Piersonia			2		
Pseudogenea	1				
Pseudohydnotrya				3	
*Stephensia	2				1
*Terfezia	10	3	6		
Terfeziopsis				1	
Tirmania			2		
*Tuber	55	3		10	
	140	6	12	25	7

Genera preceded by an asterisk (*) are British.

Habitats.

Various members of the Tuberaceae are not uncommon in England, nevertheless, the group is not infrequently entirely omitted from county lists of Fungi. Notwithstanding the fact that the Essex Field Club have for the past thirty years held annual Fungus Forays in Epping Forest, two members of the Tuberaceae were added to the list of Essex Fungi for the first time, last year.

Truffles prefer a soil consisting of clay mixed with sand and ferruginous particles, or a rich mixed alluvium. The soil must be porous to secure the proper amount of aeration. Sour soil or the presence of stagnant water is fatal. It is a prevalent opinion that truffles are met with only under beech trees, this however is a mistake. They occur in open woods of chestnut, oak, or beech, also in open woodland districts, in soil consisting of a mixture of humus and sand. The presence of truffles is often indicated in the places where they grow, by a slight cracking or upheaval of the soil, more especially under the drip of trees. The late C. E. Broome, to whose researches first and only records of many of our British species are due,

used a short-handled, strong, long-toothed iron rake when hunting for members of the Tuberaceae.

Tuber aestivum is our best native truffle from an edible standpoint. It occurs in fair abundance under beeches on Salisbury Plain. The neighbourhood of Patching, near Arundel, Sussex, is or was also noted for its truffles.

It is known that the mycelium of certain species of *Elaphomyces* forms mycorhiza on the roots of conifers. It is also known that success in the cultivation of edible truffles in France depends to a marked extent on keeping the roots of the oaks, under which they grow, near to the surface of the soil. This implies some connexion between the truffles and the oak roots, and further suggests that the truffles benefit to some extent by the formation of mycorhiza.

TUBERACEAE.

Tuberoideae, Vitt., Mon. Tub., p. 12 (1831)

Ascophore subglobose, irregularly nodulose or sulcate, indehiscent, wall continuous or variously perforated, fleshy or coriaceous, rarely becoming hard and woody. Asci varying from cylindrical to globose, 1–8-spored. *Spores* continuous. Subterranean or rarely more or less exposed.

Key to the Families.

Gleba breaking up into a loose mass of spores at maturity—Elaphomyceteae.

Gleba not breaking up into a powdery mass, but having persistent dissepiments and becoming lacunose or cavernose—Tubereae.

Fam. 1. ELAPHOMYCETEAE.

Elaphomycei, *Tub.*, Fung. Hypog., p. 100 (1851).

Ascophore subglobose, wall not perforated, firm, becoming hard when dry. *Gleba* without permanent dissepiments, containing numerous silky filaments. *Asci* 1–8-spored. *Spores* globose, coloured, forming a powdery mass at maturity. Subterranean.

Elaphomyces, Nees, Pl. Myc., p. 58 (1820); Sacc., Syll., viii, p. 803 (1889); Cooke, Brit. Fung., p. 749 (1871).

Ascophore subglobose, wall hard, *Asci* subglobose. *Spores* coloured, filling the ascophore with a dusty mass at maturity.

Elaphomyces authracinus, Vitt., Mon. Tub., p. 66, tab. 3, fig. 8 (1831); Sacc., Syll., viii, p. 866 (1889); Cooke, Brit. Fung., p. 749 (1871).

Ascophore subglobose, blackish-brown, rigid, very minutely corrugated, flesh whitish, mycelium usually copious, brownish, 2–4 cm. diam. *Asci* subglobose. *Spores* globose, blackish-brown, opaque, very minutely granulated or sometimes smooth, 16–20 μ diam., mixed with colourless cobweb-like threads.

Hab. In clay soil under trees.

Distr. Britain; France; Italy.

Exsicc. Speg., Dec. Myc. 4.

Described from a specimen from Vittadini in Herb. Kew., rare in this country. Smell weak, somewhat resembling radishes.

Elaphomyces variegatus, Vitt., Mon. Tub., p. 68, tab. 4, fig. 4 (1831); Cooke, Brit. Fung., p. 749 (1871); Sacc., Syll., viii, p. 867 (1889); *El. muricatus,* Berk., Engl. Flora, v, p. 307 (1836).

Ascophore subglobose, thick, hard, golden- or ochraceous-yellow or brownish, densely covered with pointed pyramidal warts, flesh whitish, mottled or streaked with reddish-brown, finally becoming altogether brown, mycelium yellow, scanty. *Asci* subglobose, 1–4-spored. *Spores* globose, blackish-brown, sometimes with a violet tinge, opaque, rather coarsely corrugated, 16–21 μ, when free mixed with a copious cobweb-like mass of capillitium threads.

Hab. Under trees, mostly in upland woods.

Distr. Britain; France; Italy; Germany; Belgium; Sweden and Finland; United States.

Exsicc. Vize, Fung. Brit., 147; Cooke, Fung. Brit., 418; Berk., Brit. Fung. 306; Sacc., Myc. Ven., 1390; Roum., Fung. Gall., 2170 and 2386; Thüm., Myc. Univ., 524.

Specimen from Vittadini in Herb. Kew., examined. Distinguished from its nearest ally, *E. granulatus,* Fr., by the pointed pyramidal, usual four-sided warts on the ascophore, and by the marbled or variegated flesh, hence the specific name. Smell variable, sometimes quite weak at maturity, at others suggesting that of *Ruta graveolens* or burnt hoof. *Claviceps ophioglossoides,* Fr., is often parasitic on this species.

Elaphomyces granulatus, Fr., Syst. Myc. 3, p. 58 (1821); Cooke, Brit. Fung., p. 750 (1871); Sacc., Syll., viii, p. 868 (1889); *El. leocarpus,* Vitt., Mon. Tub., p. 72 (1831).

Ascophore globose or ellipsoid, firm, yellow then tawny or brownish, densely covered with minute rounded warts, flesh tinged red but not marbled when moist, pure white when dry, 2–6 cm. diam., mycelium yellow, scanty and soon disappearing. *Asci* subglobose, 1–8-spored. *Spores* globose, blackish-brown with a purple tinge in the mass, opaque, 20–30 μ diam., mixed in the gleba with a dense mass of silky capillitium threads.

Hab. In woods, especially in dry heathy ground.

Distr. The whole of Europe; N. America.

Exsicc. Berk., Brit. Fung., 279; Moug. and Nest., Fung., 282; Thüm., Fung. Austr., 624; Klotzsch-Rab., Herb. Myc., 174; Karst., Fung. Fenn., 375; Fckl., Fung. Rhen., 1075; Syd., Myc. March., 341; Roumeg., Fung. Gall., 164; Desmaz. Crypt. Fr., sér. 1, 672.

Our commonest species, general throughout England, and has been met with in Scotland, as far north as Glamis. When large the ascophore is sometimes more or less sulcate or nodulose. Smell generally weak, but sometimes pungent.

Parasitic on the roots of trees, and is in turn the host of another fungus, *Claviceps capitata,* Fr.

Elaphomyces leucosporus, Vitt., Mon. Lycop., p. 71, tab. 3, fig. 1 (1841); Tul., Hypog., p. 104 (1851); Sacc., Syll., viii, p. 865 (1889).

Ascophore irregularly globose, usually deeply umbilicate at the base, wall thin, blackish-brown, smooth, flesh dark coloured, about 0·5 cm. diam., mycelium scanty, greenish. *Spores* globose, minutely granulated, at first almost colourless, becoming blackish-brown and almost opaque, 15–20 μ diam., showing a tinge of purple in the mass, held together by very slender hyaline capillitium threads.

Hab. Under oaks.

Distr. Britain; Italy.

This is our rarest species, having been only once found by Broome, near Chudleigh, Devon. The spores are described by Vittadini as white at first, becoming tinged yellow. This, however, only applies to the immature stage, as a specimen sent by Vittadini to Berkeley, and now in the Kew herbarium, has the spores almost black as described above; the same is the case with Broome's British specimens. Hence the specific name is not quite applicable, but must stand. The smell is weak, suggesting that of *Tuber brumale*, Vitt., and is very fugacious.

[**Elaphomyces citrinus,** Vitt., Mon. Tub., p. 65, tab. 4, fig. 16 (1831); Sacc., Syll., viii, p. 865 (1889).

Ascophore irregularly globose, blackish-brown or brownish-olive, smooth, rather soft, covered with a copious weft of persistent lemon-coloured mycellium, flesh thick, soft, greyish-white with a tinge of green, then brownish-purple becoming whitish when dry, 0·5–1·5 cm. diam. *Spores* globose, very minutely granulated, smoky green, becoming darker and almost opaque when mature, 8–10 μ diam.

Hab. Under oaks.

Distr. Britain?; Italy.

This species has been recorded as occurring in England, but I have not been able to corroborate its occurrence, and have seen no British specimens. There is a specimen from Vittadini in the Kew herbarium.]

TUBEREAE.

Tuberei, Fries, Summa Veg. Scand., p. 437 (1849).

Ascophore fleshy, becoming hard. *Gleba* furnished with veins, solid cavernose or lacunose, rarely without veins but always lacunose. *Spores* not forming a powdery mass at maturity. Subterranean or rarely more or less exposed.

Key to the Genera.

A. Gleba without veins, but having one or more cavities,

† Asci cylindrical.

Spores warted, subglobose.

Genea.

†† Asci broadly oblong or subglobose.

Spores elongated, smooth.

Balsamia.

Spores globose, reticulated.

Hydnobolites.

Spores globose, warted.

Hydnotrya.

B. Gleba furnished with veins; solid or lacunose.

Spores globose, smooth; asci cylindrical.

Stephensia.

Spores globose, warted; asci elliptic-oblong.

Pachyphlaeus.

Spores elliptical or globose, reticulated, warted or spinulose; asci subglobose.

Tuber.

Spores globose, with elongated blunt warts; asci elliptic-oblong.

Choeromyces.

Ascophore with a distinct obconic base; spores globose, warted; asci subglobose.

Terfezia.

Spores globose, hyaline, with long, very slender spreading spines.

Amylocarpus.

Genea, Vitt., Mon. Tub., p. 27 (1831); Cooke, Brit. Fung., p. 747 (1871); Sacc., Syll., viii, p. 873 (1889). *Hydnocaryon*, Wallr., Fl. Crypt, Germ., ii, p. 860 (1831).

Ascophore fleshy, warted, with a perforation at the apex and a tuft of mycelium at the base. *Gleba* broken up by walls into irregular cavities in communication with the apical opening. *Asci* cylindrical, 8-spored. *Spores* subglobose, colourless.

Genea verrucosa, Vitt., Mon. Tub., p. 28, tab. 2, fig. 7 (1831); Cooke, Brit. Fung., p. 748 (1871); Sacc., Syll., viii, p. 873 (1889). *G. papillosa*, Berk., Ann. Nat. Hist., xiii, p. 356 (1844); not of Vittadini.

Ascophore very variable in shape, sulcate or variously lobed, black, minutely warted, apical opening often large, 0·5–1·5 cm. diam. *Asci* narrowly elliptical, apex obtuse, spores 8, uniseriate. *Paraphyses* filiform, septate. *Spores* globose or very broadly elliptical, colourless, warted, 25 × 20 μ, or 25–30 μ diam.

Specimen in Kew herbarium from Vittadini examined. *Gleba* white, basal mycelium short and scanty. Smell strong, unpleasant, somewhat resembling that of *Balsamia vulgaris*, Vitt.

Hab. Clay soil, under oaks and chestnuts.

Distr. Britain; France; Italy; United States.

Genea Klotzschii, Berk. and Br., Ann. Nat. Hist., xviii, p. 78 (1846); Cooke, Brit. Fung., p. 748 (1871); Sacc., Syll., viii, p. 847 (1889). *G. verrucosa, Klotzsch*, in Dietr. Fl. Preuss., vii, no. 474 (1833) not of Vitt., Mon. Tub., p. 28, tab. 2, fig. 7 (1831).

Ascophore irregular in form, subplicate, black, warted, attached by black fibrils springing from the base, mycelium abundant, white, floccose, 1–2 cm. diam. *Asci* cylindrical, apex obtuse, spores 8, uniseriate. *Paraphyses* very slender, longer than the asci, septate. *Spores* subglobose, colourless, rather coarsely warted, 25–30 μ diam.

Hab. Nearly on the surface of the soil.

Distr. Britain; Germany.

Gleba white or tinged yellow. Smell foetid. Most nearly allied to *G. sphaerica*, Vitt., a species not met with in Britain.

The mycelium spreads for several inches on the surface of the ground like a cobweb, being densest in the centre beneath which two or three ascophores are found.

'The mycelium spreads for some distance on or within the soil, so that the plant is easily detected when the leaves are raked off. This vanishes when the peridia [ascophores] are perfect. One or more individuals are found in each patch of mycelium. In the young peridium [ascophore] the point of attachment is lateral. The sporidia [spores] are large, coarsely granulated, and much exceed in volume those of *G. verrucosa*.' Berk.

Described from Berkeley's type specimens, now in Herb. Kew.

Genea hispidula, Berk., in Tul. Hypog., p. 121, tab. 12 fig. 2; tab. 3, fig. 3 (1851); Cooke, Brit. Fung., p. 748 (1871); Sacc., Syll., viii, p. 875 (1889). *G. papillosa*, Berk., Ann. Nat. Hist., xviii, p. 76 (1844); not of Vittadini.

Ascophore subglobose, everywhere densely covered with bright brown, long tapering, thick-walled septate hairs, apical opening almost hidden, about 1 cm. diam. *Asci* cylindrical, apex obtuse, base abruptly narrowed into a short pedicel, spores 8, uniseriate. *Paraphyses* slender, septate. *Spores* globose or broadly elliptical, 25–30 × 20–25 μ, or 20–26 μ diam.

Hab. In the ground under chestnut trees.

Distr. Britain; France; United States.

The internal cavity is often almost simple.

Described from type specimen in Herb. Kew.

'The whole peridium [ascophore] is of a rich brown, and is densely clothed with brown bristles wherever it extends. The sporidia [spores] are very much larger and more coarsely granulated than in *G. verrucosa*; the granules indeed being often bifid; they often contain two nuclei [oil globules], but sometimes there is but one. From the size of a pea to that of a filbert. Odour faint, not peculiar.' Berk.

Berkeley's remark that the warts are often bifid, means that when sufficiently magnified, they are of various irregular shapes, sometimes curved, at others forked or bifid.

Balsamia, Vitt., Mon. Tub., p. 30, tab. 1, fig. 2, and tab. 5, fig. 6 (1831); Cooke, Fung. Brit., p. 747 (1871); Sacc., Syll., viii, p. 877 (1889).

Ascophore not perforated, rather soft, warted, cavernose. *Asci* elliptic-oblong or subglobose, narrowed at the base into a long, slender pedicel. *Spores* 8 in the ascus, elliptic-oblong, smooth, colourless.

Balsamia vulgaris, Vitt., Mon. Tub., p. 30, tab. 1, fig. 2, and tab. 5, fig. 6 (1831); Corda, Icon., vi, p. 59, tab. 10, fig. 99 (1854); Tul., Hypog., p. 123, tab. 4, fig. 4, and tab. 15, fig. 1 (1851).

Ascophore irregular in form, with wavy ridges and here and there deep depressions, very minutely warted, sometimes almost or quite smooth, dark coloured 2–4 cm. diam., cavities of gleba large, sinuous. *Asci* ovate-oblong or very broadly ovate, obtuse. *Spores* cylindric-oblong, smooth, colourless, 25–35 × 9–12 μ. *Paraphyses* slender, numerous.

Hab. Under trees.

Distr. Britain; France; Italy.

Smell very strong and unpleasant. Apparently rare in England, having only once been collected by Broome at Chudleigh, Devon.

Described from a specimen communicated by Vittadini, and now in the Kew herbarium.

Balsamia platyspora, Berk. and Br., Ann. Nat. Hist. xiii, p. 385 (1844); Cooke, Brit. Fung., p. 747 (1871), Sacc., Syll., viii, p. 878 (1889).

Ascophore irregularly subglobose, minutely warted, yellowish, warts darker, 1–2 cm. diam., internal cavities minute. *Asci* oblong-ovate, with a slender pedicel, 8-spored. *Paraphyses* slender. *Spores* elliptic-oblong, ends rounded, smooth, colourless, 24–30 × 8–11 μ.

Hab. Under trees or bushes.

Distr. Britain; France; United States.

Type specimen in Herb. Kew., examined.

'Globose, about the size of a horse-bean, rufous, with the interstices of the warts of a light yellowish tint, from the exposure of the internal substance. Cells minute; sporidia [spores] broadly elliptic.' Berk.

Balsamia fagiformis, Tub., Hypog., p. 125, tab. 4, fig. 3 (1851); Sacc., Syll., viii, p. 878 (1889); *Balsamia polysperma,* Tul., Ann. Sci. Nat., sér. 2, xix, p. 397 (1843); not of Vittadini, Mon. Tub., p. 31 (1831).

Ascophore globose, usually regular in outline, densely covered with minute warts, which here and there become slender and hair-like, deep dusky brown or rusty, 1·5–3 cm. diam., cavities crowded. *Asci* ovate-oblong, sometimes almost subglobose, with a slender pedicel, 8-spored. *Paraphyses* slender. *Spores* elliptic-oblong, ends rounded, colourless, 16–20 × 10–11 μ.

Hab. In clay soil.

Distr. Britain; France.

Smell very strong at maturity, resembling that of *Tuber brumale,* Vitt.

Only collected in this country as far as I am aware, by Broome, at Batheaston.

Broome's specimens are now in the Kew herbarium. I have not seen a type or authentic specimen, but Broome's plant agrees well with the description and figures given by Tulasne.

Hydnobolites, Tul., Ann. Sci. Nat., sér. 2. xix, p. 278 (1843); Cooke, Brit. Fung., p. 746 (1871); Sacc., Syll., viii, p. 879 (1889); *Oogaster,* Corda, Icon. Fung., iv, p. 60, tab. 14, fig. 121 (1840).

Ascophore fleshy, wrinkled or plicate, cells cavernous, sinuous, opening to the surface. *Asci* elliptic-oblong, 8-spored. *Spores* globose, reticulated.

Hydnobolites cerebriformis, Tul., Ann. Sci. Nat., sér. 2, xix, p. 278 (1843); Cooke, Brit. Fung., p. 746 (1871); Sacc., Syll., viii, p. 879 (1889); *Oogaster cerebriformis,* Corda, iv, p. 60, tab. 14, fig. 121 (1840).

Ascophore subglobose, sinuously wrinkled especially below, base slightly depressed, at first entirely covered with delicate whitish down, soon becoming entirely glabrous and pale yellow, 1·5–3·5 cm. diam., walls of gleba sinuous, covered with white tomentum continuous with that on the outside. *Asci* elliptic-oblong, 8-spored. *Spores* globose, reticulated, tinged yellow, 18–30 μ diam.

Hab. Attached to the ground under moss or fallen leaves in woods.

Distr. Britain; France.

Exsicc. Roumeg., Fung. Gall., 2666.

Smell weak. Specimen from Tulasne in Kew herbarium examined. The

ascophore is covered with sinuous folds resembling a brain, which suggested the specific name.

Hydnotrya, Berk. and Br., Ann. Nat. Hist., xviii, p. 78 (1846); Cooke, Brit. Fung., p. 745 (1871); Sacc., Syll., viii, p. 879 (1889).

Ascophore perforated, minutely warted or velvety, cavities of gleba wavy or sinuous, often opening to the surface. *Asci* irregularly elliptic-oblong. *Spores* globose.

Hydnotrya Tulasnei, Berk. and Br., Ann. Nat. Hist., xviii, p. 78 (1846); Cooke, Brit. Fung., p. 745 (1871); Sacc., Syll., viii, p. 879 (1889); *Hydnobolites Tulasnei*, Berk. and Br., Ann. Nat. Hist., xiii, p. 357 (1844).

Ascophore globoso-depressed, base plicate, with perforations, minutely velvety, rufous, 2·5–6 cm. diam., walls of the tortuous cavities white, pubescent. *Asci* irregularly elliptic-oblong with a long pedicel, 8-spored. *Spores* globose, amber-brown and coarsely tuberculated when mature, 25–35 μ diam.

Hab. Sandy soil.

Distr. Britain.

Exsicc. Berk., Brit. Fung. (as *Hydnobolites Tulasnei*), 302; Roumeg., Fung. Gall., 2219; Rab., Herb. Mycol., ed. ii, 301.

Smell weak. The colour of the ascophore is somewhat variable, but is always some shade of rusty brown or tawny, sometimes with a more or less decided tinge of vermilion. Berkeley describes the spores as reticulated, but examination of his own material shows them to be coarsely warted. Saccardo has repeated this mistake. Superficially closely resembling *Balsamia vulgaris*, Vitt., especially when dry.

Type specimen in the Kew herbarium examined.

Stephensia, Tul., Comp. rend., xxi, p. 1433 (1845); Cooke, Brit. Fung., p. 745 (1871); Sacc., Syll., viii, p. 880 (1889). *Genea*, in part, Vitt., Mon. Tub., p. 29 (1831).

Ascophore irregularly globose, more or less depressed. *Gleba* with tortuous dissepiments or veins, becoming cavernose, deliquescent. *Asci* cylindrical, 8-spored. *Spores* uniseriate, globose, smooth, colourless.

Readily distinguished by the smooth, colourless, globose spores arranged in a single row in the elongated cylindrical ascus.

Stephensia bombycina, Tul., Hypog., p. 130, pl. 11, fig. 4 (1851); Cooke, Brit. Fung., p. 745, fig. 348 (1871); Sacc., Syll., p. 880 (1889); *Genea bombycina*, Vitt., Mon. Tub., p. 29, tab. 3, fig. 19; tab. 4, fig. 8 (1831).

Ascophore subglobose, depressed, often very irregular in form, lobed or with sinuous folds, indented more or less at the base, rather soft, floccose or minutely downy, whitish or with a yellow tinge, 3–6 cm. diam. *Gleba* whitish with yellow sinuous dissepiments. *Asci* cylindrical, 8-spored. *Spores* uniseriate, smooth, globose, colourless, 18–23 μ.

Smell at first pleasant, soon becoming very disagreeable and strong, resembling that of *Melanogaster ambiguus*, Tul. Specimen from Tulasne, in Herb. Kew., examined.

Hab. Subterranean.

Distr. Britain; France; Italy; United States.

Pachyphloeus, Tul., Giorn. Bot. Ital., ii, p. 69 (1844); Cooke, Brit. Fung., p. 743 (1871); Sacc., Syll., viii, p. 881 (1889).

Ascophore fleshy, often warted, with a more or less well-defined apical aperture, base distinct. *Gleba* marbled, dissepiments tortuous. *Asci* oblong or ovate-oblong, 8-spored. *Spores* globose, coloured, warted (in British species, irregularly 2-seriate).

Distinguished by the ascophore having a terminal aperture, and the coloured, warted spores arranged in an irregularly biseriate manner in the ascus. In certain exotic species the epispore is reticulated or echinulate.

Pachyphloeus melanoxanthus, Tul., Giorn. Bot. Ital., ii, p. 69 (1844); Tul., Hypog., pl. 4, fig. 6, and pl. 14, fig. 4(1851); Cooke, Brit. Fung., p. 743 (1871). *Choiromyces melanoxanthus*, Berk., Ann. Nat. Hist., xiii, p. 359 (1844).

Ascophore globose or angularly globose, warted, with a distinct base, yellowish-green becoming black, opening apical or somewhat lateral, 1·5–4 cm. diam. *Gleba* olive-yellow sometimes becoming dusky, marbled with dusky lines and broad, black dissepiments. *Asci* oblong or ovate-oblong, shortly stipitate, 8-spored. *Spores* irregularly 2-seriate, coloured, globose, warted, 14–17 μ diam.

The warts on the ascophore are less prominent when the fungus is dry. Smell weak when young, becoming strong and nauseous. Berkeley states that in his specimens the ascophore was black in every stage of growth.

Hab. Among humus in oak or beech woods. Sometimes attached to sticks or dead leaves, without any connexion with the ground.

Distr. Britain; France; Germany.

Specimen in Herb. Kew., from Tulasne, examined.

Pachyphloeus citrinus, Berk. and Br., Ann. Nat. Hist., xviii, p. 79 (1846); Cooke, Brit. Fung., p. 744 (1871); Sacc., Syll., viii, p. 881 (1889).

Ascophore subglobose, minutely warted, brown, powdered with lemon-coloured particles, apical orifice deep lemon-yellow, base rooting, 1–2·5 cm. diam. *Gleba* floccose, lemon-yellow. *Asci* narrowly ovate-oblong, 8-spored. *Spores* globose, coloured, finely warted, irregularly 2-seriate, 13–14 μ diam.

Allied to *P. melanoxanthus*, Tul., differing in the thinner, much more finely warted ascophore, which is of a uniform gamboge yellow when young, also in the smaller and more finely warted spores. Smell strong, resembling rotting seaweed. Saccardo says the spores are 'reticulato-alveolatis', this is a mistake. Berkeley in his diagnosis does not mention the markings on the spores.

Type specimen in the Kew herbarium examined.

Hab. Underground in woods.

Distr. Britain.

Pachyphloeus conglomeratus, Berk. and Br., Ann. Nat. Hist., xviii, p. 79 (1844); Tul., Hypog., p. 132 (1851); Sacc., Syll., viii, p. 882 (1889).

Ascophore irregularly lobed and plicate, as if composed of several confluent individuals, lobes rounded, smooth, deep rufous-brown or olive-brown, interstices of the lobes clothed with yellow fibres, 2–3 cm. diameter. *Asci* broadly ovate-oblong, 8-spored. *Spores* coloured, globose, with rather large, somewhat distant warts, 16–19 μ diam., irregularly 2-seriate.

Readily distinguished by the much lobed, smooth ascophore, which is shortly stipitate.

Hab. In woods.

Distr. Britain; Italy.

Tuber, Micheli, N. pl. Gen., p. 221, tab. 102 (1729); Link, Obs. in ordin. plant, Diss. I, p. 33, tab. 2, fig. 51 (1809); Vitt., Mon. Tub., p. 31 (1831); Cooke, Brit. Fung., p. 738 (1871); Sacc., Syll., viii, p. 882 (1889); *Lycoperdon*, pro parte, Linn., sp. Pl., II, p. 1183 (ed. i) (1753).

Ascophore irregularly globose, without a distinct rooting base, fleshy or sometimes becoming almost woody, surface glabrous or tomentose, often papillose or warted. *Gleba* marbled with veins, some white and sterile (air chambers), others coloured and producing asci on the walls. *Asci* broadly elliptical or globose, 1–12, most frequently 4-spored. *Spores* elliptical or globose, smooth, warted or reticulated, coloured.

Distinguished by the differently coloured veins in the gleba, and the more or less globose asci.

A. *Epispore reticulated.*

Tuber aestivum, Vitt., Mon. Tub., p. 38, pl. 2, fig. 4 (1831); Cooke, Brit. Fung., p. 738 (1871); Sacc., Syll., viii, p. 891; Chatin, La Truffe, p. 62, pl. 9 (1892); Hesse, Die Hypog. Deutschl., ii, p. 19 (1894).

Tuber cibarium, Sow., Engl. Fung., pl. 309 (1797).

Ascophore irregularly globose, often indented at the base, blackish-brown, covered with large, hard, pyramidal warts which are generally with the sides striated, 2–10 cm. diam. *Gleba* whitish, becoming brown, dissepiments numerous, much branched. *Spores* 2–4 in an ascus, most frequently two, brownish, elliptical or subglobose, reticulated, mesh large, rather shallow, 40–60 × 30–40 μ, or 30–40 μ diam.

Distinguished by the coarsely pyramidally warted blackish ascophore, and the large spores with a shallow, wide-meshed reticulation. Among the best of our edible truffles, but very much inferior in this respect to the Périgord truffle, *Tuber melanosporum*, Vitt., which does not occur in Britain. Taste somewhat insipid, smell somewhat resembling that of beer yeast.

Specimen from Vittadini, in Herb. Kew., examined.

Hab. In woods, especially of beech. Just below the surface of the ground, or sometimes above-ground amongst heaps of leaves, &c.

Distr. Britain; France; Italy; Germany; Bohemia.

Exsicc. Cooke, Brit. Fung. Exs., 663; Rabenh., Fung. Eur., 1425; Klotzsch-Rabenh., Herb. Myc., 246; Fckl., Fung. Rhen., 1077 and 1078; Roumeg., Fung. Gall., 2815: Vize, Fung. Brit., 86; Thüm., Myc. Exs., 312.

Tuber bituminatum, Berk. and Broome, Ann. Mag. Nat. Hist., vii, p. 183 (1851); Cooke, Brit. Fung., p. 739 (1871); Sacc., Syll., viii, p. 892 (1889).

Ascophore globose or ovate, regular, black, covered with small polyhedral warts, with a deep depression at the base, 3–7 cm. diam. *Gleba* with the dissepiments starting mostly from the base. *Asci* subglobose, with long pedicels. *Spores* globose

or broadly elliptical, translucent brown, with large, shallow reticulations, 1–4 in an ascus, 40–50 μ, or 45 × 60 μ.

Most nearly allied to *T. mesentericum*, Vitt., from which the present species differs in the larger, globose or subglobose spores, and the less tortuous dissepiments. The ridges of the network of the epispore often give off blind ends projecting into the polygons.

Type specimen in Herb. Kew. examined.

'Closely allied to *T. aestivum*, Vitt., but easily distinguished by the odour; it differs also in the general form, being much more regular, and the warts smaller, and in the existence of a basal cavity prolonged into the substance of the fungus, which is thus very light compared with *T. aestivum*. It shrinks very much in drying. The sporangia [asci] have much longer stalks than in *T. aestivum*. The sporidia closely resemble those of that species, but are slightly longer compared with their width, and have somewhat shallower cells. It ranges from the size of a walnut to that of a hen's egg. Odour bituminous and very strong of horseradish.' Berk.

Hab. In deep sand.

Distr. Britain.

Tuber foetidum, Vitt., Mon. Tub., p. 41, pl. 1, fig. 8, and pl. 3, fig. 11 (1831); Tul., Hypog., p. 140, pl. 17, fig. 7 (1851); Corda, Icon. Fung., vi, p. 80, fig. 135 (1854); Sacc., Syll., viii, p. 887 (1889); Chatin, La Truffe, p. 69 (1892).

Ascophore irregularly globose, variously nodulose or lobed, smooth or minutely corrugated, brownish, 1·5–3·5 cm. diam. *Gleba* whitish at first, becoming soft and of a reddish-brown colour, dissepiments whitish, rather thick, very much branched and anastomosing. *Asci* obovate or variable in form, 2–4-spored. *Spores* reticulated, mesh not very large, shallow, 27–36 × 20–30 μ, brown.

Smell and taste resembling that of rancid oil, with a suggestion of onions.

Specimen from Vittadini, in the Kew herbarium, examined.

Hab. Subterranean.

Distr. Britain; Italy.

Tuber macrosporum, Vitt., Mon. Tub., p. 35, pl. 1, fig. 5 (1831); Tul., Hypog., p. 139, pl. 17, fig. 8 (1851); Cooke, Brit. Fung., p. 739 (1871); Sacc., Syll., viii, p. 887 (1889).

Ascophore globose sometimes nodulose, covered with minute depressed warts and often cracked, blackish and spotted with rusty brown, 2–5 cm. diam. *Gleba* at first white, changing to purplish-brown or blackish, dissepiments numerous, mixed with finer dusky lines. *Asci* subglobose, with long, slender pedicels, 1–3-spored. *Spores* elliptical, brown, reticulated, mesh small, rather shallow, 50–65 × 35–40 μ.

Readily known by the large, elliptical spores. The network on the epispore is small-meshed, but the size and form of the openings are irregular. Smell strong, resembling onions.

Specimen from Vittadini, in Herb. Kew., examined.

Hab. Underground, under oaks, willows, poplars, &c., often in clay soil.

Distr. Britain; France; Italy; United States.

Tuber excavatum, Vitt., Mon. Tub., p. 49, pl. 1, fig. 7 (1831); Tulasne, Hypog., p. 144, pl. 6, fig. 1, and pl. 17, fig. 5 (1851); Cooke, Brit. Fung., p. 740

(1871); Chatin, La Truffe, p. 67 (1892); Sacc., Syll., viii, p. 886 (1889); *Vittadinion Montagnei*, Zobel, in Corda's Icon. Fung., vi, p. 75 (1854).

Ascophore subglobose or irregular in form, with a basal depression, ochraceous, minutely granulated, 1·5–3 cm. diam. *Gleba* tinged ochraceous, becoming hard when dry, dissepiments white, radiating from the excavated base. *Asci* elliptical to subglobose, 2–4-spored. *Spores* elliptical or subglobose, reticulated, mesh large, deep, 35–53 × 30–40 μ, yellowish brown.

Distinguished by the minutely granulated or warted ascophore, furnished with a deep depression at the base. There are most frequently three spores in an ascus.

Smell resembling radishes.

Specimen from Vittadini in Herb. Kew. examined.

Hab. Underground in woods.

Distr. Britain; France; Germany; Italy; United States.

Exsicc. Rabenh., Fung. Eur., 911.

Tuber dryophilum, Tul., Giorn. Bot. Ital., ii, p. 62 (1870); Tul., Hypog., p. 147, pl. 5, fig. 3, and pl. 19, fig. 8 (1851); Cooke, Brit. Fung., p. 742 (1871); Sacc., Syll., viii, p. 889 (1889).

Ascophore globose, generally regular in form, even, at first white and minutely downy, becoming glabrous and brownish-violet, variegated with violet, 1·5–3 cm. diam. *Gleba* reddish-brown or purple-brown with whitish dissepiments springing from various points of the cortex. *Asci* 2–4-spored. *Spores* broadly elliptical, orange-brown, reticulated, mesh rather large and deep, 40–45 × 25–30 μ.

Characterized by the even wall of the ascophore, purplish gleba, and large, elliptical spores. More closely allied to *T. Borchii*, Vitt., a species not yet recorded for Britain. Smell weak, not unpleasant. Specimen from Tulasne, in Herb. Kew., examined.

Hab. Underground, under oaks and poplars.

Distr. Britain; France; United States.

Tuber rapaeodorum, Tul., Ann. Sci. Nat., sér. 2, xix, p. 380 (1843); Tul., Hypog., p. 147, pl. 5, fig. 4, and pl. 18, fig. 1 (1851); Sacc., Syll., viii, p. 890 (1889); Chatin, La Truffe, p. 67 (1892).

Ascophore globose or irregular in form, smooth, of a yellowish tinge and with whitish spots corresponding to the dissepiments in the gleba, 1·5–2·5 cm. diam. *Gleba* white then brownish with a few white dissepiments. *Asci* obovate or subglobose, 1–2, rarely 3–4-spored. *Spores* elliptical, reticulated, mesh large and somewhat elongated in the direction of the long axis of spore, shallow, 35–55 × 24–30 μ, yellowish-brown at maturity.

The size of the spores varies considerably, depending on the number present in an ascus. Smell strong and unpleasant, resembling radishes.

Hab. In sandy soil under trees.

Distr. Britain; France; Germany.

Exsicc. Fckl., Fung. Rhen., 2668.

Tuber puberulum, Berk. and Br., Ann. Mag. Nat. Hist., xviii, p. 81 (1846); Cooke, Brit. Fung., p. 741 (1871); Sacc., Syll., viii, p. 893 (1889).

Ascophore subglobose, somewhat lobed, pinkish-brown, clothed with short, erect

down giving it a pearly appearance, often cracked, 2–4 cm. diam. *Gleba* with whitish dissepiments radiating from the base. *Asci* subglobose. *Spores* globose or subglobose, orange-brown, reticulated, mesh not very large, deep, 35–45 μ diam.

Readily distinguished by the beautiful downy surface of the ascophore, through which the pinkish-brown colour shows. The ascophores are gregarious. Odour of radishes.

Hab. In sandy ground.

Distr. Britain; United States.

Exsicc. Cooke, Fung. Brit. Exs., 480; Rabenh., Fung. Eur., 1424.

B. *Epispore warted or spinulose.*

Tuber brumale, Vitt., Mon. Tub., p. 37, pl. 1, fig. 6 (1831); Cooke, Brit. Fung., p. 740 (1871); Sacc., Syll., viii, p. 895 (1889); Chatin, La Truffe, p. 48, pl. 4 (1892). *Oogaster brumalis,* Zobel, in Corda's Icon. Fung., vi, fig. 127 (1854).

Ascophore subglobose, generally regular in outline, reddish-violet then black, rough with polygonal warts having the apex excavated and crested with points, 2–10 cm. diam. *Gleba* greyish-black with a tinge of violet, traversed by whitish, branched dissepiments. *Asci* numerous, subglobose or broadly elliptical, shortly stipitate, 3–6-spored. *Spores* elliptical or elliptic-oblong, yellowish-brown, spinulose, spines slender, 20–30 × 15–20 μ.

Distinguished amongst the species with spinulose spores, by the stout polygonal warts on the surface of the ascophore. Edible, said by Chatin to be the best kind after *T. melanosporum, T. gulonum,* and *T. montanum.* Size variable, sometimes larger than the measurements given above. Smell strong.

Specimen in Herb. Kew., from Vittadini, examined.

Hab. Underground.

Distr. Britain; France; Italy.

Exsicc. Speg., Dec. Myc. Ital., 1.

Tuber rufum, Pico, Meleth., p. 80 (1788); Vitt., Mon. Tub., p. 48, tab. 1, fig. 1 (1831); Tul., Hypog., p. 141, tab. 6, fig. 2, and tab. 18, fig. 2 (1851); Cooke, Brit. Fung., p. 741 (1871); Sacc., Syll., viii, p. 897 (1889).

Ascophore subglobose or irregular in form, minutely warted, cracked, rusty brown, 2·5–6 cm. diameter. *Gleba* reddish-brown at maturity, dissepiments whitish then livid, mixed with tawny lines. *Asci* broadly ovate, shortly stipitate, 1–4-spored. *Spores* broadly elliptical or sometimes nearly globose, brown, densely covered with short spines, 25–36 × 17–24 μ.

The wall of the ascophore is thick and somewhat cartilaginous, colour somewhat variable but always more or less rusty. Smell also variable, at times not unpleasant and somewhat acid, at others strong and nauseous. The spores are almost indistinguishable in form and marking from those of *T. nitidum,* Vitt. Sometimes there is only one spore in an ascus, when it is very large and often almost or quite globose.

Specimen from Vittadini, in Herb. Kew., also Berkeley's British specimens, examined.

Hab. In woods, underground or partly exposed.

Distr. Britain; France; Italy.

Exsicc. Roumeg., Fung. Gall., 2816.

Tuber scleroneuron, Berk. and Br., Ann. Mag. Nat. Hist., vii, p. 184 (1851); Cooke, Brit. Fung., p. 740 (1871); Sacc., Syll., viii, p. 887 (1889).

Ascophore subglobose, somewhat lobed, cartilaginous, minutely warted or sometimes almost smooth, cracked, reddish-brown, 2–4 cm. diam. *Gleba* greyish towards the centre, becoming reddish-brown towards the periphery. *Asci* broadly ovate or clavate, shortly pedicellate, 2–4- usually 3-spored. *Spores* subglobose or broadly elliptical, brown, densely bristling with slender spines 4–6 μ long, often slightly curved at the tip, 17–23 × 25 μ.

Berkeley says the spores are 'minute cellulosa', meaning that they are minutely reticulated. An examination of his type specimens in the Kew herbarium, however, shows that the epispore is spinulose, as stated above.

'This species differs from *Tuber rufum*, Vitt., in its firmer cartilaginous texture, deep red-brown colour, and in the form of its sporidia, which are ovate, not elliptic-elongate, and in its faint aromatic odour. When dried, *Tuber scleroneuron* becomes as hard as a piece of wood.' Berk.

Hab. In the ground.

Distr. Britain.

Tuber intidum, Vitt., Mon. Tub., p. 48, pl. 2, fig. 10 (1831); Cooke, Brit. Fung., p. 741 (1871); Sacc., Syll., viii, p. 897 (1889). *Tuber Berkeleyanum*, Tul., Hypog., p. 151 (1851). *Oogaster Berkeleyanus*, Corda, Icon. Fung., vi, p. 71, pl. 16, fig. 118 (1854).

Ascophore globose or slightly depressed, hard, even, shining, just tinged yellowish-red, 1·5–3 cm. diam. *Gleba* at first whitish then reddish-brown, hard, dissepiments whitish, originating from a pale point at the base. *Asci* obovate or variable in form, 2–4-spored. *Spores* broadly elliptical or subglobose, yellowish-brown, densely covered with rather long, slender spines, 20–30 × 15–24, or 20–25 μ diam.

Distinguished by the even, polished ascophore and the small, spinulose spores. The asci vary in form according to the grouping of the spores, sometimes four are arranged as a tetrahedron, sometimes two or three more or less in a straight line.

Specimen from Vittadini, in Herb. Kew., examined.

Hab. Underground, under trees.

Distr. Britain; Italy.

Exsicc. Berk., Brit. Fung., 303; Roumeg., Fung. Gall., 2171.

Tuber ferrugineum, Vitt., Mon. Tub., p. 46, pl. 3, fig. 10 (1831); Tul., Hypog., p. 141 (1851); Sacc., Syll., viii, p. 897 (1889).

Ascophore variable and irregular in shape, very soft, rusty brown, minutely warted, often sparingly cracked, 4–9 cm. diameter. *Gleba* soft, dry, granular, pale rusty brown, traversed by a few slender, sparingly branched dissepiments of a whitish colour. *Asci* numerous, subglobose or obovate, 2–4-spored. *Spores* elleptical or sometimes almost globose, brown, densely covered with slender spines, 18–25 × 15–18 μ.

This species is characterized by the very soft ascophore. Becoming hard when dry, smell somewhat strong.

Hab. Underground.

Distr. Britain; Italy.

Choeromyces, Vitt., Mon. Tub., p. 50 (1831); Tul., Hypog., p. 169 (1851); Hesse, Hypog. Deutschl., ii, p. 37 (1894); Cooke, Brit. Fung., p. 742 (1871); Sacc., Syll., viii, p. 900 (1889).

Ascophore closed, with a distinct basal portion, even, often cracked. *Gleba* with numerous branching fine veins, interstices whitish. *Asci* oblong-ovate with a long slender pedicel, 8-spored. *Spores* globose, warted.

Distinguished by the distinctly elongated asci containing eight warted, coloured spores.

Choeromyces meandriformis, Vitt., Mon. Tub., p. 51, tab. 2, fig. 1, and tab. 4, fig. 10 (1831); Tul., Hypog., p. 170, tab. 19, fig. 7 (1851); Cooke, Brit. Fung., p. 742, fig. 345 (1871); Sacc., Syll., viii, p. 900 (1889). *Rhizopogon meandriformis*, Corda, Icon. Fung., vi, p. 68, fig. 110 (1854). *Tuber album*, Sow., Engl. Fung., pl. 310 (803).

Ascophore irregularly globose, often nodulose, smooth, pale yellowish brown, becoming much cracked and presenting a tesselated appearance, interstices whitish, base plicate, 5–10 cm. diam., flesh white when fresh, becoming yellowish with age. *Gleba* with numerous fine ochraceous veins. *Asci* ovate or oblong-ovate, tapering into a slender pedicel, 8-spored. *Spores* globose, with numerous elongated blunt warts or spines, 21–26 μ diam. including warts.

Hab. On the ground, often more or less exposed.

Distr. Britain; France; Italy; Germany; Bohemia.

Exsicc. Thüm., Myc. Univ., 1507.

Smell strong, often grows to a large size.

Described from a specimen in the Kew herbarium from Vittadini.

Terfezia, Tul., Ann. Sci. Nat., sér. 3, iii, p. 350 (1846); Tul., Expl. Scient. de l'Alger., Bot. i, p. 435 (1846). *Tulasneinia*, Zobel, in Cord. Icon. Fung., vi, p. 64 (1854).

Ascophore subglobose or irregular in form, furnished with a distinct, short, obconic stem-like base, fleshy, not perforated, continuous or variously cracked. *Gleba* fleshy, fertile portion consisting of distinct small masses, sterile portion areolate, paler. *Asci* globose or subglobose, often 8-spored. *Spores* globose, warted, echinulate or reticulated.

Distinguished by the distinct, short, stem-like base of the ascophore, globose asci, and globose warted spores, so far as British species are concerned.

Terfezia leonis, Tul., Expl. Scient. de l'Alger., Bot. i, p. 432, pl. 24, figs. 22–30 (1846); Tul., Hypog., p. 173, pl. 7, fig. 5, and pl. 15, fig. 3 (1851). *Tulasneina leonis*, Zobel, in Corda's Icon. Fung., vi, p. 64 (1854). *Tuber niveum*, Vitt., Mon. Tub., p. 47 (1831).

Ascophore subglobose or piriform, often very large, with a short obconic base, whitish, becoming stained and discoloured with age, 4–10 cm. diam. *Gleba* whitish, becoming discoloured. *Asci* large, globose or very broadly elliptical, 8-spored. *Spores* globose, coarsely nodulose, remaining colourless for a long time, finally slightly coloured, 19–25 μ diam.

Very variable in size; smell weak, not unpleasant.

Hab. Altogether subterranean or partly exposed.

Specimen in Kew herbarium, from Tulasne, examined.

Distr. Britain; France; Germany; Italy; N. Africa; Palestine; United States.

Exsicc. Crypt Lusit., 30; Rab., Fung. Eur., 241; Desm., Cr. Fr., sér. 2, 670; Thüm., Myc. Univ., 525; Ellis and Everh., N. Amer. Fung., 1782.

Amylocarpus, Currey, Proc. Roy. Soc., ix, p. 119 (1857); Cooke, Brit. Fung., p. 743, fig. 346 (copied from Currey) (1871); Sacc., Syll., viii, p. 905 (1889).

Ascophore globoso-depressed, minute, fleshy, more or less convolutely wrinkled. *Asci* elliptical, soon disappearing. *Spores* globose, colourless, with scattered long, slender spines.

A genus of very uncertain affinities, retained here only because I am unable to suggest a more suitable location. The habit and structure are not at all in accordance with those of any other genus included in the present Family. It has only been met with once, and the material is limited in amount. A careful investigation of recent specimens would in all probability throw more light on this anomalous fungus.

Amylocarpus encephaloides, Currey, Proc. Roy. Soc., ix, p. 119 (1857); Cooke, Brit. Fung., p. 743 (1871); Sacc., Syll., viii, p. 905 (1889).

Ascophore globoso-depressed, with sinuous, brain-like folds, fleshy, dull yellow, about 2–3 mm. diameter. *Asci* elliptical or broadly clavate, shortly stipitate, deliquescing at an early stage. *Spores* globose, hyaline, with sparsely scattered, very slender spines as long as the diameter of the spore, 10–12 μ diam. excluding the spines.

Type specimen in Herb. Kew. examined.

Each individual presents the appearance of a small round somewhat flattened body, of a dull yellow colour, and with an unevenness of surface caused by numberless convolutions of the integument. The diameter of the largest did not much exceed one-eighth inch. Externally with a strong resemblance to *Dacrymyces deliquescens*. The integument is of considerable thickness formed of several layers of cells, the outer large and rounded, the inner long and flat. The asci are broadly clavate, with a very short stem springing from threads proceeding from the inner surface of the integument. They are absorbed at an early period, and the sporidia form a dense mass. *Sporidia* globular, colourless, furnished with long delicate sharp rays, projecting from the surface in every direction. Each sporidium with an internal nucleus, or oil-drop. *Spores* $\frac{1}{2000}$th inch diameter (Currey).

Hab. Growing gregariously on fragments of wood, on the sands by the sea shore, at Sketty, near Swansea.

Distr. Britain.

Excluded Species.

The species of *Sphaerosoma* have been transferred to the Discomycetes.

Choeromyces ganglionis, Vitt., is not a British species, and was added to our Flora owing to a mistaken identification.

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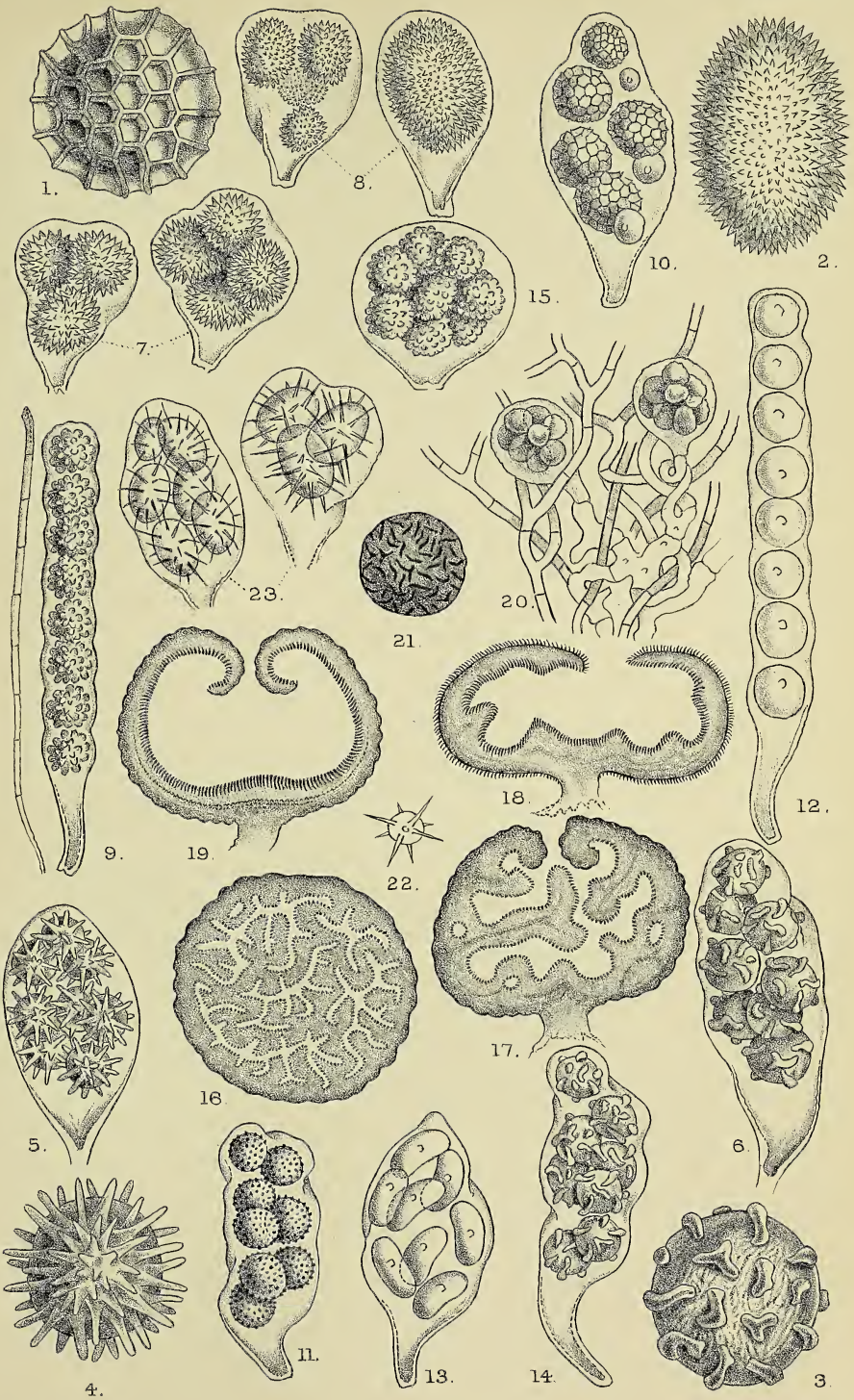
EXPLANATION OF FIGURES IN PLATE XVII.

Illustrating Mr. Massee's paper on the British Tuberaceae.

Fig. 1. *Tuber excavatum*, Vitt.
Fig. 2. ,, *melanosporum*, Vitt.
Fig. 3. *Hydnotrya Tulasnei*, B. and Br.
Fig. 4. *Choeromyces meandriformis*, Vitt.
Fig. 5. ,, ,, ,,
Fig. 6. *Hydnotrya Tulasnei*, B. and Br.
Fig. 7. *Tuber nitidum*, Vitt.
Fig. 8. ,, *melanosporum*, Vitt.
Fig. 9. *Genea Klotzschii*, B. and Br.
Fig. 10. *Hydnobolites cerebriformis*, Tul.
Fig. 11. *Pachyphloeus melanoxanthus*, Tul.
Fig. 12. *Stephensia bombycina*, Tul.
Fig. 13. *Balsamia platyspora*, B.
Fig. 14. *Hydnotrya Tulasnei*, B. and Br.
Fig. 15. *Terfezia leonis*, Tul.
Fig. 16. *Tuber mesentericum*, Vitt.
Fig. 17. *Genea sphaerica*, Vitt.
Fig. 18. ,, *hispidula*, Vitt.
Fig. 19. *Peziza vesiculosa*, L.
Fig. 20. *Elaphomyces anthracinus*, Vitt.
Fig. 21. ,, *variegatus*, Vitt.
Fig. 22. *Amylocarpus encephaloides*, Curr.
Fig. 23. *Tuber brumale*, Vitt.

The free spores are mag. 500 diam., the asci containing spores, about 250 diam., figs. 16–19 nat. size.



G. Massee del. Huth lith. et imp.

MASSEE—BRITISH TUBERACEAE.

Notes on the Life-History of Pterostylis.

BY

OSWALD H. SARGENT.

With Plates XVIII and XIX.

PTEROSTYLIS is a genus of small terrestrial herbaceous orchids. Its members are chiefly found in Australia (including Tasmania). About forty-five species are known. One of these extends to New Caledonia, and several extend to New Zealand, the home of several species not found elsewhere. So far, ten species, viz. *P. reflexa*, *constricta*, *nana*, *pyramidalis*, *recurva*, *vittata*, *Sargenti*, *rufa*, *barbata*, and *turfosa*, have been reported from Western Australia. One of these, *barbata*, has not been seen by collectors for many years. All the remaining nine I have found within a few miles of my home (at York, W.A.), and have carefully observed them for several years. I have also cultivated the majority in flower pots. Upon these observations and experiments the present paper is founded. As the genus is a very natural one, there is little doubt that my conclusions based upon the study of a part will hold good with but little modification for the whole.

During the hot, dry summer months the plant exists as a small globular succulent tuber, buried two or three inches below the earth's surface. This tuber is strangely lacking in protective covering. There is no layer of dead fibrous tissue, as in *Caladenia* or *Glossodia*, and even the cuticle is remarkably thin. Doubtless the fibrous soil in which the plants grow forms a sufficient protection against undue loss of moisture. Some dormant tubers of *P. Sargenti* rapidly became flaccid when taken out of the ground ; while on the other hand a tuber of *P. rufa* kept in a loosely covered box remained turgid right through the summer.

On the advent of the winter rains, about May, the tuber puts forth a single shoot. This grows rapidly, and as it grows develops numerous short processes all over each internode (subterranean). These processes are truncated cones of tissue whose apical layers consist of a number of thin-walled grape-shaped cells. These cells are attached at their bases only, and their function is without doubt the absorption of food salts from the soil.

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The processes on the internode next below the surface terminate in long thread-like cells, which give access to the mycelium of a fungus. This gradually penetrates to the cortex, filling its cells with a tangle of hyphae. I only discovered the presence of this fungus last year, and know very little concerning it. There is no doubt, however, that the orchids depend very largely on their fungal partners for a living. Their root system is always very scanty, only a few short roots being developed from the lowermost internode.

When the plant has spread its leaves and gained sufficient strength, a shoot is developed just above the tuber. This shoot grows straight down and ultimately thickens into a tuber, which replaces the old one, and forms the resting stage of the plant during the next summer. In the species *reflexa*, *constricta*, *nana*, and *pyramidalis*, secondary tubers are also formed in the following manner : one or more branches appear just below the fungal tissue. These grow horizontally, or slightly downwards until they attain a length of two or three inches. They then turn abruptly down and grow a little longer. The turned-down parts then thicken and become tubers. In this manner two or more plants are formed from the original one. It is doubtless, chiefly owing to this vegetative mode of reproduction, that these species are usually found in colonies, in which the plants are often very much crowded. I am sure, however, that seedlings assist in the formation of the colonies, as I have repeatedly found them therein. The other Westralian species do not form colonies, and I have never found them with long tuber-forming branches. Occasionally, however, they form two tubers instead of only one beside the old tuber. Usually only one of these produces a flowering scape the first year after formation.

The leaves of the young and non-flowering plants of all the Western Australian species are closely similar. Speaking broadly, they are ovate in outline, are borne on amplexicaul petioles (usually short), and are arranged in radical rosettes. *P. rufa* and *nana* retain this form of foliage all through their lives. The flowering plants in these species throw up scapes through the centres of the rosettes. In *P. pyramidalis*, *turfosa*, and *barbata* the lower internodes elongate at flowering time, so that the rosettes, as such, vanish, though the leaves still remain near the bases of the stems. In the remaining species the flowering and non-flowering plants are sharply differentiated. The latter, as I have stated, produce rosettes of ovate leaves ; but when a tuber of one of these species is in a fit condition to flower it throws up a strong scape bearing at intervals alternate, sessile, lanceolate leaves. There is no rosette at any time of the year in which the plant flowers.

The scape varies in height from a few inches (*P. nana*, *reflexa*, &c.) to a foot or more (*vittata* and *recurva*). The inflorescence is a loose few- or many-flowered raceme. In some species (*nana*, *pyramidalis*, *reflexa*, and *constricta*) it is reduced to a single flower, with usually the rudiment of

a second at its base. True to its ordinal character, the flower develops with its face towards the axis of the plant. In most orchids the flower is brought face forwards by torsion of the ovary. In *Pterostylis* the ovary is not twisted. In the many-flowered species the stem above the flower's insertion leans to one side, and so leaves the face of the flower free from obstruction. The flower usually leans forward and thus appears on the side of the stem opposite to that on which it is inserted.

The flower itself is remarkable and rather complicated. I think its structure and mechanism can best be explained by taking the flower of *P. reflexa* as a type, and describing it in detail with the aid of Plate XIX, prepared by my friend, Mr. Stanway-Tapp. The anterior sepal (or so-called 'dorsal' because it forms the back of the flower) and the petals together form a galea, which much resembles a boat placed 'bows uppermost' (Fig. 2, *p*, *p*, and *m.s.*). The petals are attached to the sepal in a most ingenious way (Fig. 6). Each edge of the sepal (*s.*) is sharply bent inwards, and the tongue or flange thus formed fits so tightly into a groove in the petal (*p.*), that it is difficult to separate the parts without tearing. The lateral sepals are united for the greater part of their length from the base upwards. Their free apices diverge considerably and terminate in long filamentous antennae. It will be convenient to have a short, distinctive name for this structure (the conjoined lateral sepals), so I will follow the simile of a helmet and call it the *visor*. It stands erect in front of the galea in this species, and thus the lower part of the flower is completely closed, the diverging apices of the visor leaving the upper part of the galea widely open (Fig. 2). The labellum, always an interesting member of an orchid flower, is in this genus specially interesting. In the species under discussion a short, slightly cuneate, ribbon-like claw supports a long lanceolate lamina (Fig. 1, *l*). At the base of the lamina a curious appendage arises. This appendage is a delicate strap of tissue ending in a few barbellate hairs. It is doubly curved: first transversely into a half-cylinder, thus securing rigidity; and then longitudinally so that it forms a semicircle. Its apex points upwards, the terminal brush being practically parallel with the face of the lamina (Fig. 8, *app.*). The column (Figs. 4, 5, and 10), which is arched over and completely enclosed by the galea, is a slightly curved fleshy pillar. It bears on its face about midway between base and apex two slightly sticky oblong cushions, the stigmas (Fig. 5). Just above these stigmas two large wings arise, one from each side of the column. These may be considered as originally rectangular bands of tissue, whose upper front corners have grown out into erect horns, and the lower front corners into large rounded lobes. These wings are curved, so that their edges almost meet in front of the column (Fig. 4). Thus a covered archway is formed in front of the column, the lower lobes bending outwards to touch the side of the galea. In this way the galea is divided into two chambers, an upper and a lower. The only means

of communication between the chambers is the narrow passage or tunnel between the column-wings. An inwardly directed wrinkle on each side of the galea, extending some distance upwards from the base, considerably narrows the entrance to the lower chamber. The labellum fits this doorway pretty closely (Fig. 1).

When the flower is open and ready to receive visitors, the labellum leans forward against the visor. In this position its tip protrudes a little from the flower and forms a convenient platform for insects to alight upon. The remainder of the lamina forms a sloping pathway down into the lower chamber of the galea. On the insect's arrival at the base of the lamina, the labellum suddenly springs back, hurling the insect against the stigmas. The lamina now leans against the column-wings (Fig. 1), thus closing the lower chamber's doorway, and making the insect a prisoner. The insect may remain a prisoner till the automatic door falls open, which it will do in about half an hour ; or it may at once escape by means of the tunnel between the column-wings. This course is most favourable to the flower. The tunnel narrows considerably towards the top, and once an insect arrives at this narrow part retreat is almost impossible.

The escaping prisoner makes his way through the tunnel with his back towards the column ; and, when passing through the narrow part, its back cannot fail to come in contact with the sticky face of the rostellum, which is situate at the top of the column in front (Fig. 5). The anther, which is hinged to the top of the column behind the rostellum by a very short band of connective, partly obstructs the exit from the tunnel, so that the insect in emerging must lift it slightly. Each cell of the anther contains two bacilliform pollinia lying quite loose in the cell, one above the other. When an insect lifts the anther, the part of its back rendered sticky by contact with the rostellum comes against one or two of the rods of pollen, and these, adhering, are carried away from the flower. When free from the tunnel nothing remains to hinder the insect's departure. The flowers of *P. constricta*, *nana*, *pyramidalis*, and *recurva*, differ so little from that of *P. reflexa*, that I need not describe them ; but it will be well to point out the conspicuous differences found in the flowers of the other Westralian species. In these the visor is sharply reflexed at its base, and hangs down in front of the ovary, and the labellum hangs down in front of the visor. In *P. vittata* the lamina is a small oval fleshy plate, and its appendage a mound of tissue, surmounted by a slightly hairy spike. In *P. rufa* the lamina is a fleshy rod without any specially differentiated appendage. In *P. Sargenti* the structure of the lamina is particularly remarkable. It may be described as consisting of three lanceolate segments conjoined at the base, but free above for fully half their length. Each outer segment bears at its base a large flattened clavate appendage, which leans inwards, so that the apices of the two connive. Immediately behind these appendages there is a ridge of tissue, which

I am inclined to think is the homologue of the appendage found in other species. The two large appendages are, I believe, quite peculiar to *P. Sargenti.* They seem specially designed to part the curious curtain, which closes the doorway of the galea. This curtain, also peculiar to the species, is formed by a fringe of delicate hairs arising from near the inner edge of each petal, and extending half way across the doorway. The large appendages readily push the fringes aside when the labellum flies back, and thus clear the way for the entrance of the insect when it is hurled into the flower. The fringes are sufficiently elastic to regain their former position when the labellum falls down.

The flowers of *P. turfosa* and *P. barbata* are closely similar, so a brief notice of the former will suffice for both. The lamina of the labellum is reduced almost to a thread. It ends in a clavate knob, and is rather sparsely fringed on either side with long golden hairs. Its appendage is a short flat rod, which looks like a continuation of the lamina back beyond its insertion on the claw. This strange structure leans a little away from the galea in the open blossom. When excited it moves back and forms a curtain across the entrance.

Now in all these species the labellum is highly irritable: a very light touch, while it is in the ready-to-receive-visitors position causes it to fly back and close the lower chamber of the galea. Rough trials with the finger or some heavy instrument lead one to suppose that the lamina is sensitive all over; but careful experiments with a bristle have convinced me that the sensitiveness is really localized in the appendage. Observations support this conclusion. I have seen insects alight on the labella of *P. rufa*, *Sargenti*, and *vittata*, and fly off again without having set the labella in motion. In each case the insect failed to touch the appendage, and experiment immediately after its departure showed the labellum to be in a highly irritable condition. The insects in question are tiny diptera. I weighed one like that which I saw hurled into a *P. Sargenti* blossom: its weight was just *one milligram.* So far as I could see it touched the sensitive spot with one fore-leg only, so that the pressure upon the spot must have been considerably less than a milligram. From this, some idea may be gleaned of how very ticklish the labellum is. The large appendages of *P. Sargenti* are *not* sensitive to a gentle touch. The sensitive spot lies between them at the base of the ridge, which I regard as a third appendage. This is one of my reasons for believing this ridge to be the homologue of the appendage found in other species.

Having flown back, the labellum continues to keep each flower closed for a considerable length of time. If it be pulled forward it springs back immediately on release. The duration of closure varies with the species. In *P. turfosa* it is about 25 minutes, in *P. reflexa* about 35, in *P. constricta*

about 40, in *P. Sargenti* about 35, in *P. vittata* about 2 hours, and in *P. recurva* about 3 hours, during the warmer hours of the day. These times are usually doubled before 10 a.m. and after 4 p.m., and throughout dull days. There are many variations; but good healthy specimens can generally be relied upon to keep pretty close to the times I have given. Perhaps departures from the usual time are always evidence of something wrong. The same specimen, however, often varies its time a little from day to day.

Time being up, the labellum returns to the 'ready' position sometimes abruptly, sometimes by several short jerks, and sometimes with a continuous, slow, even motion. Even the same labellum behaves differently in this respect on different occasions.

The appendage is not immediately sensitive to touch, when the labellum has reached the 'ready' position. Irritability is regained after a lapse of time, varying in different species from a few minutes to half an hour. The labellum is insensitive, I believe, all the while that the flower is closed. Tickling the appendage with a bristle while in that position seems to have no effect on the duration of closure.

Let us now leave mechanical details and examine the plants 'at home'. In my experience they are almost always found in shady situations, under bushes or amongst rocks. The flowers are remarkably inconspicuous in their natural surroundings (Pl. XVIII). Being mostly greenish-white with green longitudinal markings, or bands, they appear green. *P. reflexa* is rufous, and *P. rufa*, I believe, is usually so, though most of the specimens I have collected are green. *P. vittata* occurs in two forms, green, and deep reddish brown. *P. reflexa* blooms early in the season, and its rufous blossom is not readily seen against the background of dull brown earth and yellowish dead vegetation. Even its later flowers amongst green grass must be looked for to be seen. The flower of *P. constricta* is green; and when the plant grows amongst grass, it is decidedly difficult to see. *P. Sargenti* has a deep green flower, easily overlooked as the plant usually grows in dark coloured soil. *P. recurva* and *P. turfosa* are well hidden by the tangle of bushes, amongst which they occur. My experience of *P. rufa* is that the grey-green flowers harmonize so well with the green-grey granite inhabited by the plant, that I have often looked hard at a specimen for several minutes before seeing it.

To this lack of conspicuousness add the facts that the flower is odourless and devoid of nectar, and take into consideration the irritability of the labellum; and the conclusion that the flower is nothing but a trap is almost inevitable. But that is not all, there are other things to be reckoned with. I have often found the flowers of *P. vittata* and *P. Sargenti* with their labella missing. It seemed they had been gnawed off. This points to there being something attractive about the labellum. The behaviour of the diptera, which visit the flowers, strengthens this idea. I have seen these

insects hovering over the labella just as bees do over nectariferous blossoms. On one occasion I saw a little dipteran sucking juice from the top of a large appendage of a *P. Sargenti* labellum. I have noticed also that the diptera which visit *P. Sargenti* flowers are often in no hurry to leave their prison, when hurled into it. Quite unconcernedly they employ their time in sucking juice chiefly from the lower part of the column. One sometimes continues thus employed till the labellum falls and allows it to escape without crawling through the column wings. After such a long stay in the flower the insect always seems rather stupid and disinclined to fly, until it has spent a few minutes in the open air. Its behaviour suggests intoxication, though it walks quite steadily. A fortunate accident gave me the hint that 'blow flies' are fond of the juice of these orchids. Acting on the hint, I caught a few vigorous flies and imprisoned them under a bell-glass along with a watch-glass containing a crushed *P. recurva* blossom. Very soon the flies were greedily feasting on this. At first they were easily disturbed; but it gradually became difficult, and finally almost impossible, to drive them from their booty. Then they seemed unable to fly, though they could walk perfectly. I replaced the *Pterostylis* flower with a dish of pure water, and the flies rapidly regained their former liveliness. At another time I saw a blow fly greedily sucking the top of the ovary of a *P. recurva* flower, from which I had cut the perigone and column. This fly clung tenaciously to his prize when I tried to pull him off. As soon as I let go he sprang back, as though his legs were rubber bands fastening his body to the ovary, and recommenced sucking immediately. Even tickling his proboscis with a stiff bristle made no impression upon him. Similar experiments with *P. Sargenti* blossoms gave concordant results.

I think it is safe to conclude that the flowers contain an intoxicating principle highly attractive to the insects which visit them. I may here remark that tiny insects (diptera) are not infrequently found dead in the flowers, usually adhering to the stigma. The stigma is so slightly sticky, indeed it is scarcely more than moist, that I have always very much doubted its ability to hold a living insect, even though so small a one. I now feel sure that these little flies found dead in the flowers are victims to their unbridled appetites. Only once have I seen a visit to a flower carried completely through. I will briefly describe it. On July 17, 1907, a vase containing several racemes of *P. vittata* was standing close to my window. At about 3 p.m. I noticed a small 'gnat' hovering over the flowers apparently choosing a labellum to settle upon. Soon it did alight on one, but quickly flew off again. It shortly returned, and again settled on the same labellum. It settled near the apex and gradually moved up to the base of the lamina. I believe it then thrust its proboscis into the fleshy tissue of the appendage. Then the labellum immediately sprang back, and made the gnat a prisoner. After fluttering about for a few seconds in the lower chamber,

the gnat commenced to climb through the tunnel formed by the column wings, with its back towards the column. It had to struggle violently to get through the narrow top of the tunnel, but within three minutes after its capture it was free again. It settled on the window, where I easily caught it. After killing it with chloroform I found that it bore two pollen-masses on its back.

Thrice I have seen an insect struggle through the tunnel of a *P. Sargenti* flower, but more often these insects have shown no desire to leave their prison. Once I saw a dipteran hurled into a flower of *P. Sargenti.* It immediately set about sucking juice. I watched the flower for twenty-five minutes, at the end of which time the insect managed to escape by squeezing past the labellum, the flower having been damaged at one spot. I have not seen any other species visited. All the diptera I have seen in *P. Sargenti* flowers appear to belong to one species, and they pay no attention to other species of *Pterostylis.* The insect I saw visit a *P. vittata* blossom was very different; I should think it belongs to a separate genus. I have several times seen, what seemed yet another kind of dipteran, hovering over the flowers of *P. rufa.* A fourth kind I have seen hovering round *P. nana* flowers, and a fifth I have found dead in a flower of *P. constricta.* It seems that each species is pollinated by its own special species of dipteran, as careful examination of the flowers leads one to expect. Closely allied species are exceptions to this rule. I have found hybrids between *P. reflexa* and *P. constricta.* Rodway reports hybrids between *P. concinna* and *P. praecox* in Tasmania, and Ewart announces the discovery of hybrids between *P. concinna* and his 'var. *intermedia*' of *P. reflexa,* in Victoria.

My observations are insufficient to enable me to deal exhaustively with the pollination of these flowers, but I have formed a working hypothesis, which, because it seems to me so very probable, I will venture briefly to expound. The first time an insect is hurled into a flower it is so alarmed that it immediately seeks a way of escape. This it soon finds in the column-wings tunnel, and, as it struggles through, a load of pollen is fastened to its back. The attraction of the flower is great, and it is not long before the insect again alights on a labellum, and is a second time hurled into a flower. Its pollen-covered back strikes the stigma, which removes a portion of the load. Less alarmed on this occasion the insect probably lingers and sucks the intoxicating juice. A little suffices this time, and the dipteran seeks freedom via the tunnel. A few more flowers may be visited in a similar manner, but the time soon comes when the dipteran lingers in the blossom till the labellum falls, and then makes its exit without passing through the tunnel. Then, when next it visits a flower its stock of pollen will be comparatively small, but it will linger long, and brushing frequently against the stigma will coat that organ with a sufficient number of the precious grains.

I refrain from recounting all the observations which have enabled me to

formulate the above hypothetical account of the adventures of a dipteran, because *at present it is only a working hypothesis.*

I think I have shown that the flowers are very cunningly devised. Intoxicating juice and irritable labellum seems a remarkably good combination. But the best laid schemes do not always succeed, and the question arises: how effective is this one? How shall we judge? If seed production is to be the criterion, we shall receive an indefinite answer. Some species (e. g. *P. reflexa*, *constricta*, and *rufa*) are very shy seeders, but others seed freely, *P. recurva* sometimes gets over ninety per cent. of its flowers fertilized. But even supposing that the majority of species usually get but a small proportion of their flowers fertilized, it does not necessarily follow that the method of pollination is to blame. It might be thought that the sensitive labella would frequently be excited to close the flower by causes other than the alighting of desirable insects; but this is not the case. Very seldom is a blossom found with its labellum closing the lower chamber. Wind, unless perhaps a very strong one, does not affect the labellum. The fact that over forty species of this genus are in existence, scattered over a vast continent, is surely strong evidence that their reproductive mechanism has proved effective in the past. As to the future; five years' close observation has given me the impression that the species I know are well able to hold their own in their native wilds. I have no data for forming an opinion as to the effect civilization will have on them.

I have not exhausted the resources of my notebook, but I think I have said enough for the present. I have sought in this paper to give a clear idea of the impressions I have formed, so I have not tried to demonstrate any of my statements. Much that is interesting I have reluctantly omitted. I am continuing my observations and experiments, and I hope from time to time to deal with individual points in full detail.

In conclusion, I wish to express my indebtedness to Professor Oliver, Dr. A. B. Rendle, and Dr. R. S. Rogers (of Adelaide, S.A.), for much help and encouragement in various ways.

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EXPLANATION OF PLATES XVIII AND XIX.

Illustrating Mr. O. H. Sargent's paper on *Pterostylis*.

PLATE XVIII.

Flowering specimens of *Pterostylis reflexa* growing in their natural surroundings.

PLATE XIX.

All figures are *Pterostylis reflexa*.

Fig. 1. Side view of flower, one sepal, petal, and part of *galea* removed to show internal arrangement. *l.* the labellum in the contracted position; its appendage is seen below.

Fig. 2. Front view of flower, one sepal removed. *p.p.* lateral petals; *m.s.* median sepal—the three collectively forming the *galea*. *l.s.* lateral sepal.

Fig. 3. Side view showing petal; one sepal and part of *galea* removed. *c.* column. Sections of the petal at various heights are shown on the right.

Fig. 4. Column (front view).

Fig. 5. Column (wings spread open). *anth.* anther; *rost.* rostellum; *stig.* stigma.

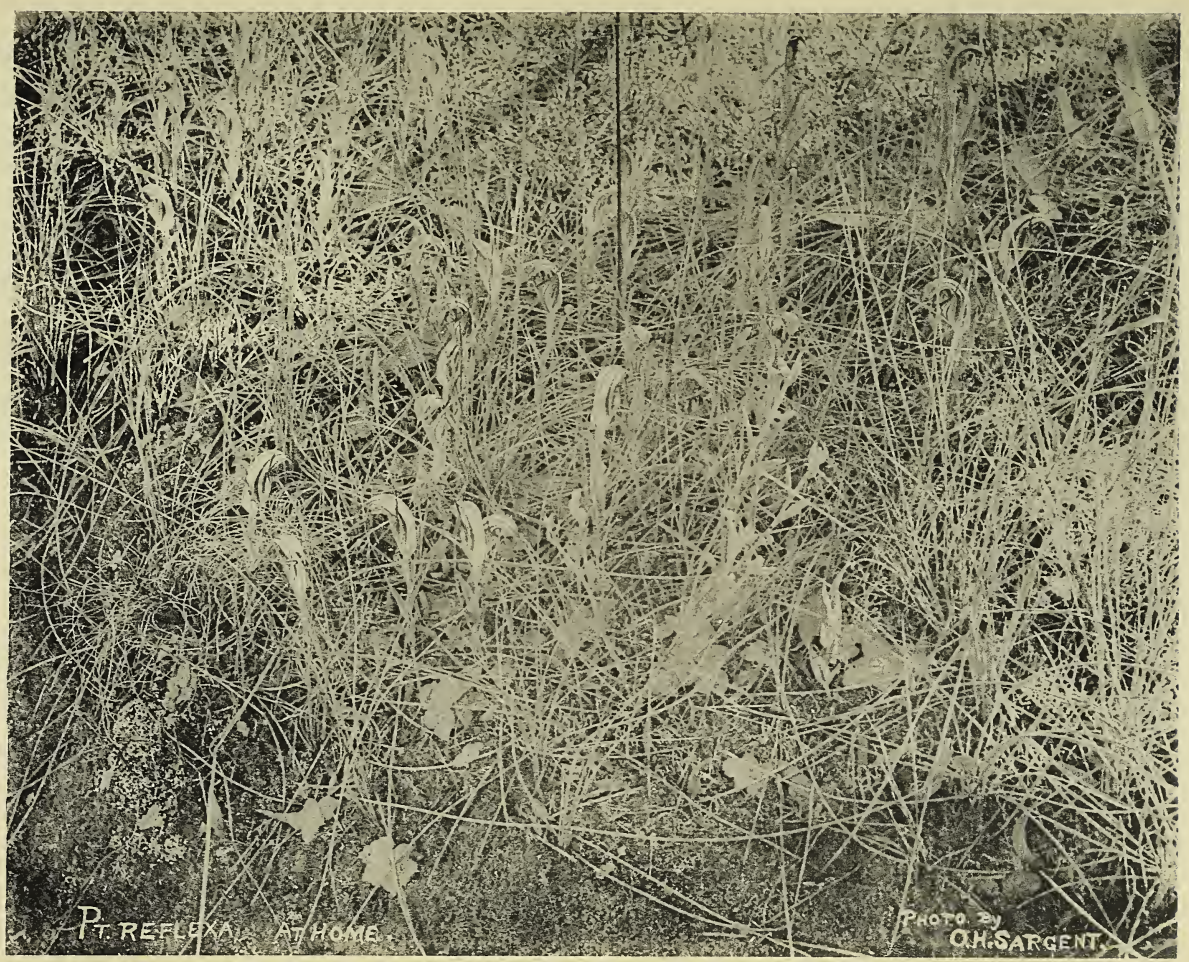
Fig. 6. Section showing attachment of petal to *galea*. *p.* petal; *s.* sepal.

Fig. 7. Labellum viewed from within. *app.* appendage.

Fig. 8. Labellum (side view). *app.* appendage; on right section of blade.

Fig. 9. Top of column and one wing.

Fig. 10. Side view of column.



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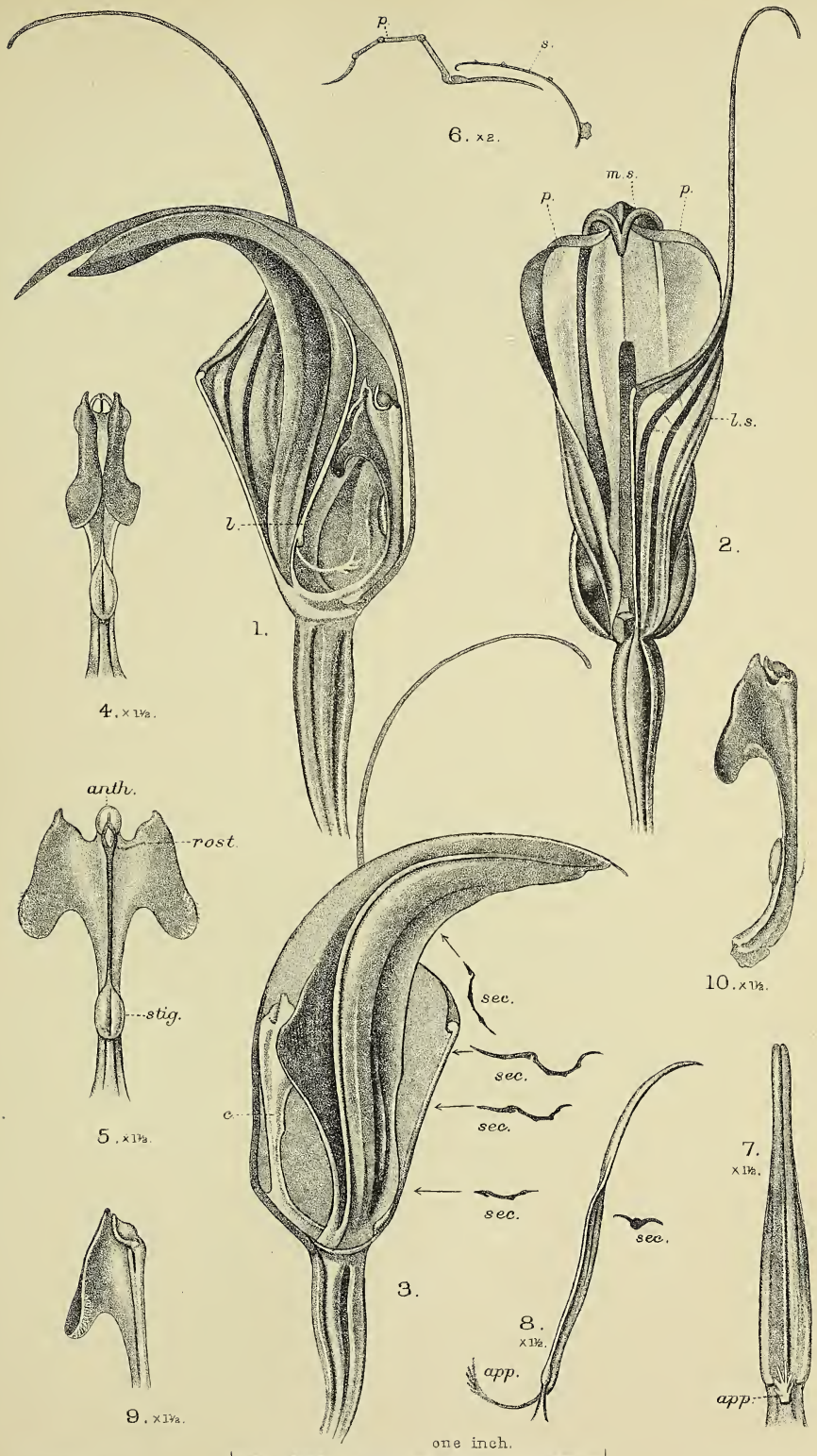
SARGENT — PTEROSTYLIS.



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SARGENT — PTEROSTYLIS.

On Stratification in the Vegetation of a Marsh,[1] and its Relations to Evaporation and Temperature.[2]

BY

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With Plate XX, and eight Figures in the Text.

IT has long been known that many marsh and bog plants possess so-called xerophytic characters. But although various theories have been put forward from time to time, to account for the existence of these 'swamp xerophytes', none of them can be said to be either established, or even generally accepted. Without dealing with the historical aspect of the question in detail, one or two of the more important theories may be mentioned.

Kihlman[3] regards these xerophytic structures as necessary in high northern latitudes, where, he points out, root-absorption may be hindered by the coldness of the wet or even frozen soil, at the same time that strong drying winds tend to increase transpiration. Goebel[4] adopts a similar explanation in the case of the swamps of the Venezuelan Paramos.

Schimper[5] regards swamp soils as being in many cases 'physiologically dry', owing to the high percentage of salts, humic acids, &c., in solution. Stenström[6] takes a similar view.

Other authors think that these xerophytic structures are primitive characters, which were originally developed under habitat conditions very different from those obtaining at present, and now survive only in the more stable species. Schwendener[7] has come to this conclusion as a result of his study of the distribution of the Carices: while Clements[8] and others hold the same view. These authors lay stress on the occurrence in nature

[1] This is equivalent to the German Flachmoor.

[2] Some of the results given here formed the subject of a paper read before Section K of the British Association, Dublin Meeting, 1908.

[3] Kihlman ('90), pp. 107 et seq.

[4] Goebel ('91), Teil II, p. 11.

[5] Schimper ('90), pp. 635 et seq., also ('03), p. 15.

[6] Stenström ('95), p. 71.

[7] Schwendener ('89), p. 73.

[8] Clements ('05), p. 126.

[Annals of Botany, Vol. XXIII. No. XC. April, 1909.]

of hydrophytic species side by side with species possessing xerophytic characters.[1]

But most authors admit that the whole question is still in a state of uncertainty.

Now although this problem has attracted a good deal of attention, the methods of attack seem to have been incomplete. For instance no one appears to have really carefully studied the habits of the plants concerned, together with their relations to each other, and to the physical conditions of their habitats.

The author has been engaged for some years on the study of marsh vegetation, in the hope that the results of a more detailed investigation of the plants, both in the field and in the laboratory, might bring us nearer to a solution of this, and possibly of other problems. The present paper treats of the structure of the vegetation of a marsh, and more particularly of the relations of the aerial shoots, both to each other and to some of the habitat factors which directly affect transpiration. It is intended in later papers to deal with other aspects of the subject of 'swamp xerophytes'.

Stratification[2] of the Aerial Shoots.

The field work which forms the basis of this paper was chiefly carried on at Wicken Fen, in Cambridgeshire, and a preliminary account of the Fen Vegetation has already been published.[3] The observations on the habits of marsh plants made there have, however, been supplemented by others on marsh vegetation in various parts of the country.

In the first place it may be mentioned that, although the herbaceous vegetation is very mixed, and composed of many species, yet so many of the plants grow to about the same height that the *facies* of the vegetation is fairly uniform. At Wicken Fen the actual height of what we may term the 'general vegetation level', or 'general shoot-level', varies, according to the dominant species (and, of course, other factors) from some eighteen inches or two feet to four or five feet above the ground.

Between this upper level of the vegetation and the soil are the aerial shoots of the plants. It will be instructive to consider the vertical distribution of the transpiring organs of the different species. In doing so, we may

[1] Cf. also Warming ('96), p. 177.

[2] 'Stratification' is used here to signify the differences found in vegetation at different vertical levels. The various stages may be called strata or layers. The German equivalents are Etagen (Schimper ('88), p. 99), Schichten or Stockwerke (Warming ('96), p. 117). Clements ('05), p. 280, uses the phrase 'vertical zonation', in the sense that stratification is used here. He defines zonation (p. 274) as 'the practically universal response of plants to the quantitative distribution of physical factors in nature'. But as Clements also includes under the term zonation, the altitudinal and latitudinal differences in vegetation, it seems to me that in practice it will be better to use another term to describe this common phenomenon of 'vertical zonation'.

[3] Yapp ('08), pp. 61–81.

first deal with the various types of plant form, and then with the stratification of the vegetation as a whole.

Now even in the case of those plants which reach the upper surface of the vegetation, all do not develop their leaves at the same vertical level. It has long been known that rhythmic variations in the size of parts occur during the development of the shoots of flowering plants. Thus in ascending a shoot formed during one vegetative season not only do the internodes show a successive increase and decrease in length, but the leaves also exhibit similar changes of dimensions.[1] The shoots of different species, however, show marked differences with respect to the relative positions on the stem at which the maximum size of leaf is attained. In some of the plants under consideration, the largest leaves are formed near the base of the stem, and their subsequent diminution in size is somewhat abrupt. In others the rise and fall in the size of successive leaves is gradual. In yet others the maximum leaf size is only attained at comparatively high levels in the vegetation. Again, while in many cases the greatest leaf-development occurs on the erect flowering shoots, in others the larger leaves are chiefly formed on lateral stolons, &c., and thus are practically radical in position. A rough classification of the plants in question according to their ecological habit,[2] may perhaps conduce to clearness. But it must be remembered that the varieties of plant form outlined below are not sharply demarcated. There is, in fact, every gradation between the different types :—

Type (1). The whole plant is more or less dwarf, and is generally found in sheltered situations. If growing with taller plants, it forms a constituent of one of the lower layers of the vegetation. The inflorescences or sporophylls are frequently inconspicuous ; e. g. *Hydrocotyle vulgaris*[3] (cf. Text-figs. 2, 4, and 6), *Lastrea Thelypteris*, *Ophioglossum vulgatum* (Text-fig. 6), &c. Various mosses and liverworts may also be included here. The following have conspicuous inflorescences, and form a transition to type (2), *Orchis incarnata*, *Epipactis palustris*, *Potentilla sylvestris*, *Menyanthes trifoliata*, and *Caltha palustris*. N.B. The two latter are but rarely found in the shade of other plants.

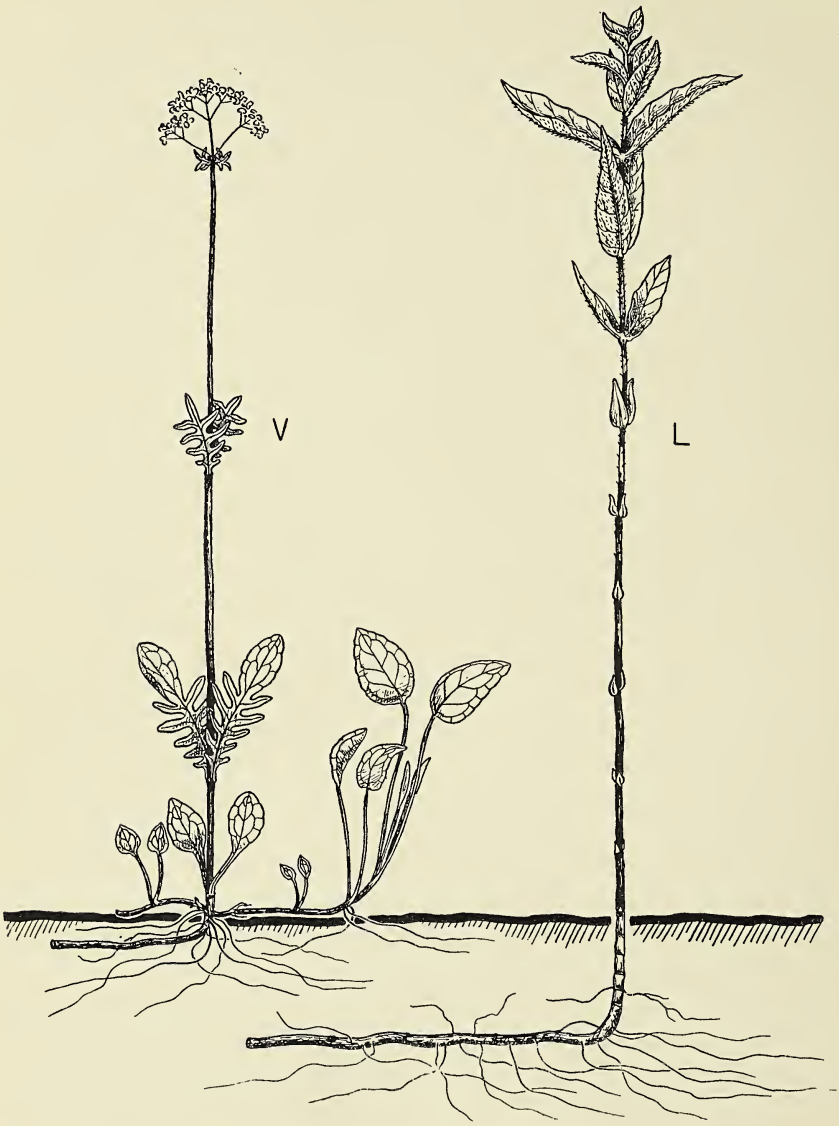
Type (2). The plants are frequently taller : their inflorescences more or less reach the general vegetation level, or even project beyond it. The leaves, however, are chiefly developed in comparatively low layers of the vegetation, and markedly decrease in size (sometimes abruptly) as the upper layers are reached ; e. g. *Valeriana dioica* (Text-fig. 1), *Carduus pratensis*, *Samolus Valerandi*, *Scabiosa Succisa* (Text-fig. 6), *Angelica*

[1] Cf. Pfeffer ('03), p. 7 ; also Groom ('08), p. 98. Pfeffer states that 'the variation in the size of leaves was known from Goethe onwards'.

[2] This refers to the aerial shoots only. In Yapp ('08), p. 69, will be found a list in which these same plants are arranged according to the degree of soil moisture preferred.

[3] The plant names used in this paper are those employed in Yapp, l. c., pp. 69–70.

sylvestris (the upper leaves are here practically reduced to leaf sheaths; cf. Text-fig. 6), &c. *Peucedanum palustre*, *Symphytum officinale*, &c., form transitions to type (3).



Text-Fig. 1. **V.** *Valeriana dioica* (Type 2). The larger leaves, whether on the flowering stems or lateral stolons, are formed at low levels.
L. *Lysimachia vulgaris* (Type 4). Larger leaves formed at relatively high levels, on both the flowering and non-flowering shoots. (May [1]). × $\frac{3}{8}$.

[1] The month in brackets after the descriptions of figures refers to the time when the field-sketches were made, from which the figures are taken.

Type (3). Inflorescences exposed as in the last type. The aerial stems are more completely foliated than in (2), and the decrease in the size of leaves (after the maximum) is, on the whole, much more gradual. As leaves of a fair size occur on most parts of the erect shoots, the transpiring organs are found in most layers of the vegetation. If the latter is dense, the lower leaves frequently die early, and the functional leaves are then comparatively high on the stem. In this state the plants approach those of type (4); e. g. *Thalictrum flavum*, *Spiraea Ulmaria* (Text-figs. 4 and 6), *Lathyrus palustris*, *Carduus palustris*, *Eupatorium cannabinum*, *Valeriana officinalis*, &c.

Type (4). Inflorescences exposed. The largest leaves are formed in relatively high layers of the vegetation. The lower leaves on the erect stem are small, frequently scale-like; e. g. *Lysimachia vulgaris* (Text-figs. 1 and 6), *Lycopus europaeus*, *Lythrum Salicaria* (Text-fig. 6), *Phragmites communis* (Text-figs. 4 and 6), &c.

Type (5). The grass or sedge type. Here the inflorescences are exposed as before. The leaves are formed at low levels, but owing to their length and vertical growth usually attain to the general vegetation level. On account of the numerical superiority of the plants of this type, they dominate the vegetation here dealt with, and impart to it its characteristic physiognomy[1]; e. g. *Cladium Mariscus* (Text-fig. 4), *Schoenus nigricans*, *Carex* spp. (Text-fig. 6), *Molinia coerulea*, and other grasses, *Juncus* spp. (Text-fig. 6), *Iris Pseudacorus*, &c.

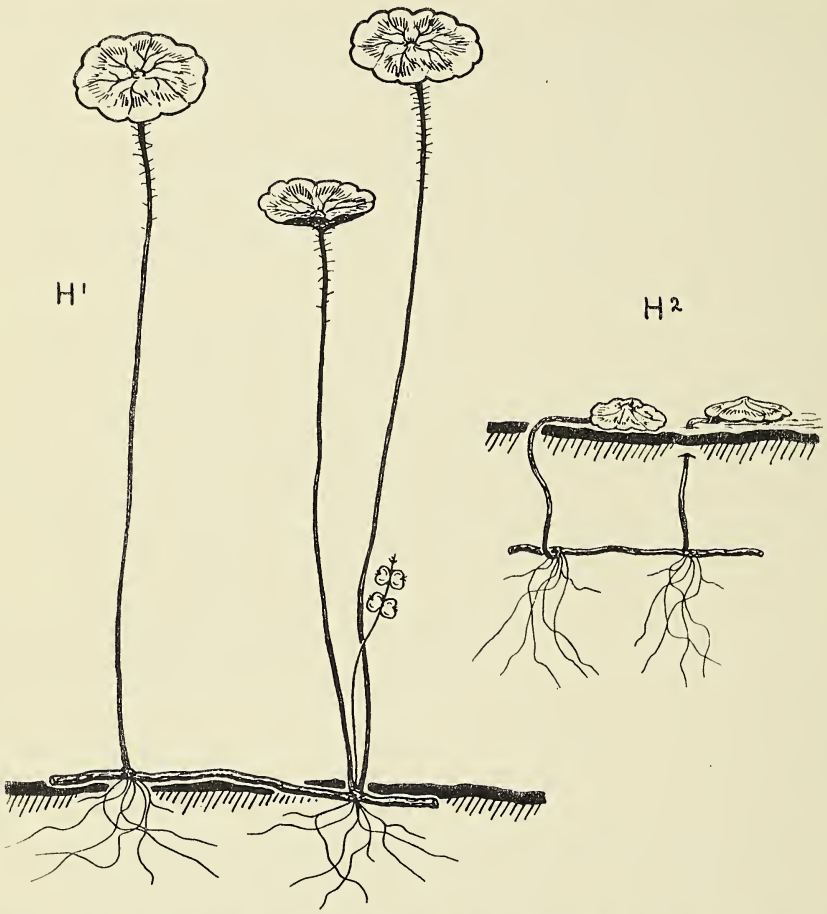
To sum up, the inflorescences of the great majority of the species are placed at or near the general vegetation level (sometimes above or sometimes just below). This is, of course, to be correlated with pollination requirements. On the other hand, the transpiring organs are found at very various levels, but rarely project far above the general vegetation level. Roughly, one may say that, of the above groups, (1) and (2) habitually have their leaves in sheltered, often shady positions; while the leaves of the plants of the remaining groups are, in varying degrees, more exposed. As examples of plants which usually have more or less exposed leaves may be mentioned *Spiraea Ulmaria*, *Lythrum Salicaria*, *Lysimachia vulgaris*, *Juncus* spp., *Cladium Mariscus*, and *Phragmites communis*. Amongst the habitually sheltered plants are *Lastrea Thelypteris*, *Ophioglossum vulgatum*, *Orchis incarnata*, *Hydrocotyle vulgaris*, *Valeriana dioica*, &c.

Although the plants of the lowest strata often exhibit shade characters in their leaves, it is important to distinguish between 'shelter' and 'shade' plants. Many of the usually sheltered plants are not necessarily shade plants at all, as they may grow exposed to full sunlight.[2] They then form

[1] Yapp, l. c., p. 66.

[2] Cf. Leist ('90), pp. 182 et seq. This author found that there is a general agreement in size and structure between the leaves of alpine plants, in open, sunny situations, and those of lowland

low, compact growths, which are sheltered by their position (cf. p. 298) though not directly by other plants. Thus *Hydrocotyle*, when growing in rank vegetation, forms erect petioles, often a foot or more in length. When growing, however, on (say) an exposed mound of peat, the petioles remain short, and on reaching the surface of the soil bend horizontally, and



TEXT-FIG. 2. *Hydrocotyle vulgaris*. H^1, from a shady, lower stratum of marsh vegetation. H^2, a plant growing on an exposed, fairly dry peat bank. The leaves are fully insolated. (September.) × $\frac{1}{3}$.

so place the laminae close to the ground (cf. Text-fig. 2). This behaviour of *Hydrocotyle* can be compared to that of many rosette-forming plants. The leaves of the latter may vary from a horizontal to a more or less erect position, according to the height of the surrounding vegetation. It is probable that in these cases relative humidity plays a very important part in determining the proper position for the leaves (cf. p. 298).

shade plants. He sought to connect these similarities with reduced transpiration; due to relatively great air and soil moisture (p. 200).

Thus the general vegetation of the Fen is made up of a number of species of very varied ecological habit. Although the distribution of these species depends to a great extent on the relative dampness of the soil,[1] this factor alone will not account for the preferences of the plants in all cases. For instance, certain species are rarely found in the denser mixed vegetation of the marsh, even when the moisture conditions are apparently suitable. These seem to prefer the more open banks of ditches or streams. Such are *Caltha palustris*, *Epilobium hirsutum*, *Samolus Valerandi*, &c.

From what has been said above, of the habits of the different species, it will be seen that where the vegetation is rank and dense, marked stratification (at least of the transpiring organs) will occur. Although such strata are more obvious in woodland than in other formations, yet they are found to a greater or less extent in most vegetation. Warming,[2] in fact, says that layers may be distinguished in every plant association. Clements, however, does not appear to recognize the existence of stratification in his 'co-ordinate associations'. In the latter, with which he classes most grassland and herbaceous formations, 'the individuals are on the same level, or nearly so.'[3]

In the mixed vegetation under consideration the number and composition of the strata vary according to the species present, the height of the vegetation, the season of the year, &c. Moreover, the different strata shade into each other more, and are in consequence less distinct than those of a forest. The following, however, shows the kind of stratification which may be observed (cf. Text-figs. 4 and 6):—

(*a*) The ground or bottom stratum consists of liverworts and mosses, and the young seedlings of many plants.

(*b*) The next stratum will contain the lower parts of the leaves of Carices and other plants of type (5). Also the taller young plants, *Hydrocotyle* and *Ophioglossum*; the larger leaves of *Orchis incarnata*, *Valeriana dioica*, *Scabiosa Succisa*, *Carduus pratensis*, &c.; the lower leaves (formed in springtime) of many plants, e.g. *Spiraea Ulmaria*.

(*c*) A still higher stratum will comprise the middle portions of the grass and sedge leaves; *Lastrea Thelypteris*; the larger leaves of such plants as *Angelica sylvestris*, *Peucedanum palustre*, *Thalictrum flavum*, *Spiraea Ulmaria*, *Symphytum officinale*, &c. Also the inflorescences of a few species, e.g. *Orchis incarnata*.

(*d*) The surface stratum contains the upper parts of the leaves of the sedge type; many of the larger cauline leaves of *Lysimachia vulgaris*, *Lythrum Salicaria*, *Phragmites communis*, &c.; the smaller cauline leaves of *Spiraea Ulmaria*, *Thalictrum flavum*, &c. Also the inflorescences of many plants.

[1] Yapp, l. c., pp. 68 et seq. [2] Warming ('96), p. 117. [3] Clements ('05), pp. 206–7.

(*e*) Projecting above the general vegetation level may be seen stray shoots of *Phragmites communis* (N.B. This species forms its own general level when social), and, if the vegetation is dwarfed, parts of the leafy shoots of other species such as *Lythrum*, *Lysimachia*, *Spiraea*, &c. Many inflorescences, accompanied only by the smallest cauline leaves (e. g. *Angelica*, *Spiraea*, *Calamagrostis*, &c.), also project above the general level.

From the foregoing it follows that not only are there fairly definite strata in the vegetation, but also that many of the plants themselves may be said, in a sense, to be more or less stratified. In the case of erect shoots, as pointed out above, great differences of leaf-development are seen at different levels on the same shoot. But the leaves formed at these various levels often differ in character as well as in size. This point will be dealt with more in detail in a future paper, but the case of species with hairy leaves may be mentioned here. In *Spiraea Ulmaria*, for example, the lowest leaves (formed in the springtime), are glabrous, while those formed at higher levels are more and more densely hairy. *Lysimachia vulgaris* (Text-fig. 1), *Mentha aquatica*, and other hairy species, show similar relations as regards the position and time of development of glabrous and hairy leaves.[1] Thus, as regards the vertical distribution of the transpiring organs, even in a plant association where the majority of the individuals attain to more or less the same height, differences in habit of the individual plants give rise to a marked stratification of the vegetation. It is, of course, a commonplace that the organs of the plant, stems, petioles, &c., can readily adjust themselves by growth, so that the leaves may be placed in each case in the appropriate ecological layer of the vegetation (cf. Text-fig. 2).

Evaporation as a Measure of the Atmospheric promotion of Transpiration.

It is obvious that the physical conditions, i. e. light, humidity, &c., must vary to a greater or less extent, in the different strata of the vegetation. Further, it would seem probable that such differences, if sufficiently great, might have an important bearing on the question of xerophytism in marsh plants. So far as I know, no quantitative experiments had been previously conducted, such as would afford any definite idea of the actual physical conditions obtaining at different levels in a vegetation like that dealt with in this paper. It was therefore decided to investigate these conditions, in so far as the factors directly affecting transpiration are concerned.

Apart from the question of control by the plant, the physical process of transpiration from the external part of the wall of a transpiring cell is

[1] Yapp ('07), p. 691.

merely one of evaporation. The chief external factors which exert a direct influence on transpiration are therefore those which affect the evaporation of water in air. These are the relative humidity of the air, temperature, air-movements and air pressure.[1] Temperature and wind act more especially by inducing changes, general or local, in the relative humidity. This is of course apart from the action of light, &c., on the movements of stomata.

The action of these factors may be determined either separately or in combination. Clements,[2] Hesselman,[3] and others, in studying plant habitats, have investigated them separately. But so far as transpiration problems in the field are concerned, the combined effect of these factors will present to the mind a much clearer picture than a consideration of each factor taken separately. This combined effect may be determined by measuring the evaporating power of the air, which will afford a good indication of the extent to which it promotes transpiration.

Hitherto evaporation determinations have been little used in field studies of vegetation. Schimper[4] suggested their use, Clements[5] mentioned atmometers, and Blackman and Tansley[6] pointed out their superiority to a separate measurement of the individual factors affecting transpiration. Leist,[7] in investigating alpine plants, calculated the evaporation at different altitudes, by combining the various meteorological factors according to the formula of Hugo Meyer.[8] But the only published records I have met with of actual evaporation experiments in connexion with ecological studies are those of Livingston and Transeau.

Livingston[9] published in 1906 a very interesting paper on the results of evaporation experiments he had carried out at the Arizona desert laboratory. He determined the evaporating power of the desert air under various conditions, and also compared this with the actual transpiration from potted plants. Reference will be made later to the evaporimeter used by this author.

Transeau[10] has recently determined, in a preliminary way, the relative evaporation in various plant associations. The differences obtained were considerable ; the greatest evaporation, i. e. that from a salt marsh, being about twelve times that recorded in the most humid association, a swamp forest.

Evaporation has been studied chiefly from the meteorological point of view. A few meteorologists, however, have worked at it in relation to vegetation. Thus Miller,[11] and later Wollny,[12] have shown that the covering of the soil by living plants considerably increases the amount of evaporation.

[1] Cf. Livingston ('06), p. 24. [2] Clements ('05), Ch. II. [3] Hesselman ('04), p. 347.
[4] Schimper ('03), p. 176. [5] Clements ('05), p. 46.
[6] Blackman and Tansley ('05), p. 239. [7] Leist ('90), p. 195.
[8] Meyer ('85), p. 154. [9] Livingston ('06). [10] Transeau ('08).
[11] Miller described a new evaporimeter for soils, in Symons's British Rainfall ('72), p. 206. In Miller and Skertchly ('78), p. 270, he records his chief results.
[12] Wollny ('96), p. 363.

Houdaille [1] also refers to the injurious effect of high evaporation on the development of vegetation in the south of France.

Meteorologists seem to have been much impressed by the difficulties of obtaining reliable comparative records, and of devising satisfactory evaporimeters. According to Hann [2] the Wild evaporimeter is the best.[3] In most evaporimeters used up to the present time evaporation is allowed to take place from a free, open surface of water.[4] The instrument is either sheltered from rain (and therefore, of course, from sun and wind), or else a rain gauge is used to indicate the amount of replenishing which occurs naturally. But the results obtained have varied greatly, according to the size of the vessel used, the degree of exposure, the depth of the water, the distance of the water-level below the edge of the vessel, and so on.

British meteorologists, who aim at measuring the natural loss, by evaporation, from large surfaces of water, have now adopted a standard evaporation tank. This is 6 feet square, and 1 ft. 6 in. deep, with a rim rising 3 inches above the ground.[5] They regard the results obtained from small exposed evaporimeters as quite worthless, owing to the greatly increased rate of evaporation induced by the heating of the water in direct sunshine.[6]

But it must be strongly emphasized that the object of the ecologist is a totally different one from that of the meteorologist. The former requires an instrument which can be placed under practically the same conditions as those which the plant itself is called upon to endure. For this purpose a small evaporimeter is not only unobjectionable, but in all respects desirable.

As there were obvious objections to the use of any small evaporimeter with an open water surface, I had already, in 1906, conducted some preliminary experiments with the view of devising a suitable instrument, when Dr. F. F. Blackman called my attention to the then recent paper of Livingston. The instrument described by this author [7] seemed distinctly superior, for biological purposes, to anything previously tried, and its main principle was

[1] Houdaille ('92), pp. 59 et seq. [2] Hann ('03), p. 73.

[3] This is a small instrument in which the loss of water is determined by weighing. It is described in Wild ('74), pp. 440–5, and a much more elaborate self-recording instrument in Wild ('90).

[4] Many forms of open-water gauge are described in Symons's British Rainfall, 1869–70. In the former year Leslie's atmometer is also described, pp. 169–71. This instrument has an evaporating surface of porous earthenware, but the principle on which it was constructed is quite different from that of the evaporimeter about to be described. In France the Piche gauge, and an improved form of this devised by Houdaille ('90, pp. 31 et seq.) have been much used. In both of these the evaporating surface consists of a circular disk of blotting paper placed in a horizontal position.

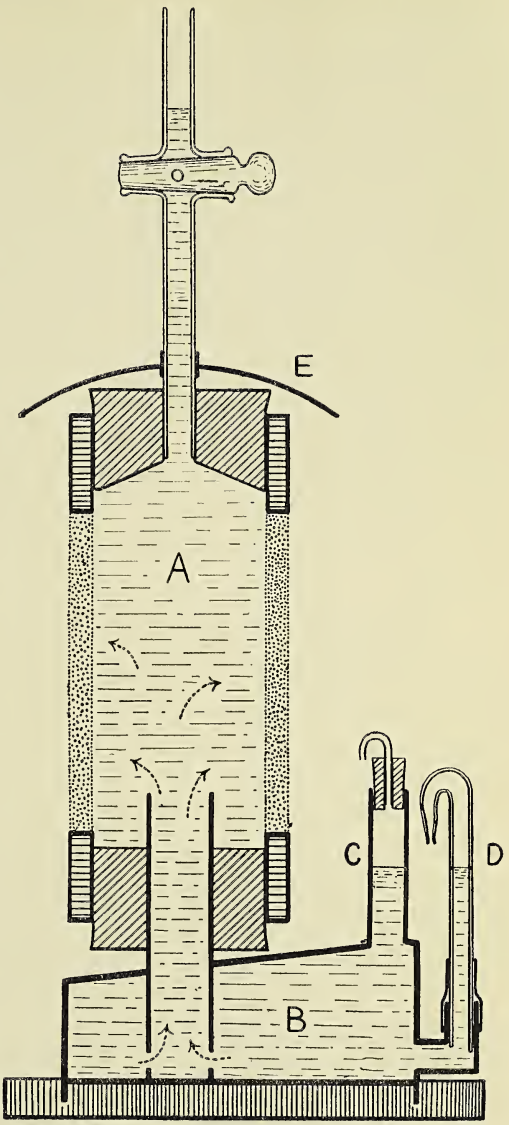
[5] Mill ('06), p. 35.

[6] Field and Symons ('69), p. 153; also Mill ('07), p. 47. On the other hand, the Wild evaporimeter, which has been widely used in Russia, the Piche gauge, which has been used at most French meteorological stations, as well as Houdaille's evaporimeter, are all small instruments, and contain no large body of water.

[7] Livingston ('06), pp. 24 et seq., and Fig. 4, p. 26.

therefore adopted. But the evaporimeter as finally used in my experiments was different in many respects from that of Livingston, and it may therefore be described here.[1]

Text-fig. 3 represents a vertical section of the evaporimeter in question. It consists essentially of a porous cylinder of earthenware (A), filled with distilled water.[2] As the water percolates through the pores of the earthenware, and evaporates from its surface, it is replaced by water drawn up from the reservoir (B) below. This reservoir is filled through the tube (C), until the water reaches a certain mark on the glass reading-tube (D). The porous cylinder remains constantly full of water, the latter being supported by air pressure from below, as in a water barometer. But as evaporation proceeds the water-level in the reservoir sinks. On again filling up to the original level by means of a burette, the loss of water is directly measured in c.c. The last parts to be filled are the tubes (C) and (D), both of which have a small sectional area. It is thus possible to read the water-loss to a single drop, i. e. to about 0·05 c.c.[3]



Text-Fig. 3. Vertical section of Evaporimeter: half actual size. For description see text.

[1] I wish to express my warmest thanks to Dr. F. F. Blackman, not only for suggesting a number of the more important features adopted in the evaporimeter, but also for the helpful interest he has taken in the progress of the work generally.

[2] Porous battery pots of good quality were used, the closed end being sawn off. About 2·5 cm. at each end was then soaked in a mixture of melted paraffin wax and resin, and finally coated with enamel. This limited the actual evaporating surface to a definite known area, about 18·0 by 7·9 cm. The size of the cylinders now in use is about 13 cm. long, with a diameter of 5·8 cm., and a thickness of ·5 cm. The method of calibrating the various cylinders is given in the Appendix, pp. 37 et seq.

[3] Transeau ('08, p. 219), used the same porous pots as Livingston, but for a water reservoir

The top of tube (D) is bent to allow air to enter, but not rain. The rubber cork closing tube (C) is fitted with a bent glass tube for the same purpose.

The open ends of the evaporating cylinder are closed by rubber corks. Through the upper cork passes a glass tube with a stop cock.[1] This facilitates the preliminary filling of the cylinder, by suction, and would also serve to indicate the presence of any air bubbles which might find their way into the cylinder. A metal hood (E) prevents rain collecting on the upper part of the apparatus. Through the lower cork a brass tube passes to the bottom of the reservoir, the tube being pierced with holes just above its base. Water can thus pass readily from the reservoir to the cylinder, while at the same time no air can enter the latter, unless the supply of water in the reservoir is practically exhausted.

The capacity of the reservoir is 200 c.c. It is made of copper, enamelled white outside to reflect as much heat as possible.[2] Its roof slopes upwards from all parts to the point of insertion of the filling tube (C); this facilitates the exit of air during filling. The reservoir is fixed to a massive base of lead, so that the evaporimeter is quite stable, even when exposed to strong winds.[3]

Such an instrument is portable, and can be readily used in the open in all weathers except during severe frosts. It will be seen later that the evaporimeter is, like the similar one of Livingston,[4] very sensitive to even slight changes in the atmospheric conditions. Being rainproof, it may be used during rain, wind, sunshine, &c., and, in short, exposed to precisely the same conditions as the plants themselves in nature. But while the evaporimeter can be subjected to the same external conditions as the plant, comparison between the two must not be carried too far. The evaporimeter lacks the controlling mechanisms and the osmotic cell-sap of the plant. It is, in fact, merely a physical instrument for recording the cumulative effect of the constant or frequent local changes in the relative humidity of the air.

For use in the field, a light but rigid iron stand, fitted with wire guys, was made (Pl. I, Fig. 2). The stand was provided with movable brackets, on which three evaporimeters were placed. Two of these were at different levels in the vegetation, and the third above it (Text-figs. 4 and 6). Maximum and minimum thermometers (Centigrade) were fixed on the opposite

employed a 'pint jar'. He states that 'because of the large water-area in the jar, the error in these readings is estimated to be more or less 1 c.c.'

[1] A thermometer might with advantage be also inserted through the upper cork, with its bulb in the water. This has not been done hitherto, but it would be useful to know the temperature reached by the water under different conditions.

[2] For the effect of different surface colours on temperature, see Hann ('03), p. 42.

[3] The evaporimeters were made for me by Mr. Bellingham, of the Physics Department, University College of Wales, Aberystwyth.

[4] Livingston ('06), pp. 30–33.

side of the stand, the point midway between a pair of thermometers being at the same level as the centre of the porous cylinder of the corresponding evaporimeter (Text-figs. 4 and 6). The thermometers were always read at the same times as the evaporimeters. Soil temperatures were also taken. The thermometers were fully exposed and not shaded as is usual in meteorological observations. On account of the impossibility of comparing the actual readings obtained, when thermometers by different makers are employed, meteorologists regard 'temperatures in the sun' as quite unreliable, at all events unless the black bulb thermometer in vacuo is used.[1] But here again the objects of the biologist and the meteorologist are different. Plants are in nature exposed to the effects of direct insolation and radiation, both of which are practically cut off if a screen is used. So that whether the object is to trace the connexion between temperature and the loss of water from an exposed evaporimeter, or to ascertain the relative temperatures in different layers of the vegetation, it is as well to use exposed thermometers.[2] So long as a set of quite similar thermometers is employed for any given experiments, the relative results obtained will be comparable; the absolute temperatures are of little importance.[3]

Experiments in the Field.

Field observations on evaporation and temperature were carried on during the summers of 1907 and 1908. The 1907 experiments were as follows:—

1st series July 9th to 15th.[4]
2nd „ July 18th to 21st.
3rd „ August 13th to 16th.
4th „ September 3rd to 8th.

During 1908 continuous observations were made from July 30th to August 9th.

(*a*) Evaporation in 1907.

During this year the experiments were confined to the so-called 'Sedge' vegetation.[5] In this *Cladium Mariscus* is dominant, though it is everywhere mixed with other species. In the part selected the herbage had not been cut for about four years, and in consequence formed a dense growth, with the 'general vegetation level' about 4 ft. to 4 ft. 6 in. above the soil (see Pl. I, Fig. 1, and Text-fig. 4).

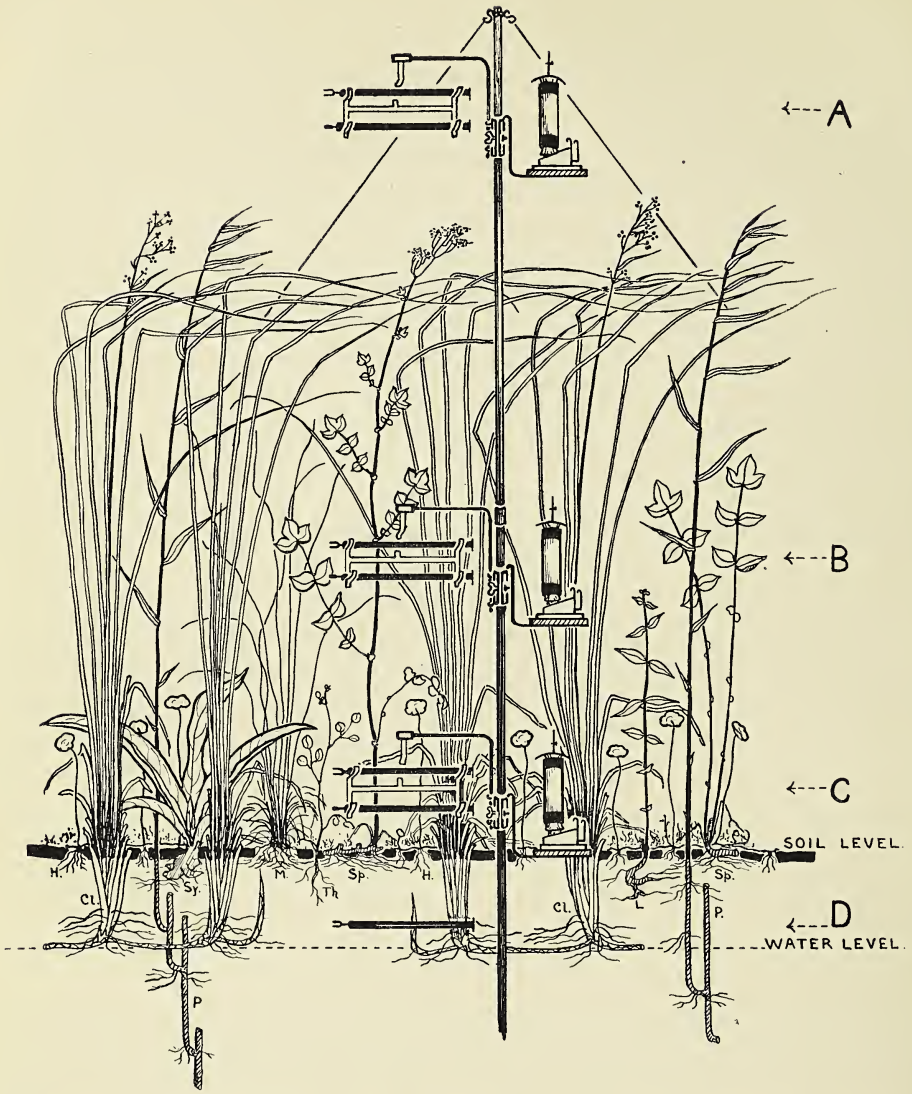
[1] Hann ('03), p. 39.

[2] Clements ('05, p. 66) says that he uses unsheltered thermometers for ecological work.

[3] The maximum were mercury, and the minimum alcohol thermometers. They were supplied by Messrs. Gallenkamp & Co. of Finsbury Square, London.

[4] Of all the months of the year, evaporation is usually greatest in July. June and August are also, as a rule, months of high evaporation; cf. Mill ('08), p. 47.

[5] Cf. Yapp ('08), p. 68.



TEXT-FIG. 4. Diagrammatic section through 'Sedge' vegetation, showing stratification of both shoots and roots. The field sketch was made on a windy day (August 15, 1907). Note that the direct effect of the wind is confined to the upper strata. The instruments (**A**, **B**, **C** and **D**) are as arranged for the August and September Experiments, 1907.[1]

Cl. *Cladium* (5)[2]; **H.** *Hydrocotyle* (1); **L.** *Lythrum*; **M.** *Molinia*; **P.** *Phragmites* (4); **Sp.** *Spiraea* (3); **Sy.** *Symphytum*; **Th.** *Thalictrum* (young). × $\frac{1}{16}$.

[1] N.B. Though here shown vertically over one another, each set of instruments was really arranged at an angle with the others.

[2] The numbers in brackets after plant-names refer to the ecological type of habit (see p. 277).

The vegetation was disturbed as little as possible when taking the readings, but it was judged advisable to place the instrument-stand in a fresh, though similar, position, for each series of observations.

For the first and second series the instruments were disposed of as follows:—

(1) Set **A**,[1] 4 ft. 6 in. above the soil-level. This was just above the general vegetation level; and on about the same level as the stray projecting shoots of *Phragmites*, *Cladium*, &c. The instruments were exposed to both sun and wind.

(2) Set **B**, 2 ft. 2 in. above the soil, i. e. at about the middle of the vegetation. In this position the instruments were lightly shaded, and sheltered from wind.

(3) Set **C**, five inches above the soil, i. e. at the bottom of the vegetation. Here the instruments were almost completely in shade, and quite sheltered from wind.

(4) Set **D** (thermometers only), six inches below the surface of the soil.

For the third and fourth series of observations set **A** was placed at a height of 5 ft. 6 in., i. e. quite clear of the vegetation. The positions of sets **B** and **C** remained practically unchanged (see Text-fig. 4).

It seemed desirable to know not only the aggregate differences of evaporation in the various positions, but also something of the extremes which might occur. Readings were therefore made at first twice a day, at about 10 a.m. and 6 p.m. The 'day' period of eight hours would of course include the hours of maximum evaporation. Subsequently an additional reading was made at 4 p.m., as the rate of evaporation was often observed to fall rapidly after about that time. On certain days readings were taken at more frequent intervals. It is unnecessary to give all the actual readings in detail. Table I gives for a number of days (twenty-four hours) the total evaporation from each instrument, and also various other meteorological data. The records of relative humidity, sunshine, and rainfall were made at the Cambridge Botanic Garden, distant some eleven miles from Wicken Fen. These probably represent fairly nearly the conditions at Wicken on the corresponding days. I am indebted to Mr. R. I. Lynch for kindly furnishing me with these Cambridge readings.

The effect of the various meteorological factors on evaporation can be seen to some extent from the following table; but this question will be more fully discussed after the 1908 results have been given.

[1] Each set (**A**, **B**, and **C**) included one evaporimeter and a pair of thermometers. The measurements refer in each case to the height above the soil, of the middle point of the porous cylinder of the evaporimeter, and of the point midway between the two thermometers. In the second series the height of set **A** was actually 4 ft. 8 in., but the relative position was the same, the vegetation being slightly taller.

TABLE I.

Date 1907	*Recorded at Wicken Fen.*							*Recorded at Cambridge.*		
	Total evaporation in c.c.[1] *for periods of 24 hours.*[2]			*Maximum and minimum temperatures in degrees Centigrade.*			*Wind direction, and velocity (estimated acc. to the Beaufort scale).*	*Relative humidity.*	*Rainfall in inches.*	*Number of hours sunshine.*
	A	B	C	A	B	C				
Series 1 July 10	18·0	3·15	2·0	max. 18·0 min. 2·3	18·0 —[3]	14·6 3·5	N. 1	9 a.m., 89 9 p.m., 92	0·6	0·75
July 11	70·4	24·0	5·45	21·5 2·0	22·8 —	19·0 2·5	S.W. 1	65 94	0	12·75
July 14	40·1	13·5	2·45	22·6 5·9	26·8 —	21·2 5·8	N.W. 0 to 1	77 88	0	0·5
Series 2 July 19	54·5	24·0	6·95	24·5 2·8	26·3 2·5	17·8 3·6	N.E. 1 to 2	76 88	0	12·0
July 21	53·05	19·6	7·5	21·8 6·2	23·4 5·7	19·7 6·5	N.E. 0 to 1	80 76	0	1·0
Series 3 Aug. 15	86·5	24·75	2·0	22·5 10·5	23·3 11·5	16·7 13·0	S.W.byW. 3 to 6	67 93	0·08	8·5
Series 4 Sept. 5	49·55	11·35	nil	20·9 8·0	20·0 —	17·4 8·5	S.W. 2 to 3	77 78	0·07	1·63
Sept. 7	49·5	13·0	0·7	25·2 10·3	24·9 —	18·3 9·2	S.W. 1	74 94	0	3·5
Sept. 8	66·7	23·85	4·75	23·8 11·9	23·4 —	18·1 11·7	N.E. 1 to 2	85 79	0	5·8

After deducting a few occasions when one or other of the evaporimeters had to be removed for slight repairs, the time occupied by the first two series of experiments (in July, 1907) amounted to between seven and eight days. During this time the total evaporation from the different instruments was as follows:—

A. 301·6 c.c.
B. 113·35 c.c.
C. 30·45 c.c.

Taking **A** = 100, the relative evaporation in the three positions was according to the following ratios:—

A : **B** : **C** = 100 : 37·6 : 10·1.

[1] All the evaporation readings in this table have been corrected according to the method given in the Appendix (p. 39).

[2] On the following occasions the periods were not precisely 24 hours:—July 10th and 21st ($23\frac{3}{4}$ hours each), and July 11th ($24\frac{1}{4}$ hours).

[3] The **B** minimum thermometers were defective during Series 1 and 4.

Of course the ratios varied somewhat on different days, e. g. on July 11 they were 100 : 34·1 : 7·7, and on July 21, 100 : 37 : 14·1. But on the whole, the differences for the three positions are not only very considerable, but also moderately constant.

Treating the third and fourth series (the total time being again between seven and eight days ; in August and September) in the same way, position **A** being now clear of the vegetation, the totals are :—

A. 409·1 c.c.
B. 114·65 c.c.
C. 12·9 c.c.

And the corresponding ratios :—

A : **B** : **C** = 100 : 28 : 3·2.

Thus the differences of evaporation for the three positions are, on the whole, even greater than in the first two series. This is no doubt due in part to the raising of the evaporimeter **A**. In addition to this, however, it must be noted that while the actual evaporation from **A** is higher, that from **C** is lower than in the first two series. **B** is almost exactly the same in both cases.

Combining the whole of the 1907 observations, the ratios work out at:

A : **B** : **C** = 100 : 32·8 : 6·6

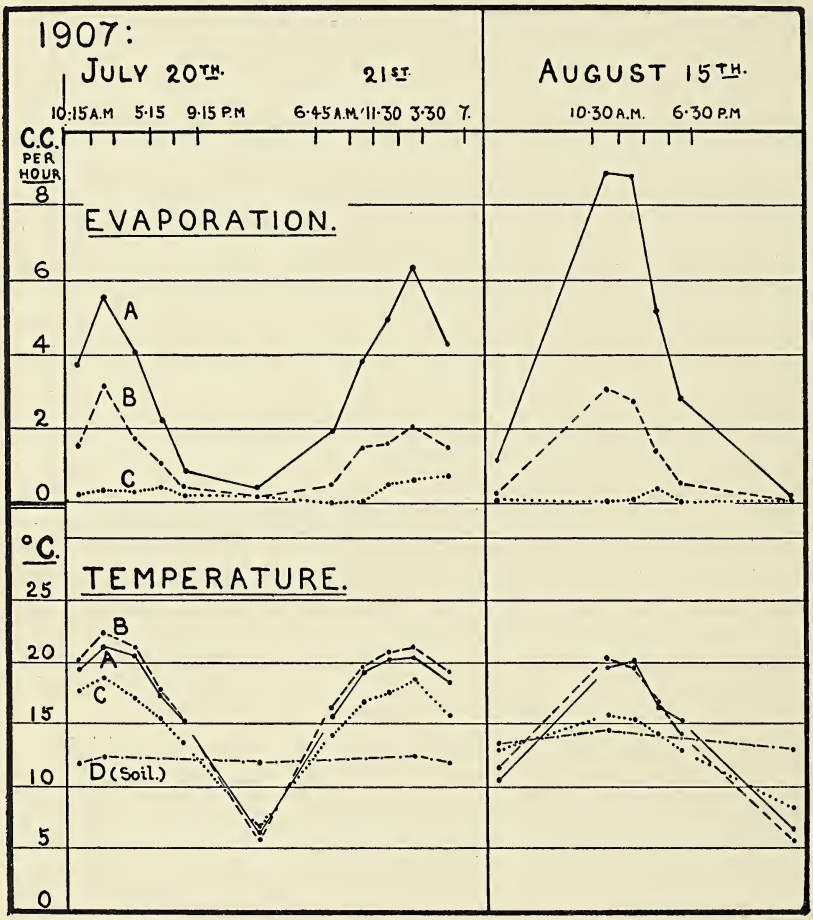
That is, for the vegetation in question, the promotion of transpiration by the atmosphere, as regards shoots which project above the general vegetation level, is something like three times as great as the effect produced in the middle layer of the vegetation. Further, the average effect on these projecting shoots is some fifteen times, and on the shoots of the middle layer about five times, as great as that experienced by plants occupying the lower layers of the vegetation.

August 15, 1907, is of especial interest, on account of the high evaporation from the exposed evaporimeter **A**. The water-loss from this instrument was, on this occasion, the greatest recorded (for twenty-four hours) during either year.[1] That from **B** and **C** was, however, not exceptional (cf. Table I). Further, the wind-velocity was greater on this day than on any other during the field observations. Readings were taken at intervals of two hours through most of the day. Text-fig. 5 shows the curves of evaporation and temperature on this occasion. July 20 and 21 are added for comparison, and the soil temperatures are also included.

It may be noted that during the four hours from 10.30 a.m. to 2.30 p.m. the loss of water from **A** averaged nearly 9 c.c. per hour, which again was the highest rate for either year (cf. curves in Text-figs. 5, 7 and 8). For the same four hours the *total* evaporation from **C** was only 0·1 c.c. During the

[1] This was 86·5 c.c. (see Table I), or, in linear evaporation, 0·169 inch ; which is almost exactly one-third of the highest evaporation (for 24 hours) recorded by Livingston during his desert experiments (see Appendix, p. 316).

earlier part of the day, all the conditions were favourable to evaporation. Although the temperature was not very high, the relative humidity was low, the wind strong, and the duration of sunshine considerable (see Table I). In the course of the afternoon the wind moderated, and between 3 and 4 p.m. there was heavy rain for about twenty minutes ; the temperature also dropped



TEXT-FIG. 5. Curves [1] of Evaporation and Temperature for July 20 and 21, and August 15, 1907.

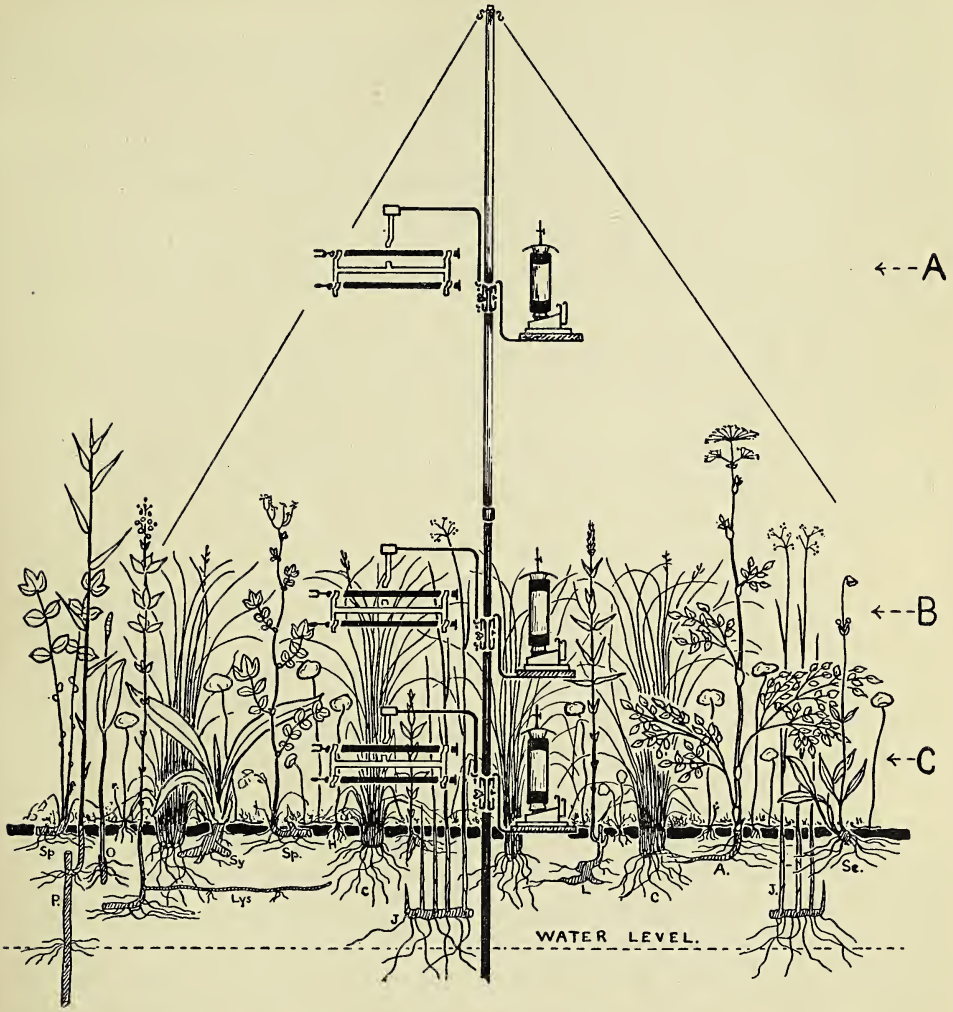
suddenly. The effect of these changes on the evaporation is easily seen in Text-fig. 5.

(*b*) EVAPORATION IN 1908.

Having completed many of the field observations which had taken me to the more distant parts of the Fen, I was, in 1908, at liberty to take more

[1] The methods used in the construction of these curves are explained on p. 319.

frequent and regular readings. In consequence, the instruments were observed regularly every two hours, from 8 a.m. to 8 p.m. each day from July 31 to August 9. Moreover, it was thought desirable to see how



TEXT-FIG. 6. Diagrammatic section through 'Litter' vegetation, showing stratification of aerial shoots and subterranean parts. The instruments (**A**, **B** and **C**) are in the positions used during the 1908 experiments.

A. *Angelica* (2)[1]; **C**. *Carex* (5); **H**. *Hydrocotyle* (1); **J**. *Juncus obtusiflorus* (5); **L**. *Lythrum* (4); **Lys**. *Lysimachia* (4); **O**. *Ophioglossum* (1); **P**. *Phragmites* (4); **Sc**. *Scabiosa Succisa* (2); **Sp**. *Spiraea* (3), both flowering and non-flowering shoots; **Sy**. *Symphytum*. (August, 1908.) × $\frac{1}{16}$.

evaporation in vegetation of a more dwarf and less dense character would compare with the results obtained in 1907. Accordingly, the instruments

[1] The numbers in brackets refer to the ecological type of habit (see p. 277).

were placed in 'litter' vegetation.[1] *Cladium* was absent, being largely replaced by *Carices*. Mingled with the *Carices* were *Phragmites* (dwarf), *Molinia*, &c., and a number of species of dicotyledonous herbs (see Pl. I, Fig. 2, and Text-fig. 6). The general vegetation level was about two feet above the ground.

The instruments were now arranged as follows (see Text-fig. 6) :—

(1) Set **A**. 4 feet above the soil. In this position the instruments were clear of the vegetation, and quite exposed.

(2) Set **B**. 1 ft. 7 in. above the soil level. Here the conditions were those of the surface layer of the vegetation. The instruments were fairly exposed to the sun, but the wind effect was lessened.

(3) Set **C**. $5\frac{1}{2}$ inches above the soil, i. e. in the lower layers of the vegetation. Instruments lightly shaded, and almost completely sheltered from the wind.

Table II gives the evaporation and other meteorological data during the whole period occupied by the 1908 experiments. Cf. also the curves in Text-figs. 7 and 8.

The total evaporation during the whole of the ten days, from the three instruments respectively, was as follows :—

A. 532·85 c.c.
B. 299·25 c.c.
C. 78·45 c.c.

These amounts are in the ratios :—

A : **B** : **C** = 100 : 56·2 : 14·7

as compared with the average relative evaporation during the 1907 experiments of :—

A : **B** : **C** = 100 : 32·8 : 6·6.

As was to be expected, by far the greater part of the total evaporation occurred during the daytime. Thus in the case of **A** about 90 per cent. (on an average) of the total evaporation for the whole twenty-four hours took place during the twelve hours between 8 a.m. and 8 p.m. The corresponding averages for **B** and **C** were 92 per cent. and 76 per cent. respectively. Further, the day hours themselves are not alike in evaporating power. On favourable days evaporation was usually most rapid between the hours of 10 a.m. and 4 p.m.[2] Thus, again taking averages, it was found that the following percentages of the total evaporation (for twenty-four hours) were recorded for the six hours between 10 a.m. and 4 p.m. :—**A**, 55 per cent. ; **B**, 58 per cent. ; **C**, 50 per cent. (cf. evaporation curves in Text-figs. 5, 7 and 8).

[1] Yapp ('08), p. 68.

[2] Houdaille ('92) found that at Montpellier the maximum rate of evaporation occurs between 2 and 3 p.m.

TABLE II.

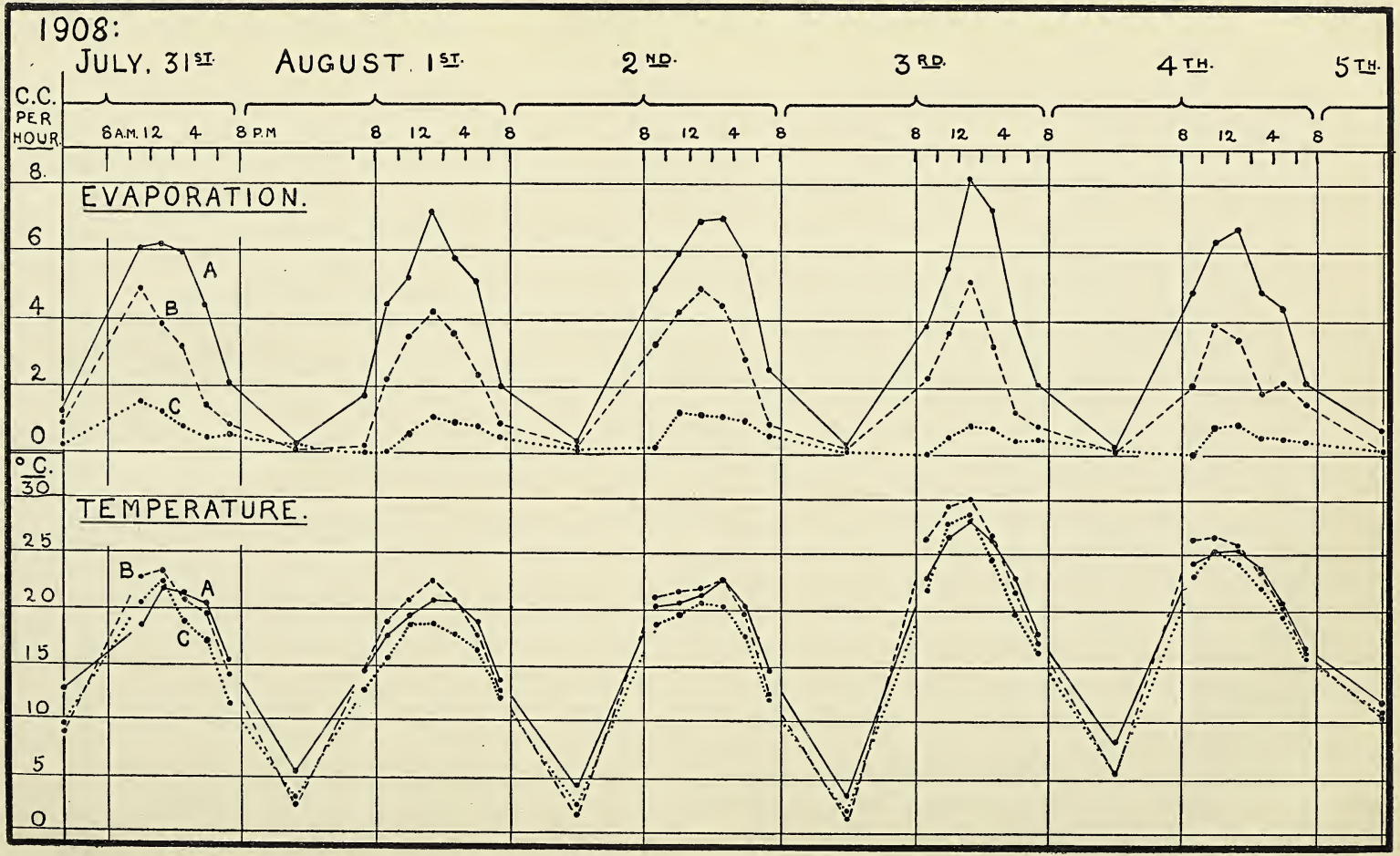
Date 1908	*Recorded at Wicken Fen.*							*Recorded at Cambridge.*		
	Total evaporation in c.c.[1] *for 24-hour periods.*			*Maximum and minimum temperatures in degrees Centigrade.*			*Wind direction, and velocity (estimated acc. to the Beaufort scale).*	*Relative humidity.*	*Rainfall*[2] *in inches.*	*Number of hours sunshine.*
	A	B	C	A	B	C				
July 31	67·6	40·15	12·85	max. 24·5 min. 12·4	26·7 9·0	24·0 9·5	W. 1 to 3	9 a.m., 64 9 p.m., 71	0	10·41
August 1	64·05	34·7	10·3	22·8 5·5	26·0 2·3	20·3 3·0	N.W. 0 to 3	64 76	0	11·58
,, 2	70·3	43·35	12·8	23·4 4·1	25·3 1·6	21·8 2·4	N. 0 to 3.	58 84	0	12·50
,, 3	64·7	34·85	7·45	28·5 3·1	31·2 1·2	29·5 1·8	W.toN.W. 0 to 2	67 76	0	12·08
,, 4	60·35	30·05	8·3	26·9 8·2	30·0 5·6	27·7 5·5	W.toN.W 1 to 2	65 78	0	7·00
,, 5	22·9	9·4	2·95	15·7 11·8	15·7 10·4	14·8 10·6	W. to N. 3 to 4	76 92	0·01	0·16
,, 6	46·7	27·8	5·95	21·4 12·4	22·0 12·2	19·8 12·5	N. 3 to 4	75 85	0·15	1·91
,, 7	62·0	39·05	9·5	23·7 12·3	25·5 7·7	23·8 10·1	N.W. 0 to 4	81 93	0	9·00
,, 8	15·75	8·0	2·6	20·6 7·7	21·8 5·4	18·3 7·5	Calm. 0	91 99	0	0·50
,, 9	58·5	31·9	5·8	27·5 7·6	31·7 5·2	22·5 6·5	N.W. 0 to 1	63 81	0	8·91

The results of the 1908 observations show that shoots at, or just below, the general vegetation level are exposed to transpiration conditions little more than half as severe as those which exist at a level well above the vegetation. Also, although the herbage is only about two feet in height, its lower strata have an atmosphere which on the average is about four times as humid as that of the upper strata, and seven times that two feet above the vegetation.

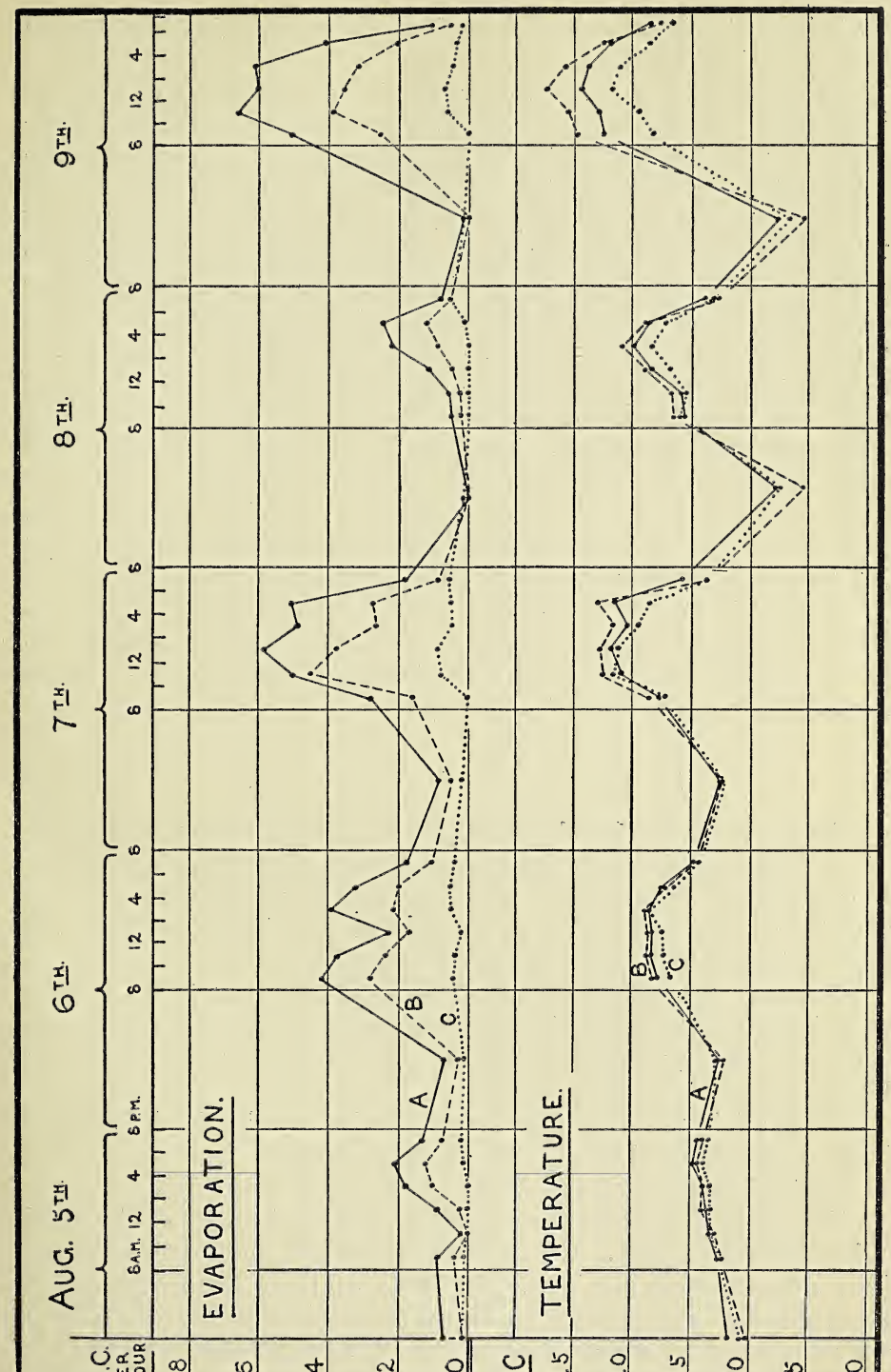
It will be noted that evaporimeters **B** and **C** recorded, on the whole, not only relatively, but actually, much greater water-loss in 1908 than in 1907. This is to be accounted for partly by the differences in the character of the

[1] These are the corrected readings; see Appendix, p. 313.

[2] Note that the 24-hour periods in this table are reckoned from 8 p.m. to 8 p.m. On the other hand, the Cambridge rainfall readings are taken at 9 a.m. each day. From notes made at Wicken I think part of the rainfall on August 6th should be credited to the 5th.



TEXT-FIG. 7. (See p. 319 for explanation.)



TEXT-FIG. 8. (See p. 319 for explanation.)

vegetation, but chiefly by the fact that in 1908 both these instruments were much nearer its upper level.

In general, the results of the evaporation experiments show that the lower strata of the vegetation possess an atmosphere which is continually very much more humid than that of the upper strata ; and further, that the higher and denser the vegetation, the greater these differences are.

Owing to evaporation of water from the soil, the frictional resistance of the latter to the wind, &c., the layers of air immediately above even bare ground are doubtless in general more humid than those at somewhat higher levels. But on dry days these differences are in all probability very much less than those recorded above for different vegetational layers. It was intended to make some experiments on this point during the summer of 1908, using evaporimeters in the open. Want of time has so far prevented these from being carried out. Professor F. W. Oliver has, however, very kindly allowed me to make use of some observations made by him in April 1908, on the salt marshes at Erquy in Brittany. Humidity readings were taken in five separate localities, while a wind was blowing. It was found that the average relative humidity at the ground-level was about 70 to 73 per cent., while at five feet above the ground it was about 61 per cent. These results tend to support the suggestion made on p. 280 with respect to the part played by humidity in determining the proper position for the leaves of *Hydrocotyle*, &c. (cf. Text-fig. 2).

(*c*) Air and Soil Temperatures.

As previously mentioned, unscreened thermometers were used throughout. In the case of the air thermometers, readings were taken each time the evaporation was measured. The soil temperatures were taken at first twice, but subsequently only once, a day. The more important results are given (in degrees Centigrade) in the following tables.

Table III is compiled from the whole of the 1907 observations (series 1 to 4) ; but owing to defects in the **B** minimum thermometers during the first and last series, results involving the use of **B** minima are omitted from this table.

Table IV, however, which refers only to the second and third series of the 1907 experiments, includes data for all positions.

Table V gives the results obtained from the 1908 readings.

The methods by which these results have been calculated will be found in the Appendix (p. 316). Except in the case of the mean daily temperatures, these methods are much the same as those in general use by meteorologists. The positions (**A**, **B**, and **C**) occupied by the different sets of air thermometers are explained above (cf. Text-figs. 4 and 6, and Pl. I, Fig. 2). The soil temperatures (**D**) were taken six inches below the surface.

This depth was chosen because the roots of the majority of species rarely penetrate, in this wet soil, to a much greater depth than this.

TABLE III.

Temperatures, 1907 (Series 1 to 4).

Position.	*Mean daily temperatures.*	*Mean of daily maxima.*	*Mean of daily minima.*	*Mean diurnal range.*	*Absolute extremes.*	
A.	16·5° C.	22·1	6·6	15·5	25·5	2·0
B.	—	23·0	—	—	26·8	—
C.	14·1	18·0	7·1	10·9	21·2	2·5
D (soil).	11·8	12·4	11·2	1·2	14·7	9·2

TABLE IV.

Temperatures, 1907 (Series 2 and 3 only).

Position.	*Mean daily temperatures.*	*Mean of daily maxima.*	*Mean of daily minima.*	*Mean diurnal range.*	*Absolute extremes.*	
A.	17·0° C.	23·4	7·5	15·9	25·5	2·8
B.	17·2	24·4	7·2	17·2	26·3	2·5
C.	14·7	18·8	8·5	10·3	20.2	3·6
D (soil).	12·7	13·3	12·2	1·1	14·7	10·5

TABLE V.

Temperatures, 1908.

Position.	*Mean daily temperatures.*	*Mean of daily maxima.*	*Mean of daily minima.*	*Mean diurnal range.*	*Absolute extremes.*	
A.	16·2° C.	23·5	8·5	15·0	28·5	3·1
B.	16·3	25·6	6·0	19·6	31·7	1·2
C.	14·9	22·2	6·9	15·3	29·5	1·8
D (soil).	14·8	15·3	14·3	1·0	156	12·8

Perhaps the most striking of the results shown in the above tables is the relatively wide range of temperature recorded for position **B.** Almost

without exception both the highest and the lowest temperatures for any given day occurred in this position (cf. also the temperature curves in Text-figs. 5, 7 and 8). Comparing **A** and **B**, this difference of diurnal range was considerably greater in 1908, when **B** was only just below the general vegetation level, than in 1907, when it was situated about two feet below the top of the vegetation (see Text-figs. 4 and 6). Nocturnal radiation is no doubt largely responsible for the low night minima of **B**, especially on clear nights.[1] The high day maxima are not quite so easily explained. Perhaps they may be partly due to a greater loss of heat from **A**, by convection during the daytime, owing to the fact that air-currents have freer play in this position than in **B**.

The mean diurnal range of temperature at **C** was in both years much less than at **B**, but while in 1907 it was also considerably less than at **A**, in 1908 it was slightly greater. This apparent discrepancy is probably due to the greater height and density of the vegetation in the former year.

It is interesting to note that the mean daily temperature in the positions **A** and **B** was nearly identical, while that of **C** was lower, and approached much more nearly the mean temperature of the soil. In fact, in 1908 the mean temperatures of **C** and **D** were practically the same, though the divergence was greater in 1907.[2]

From the foregoing it seems that the greatest diurnal range of temperature (when unscreened thermometers are used) is at or just below the general vegetation level. Further, it would appear that this daily range diminishes as the vertical depth below the superficies increases,[3] until it approximates to that in the free air above the vegetation. Below this point the range still further diminishes, and the temperature conditions become, in consequence, more uniform. But even in the lowest strata of the vegetation the diurnal range is still, of course, much greater than that of the soil itself, though the mean temperatures of the two may be similar.[4]

Though the evidence available certainly points in the direction of the above conclusions, it must be remembered that they are drawn from observations taken, in each year, at three levels only.

But at all events it seems clear that not only are the upper strata

[1] Cf. Hann ('03), p. 41.

[2] In connexion with this divergence it is to be noted that, apart from the generally cooler summer of 1907, not only was the vegetation employed in this year taller and less open, but the soil was considerably wetter than in 1908. Further, the soil temperatures were not taken, in the latter year, at exactly the same spot as that where the instrument-stand was placed. This may have made some difference.

[3] Probably also the relative density of the vegetation is important.

[4] In considering these temperature relations, due allowance must be made for the fact that the thermometers in position A were exposed to direct sunshine. Those at B varied in this respect in the two years; while C was scarcely, and D not at all, under the influence of direct insolation. Of course the same is true of the various plant organs which occupy similar positions.

of the vegetation subjected, during the summer months, to far greater humidity variations than the lower, but also to a considerably greater diurnal range of temperature.

Effect of Temperature, etc., on Evaporation.

We are now in a position to inquire into the effect of the various meteorological factors on evaporation. This effect can be very well seen by reference to Tables I and II, and the curves in Text-figs. 5, 7 and 8. But the more important factors may be referred to individually.

(1) Temperature. The curves show at a glance the connexion between evaporation and temperature. In a general way, the evaporation rises and falls, *pari passu* with the rise of temperature by day and its fall by night.[1] But that temperature is not the only factor concerned is shown by the fact that the highest temperature is recorded at **B**, but the maximum evaporation at **A**. Again, the curves of evaporation diverge much more widely during the daytime than do the temperature curves (Cf. Text-figs. 7 and 8).

(2) Relative Humidity is, after all, the most important factor; for most of the others act by influencing this. Relative humidity and temperature are intimately connected, the former reaching a maximum during the coldest, and a minimum during the warmest, hours of the day.[2] High evaporation with low relative humidity (in the morning) was recorded on July 11 and August 15, 1907, and from July 31 to August 4, 1908 (see Tables I and II).

(3) Wind. The effect of wind is well seen on August 15, 1907 (Text-fig. 5). The breezes, though fairly light, materially assisted the evaporation from July 31 to August 3, 1908. Thus, on August 3, the early morning was calm, and, in spite of the high temperature, the evaporation between 8 and 10 a.m. was lower than on several of the preceding days. A light breeze sprang up soon after 10 a.m., and increased in strength till about 1 p.m. The effect of this breeze on the rate of evaporation was at once apparent (see Text-fig. 7).[3]

In general (though they can, of course, only act by influencing the local relative humidity), it can scarcely be doubted that air-currents are the chief factor in determining the high rate of evaporation in the exposed position **A**. Conversely, the stagnation of the air in the lower layers of the vegetation, and its consequent high relative humidity, even on dry days, no doubt account to a large extent for the flatness of the **C** evaporation curve.[4]

[1] Cf. Livingston ('06), p. 32, and the curves on p. 30.

[2] Sprung ('85), p. 352.

[3] Cf. also Livingston, l. c., pp. 32 and 33.

[4] Of course much of the actual air-moisture in the vegetation is that transpired by the plants; but some, no doubt, is due to direct evaporation from the damp soil.

Dr. Mill[1] found at Camden Square that the curve of wind velocity has apparently little relation to that of evaporation. But air-currents have much freer play around the vertical evaporating surface of the instrument used in these experiments, than on the horizontal water surface of a sunken evaporating tank. It is therefore to be expected that the influence of wind would be considerable. This is also true of the plants themselves, especially of the more exposed ones.

The effect of wind on the vegetation will be referred to again later.

(4) Duration of Sunshine. It is noteworthy that the total evaporation was high on all days when there was long continued sunshine (Tables I and II). This agrees with the results of Dr. Mill,[2] who states that on the whole, at Camden Square, the evaporation curve follows, in summer, the curves of duration of sunshine and black-bulb temperature; and in winter the mean temperatures of water and soil (at one foot).

(5) Rainfall. The effect of rain is well seen on July 10 and August 15, 1907, and August 5 and 6, 1908, &c. On the two latter occasions heavy rain after 10 a.m. on August 5, and slight showers after 12 noon on August 6, may be seen to have immediately depressed the evaporation curves (Text-fig. 8).

Of course the greatest effect is produced when several of the factors act in the same direction, as was the case on July 11 and August 15, 1907; and from July 31 to August 3, 1908. Thus, on August 15, 1907, a strong wind, reinforced by low relative humidity, 8·5 hours of sunshine, and so on, resulted in the highest total evaporation recorded for any single day. It must be noted that part of this total was due to high night evaporation; the night also being unusually windy. On the other hand, on August 5, 1908, in spite of a fairly strong wind, the total was one of the lowest of the whole series. But in this case the temperature was low, the relative humidity rather high, and it was rainy and dull.

The above results show, I think, that the evaporimeters employed, like the similar ones of Livingston, are highly sensitive to even slight changes in the atmospheric conditions.

With regard to the two particular years during which the experiments were carried on, 1907 had an unusually wet and cold summer. Dr. Mill recorded[3] less total evaporation for that year than for any year since 1902. The deficiency was chiefly from April to August. September, on the other hand, was exceptionally dry. 1908 was fairly normal. Dr. Mill has kindly furnished me with the following information (not yet published) regarding his records for the latter year. The total evaporation for the year almost exactly coincided with the average for the last twenty-three years. Further, the evaporation for August, during which most of my experiments were made, was slightly in excess of the average for that month.

[1] Mill ('08), p. 45. [2] Mill, l. c., p. 45. [3] Mill, l. c., p. 45.

The days on which observations were made at Wicken included a considerable variety of weather. Almost the only kind lacking was a really strong gale. But gales during the summer months are infrequent in the Fen country.[1]

It would seem, then, that the results obtained indicate fairly closely the normal range of evaporation under varying atmospheric conditions. Of course, even greater evaporation would probably occur on exceptional days in other summers. But in a climate such as ours the plants would only rarely be called upon, during the summer months, to endure much more severe transpiration conditions than on some of the days when the above records were made. In any case, there would still be great differences of evaporation in the different strata of the vegetation.

The Mutual Protection of Shoots.

The action of air-currents in increasing evaporation has been discussed above ; but the general relations of the vegetation to wind may be considered a little more fully. There can be no doubt that wind is an ecological factor of the greatest importance. This point has been emphasized by many authors ; more especially with regard to the effect of wind on tree-life.

The action of wind in increasing transpiration has been proved by Wiesner,[2] Livingston,[3] and others.

Hansen [4] has shown that wind frequently causes considerable injury to leaves. According to him, the withering of leaves at the apices or edges, which is so commonly seen, is due to this cause. It is important to note that Hansen's experiments prove that a strong wind is not necessary to cause this injury, provided that the wind is allowed to blow through the plant.[5] In another paper Hansen [6] affirms that all plants are sensitive to the drying action of wind.

Again, Hansen [7] correlates the great prevalence of plants of low growth in the East Frisian Islands with the strong winds which regularly occur there.

Kihlman,[8] Warming,[9] Schimper,[10] and others, have discussed the influence of strong winds in determining the configuration of trees and shrubs.

Well-defined contours are characteristic of many plants which grow either in very windy localities, or in general under conditions which greatly favour transpiration. Examples are the dune-like trees on our sea-coasts ;

[1] Miller and Skertchly ('78), p. 284.
[2] Wiesner ('88), pp. 182–214.
[3] Livingston ('06), p. 30.
[4] Hansen('04), pp. 38 et seq.
[5] Hansen (l. c., pp. 33 and 38) found that a continuous breeze, of a strength not exceeding 1–2 (Beaufort scale), was sufficient to wither the edges of the leaves of young tobacco plants.
[6] Hansen ('01), p. 66.
[7] Hansen, l. c., pp. 26 et seq.
[8] Kihlman ('90), pp. 61 et seq.
[9] Warming ('96), pp. 37 et seq, also ('02).
[10] Schimper ('03), pp. 76 and 347. Cf. also references given by these authors.

the characteristic rounded outlines of desert shrubs[1]; the cushion and rosette plants of alpine regions, &c. All these have long been known. Their regular outlines are, of course, due to the growth of the individual twigs or leaves to about the same external level. Under extreme conditions all twigs which project beyond this general shoot-level may be killed off.[2] This adds further to the severity of the general contour.

Now a great deal has been written on the subject of competition amongst plants. But, so far as I am aware, with the exception of the cases already mentioned (i. e. of plants growing under unusually severe conditions), comparatively little on the mutual protection afforded by the association or massing of shoots at the same level.

Warming,[3] in contrasting animal and plant associations, says, 'Nur im uneigentlichen Sinne kann man sagen, dass gewisse Individuen einander beschützen', and he instances the case of wind-swept trees. Later on, however, he says further, 'Es giebt in den Pflanzenvereinen ganz gewiss oft (oder immer) eine gewisse natürliche Abhängigkeit und eine gegenseitige Rücksicht der vielen Glieder eines Vereines von- und aufeinander.' I am inclined to think the latter statement deserves more prominence than is sometimes assigned to it. Thus, at least in the more extreme cases already instanced, it is obvious that the mutual protection derived from the close association of shoots at a given level is not only advantageous, but necessary. But I shall now attempt to show that even in the case of more favoured vegetation the difference is merely one of degree.

It may be said that in general shoots exhibit, in nature, a more or less gregarious habit. Thus it is a matter of common observation that herbaceous plants, as well as shrubs and trees, tend to assume a more compact form when solitary than when growing in close proximity to other plants.

But what is true of isolated individual plants is true also of vegetation. A wood or forest, for instance, generally exhibits a very regular undulating superficies: and a field of corn, where the dominant plants are all of one species, clearly shows a similar general vegetation level. It has also been shown that even in the mixed vegetation dealt with in this paper most of the shoots grow to about the same height (see Pl. I, Fig. 1, and Text-figs. 4 and 6).

Of course the uniformity of contour is much less in the case of mixed associations growing under more favourable conditions than in the extreme cases cited above. For instance, the general outline is frequently obscured by projecting inflorescences, or vegetative shoots of the (presumably) more hardy species. Further, where e. g. shrubs and herbs are intermingled, the

[1] Cf. photos of desert shrubs; Weiss and Yapp ('06), Pls. V, VI, and VII.

[2] Many figures showing this have been published. It can be readily observed in the case of wind-swept Hawthorns, &c., round our own coasts.

[3] Warming ('96), p. 110.

taller plants (which, however, generally form their own independent shoot-levels) tend to distract attention from the uniformity of level of the shorter vegetation.[1] But even in the less obvious cases, a general tendency to the formation of 'twig or shoot associations' may usually be traced.

With the *causes* of this phenomenon[2] we are not concerned here. But its *effect* on the transpiring organs have been seen throughout this paper; at least in the case of two slightly differing types of marsh vegetation. This effect is to very markedly lessen the atmospheric promotion of transpiration, even in the upper strata of the vegetation. Further, it is important to note that the majority of the transpiring organs (especially the larger ones) are placed below the general vegetation level. It also limits the influence of wind. Text-fig. 4 attempts to show how the direct action of wind is practically limited to the highest strata of the vegetation. Even when strong winds are blowing, the plants of the lower layers are scarcely disturbed at all.

The fact[3] that the exposed tips of the leaves of the sedges and grasses die early affords additional evidence of the relative severity of the atmospheric conditions at or above the upper level of the vegetation. In one of his papers Hansen[4] describes an experiment in which he trained vines against open espaliers in a garden. The winds were only light, but usually just sufficient to keep the leaves slightly moving. By the end of July, however, many of the leaves had a complete edge of withered brown tissue. On the other hand, the wind had practically no injurious effect on vines growing against a wall.

To sum up, there seems to be a general tendency in nature for vegetation, as well as isolated individual plants, to form more or less definite 'general shoot-levels'. This close proximity of the shoots to each other results in a very real (and at least in many cases necessary) mutual protection, both against excessive transpiration, and also against the mechanical effects of wind. Further, the contours to which such shoot associations give rise depend to a considerable extent for their degree of regularity on the relative severity of the environmental conditions.

Thus the structural peculiarities of vegetation, no less than morphological or anatomical modifications of leaves and other transpiring organs, may be effectual in securing immunity from the dangers of excessive transpiration.

In a later paper it is intended to deal with the anatomical structure of the transpiring organs, in relation to the stratification of the vegetation.

[1] e. g., the bushes on a marsh; cf. Yapp ('08), p. 65, also Pl. IV, Fig. 1. Gorse bushes on a mountain pasture are another example.

[2] e. g., the relative parts played by light, humidity, &c., in determining these growth relations of shoots.

[3] Mentioned in Yapp, l. c., p. 65.

[4] Hansen ('04), pp. 33–4.

I have been to some extent guilty of arguing from the particular to the general: the differences, therefore, between the particular vegetation described in this paper and other types must not be lost sight of. For instance, the substratum, in the case of marsh vegetation, is unusually damp, and this may of course influence the humidity of the air in the lower strata. But it can scarcely be doubted that in all dense vegetation physical differences more or less comparable to those described above exist at various levels. To take a single instance: Schimper [1] distinguished three layers of epiphytes in the primaeval forests of tropical America. The epiphytes of the upper layer, i. e. on the tree-crowns, are much more xerophytic in structure than those of the two lower layers in the forest. Schimper correlated this with the differences of light, and air moisture. It would, however, be of interest to determine the actual evaporation at various levels in a woodland, as well as in other types of vegetation.

The Problems of Different Species.

From the general results recorded in this paper it is obvious that the problems which confront any given plant must vary considerably according to the habit of growth of the species. It has been shown in an earlier paper that the subterranean parts of marsh plants place themselves at various levels, according to the degree of moisture appropriate to the species.[2] We can thus speak of a distinct stratification of the roots, in addition to that exhibited by the shoots (cf. Text-figs. 4 and 6). The latter has already been discussed, but the former is also important. For instance, in the case of a very wet soil all except quite the upper layers are but poorly supplied with oxygen. It is to be noted that a very large number of marsh plants are 'surface rooted'.

Now, apart from its specific structure and the length of its vegetative period, the physiological problems of any given plant will depend to a large extent on:—

1. The depth of its root-system.
2. The height above the soil to which its shoots reach.
3. The layer of the vegetation in which its larger leaves are placed.

It has been shown that different species vary greatly in all these respects. One has only to consult Text-fig. 4 to realize the very considerable environmental differences of such plants as (say) *Hydrocotyle* on the one hand, and either *Cladium* or *Phragmites* on the other.

Hydrocotyle has its leaves in the lower, more humid strata of the vegetation; its roots are practically on the surface of the damp soil; and it is sheltered from the wind. Thus, not only are the diurnal fluctuations of both temperature and evaporation reduced to a minimum, but whatever

[1] Schimper ('88), chap. iii, pp. 89 et seq.

[2] Yapp ('08), pp. 68 et seq.

the physical conditions may be at a given moment, they are fairly uniform for the entire plant.

On the other hand, both *Cladium* and *Phragmites* are, relatively speaking, plants of extremes. Their roots are fairly deeply placed in permanently wet soil, while their leaves reach levels where the effect of wind and the range of temperature and evaporation are at a maximum. Not only are the leaves themselves liable to frequent and sudden fluctuations of conditions, but the absorbing and transpiring organs will often be, at the same time, under widely differing conditions. But the problems of even these two plants are not the same, for while *Cladium* is evergreen, the aerial shoots of *Phragmites* are annual.

In fact, it is scarcely going too far to assert that few of the species of plants forming the vegetation of a marsh have to face precisely the same set of physiological problems.[1]

It may be pointed out further, that in the case of such plants as *Cladium*, &c., the task of securing complete co-ordination between the functions of absorption, conduction, and transpiration, must be a complicated one. Several authors[2] have drawn attention to this point; more especially with regard to the effect on transpiration, of warm air or strong winds, coupled with low soil temperatures. Such a combination often occurs on sunny days in winter or spring.

But the converse simplification of the problem of transpiration, in the case of small plants, appears to have attracted less attention. Here all the various organs of the plant will be, at a given time, under fairly uniform conditions of temperature, &c. Schimper,[3] however, in one of his papers, referred to the wintering of delicately formed plants, such as fungi, algae, &c., and herbs like *Stellaria media*, &c. These, he said, behave like the soil, as far as temperature fluctuations are concerned, and so do not need any protection against transpiration.

In our country, the contrast in winter time between delicate green seedlings, dwarf grasses, &c., on the one hand, and the tall bare trees or strongly xerophytic evergreen shrubs, on the other, is a striking one.[4]

The authors cited above have dwelt more particularly on the dangers of excessive transpiration in winter or spring. But though in our climate these dangers are perhaps the most serious, they are not the only ones. All the evidence adduced in this paper tends to show that even in summer time the atmospheric conditions may be unfavourably severe, as regards the most exposed shoots of the vegetation in question. Thus it is perhaps

[1] cf. Warming ('96), p. 119.

[2] e. g. Schimper ('90), pp. 647 et seq. Also Kihlman ('90), p. 107.

[3] Schimper, l. c., p. 649.

[4] A good many authors have, of course, remarked on the shelter from wind, &c., afforded by a low habit of growth; e. g. Meigen ('94), p. 408, Hansen ('01), &c.

scarcely to be wondered at that, for example, reed swamps should retain their peculiar physiognomy, even in warm climates.[1]

As mentioned at the beginning of this paper, some authors have regarded the fact that swamp plants both with and without xerophytic structures may grow side by side, as evidence that such characters cannot be a necessity to those species which possess them. Warming [2] also, in discussing the whole question of swamp xerophytes, concludes by pointing out that there are some swamp plants which apparently have no xerophytic characters, and which cannot be brought into agreement with such a habitat. He instances *Hydrocotyle*, *Caltha*, &c.

Whether such xerophytic structures are or are not present-day necessities to their possessors, I think it has been shown that any argument drawn from mere proximity of position, without reference to the varying physiological problems of the different species, is entirely inconclusive.

Summary of Results.

1. The primary aim of these researches is to throw light on the problem of 'swamp xerophytes'. The present paper, which deals with one aspect of the subject, describes the structure of the vegetation of a marsh. In particular, the relations of the aerial shoots, both to each other and to the external factors which directly influence transpiration, are discussed.

2. In considering the vertical distribution of the transpiring organs of the various species, the plants in question are grouped into some five ecological types (p. 277). These types depend to a large extent on the relative positions, on the stem, of the larger leaves. It is shown that while rhythmic variations in the size of leaves is common to all the species, yet specific differences result in the attainment of the maximum leaf-size at different vertical levels, in various cases (cf. Text-figs. 1, 4, and 6).

3. This difference of ecological habit results in a marked stratification of the vegetation, which varies in height from about two to five feet. But the strata or layers merge into one another more, and are therefore less distinct, than the very obvious vegetational strata of a forest. The subterranean parts also exhibit a stratification no less marked than that of the aerial shoots (Text-figs. 4 and 6).

4. Evaporation as a means of determining the extent to which the atmosphere promotes transpiration under various conditions is discussed (p. 282). Also the relation of evaporation to other meteorological factors (p. 301). An evaporimeter is described (see Text-fig. 3), which is really

[1] cf. Warming ('96), p. 176. This question will be considered further in a later paper. It is merely mentioned here on account of a difficulty raised by Warming. He accepts the explanation of Kihlman and Goebel, with regard to the 'swamp xerophytes' of high northern and alpine regions, but remarks that it will not suffice in the case of warmer climates.

[2] Warming, l. c.

a modified form of one previously used by Livingston at the Arizona desert laboratory.

5. In the field, both evaporation and temperature readings were taken at different levels in and above the vegetation. For this purpose, two types of marsh vegetation of differing heights and densities were selected.

6. In 1907, the vegetation employed was about five feet high. The average evaporation at three different levels was according to the ratios :—

$$\mathbf{A} : \mathbf{B} : \mathbf{C} = 100 : 32{\cdot}8 : 6{\cdot}6.$$

Position **A** was above the vegetation, **B** about the middle of it, and **C** in the lower strata (see Text-fig. 4).

7. In 1908 the vegetation selected was only about two feet in height. The corresponding ratios were :—

$$\mathbf{A} : \mathbf{B} : \mathbf{C} = 100 : 56{\cdot}2 : 14{\cdot}7.$$

Here position **A** was clear of the vegetation, **B** only just below the upper level of the vegetation, and **C** at the bottom (Text-fig. 6).

8. Thus the air in the vegetation is on the whole very much more humid than that outside it. Further, the higher and denser the vegetation, the greater the differences in atmospheric humidity between the upper and lower strata.

9. The temperature results show that the highest layers of the vegetation possess a greater diurnal range of temperature than either the free air above or the lower layers of the vegetation. Regularly, especially in clear weather, both the highest day, and the lowest night temperatures were recorded in this position.

The transpiring organs which occupy the lower strata of the vegetation are thus not only in more humid air, but in general under considerably more uniform conditions than those which reach what is called in this paper the 'general vegetation level'.

The general results of the experiments are given in Tables I to V, and in the form of curves in Text-figs. 5, 7 and 8.

10. It is shown that the great majority of the shoots of the different species attain to very much the same height, thus giving rise to a fairly uniform 'general vegetation or shoot-level'. Moreover, the shoots of isolated individual plants, and also of most kinds of vegetation, show a gregarious habit, which results in a greater or less degree of uniformity of external contour. When the external conditions are of great severity, the contours thus produced are exceedingly regular (e. g. desert shrubs, wind-swept trees on sea-coasts, &c.). But there is also evidence that even in the case of the vegetation dealt with in this paper, the atmospheric conditions may be, at least at times, unfavourably severe, as regards the upper strata of the vegetation. This is suggested by the early death of the projecting, exposed ends of the grass and sedge leaves (cf. Hansen's experi-

ments referred to in the paper). The general reduction in the size of the transpiring organs in the upper layers of the vegetation is also suggestive, though it is merely the effect of this on transpiration, and not the causes of the diminution in leaf-size, which is dealt with in this paper.

11. Thus the mutual protection, both from excessive transpiration and also from the mechanical effects of wind, derived from this very general 'association of shoots', is probably beneficial, even apart from the more obvious cases where the climate is exceptionally rigorous. The structural peculiarities of vegetation then, no less than morphological or anatomical modifications of leaves and other transpiring organs, may be effectual in securing immunity from the dangers of excessive transpiration.

12. It is shown that the different species vary as regards the depth of their root-systems; the height of their shoots; the relative positions of their transpiring organs with respect to other plants; the length of their vegetative period, &c. In fact, few of the species of plants forming the vegetation of a swamp-moor have to face precisely the same set of physiological conditions. Thus the arguments of authors, who insist that the so-called xerophytic structures of marsh plants can have no reference to present-day conditions, because both xerophytic and non-xerophytic species often grow side by side in nature, are entirely inconclusive.

. . . . . .

In addition to the acknowledgements made in the course of this paper, I wish to express my thanks to Dr. H. R. Mill for information respecting his evaporation records at Camden Square, which he has placed at my disposal; to Dr. G. A. Schott and Mr. A. A. Robb for criticisms and assistance with respect to the physical portions of this paper; and finally to Professor A. C. Seward for permission to use the Cambridge Botanical Laboratory as my head quarters during the investigations in the neighbouring Fenland. I have also to acknowledge grants to defray the cost of instruments, &c., made by the British Association for the Advancement of Science.

APPENDIX

It was thought advisable to remove certain details respecting the evaporimeters used, the methods of calculating results, &c., from the paper itself, and to insert them at the end, in the form of an appendix. Such details do not affect the main argument of the paper, but may be useful to those who happen to be working at the same or cognate subjects.

Calibration of the Evaporimeters.

As stated in the foregoing paper, the evaporating surface of each instrument consisted of a porous earthenware cylinder. To prevent fluctuations in the area of this surface, in the event of the rubber corks being inserted to different depths on different occasions, the ends of the cylinders were made impervious for distances a little beyond those reached by the corks (see Text-fig. 3).

Before filling, the cylinders were always first placed in water, and the air removed from their pores under an air-pump.

It was also necessary to calibrate the instruments, in order that the results obtained might be comparable. The calibration methods were very much the same as those employed by Livingston.[1] The three instruments were compared with each other as follows:—

(*a*) In a laboratory with windows facing the north: both doors and windows being kept closed. Direct sunlight was thus excluded, and air-currents reduced to a minimum. The three evaporimeters were arranged triangularly on a table, about 3 ft. 6 in. apart. In the centre was a crystallizing dish, filled with distilled water to about 3 mm. below its upper edge. The free surface of water was at the same height as the centre of the evaporating surface of the cylinders.

(*b*) The instruments were afterwards placed on the roof of the Botanical Laboratory at Cambridge. Here they were exposed to both sun and wind.

In both cases readings were taken at intervals and in the laboratory the loss of water from the crystallizing dish was ascertained at the same time by weighing. After each reading the positions of the instruments were interchanged, in order to neutralize any differences due to the positions themselves.

The respective evaporimeters may be designated I, II, and III: and as different porous cylinders were used during the second year, the year may be added.

[1] Livingston ('06), p. 26.

The 1907 laboratory experiments may be taken first. The total evaporation, as obtained from a number of consecutive readings, was as follows :—

Evaporimeter, 1907, I	37·75 c.c.
„ „ II	35·2 c.c.
„ „ III	40·15 c.c.
Crystallizing dish, 1907	13·9 grams.

The individual readings were not always precisely in the same ratios as the above totals ; but the maximum variation did not exceed 10 per cent. on either side of the mean.

We may first compare the evaporation from the porous cylinders with that from the open surface of water in the dish. For this purpose 1907, III, may be taken as a standard. Then :—

(1) Comparing the areas of the two evaporating surfaces, we have the ratio :—

$$\frac{\text{1907, III}}{\text{1907 cryst. dish}} = \frac{149{\cdot}40 \text{ sq. cm.}}{58{\cdot}09 \text{ sq. cm.}} = \frac{2{\cdot}57}{1{\cdot}00}$$

(2) From the above readings we find that the total evaporation for a given time, from the standard instrument and the crystallizing dish respectively, is in the ratio :—

$$\frac{\text{From 1907, III}}{\text{From 1907 cryst. dish}} = \frac{40{\cdot}15}{13{\cdot}90} = \frac{2{\cdot}89}{1{\cdot}00}$$

(3) Therefore, the different rates of evaporation, per unit area, from the respective evaporating surfaces are according to the ratio :—

$$\frac{\text{Porous earthenware}}{\text{Open water surface}} = \frac{2{\cdot}89}{2{\cdot}57} = \frac{1{\cdot}12}{1{\cdot}00}$$

For the 1908 experiments new porous cylinders, made of earthenware of a finer grain, were used. Leaving, for the moment, the comparison between the evaporimeters themselves; the 1908 calibration data, and the corresponding comparison between the evaporation from open water and earthenware, may be given.

Taking combined readings as before, we have the following totals :—

Evaporimeter, 1908, I	50·4 c.c.
„ „ II	52·2 c.c.
„ „ III	50·45 c.c.
Crystallizing dish, 1908	15·51 grams.

Here the maximum variation for individual readings was less than 7 per cent. on either side of the mean.

This time, 1908, I, was taken as the standard instrument.

Then :—

(1) Comparing the respective areas of the two evaporating surfaces, we have the ratio :—

$$\frac{\text{1908, I}}{\text{1908 cryst. dish}} = \frac{\text{141·20 sq. cm.}}{\text{62·07 sq. cm.}} = \frac{2{\cdot}27}{1{\cdot}00}$$

(2) From the calibration data given above we find that the total evaporation for a given time, from the standard and the dish respectively, is in the ratio :—

$$\frac{\text{From 1908, I}}{\text{From 1908 cryst. dish}} = \frac{50{\cdot}40}{15{\cdot}51} = \frac{3{\cdot}25}{1{\cdot}00}$$

(3) Therefore, the different rates of evaporation per unit area from the two evaporating surfaces are according to the ratio :—

$$\frac{\text{Porous earthenware}}{\text{Open water surface}} = \frac{3{\cdot}25}{2{\cdot}27} = \frac{1{\cdot}43}{1{\cdot}00}$$

It will be interesting to compare the rate of evaporation from the porous cylinders employed in my experiments with the rate in the case of Livingston's instruments. The latter author used unglazed porcelain cylinders, with one end closed and rounded.[1] From the measurements he gives, the area of the evaporating surface of one of his cylinders may be calculated as about 86 sq. cm. The average of his calibration experiments showed that the evaporation from one cylinder was equivalent to that from 99·26 sq. cm. of open water surface. Therefore the different rates of evaporation, per unit area, from the respective evaporating surfaces, were in the case of his cylinders according to the ratio :—

$$\frac{\text{Porcelain}}{\text{Open water surface}} = \frac{99{\cdot}26}{86{\cdot}00} = \frac{1{\cdot}15}{1{\cdot}00}$$

That is, the rates of evaporation per unit area from the porous cylinders used in the three cases were as follows :—

(*a*) From Livingston's cylinders, 1·15 times as fast as from an open water surface.

(*b*) From my 1907 cylinders, 1·12 times as fast as from open water.

(*c*) From my 1908 cylinders, 1·43 times as fast as from open water.

These differences in the rate of evaporation are probably due, as will be seen later, to differences in the grain of the earthenware of which the cylinders are composed.

We may now compare the cylinders with each other. The calibration data obtained from the sum total of the laboratory readings enabled multiplication factors to be calculated by which all the field readings could

[1] Livingston ('06), p. 20.

be reduced to a common standard. Using evaporimeter 1908, I, as the standard instrument, these factors were as follows :—

1907, I	. . . .	multiply by 1·32
„ II	. . . .	„ „ 1·39
„ III	. . . .	„ „ 1·27
1908, I	. . . .	„ „ 1·0 (standard)
„ II	. . . .	„ „ ·965
„ III	. . . .	„ „ 1·0 (equivalent to standard).

All the evaporation results given in the main paper have been corrected according to these factors.

Porosity of the Earthenware Cylinders.

As earthenware is practically a new (and apparently a highly satisfactory) material, so far as evaporation experiments are concerned,[1] it may be well to consider the relative porosity of fine and coarse-grained earthenware somewhat in detail.

An important question is whether, when the rate of evaporation is high, water can percolate through the pores at a sufficient rate to keep pace with evaporation from the surface. Apparently in all the field experiments, the instruments were equal to the demands made upon them; as the cylinders were always cool and moist to the touch, even when evaporation was greatest. One cylinder alone proved inadequate when evaporation was high, but as this was discovered during the calibration experiments, it did not affect the results obtained in the field. The exception was 1907, II. When exposed on the laboratory roof this instrument exhibited a very marked incapacity to keep pace with the other two evaporimeters, if evaporation was great. The following actual readings will show the lag of this cylinder :—

	1907, I.	1907, II.	1907, III.
(*a*)	70·7 c.c.	40·9 c.c.	71·2 c.c.
(*b*)	76·9 „	29·7 „	79·9 „
(*c*)	96·4 „	31·2 „	100·4 „
(*d*)	17·05 „	15·5 „	17·3 „

The periods for which these readings were recorded varied in length from twenty-one to twenty-eight hours. The first three were windy and sunny (during the daytime). During the fourth, however, the weather was dull and damp. Careful comparison showed that 1907, II, seemed to be incapable

[1] cf. references given in the main paper.

of evaporating more than some 2 c.c. per hour ; but that up to that amount it would behave very much like the other two instruments. This evaporimeter was therefore kept in the lowest position, **C**, during the field experiments. Here the actual evaporation was always so small that the instrument was quite capable of meeting the demands made on it. In all other cases, whether on the roof, or in the laboratory, the evaporation ratios for the different cylinders remained fairly constant.

It has been shown that, per unit area, the fine-grained porous cylinders used in 1908 had an evaporating capacity some 1·28 times as great as the somewhat coarser ones of 1907. This is probably due, partly to the finer earthenware possessing a larger number of pores per unit area, and partly to the greater capillary effect of the finer pores. So long as there is equilibrium between the evaporation outside, and the flow of water through the capillary pores, so long will the rate of evaporation increase with the fineness of the earthenware. But if evaporation should become very excessive, this state of equilibrium may be destroyed, owing to the counteracting of the greater capillary effect by the increased frictional resistance in the pores. On the other hand, the viscosity of water diminishes as the temperature rises. Thus a high temperature would not only increase evaporation, but would at the same time lessen the frictional resistance due to the viscosity of the water, and so decrease the risk of the steady state not being reached. In point of fact, it is probable (as seen above) that this state of equilibrium between evaporation and the flow of water through the pores was reached in all the field experiments recorded in this paper. But the case of 1907, II, shows the advisability of testing the individual cylinders before using them in exposed positions.

On the whole, the above considerations, as well as the greater uniformity shown by the 1908 cylinders (cf. the calibration data given above), point to the distinct superiority of earthenware of a fine, even grain.

To convert Cubic into Linear Evaporation.

In this paper I have given the evaporation results in cubic centimetres. But as most published observations are recorded in linear measure, it may be useful to give multiplication factors whereby my results may be converted into either centimetres, millimetres, or inches.

It has been seen that the standard evaporimeter (1908, I) had an evaporating surface of 141·2 sq. cm.: and further, that 1·0 sq. cm. of the porous cylinder was equal in evaporating power to 1·43 sq. cm. of open water surface.

Therefore $1{\cdot}43 \times 141{\cdot}2$ c.c. will evaporate from the whole cylinder during the time that it takes 1·0 c.c. to evaporate from 1·0 sq. cm. of open water surface.

So, to convert cubic into linear evaporation, it will be necessary to multiply the corrected readings (in c.c.), by the following factors :—

(*a*) To convert into centimetres the multiplication factor will be :

$$\frac{1}{1{\cdot}43 \times 141{\cdot}2} = \frac{1}{202}$$

(*b*) To convert into millimetres :

$$\frac{10}{1{\cdot}43 \times 141{\cdot}2} = \frac{1}{20{\cdot}2}$$

(*c*) To convert into inches :

$$\frac{1}{1{\cdot}43 \times 141{\cdot}2 \times 2{\cdot}54} = \frac{1}{513}$$

Using the last factor (*c*), the greatest evaporation for twenty-four hours recorded at Wicken Fen in 1908 (i. e. on August 2) by the exposed evaporimeter in position **A** was 0·137 inches. On one occasion this was exceeded in 1907 (i. e. on August 15), when the evaporation for the same position was 0·169 inches. Dr. Mill informs me that the highest evaporation from the standard tank at Camden Square, London, during the whole of 1908, was 0·21 inches (on June 5). Also, that on only four days during the whole year did the evaporation reach 0·20 inches.

We may now compare these results with those obtained by Livingston. In the desert air at the Arizona Laboratory, the evaporation was of course very much greater. The highest recorded by Livingston for any one day was 0·504 inches, while the average for twenty-nine days was 0·304 inches.[1]

Methods of calculating Temperature Results.

The temperature results given in Tables III to V (p. 299) were obtained by the following methods :—

(*a*) *Mean daily temperature.* As stated in the main paper, maximum and minimum temperature readings were taken each time that the evaporation was measured. From the data thus obtained, it was not possible to calculate the mean daily temperatures by the same methods as those employed by meteorologists.[2] The following was the method adopted. The mean was first taken of all the day readings for the period in question. Then the mean of all the night readings. Finally, the mean of the day and night means was found, and this was regarded as the mean daily temperature.

(*b*) *Mean of daily maxima.* Obtained by taking the means of all the absolute maxima for the days in question. The lower maxima from the other daily readings were, for this purpose, ignored.

[1] Livingston ('06), p. 29.

[2] Hann ('03), pp. 7 and 8.

(*c*) *Mean of daily minima.* Obtained in a similar manner to (*b*), by taking the mean of all the absolute minima.

(*d*) *Mean diurnal range.* This is calculated by first finding the daily range, i. e. the difference between the maximum and minimum for each day. The mean of all these is here called the mean diurnal range: it corresponds to the 'non-periodic amplitude' of meteorologists.

(*e*) *Absolute extremes* are the actual maximum and minimum recorded during the whole of any series of experiments.

Comparison between Sun and Shade Temperatures, etc.

A comparison may be made between the 'temperatures in the sun taken at Wicken and the shade temperatures for the corresponding days taken at the Cambridge Botanic Garden. For this purpose the 1908 experiments will suffice. In Table V the means of the daily maxima were seen to be:—

A, 23·5° C. : **B**, 25·6° C. : **C**, 22·2° C.

The corresponding mean of the daily maxima at Cambridge was 21·5° C. Thus the mean maximum at **A**, four feet above the ground, and fully exposed to the sun and wind, was 2·0° C. higher than at Cambridge, also taken at four feet above the ground, but screened in the usual way. This may give some indication of the kind of differences that may be expected when sun temperatures are taken. But of course it is at best only a rough approximation.

The means of the daily minima at Wicken, for the same period, were respectively:—

A, 8·5° C : **B**, 6·0° C : **C**, 6·9° C.

The mean of the Cambridge minima, taken on the grass, and therefore comparable to the Wicken minima, was 8·8° C. It was to be expected that the minima on low-lying marsh land should be lower than those on somewhat higher, drier land.

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EXPLANATION OF FIGURES IN PLATE XX, AND TEXT-FIGURES 5, 7 AND 8.

Illustrating Professor Yapp's paper on the Vegetation of a Marsh.

PLATE XX.

FIG. 1. 'Sedge' vegetation, about four feet high. *Cladium Mariscus* is dominant, but *Phragmites communis*, *Thalictrum flavum*, *Hydrocotyle vulgaris*, *Valeriana officinalis*, *Lysimachia vulgaris*, &c., are present.

This photograph was taken in July, 1907, near the stand of instruments. Note the density of the vegetation, and the 'general vegetation level'. Only the tips of the leaves of *Cladium*, and a few shoots of *Phragmites*, &c., project above this general level. During a wind, the tips of the leaves are beaten down, and the general vegetation level then becomes remarkably uniform (cf. Text-fig. 4).

FIG. 2. 'Litter' vegetation (about two feet high), in which the 1908 experiments were conducted. Note the **A** and **B** sets of instruments. **C** is hidden in the lower strata of the vegetation. *Carices* are the most abundant plants in this society, but many other species are freely intermingled with them.

TEXT-FIGURES 5, 7 and 8.

TEXT-FIGS. 7 and 8. Curves of evaporation and temperature for the period July 31 to August 9, inclusive.

The curves are plotted with intervals of time as abscissae; and as ordinates either (1) the rates of evaporation in c.c. per hour, or (2) temperature in degrees centigrade.

Readings were taken regularly every two hours from 8 a.m. to 8 p.m. throughout the period. The only exception to this was on July 31, when the first reading was at 10 a.m. There was also an additional reading at 6 a.m. on August 1. The times of observation are indicated by the short vertical lines below the numbered hours.

The letters **A**, **B** and **C** refer to the respective positions of the three sets of instruments (see Text-fig. 6).

(*a*) *Curves of Evaporation.* These are constructed by taking the average rate of evaporation per hour for the period between two observations, and plotting this in the middle of the period. The same method is employed for both day and night. The night periods, however, were twelve hours in length, as compared with the two-hour periods during the day, and the curves are therefore proportionately less accurate. The true curves of night evaporation, especially for the **A** position, should be somewhat flatter than represented here.

(*b*) *Curves of Temperature.* So far as the day periods are concerned, the curves are constructed in the same way as those of evaporation. The average temperature for the period is found by taking the mean between the maximum and minimum readings. For the night periods, however, the means would be too high, owing to the usually great rise of temperature shortly before 8 a.m. The absolute minimum for the night was therefore taken, and plotted midway between 8 p.m. and 8 a.m. This double method gives curves which represent the actual variations of temperature more nearly than if the means had also been used for the long night periods. The difference in method is indicated by a break in the continuity of the curves between day and night.

The curves forming Text-fig. 5 are constructed in the same way, except that soil temperatures are added. In the case of the latter, the diurnal range is so small, that the absolute maxima and minima (and not the means) are used for both day and night.

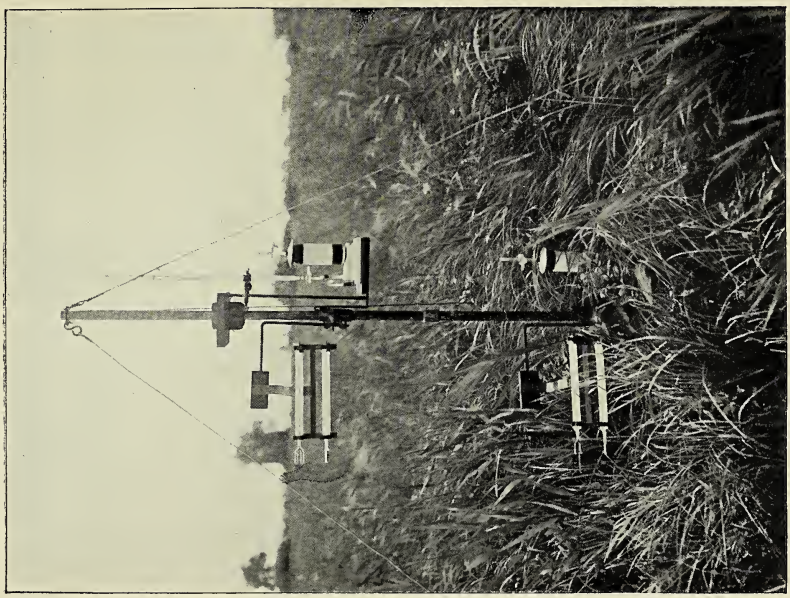

FIG. 2.

FIG. 1.

The Seedling Structure of Araucaria Bidwillii.

BY

F. J. F. SHAW, B.Sc. (Lond.), A.R.C.S.

With Plate XXI and six Diagrams in the Text.

THE seedling structure of Gymnosperms has till lately been somewhat neglected. In a recent paper, Hill (2) has described the transition phenomena in some species, chiefly Cupressineae and Taxaceae, and at an earlier date Seward (4) has given a partial account of the seedling anatomy of two species of Araucariae, the two species dealt with being *A. imbricata* and *A. Bidwillii*. The present paper is concerned only with *Araucaria Bidwillii*, which was not treated in detail by Seward and Ford. In view of the uniformity found to exist in seedling Gymnosperms, so far as at present investigated, the wide range of variation in the number of cotyledonary bundles and in the structure of the root lends considerable interest to this species.

The specimens were about 16 cm. long and 1·5 cm. broad at the widest part of the hypocotyl. In normal cases a seedling consists of a swollen or tuberous hypocotyl passing gradually into a thin root (Pl. XXI, Fig. 1); none of the specimens showed cotyledons. The whole of the swollen portion, from the point at which the cotyledonary tube joins the axis, to a level at which the main axis runs on without any essential change in diameter, is considered to be the hypocotyl. In Diagram IV, Fig. 13, Sections 3 to 8, corresponding to the Figures of same number in Diagrams I and II, are in the hypocotyl. The plumular shoot at the apex of the seedling is enclosed in a cotyledonary tube formed from the persistent fused bases of the cotyledons, the whole external surface is covered with cork.

Transition Phenomena.

For the elucidation of the course of the vascular bundles, series of transverse sections were cut by hand from the apex to the base of numerous seedlings. Longitudinal sections were taken from certain portions to check the results.

At the apex of the seedling the plumular bundles (*pl. b.*) form a ring which runs downwards as far as the point at which the cotyledonary tube joins

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the hypocotyl. At this level they spread outwards, allowing for the insertion of the numerous bundles (*c.b.*) from the cotyledons (Diagram I, Figs. 1, 2). The number of bundles (*c.b.*) entering the hypocotyl from the cotyledonary tube is subject to great variation. In the tube itself there are usually from 12–16 small collateral bundles. These bundles consist chiefly of secondary xylem (*x.*), the primary xylem (*p.x'.*) being practically restricted to the endarch protoxylem. As the bundles enter the hypocotyl, they become associated, sometimes in pairs, sometimes in threes, and these groups pass

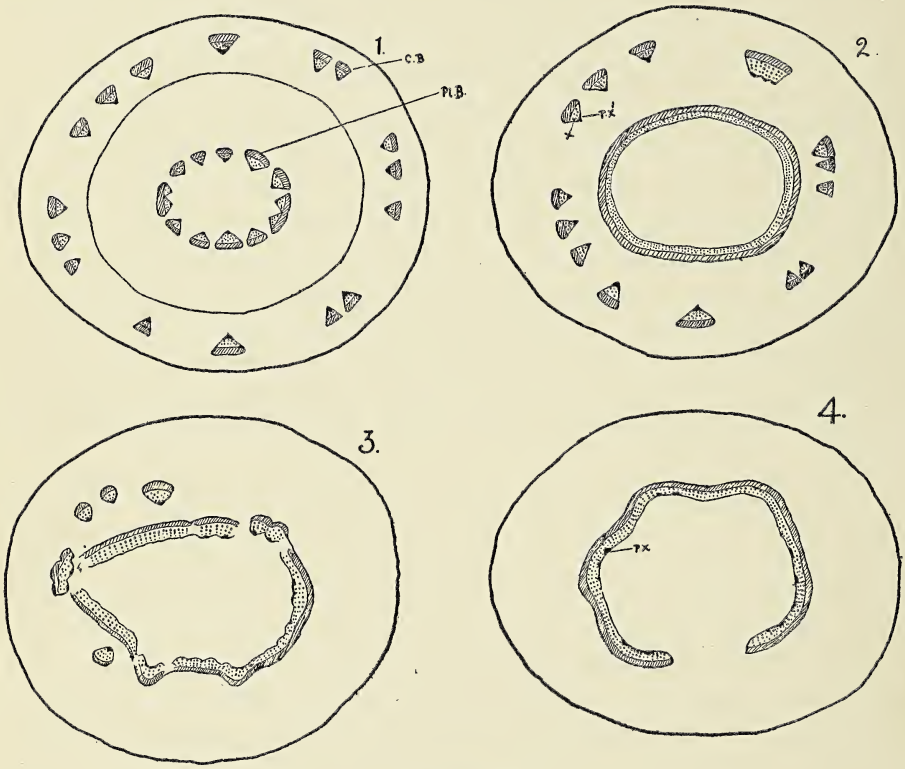

Diagram I.

inwards and fuse with the vascular tissue from the plumule (Diagram I, Fig. 3). The bundles derived from the cotyledons fuse with those from the shoot to form a continuous ring in which it is impossible to discern the limits of the vascular tissue from either source (Diagram I, Fig. 4). Lower down, this ring breaks up into from 5–7 curved segments (*s.*), with the concavity outwards, and each segment then divides into two bundles which divaricate and approach those derived from adjacent segments (Diagram II, Figs. 5, 6). In this way there arises in the upper portion of the hypocotyl a ring of from 5–7 pairs of vascular bundles, the two individuals of any one pair being inclined towards each other, and originally derived from different segments.

The above account only represents the course of the vascular bundles in a general sense; as a matter of fact, hardly any two seedlings seem to agree precisely in the manner in which the bundles in the hypocotyl are differentiated out of those from the cotyledons and plumule. Numerous specimens were investigated in the hope of establishing the relationships between the bundles appearing in the hypocotyl and those entering from the cotyledonary tube. As a general rule, owing to the complete fusion of cotyledonary and plumular strands, and the large amount of secondary thickening, it is

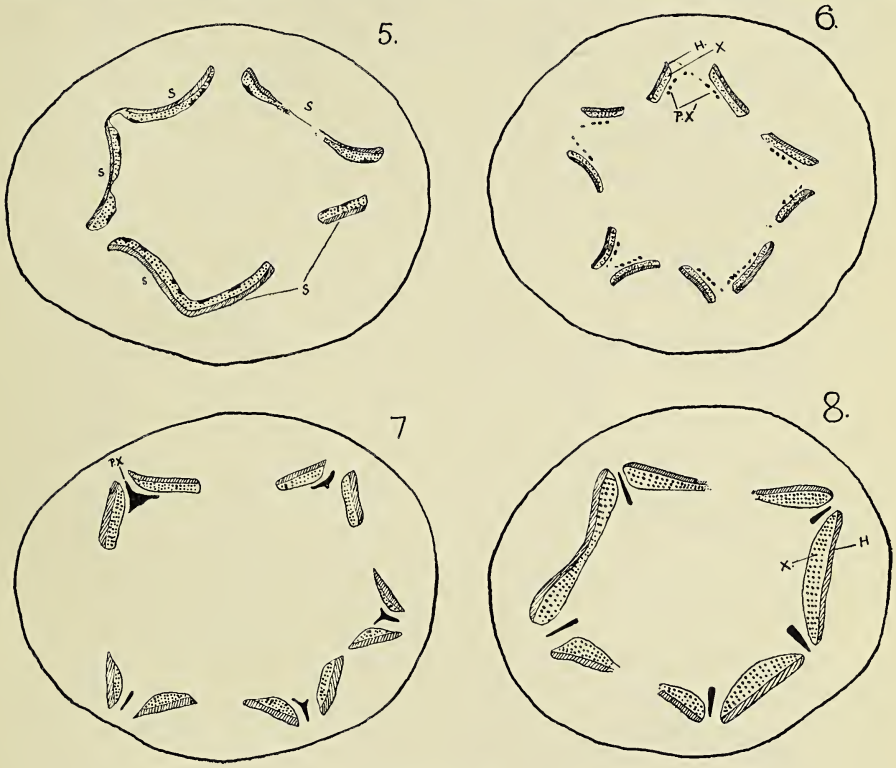

Diagram II.

impossible to trace any one bundle in the hypocotyl back to a particular bundle in the cotyledonary tube. In two specimens, however, the cotyledonary bundles seemed to preserve a certain amount of individuality throughout their fusion with the vascular tissue from the plumule; here, accordingly, it was possible to trace them into connexion with those in the hypocotyl.

In one of these seedlings (Diagram III, Fig. 1) fourteen bundles (*c.b.*) entered the hypocotyl from the cotyledonary tube; they were arranged in a ring of four groups of three bundles and one group of two bundles. These five groups of bundles fuse with the plumular strands, each group as it does

so forming an arc-shaped strand with the concavity inwards (Diagram III, Fig. 2) ; each of these strands subsequently splits into two bundles with their xylems obliquely facing each other (Diagram III, Fig. 3). Thus a ring of

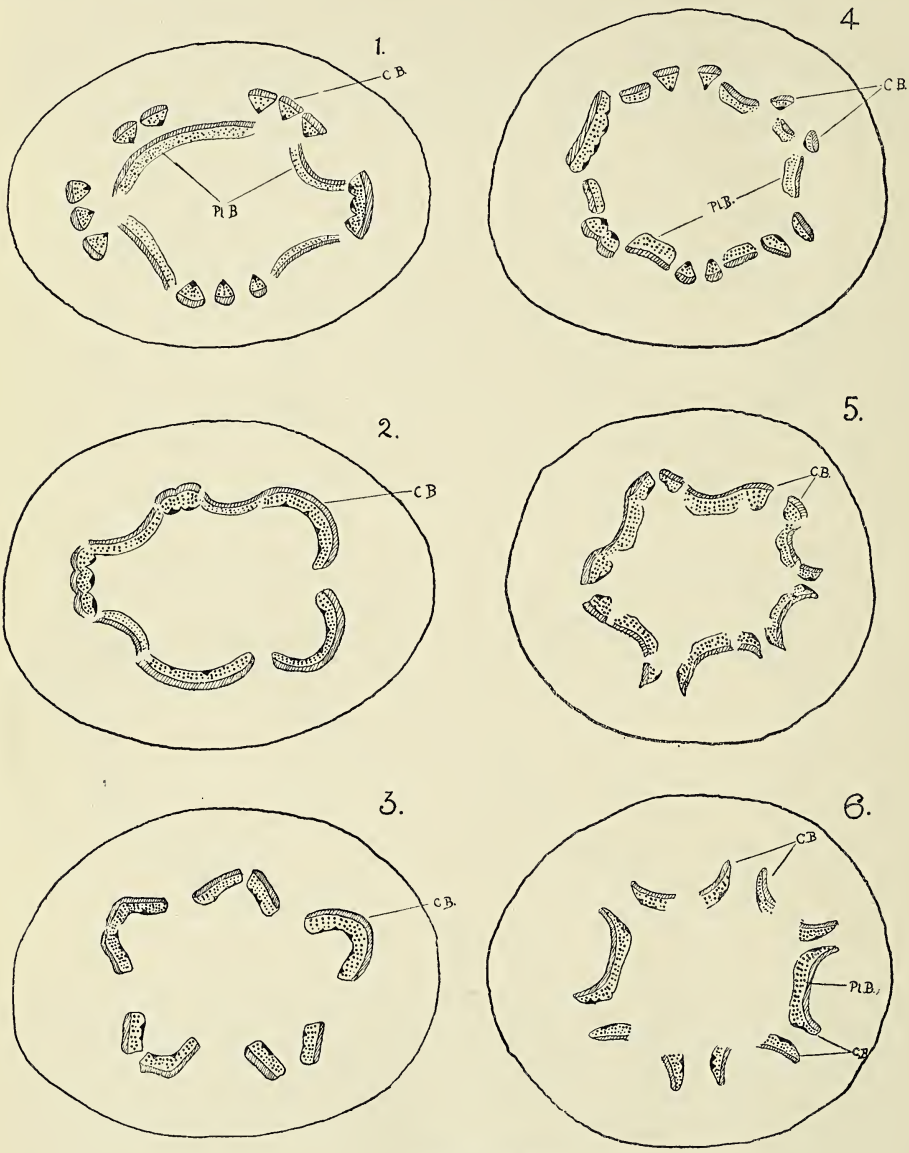

Diagram III.

five pairs of bundles is formed, as in the previous case, with this difference : that each pair of bundles is derived from the same segment and the segments themselves can be definitely traced back to groups of cotyledonary bundles.

In the other seedling, the bundles (*c.b.*) from the cotyledons entered the plumular ring in pairs in such a way that the individual bundles of a pair form the 'horns' of different segments, which, as in the first case, are curved with the concavity outwards (Diagram III, Figs. 4, 5). When, therefore, the segments divide to form the paired bundles in the hypocotyl, each pair is identical with a pair of cotyledonary bundles (Diagram III, Fig. 6).

Thus in both these cases there is evidence that each pair of bundles in the hypocotyl is identical either with a pair of bundles in the cotyledonary tube or with a group of bundles which fuse to form an arc-shaped strand.

The bundles in the hypocotyl pass down, remaining practically unchanged, through 2–3 cm. of the hypocotyl, which is extremely swollen and filled with starch. About the middle of the swollen hypocotyl a change takes place in each bundle. The primary xylem (*p.x'.*), which is practically identical with the protoxylem, begins to become separated from the secondary xylem (*x.*), and follows the course of the bundle at some little distance from the inner face of the latter (Diagram II, Fig. 6 ; Pl. XXI, Fig. 2). A longitudinal section shows the annular and reticulate elements of the protoxylem separated from the parent bundle by two or three layers of parenchyma. A little lower down, the primary xylems of any two bundles, forming a pair, can be seen curving towards a point midway between the respective bundles ; ultimately they fuse and form a single protoxylem group (Pl. XXI, Fig. 3). Thus at the base of the hypocotyl there are half as many separate and distinct primary xylem groups as there are bundles, each group being situated between the two bundles with which at a higher level it was associated (Diagram II, Fig. 7).

There are, therefore, five, six, or seven protoxylems, and these alternate with twice as many bundles consisting only of secondary xylem (*x.*) and phloem (*h.*), and thus the structure is essentially that of a pentarch, hexarch, or heptarch root, in which secondary thickening has already taken place. As the hypocotyl decreases in diameter, the separate bundles of secondary xylem and phloem fuse to form continuous tangential bands between the primary xylem groups (Diagram II, Fig. 8) ; it should be noticed that this union is between bundles originally belonging to separate pairs at a higher level. The numbers of seedlings showing heptarch, hexarch, or pentarch structure respectively, were about equal.

The further changes in the vascular system of the root will be described in a specimen which showed pentarch structure in the base of the hypocotyl, though the following remarks, *mutatis mutandis*, would apply equally to those with hexarch or heptarch structure.

As a rule, the pentarch structure persists for some distance, running through the rapidly narrowing hypocotyl and into the main root ; its extent

can be judged from Diagram IV, Fig. 13, where it runs from Section 7 to about Section 9. At length one side of the pentarch stele appears shorter than the others, owing to two adjacent protoxylem groups curving towards one another in a tangential plane (Diagram IV, Fig. 9). Then these xylems ultimately fuse, giving rise to a tetrarch root. In the process of fusion one protoxylem is commonly dominant, retaining its original size and not undergoing any marked change in position, whilst the other becomes considerably smaller and curves over to fuse with the more central portion of the larger

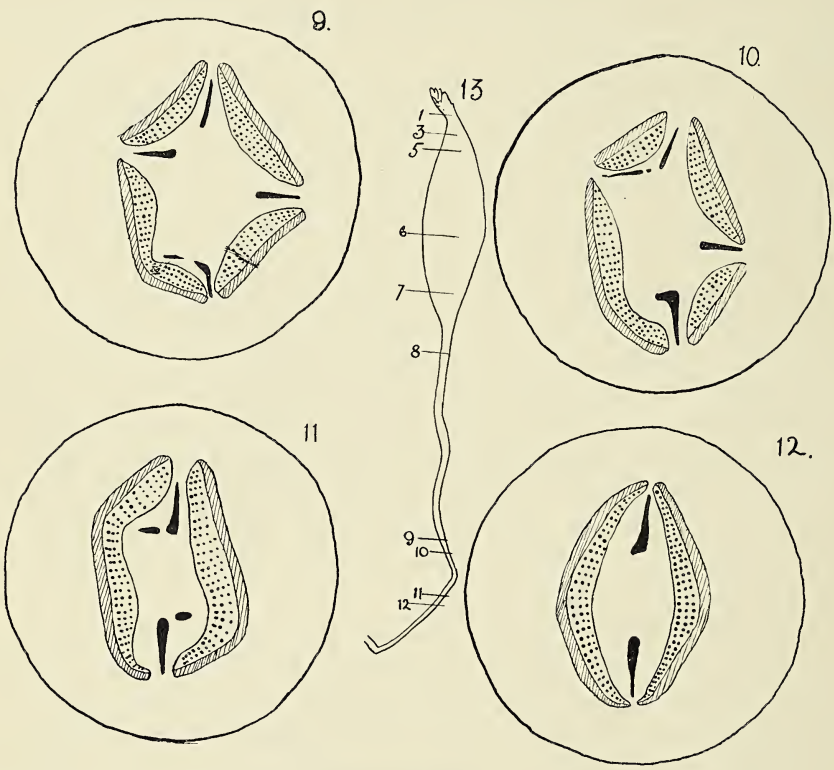

Diagram IV.

group (Pl. XXI, Fig. 4). A similar process now takes place on the opposite side of the root, reducing the structure to triarch, and this passes finally to diarch (Diagram IV, Figs. 10, 11, 12); the last two changes take place almost simultaneously. In this way the young tap-root is invariably diarch, and consists of a single primary xylem plate flanked by two masses of secondary xylem and phloem, the sole remnants of the former numerous bundles (Diagram IV, Fig. 12). Throughout the transition, the primary xylem is the only vascular tissue which has undergone any marked change in position, the primary xylem of each bundle in the hypocotyl having moved sideways

to fuse with that of an adjacent bundle. The secondary xylem and the phloem of the root is linearly continuous with the secondary xylem and phloem of the bundles in the hypocotyl.

The reduction of the root to the diarch type ultimately takes place whether the original structure was pentarch, hexarch, or heptarch. In some specimens, however, the youngest portions of the root still showed triarch structure, the diarch stage not having yet been reached. Had the plants been older the diarch appearance would doubtless have been shown.

Secondary roots are always diarch, and derive their primary vascular tissue from a single primary xylem group lying between two masses of secondary xylem. This was particularly clear in one specimen examined, in which the main tap-root had decayed away; the base of the hypocotyl, therefore, terminated in a blunt projection, on one side of which a thin adventitious root arose. This root was diarch; a transverse section of the hypocotyl, however, showed pentarch structure. On tracing the adventitious root into the hypocotyl, it was found that it arose from a single primary xylem group lying between two bundles; had each primary xylem group given rise to an adventitious root, there would have been five roots in a ring at the base of the withered tap-root.

An Anomalous Seedling.

One specimen in the material first attracted attention from its abnormal external appearance, a variation which was found to be accompanied by even stranger diversities in the vascular tissue. The seedling in question (Diagram IV, Fig. 9) had a longer, more tuberous hypocotyl than usual; at the base of the swollen portion of the hypocotyl the seedling took a sharp bend, running at right angles to its former course for some 3 cm.; it then became sharply constricted and continued as a very thin root.

The vascular structure of the seedling was normal down to a point (*p.*) about half way along the horizontal portion of the root, here hexarch in structure, derived from six pairs of bundles in the hypocotyl. A section taken at the constriction (*d.*) in the root, about 1·5 cm. lower down, showed diarch structure; the transition from hexarch to diarch in the intervening portion (*t.*) was marked by some curious abnormalities.

The hexarch stele passes quickly into pentarch in the normal way; during this process a series of cell-divisions takes place in the pith. The divisions occur tangentially in a zone forming a closed circle; they give rise to a circular cambium (*c.*) in the pith, and this cambium produces xylem externally and phloem internally. Thus a ring of vascular tissue with inverted orientation is formed inside the root (Diagram V, Fig. 1). While this is proceeding, changes take place in the five primary xylem groups; each splits up into several smaller groups which return to the positions which they formerly occupied in the hypocotyl. That is to say, they

curve away from one another and attach themselves to the inner side of the secondary xylem (Diagram V, Figs. 1 and 2). At this stage, therefore, the root contains an outer ring of vascular tissue with normal endarch protoxylem and an inner ring with inverted orientation.

A gap now appears in the outer ring, and its cambium becomes continuous with that of the inner round the edges of the gap, the inverted xylem also becoming continuous with the normal (Diagram V, Fig. 2). In this way a stele is formed consisting of a U-shaped mass of xylem

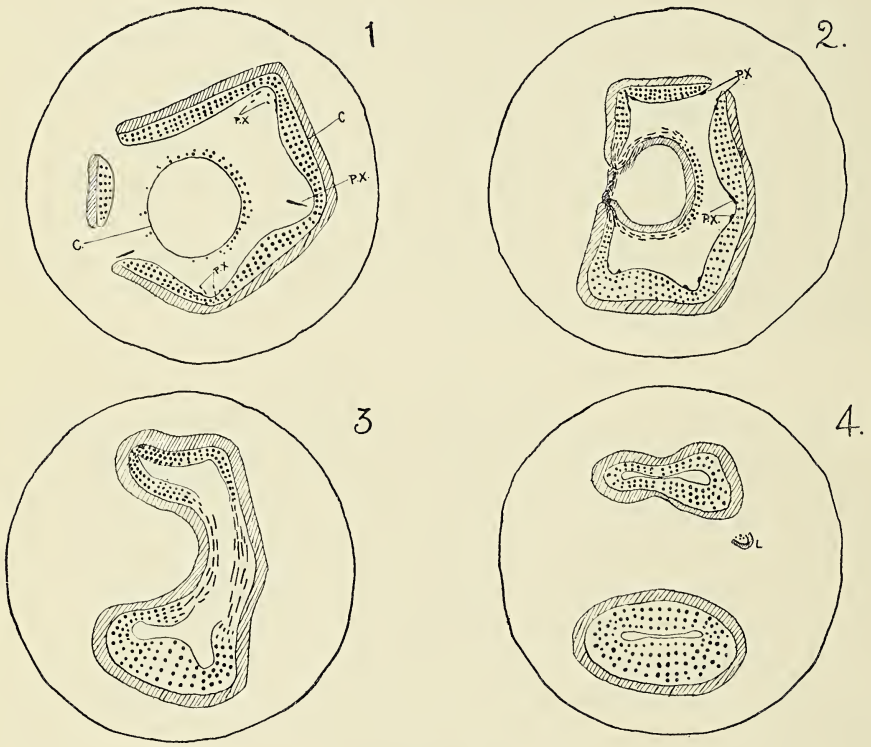

Diagram V.

enclosing a pith and surrounded by phloem (Diagram V, Fig. 3). Lower down in the root, the stele tends to straighten out, and it finally splits in a plane at right angles to its greatest breadth. The two segments immediately close up, each forming a single concentric stele, one of which gives off a small strand (*l.*) ending blindly in the parenchyma at a lower level (Diagram V, Fig. 4).

Both of the steles now divide in a plane at right angles to the first division, and thus give rise to four steles in the root (Diagram VI, Fig. 5). Of these four steles, one (*k.*) appears to die away at a lower level, while the other three go through a complicated series of changes to form the diarch

root. All three fuse, giving rise to a triangular mass of xylem and phloem enclosing a pith (Diagram VI, Fig. 6); from each angle of this triangle a branch is sent out laterally (Diagram VI, Fig. 7). The exact fate of these branches was not quite established; it is believed that they simply died out in the parenchyma, although the possibility is not excluded that they went to supply secondary roots. At all events, the remainder of the triangular mass rounds itself off to form a single concentric oblong stele. In the centre of this stele a little diarch xylem plate now appears; it seems to arise

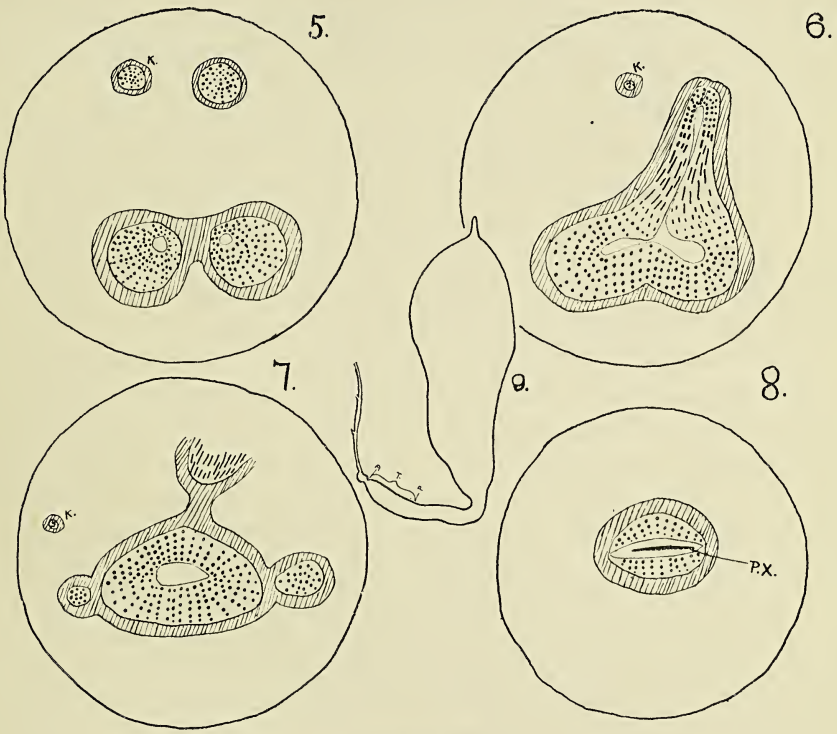

Diagram VI.

by the separation of the protoxylem from the inner surface of the secondary xylem of the stele (Diagram VI, Fig. 8). Throughout the whole of the seedling enormous resin ducts run longitudinally in the cortex, and at the point at which the root becomes diarch frequent anastomoses occur.

Conclusions.

The essential points of the above type of seedling structure seem to lie in the variation in the number of cotyledonary bundles entering the hypocotyl, in the number of protoxylem groups in the root, and in the reduction of the structure in the younger portions of the latter.

As mentioned above, it was not possible, as a general rule, to trace the relationship of the cotyledonary bundles to those appearing in the hypocotyl. This was probably due to the large amount of secondary thickening in all the specimens; had younger seedlings been available there is no doubt that the connexion, of which there were strong indications in two specimens (Diagram III), would have been substantiated. It will be remembered that in these two seedlings each pair of bundles in the hypocotyl was traced back, in the one case to a pair of bundles in the cotyledonary tube, in the other to a single arc-shaped strand which divided. The protoxylems of each pair then fuse, the secondary xylems and phloems divaricating to fuse with those from adjacent pairs. Ultimately diarch structure is obtained in the root.

Now this does not differ essentially from the type described by Hill and de Fraine in the Cupressineae. Here a single bundle enters the hypocotyl from each cotyledon and splits into a pair of bundles. The xylems of each pair then twist towards one another and fuse, with their protoxylems external; the phloems divaricate to fuse with those of adjacent pairs. Thus, if the seedling had three cotyledons, a triarch root should arise; if two cotyledons, a diarch root is formed.

Now *Araucaria Bidwillii* has two cotyledons and ultimately forms a diarch root, each pair of bundles in the hypocotyl behaving very much as a pair of bundles in the hypocotyl of a seedling *Cupressus*. If the remaining Araucarieae agree essentially with this type, then the transition phenomena in Coniferae will show a strong general uniformity. *Araucaria Bidwillii*, however, while agreeing with the general plan, shows interesting numerical variations—numerous pairs of bundles derived from the cotyledons enter the hypocotyl, and diarch structure is attained in the young taproot by reduction from an earlier (embryonic) stage, when numerous protoxylems are present. Thus, in a species remarkable for its swollen hypocotyl, there is in the root-structure at the base of the hypocotyl a large increase in the number of protoxylems, the primary root becoming diarch by reduction.

This at once suggests a comparison on anatomical grounds with the seedling of *Eranthis hyemalis*. In this plant there is a tetrarch structure in the tuberous hypocotyl at the base of the cotyledonary tube, this structure becoming reduced to diarch as the hypocotyl merges into the main root. As is well known, this tetrarch structure has been compared with that occurring in *Anemarrhena*, and it has been made the basis of wide phylogenetic speculation. It was, however, early suggested (Tansley, 5) that, since diarch structure prevailed almost exclusively in the hypocotyl of non-tuberous species of Ranunculaceae, the increase in number of xylem groups in the tuberous *Eranthis* should probably be correlated with the increased need for vascular tissue in such a structure rather than be regarded as an ancestral trait.

In *Araucaria Bidwillii* the same variation in internal structure is associated with a similar abnormality in the external morphology. In other words, numerous protoxylem groups prevail at the base of a tuberous hypocotyl. Moreover, the number of these groups shows considerable variability, seedlings with heptarch, hexarch, or pentarch structure being equally common. It was not possible to trace any relationship between the degree of tuberosity of the hypocotyl and the variation in the number of protoxylem groups in the root structure at its base, although this variability may be looked upon as evidence that the structure at this point is the result of the operation of biologic factors. In the Cupressineae an increase in the number of bundles in the hypocotyl is caused by an increase in number of cotyledons. Here, however, certain bundles behave as though coming from half-cotyledons, and there is no deviation from the normal character of the root structure. But the occurrence of heptarch, hexarch, or pentarch structure in *A. Bidwillii* obviously cannot be correlated with any splitting of cotyledons.

Some seedlings, in which the main tap-root was rather shorter than the average for the specimens, did not attain to diarch structure, remaining triarch, or even pentarch in the case of specimens beginning with heptarch structure at the base of the hypocotyl. It takes, therefore, some time for the seedling to obliterate the influence of its swollen hypocotyl and to reduce its structure to the normal. In very young seedlings, of course, the root would show no such reduction. This circumstance perhaps accounts for the statement by Borzi (1) that the root was always pentarch. Another tuberous species, *A. imbricata*, shows, as far as can be gathered from the existing description, an essential similarity to *A. Bidwillii*, although numerous protoxylems in the base of the hypocotyl do not seem to be so distinct as in the latter species.

Thus the relationship between the seedling structure of *A. Bidwillii* and the usual type shown by seedling Conifers seems to be exactly paralleled by that subsisting between *Eranthis* and the remaining Ranunculaceae. It is difficult to avoid the conclusion that the similar peculiarity in habit is directly connected with the corresponding abnormalities in structure. While in any one natural group of plants the transition phenomena may show a unity in the general method, yet the actual number of separate xylem groups in the root seems to depend upon the particular character of the individual. Any variation from the normal number of protoxylem groups in an aberrant species seems not likely to be of serious taxonomic value, but is more probably to be correlated with the habit of the seedling. The study of seedling anatomy from a biological standpoint probably offers a more fruitful subject for investigation than is likely to be afforded by the attempts made to found far-reaching conclusions upon the vagaries of seedling structure.

There yet remains to be considered the seedling which showed the anomalous structure. Two points stand out clearly in this—the occurrence of inverted vascular tissue, and the production of several steles. In another group of plants, the Cycads, Worsdell (6) considers that the presence of inverted vascular bundles is evidence of the derivation of these plants from polystelic ancestors, the inverted vascular bundles in modern Cycads being interpreted as the remnant of the interior portion of a formerly concentric stele whose peripheral segment forms part of the normal vascular ring. It is interesting to note that the polystelic stage in *Araucaria Bidwillii* is derived from a stage with inverted vascular tissue, this inverted vascular tissue forming the segment of each stele lying nearest the centre of the root. Thus the inverted vascular tissue gives rise to that part of each stele which it should do if Worsdell's theory of the Medullosean origin of Cycads is correct.

The value to be attached to this abnormal anatomy depends upon the value which we attach to seedling anatomy as a phylogenetic criterion, and not less on the still wider consideration as to the phylogenetic value of teratological phenomena in general.

As a rule it has been shown that deviations from the normal number of protoxylems and of cotyledonary bundles should not be made the basis of phylogenetic speculation; they are probably correlated with other, and more local, factors in the development of the seedling. The question remains how far the habit of the seedling can influence the development of its vascular tissue; that variable conditions can under normal circumstances influence the amount produced seems clear, but whether the more striking abnormalities in structure are due to the same cause is doubtful.

Worsdell postulates that of all the regions in a plant likely to show ancestral traits the cotyledonary or first node is one of the most favourable. He cites the case of *Encephalartos Barteri*, which showed three steles in the hypocotyl, a structure which he considers as recalling Medullosean ancestry. Are we to attach the same meaning to the polystely of *Araucaria Bidwillii*? The part played by the inverted vascular tissue in the formation of the stele certainly favours this view. On the other hand, is there any external feature in the seedling which distinguishes it from the normal type and would point to its having grown under different conditions? A reference to Diagram VI, Fig. 9, shows at once the peculiarity of the specimen which has already been described (p. 327). The polystelic zone (*t.*) occurs in the upper part of the root, which is here horizontal, the base of the hypocotyl having been sharply bent, and immediately precedes a sharp constriction beyond which the root is much thinner and of the normal diarch type. Whether this constriction necessitates diarch structure in the subsequent and thinner part of the root, and therefore the rapid production of this from a closely adjoining part with hexarch structure leads to the

complicated series of fusions and divisions described, is a matter on which it is hardly safe to express an opinion. The fact that several of the independent strands seem to end blindly in the parenchyma suggests that there are some definite factors which operate in the rapid reduction in amount of the vascular tissue before it reaches the constricted zone. In fact the phenomenon is perhaps to be attributed to the indirect effect of an injury, or to the action of some unfavourable conditions under which the seedling grew.

Summary.

(1) The transition phenomena in *Araucaria Bidwillii* follow in broad outline the general method already described for other Gymnosperms.

(2) The number of bundles entering the hypocotyl from the cotyledons is very numerous and variable.

(3) The number of protoxylem groups in the root structure at the base of the hypocotyl is also numerous and variable.

(4) There is a reduction in the number of protoxylem groups in the younger parts of the root, diarch structure being finally attained.

(5) The differences between the normal type of transition in Gymnosperms and that in *Araucaria Bidwillii* are probably due to the tuberous nature of the latter, and are to be compared with those between the normal type of transition in Ranunculaceae and that in *Eranthis*.

(6) A single specimen with abnormal external features showed aberrant internal anatomy producing numerous steles in the root.

In conclusion, I desire to express my thanks to Professor Farmer, F.R.S., for his constant help and valuable advice throughout the progress of the research.

Royal College of Science.
February, 1909.

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EXPLANATION OF FIGURES IN PLATE XXI.

Illustrating Mr. F. J. F. Shaw's paper on the Seedling Structure of *Araucaria Bidwillii.*

Abbreviations used: *c.* cotyledonary tube; *pl.* plumule; *p.x.* fused primary xylems; *p.x'.* primary xylems of bundles; *x.* secondary xylem; *h.* phloem.

Fig. 1. Seedling.

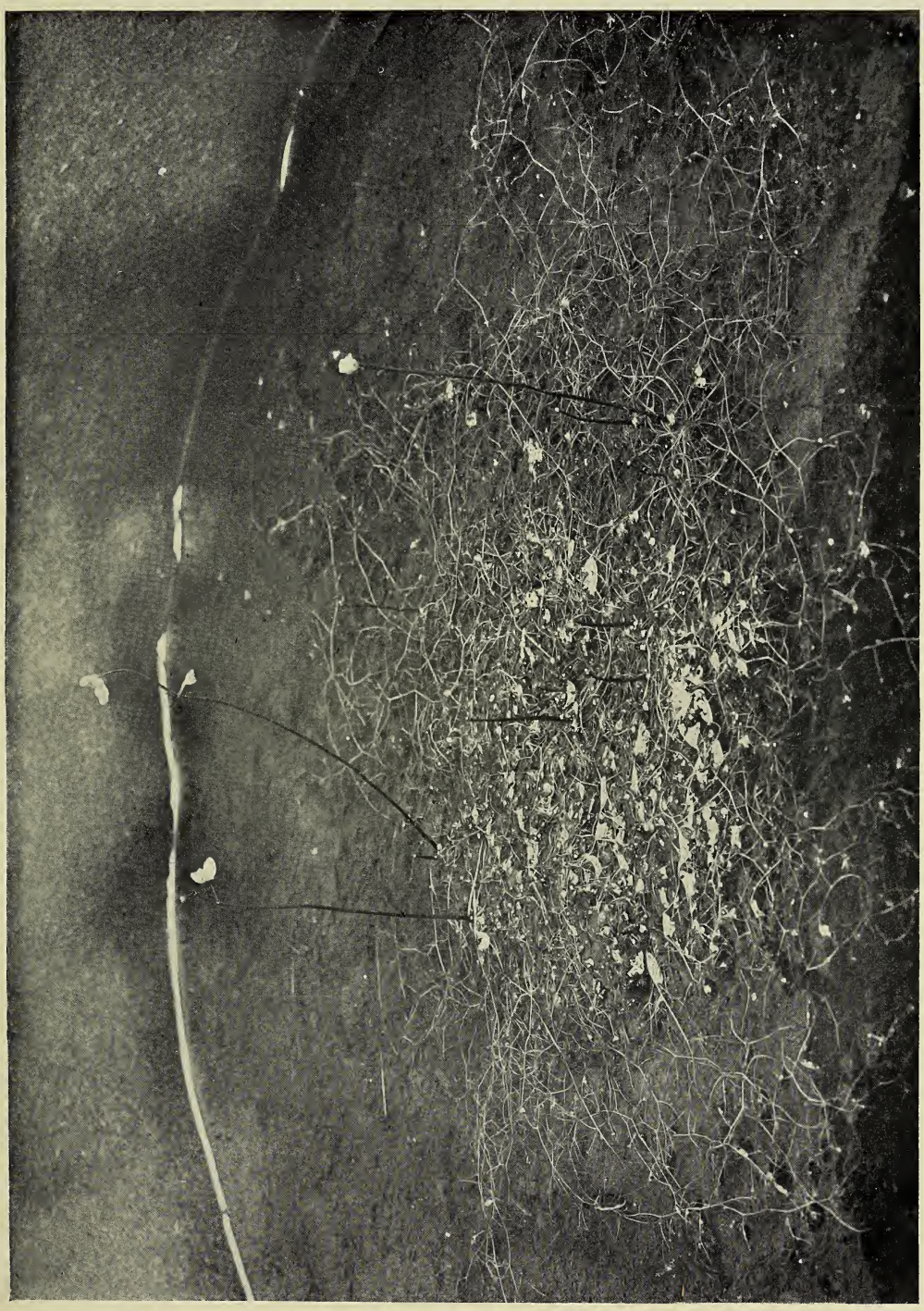
Fig. 2. Bundle in the hypocotyl showing separate primary xylem. × 100.

Fig. 3. Fusion of primary xylems of a pair of bundles in the hypocotyl. × 100.

Fig. 4. Fusion of two protoxylem groups in the root. × 100.

F.J.F.S. del. Huth. lith. et imp.

SHAW–SEEDLING OF ARAUCARIA BIDWILLII.



UTRICULARIA EMARGINATA, BENJ.

NOTES.

ON A NEW GENUS OF ASCOMYCETES.—'During the present summer a quantity of fungus material was sent to me for examination by the Lancashire County Council, with the information that it was causing blockage in certain drains in an estate near Preston. The material on examination was found to consist largely of mycelia of members of the Saprolegniaceae, especially *Saprolegnia androgynia* and *Leptomitus* (*Apodya*) *lacteus*. There were present, however, indications of another fungus which could not be identified with the means at my disposal. I submitted some of the material to Mr. Massee, of Kew, for his opinion. He finds that

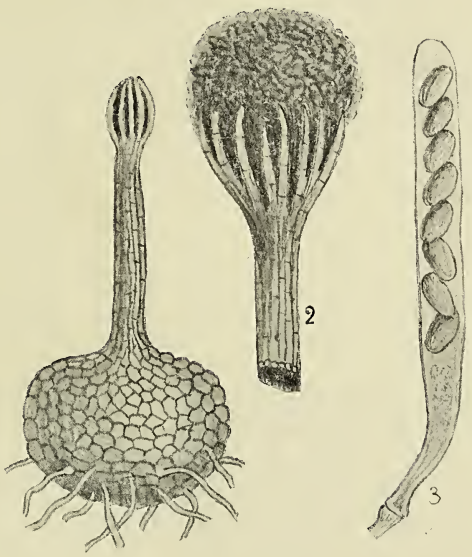


Fig. 1. Perithecium. × 60.
Fig. 2. Upper portion of beak of perithecium showing extruded mass of mucilage, containing spores. × 200.
Fig. 3. Ascus containing spores. × 400.

the unknown fungus is a genus and species new to Science. Mr. Massee has been so kind as to examine the structure of the fungus in detail, and has favoured me with the following diagnosis and description.

R. J. Harvey-Gibson.

University of Liverpool.'

GIBSONIA, Mass. (nov. gen.).

'Perithecia subglobosa, superficialia, membranacea, olivacea, in rostrum longum cylindraceum apice fimbriata attenuata; ascis evanescentibus, octosporis; sporis continuis, brunneis, ellipsoideis, demum in massa mucilaginosa ex ore rostri eiectis.'

This genus is almost the exact parallel of the genus *Spumatoria*, Mass. and Salm., in the Hyalosporae. Its characteristic features are the very long neck of the perithecium and the continuous, dark-coloured spores. The asci deliquesce into a mucilaginous mass in the perithecium immediately the spores are mature. This mucilage is highly hygroscopic and absorbs water through the wall of the perithecium. The tension thus caused forces the mucilage, in which the spores are imbedded, up the canal of the neck or beak of the perithecium, where it forms a globular dark-coloured mass, supported by the long hyphae terminating the beak, which spread out like the ribs of an umbrella. The mass of mucilage and its contained spores are finally dispersed by water.

GIBSONIA PHAEOSPORA, Mass. (sp. nov.).

'Peritheciis sparsis, circa 1 mm. altis, subglobosis, olivaceis, contextu parenchymatico e cellulis polygonis composito, glabris, basi hyphis repentibus instructis, in rostrum longissimum cylindricum abrupte attenuatis; ascis cylindraceis; sporis monostichis, ellipsoideis, brunneis, continuis, 14–15 × 7–8 μ.'

Found sparingly on a decomposing mass of Saprolegniae, &c., in a drain in North Lancashire.

GEORGE MASSEE.

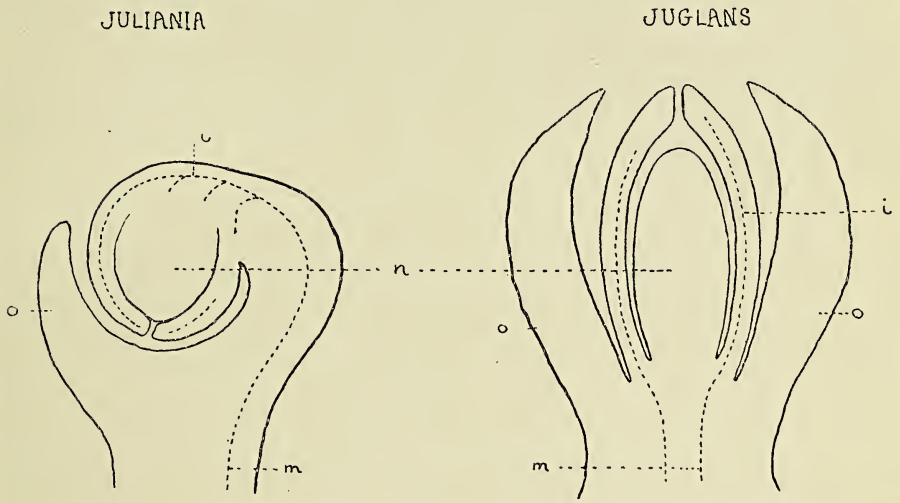
Kew.

NOTE ON THE RELATIONSHIP OF THE JULIANIACEAE.—In his description of the ovules of the Julianiaceae Mr. Boodle[1] mentions certain peculiarities of structure, on which, however, Mr. Botting-Hemsley has not laid stress in his discussion of the relationship of this new order to the Juglandaceae and other allied families. It seems to me that a good deal of support for Mr. Hemsley's views might be obtained from a more detailed comparison of the ovule of *Juliania* with that of *Juglans* as described by Nicoloff.[2] In the first place the ovules of both *Juliania* and *Juglans* contain a vascular supply in the integument, a feature not common in Angiosperms though present in some of the older Gymnosperm seeds, e. g. *Trigonocarpus* and *Polylophospermum*. A similar vascular supply occurs in the integument of *Myrica Gale*, a full account of which I hope to publish shortly. The presence of this vascular supply in *Juglans* and *Myrica*, and in some of the fossil seeds should, I think, be correlated with the fact that the integument is quite free from the nucellus

[1] Botting-Hemsley ('06): On the Julianiaceae: A new natural Order of Plants. Phil. Trans. Roy. Soc., vol. cxcix.

[2] Nicoloff: 'Sur le Type floral et le Développement du Fruit des Juglandées.' Journal de Botanique, tom. xxviii–xxix.

in these forms. In the anatropous ovules of other Amentiferae, where the integument is closely united with the nucellus, the vascular supply may have been lost, while in the hemi-anatropous ovule of *Juliania* it has still been maintained. Another feature which *Juliania* has in common with *Juglans* is the curious outgrowth at the base of the ovule—the obturator. In *Juglans*, where the ovule is orthotropous, there are two such outgrowths (see Nicoloff's[2] Figs. 24 and 25), and it is obvious that in becoming hemi-anatropous there might be a tendency in *Juliania* for one of them to disappear. Whatever their morphology or their physiological or biological significance may be,



TEXT-FIG. Diagrams of young ovules of *Juliania* and *Juglans*, reconstructed from Boodle's and Nicoloff's figures respectively. The obturator *o*, single in the case of *Juliania*, is seen to be paired in *Juglans*. *i* = integumentary vascular supply; *n* = nucellus; *m* = main supply bundle to ovule.

it seems to me that their occurrence, paired in the case of *Juglans* and single in the hemi-anatropous ovule of *Juliania*, strongly supports the close affinity of these two plants. (See the Diagrams above.)

The differences between the ovules of the two plants are explicable by the change in the direction of the ovule, while the characters they have in common are striking, and one of them, the possession of a vascular supply, is undoubtedly of considerable phylogenetic importance.

On the other hand it should be mentioned in support of the affinities of the Julianiaceae with the Anacardiaceae, which seem so strongly indicated by the similarity of their anatomical structure, that the ovule of *Mangifera* also possesses a vascular supply in its single integument, though there is no clear indication of the obturator.

E. M. KERSHAW.

MANCHESTER UNIVERSITY.

BRANCHING PALMS.—In the Annals of Botany, vol. xxi, p. 415, I gave an account of the branching of palms as far as I had observed variations of this character; since then I have come across three more instances which seem to me to be worthy of record.

Two of these are remarkable instances of branching in the two cultivated species of *Metroxylon*, viz. *Metroxylon Sagus*, Rottb. and *M. Rumphii*, Mart.

The Sago palms, as is well known, are rhizomatous palms possessing a stout branching stem which creeps half buried in the damp soil and sends up from its leaf-axils huge, lofty, erect branches which are eventually terminated by the flower panicle.

In clearing a thick patch of scrub in the Singapore Gardens, in which about twenty-five years ago a quantity of both kinds of Sago had been planted, the remarkable abnormalities, one of *M. Sagus* and the other of *M. Rumphii* growing at a few feet distant from each other, were found. In both the abnormality was almost identical, the only difference being that one had developed a little more than the other, so that one description will serve for both.

From the ordinary rhizome is growing an erect stem, now about two feet round and six feet tall; at this height the stem bears a clump of four shoots, one of which is large and grows at an angle with the top of the stem, and the three smaller shoots surround it. Below this clump is a mass of roots about a foot and a half long, so that the whole has the appearance of a rhizome with the ordinary shoots, placed on the top of a bare cylindric stem. This is *M. Sagus*. The other plant (*M. Rumphii*) is very similar except that the stem is stouter and the clump at the top is not bent at an angle, but stands exactly on the top of the bare stem. The main shoot is larger and there are four other side-shoots. The mass of aerial roots, too, is larger. The abnormality is probably due to some accident, such as the falling of a tree on the top of the two erect stems, and the big shoots probably represent the original stem shoots, which have taken on the form of a rhizomatous portion, and thrown up lateral shoots and emitted roots after the manner of a rhizome.

I have never before seen an erect stem of the Sago palms showing any signs of branching whatever, or emitting buds, and think that this is worth recording.

Korthalsia ferox, Becc. A remarkable branched stem of this rattan was brought from the Rantau Panjang Reserve in Selangor to the Agricultural Exhibition in Kuala Lumpur in 1908. The base of the stem for about six feet from where it is cut off is quite normal, but at that height is emitted a lateral shoot incomplete, and about eight inches further, a long one three feet in length. This shoot is extruded through the sheaf of a leaf, which is split three inches above the mode. It is half an inch thick, and at the base, where it is extruded, are three short annular sheaths. The branch consists of eight internodes, and produces no other branches. Above the point at which this branch is produced the main stem continues, and above the next node is emitted another branch two feet long (the complete top is missing). This is similar in form and in the basal sheaths to the preceding one, but this branch emits two others. The lowest appears above the second internode; above the next internode appears another branch, which again emits another branch thirteen inches long. The branches are alternate, not quite regularly arranged, but approximately a quarter of the circle apart. The branching of rattans to a certain extent has been mentioned in the paper

above quoted, but in this case the branching is very much more extensive. It does not appear that these ramifications are due to any such accident as the trampling on the branch by animals, as there are no signs of rooting at any of the internodes, and indeed the branching is too extensive to render this a probable cause.

The proliferous *Calamus* described and figured in the previous paper from Matang in Borneo, p. 421, Fig. 39, proves to be *Calamus pygmaeus*, Becc.

Daemonorops longipes, Mart. A plant in the Botanic Gardens has emitted a number of the long stems which lie upon the ground, the tips ascending. In one of these, which has probably been bruised or injured somehow, three shoots have been produced below the terminal one. This is somewhat similar to the production of buds on the rattan of *Plectocomia* previously mentioned, and will probably not develop much further. They are quite young as yet. The branching of the rattans of *Daemonorops* has not been observed before.

HENRY N. RIDLEY.

Botanic Garden, Singapore.

UTRICULARIA EMARGINATA, BENJ.—Although much has been written in recent years upon the structure and development of species of *Utricularia*, there is still much to learn regarding them, and the interpretation of the homologies of the parts in these aquatic heterotrophic plants cannot be said to be yet determined beyond question; indeed, an approach to such determination will only be possible after many more species have been thoroughly studied. As a contribution to our knowledge of the genus I give the following preliminary note upon *Utricularia emarginata*, a Mexican species first described by Benjamin,[1] which is now flourishing in the Royal Botanic Garden, Edinburgh, and which, so far as I know, has not been examined carefully before now. It was introduced to Edinburgh in 1906 by means of seeds obtained from a specimen in Pringle's Mexican collections of 1904. A free-growing plant, as shown in Plate XXII, reproduced from a photograph by Mr. R. Adam, in a moderately warm temperature it flowers and seeds abundantly almost throughout the year, and these characters, along with its ready propagation from seed, make it a peculiarly favourable subject for study in the laboratory of the germination and subsequent history of this type of plant. In fact it may well become a useful plant for teaching purposes.

Mr. Laurence Stewart, foreman in the Glass Department of the Royal Botanic Garden, gives the following account of his experience in the cultivation of the plant:—

'Seeds should be sown in a shallow pan, having a thin layer of mud at the bottom, with enough water to cover the mud. Germination begins in the sixth week.

'The plant grows submerged. Experiment has shown that the best method of cultivation is to keep the plant in partial shade in still water. When plants are cultivated thus the water soon becomes alive with micro-crustacea, which when they enter the bladders are absorbed by the plant as food. Once the water is infested with the micro-crustacea, the plant grows quickly and requires room; it will cover an area of a square yard in a short time.

[1] Linnaea, xx (1847).

'Mr. W. Evans, F.R.S.E., has identified the micro-crustacea which were collected in the water in which this plant is growing, as *Pionocypris vidua*—(Ostracoda), *Chydorus sphaericus*—(Cladocera), *Cyclops viridis*—(Copepoda) and *Cyclops serratulus*—(Copepoda).

'Plants from seed which germinated in 1906 began to show signs of flowering in March, 1907, and the first flower opened on April 27. The inflorescences were from six to seven inches in length, each bearing two to three small yellow flowers.

'After fertilization the inflorescence bends down to the water, the peduncles of the fertilized flowers twist and enable the ovary to be submerged. Each flower in turn, after being fertilized, bends down in the same way until all the flowers of the inflorescence are submerged. The seed soon ripens and can be seen through the transparent capsule. The placenta then absorbing water begins to swell and becomes mucilaginous; it bursts the capsule and allows the seed to float out into the water, when it is then ready for germination.'

My own study of the germination has shown me that the oval discoid protocorm (which has a pale green colour) of *Utricularia emarginata* produces at one end a couple of subulate outgrowths which rapidly develop and reach a length ultimately of twice that of the body of the protocorm. Between these outgrowths two watershoots are formed in succession; one of them grows more quickly than the other, and becomes the chief watershoot having circinate ptyxis, showing successively forked branchings, and bearing linear lateral appendages ('leaves' of authors). The first bladder arises on this shoot in a superaxillary position to the first formed of these appendages. The younger shoot also elongates, though much more slowly, and in its turn branches and produces lateral appendages and bladders. Glück,[1] who has himself made no observations upon the germination, arranges the species of *Utricularia*, of which the features of germination are known, in three groups :—

1. That of *U. vulgaris*, *U. oligosperma*, *U. reniformis* :—With many primary *leaves*.

2. That of *U. exoleta* :—With two primary *leaves*.

3. That of *U. bifida*, *U. lateriflora*, *U. montana* :—Without primary *leaves*.

Whether these are definite types and cover all the forms is a matter for future investigation to decide. *Utricularia emarginata* apparently falls into the second of Glück's categories.

My paper dealing in detail with the life-history of *Utricularia emarginata* will appear soon, and in it will be given illustrations of the various stages of development, as well as the results of experiments in cultivation. This note may call attention meanwhile to this easily-grown plant as an object for demonstrations. In the laboratory, seedlings grow vigorously in a jar of water stood on top of a jacketted embedding oven.

BERTHA CHANDLER.

EDINBURGH.

[1] Glück, Biologische u. morphologische Untersuchungen über Wasser- und Sumpfgewächse. Teil II, Jena, 1906.

NOTE ON THE NUCLEI OF SOME UNISEXUAL PLANTS.—In 1907–8, at the suggestion of Mr. Gregory, I examined the nuclei of some unisexual plants with the object of studying any differences which might be detected between the constituents of the nucleus in the two sexes.

Hydrocharis Morsus-ranae, *Bryonia dioica*, *Lychnis dioica*, *Mercurialis perennis*, *Sagittaria montevidensis*, and *Cucurbita Pepo* were investigated. The nuclei of the male and female plants were in all cases apparently identical, in both the number and the characters of their chromosomes.

Some further points of interest seem, however, worth recording.

1. A paired arrangement of the constituents was obvious in the somatic nuclei of both sexes of all these plants. In *Hydrocharis* and *Bryonia* very convincing examples of double reticula were seen, while in *Lychnis* and *Sagittaria* the fully formed chromosomes were seen lying side by side in pairs. These observations extend the account already given of a double structure in the somatic nuclei of *Funkia ovata* and *F. Sieboldiana*.[1] It is also worthy of note that while the material of *Funkia* was all preserved by one method, several different methods were used in the fixing of the above material.

2. In *Lychnis* an irregular number of paired aggregations were found in the prophases of the heterotype division of the pollen mother-cells. In the early stages of synapsis there are usually six pairs of these aggregations, the number then according with that of the somatic chromosomes (twelve).

3. In the rudimentary ovule of the male flower of *Sagittaria*, one of the hypodermal cells becomes differentiated from the rest, and its nucleus goes through all the stages of a normal heterotypic division. It develops no further, but later becomes surrounded by a thick wall.

4. In *Cucurbita* two cells were seen in the same nucellus with nuclei in process of heterotypic division.

5. In *Lychnis* the embryo sac shows no trace of polarity up to the stage at which it contains four nuclei. The final arrangement is quite normal.

M. G. SYKES.

Botany School, Cambridge. 1909.

[1] Sykes, M. G., 'Nuclear division in *Funkia*': Archiv für Zellforschung, B. i, 1908, pp. 392–3, and 'Note on the number of somatic chromosomes in *Funkia*,' ibid., p. 526. Double structures in the somatic nuclei have since been recorded by Overton in *Thalictrum* and *Calycanthus*. Overton, J. B. 'On the organization of the nuclei in the pollen mother-cells of certain plants, with especial reference to the permanence of the chromosomes': Annals of Botany, Jan. 1909, p. 19.

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On the Prophases of the Heterotypic Mitosis in the Embryo-sac Mother-cell of Lilium.

BY

DAVID M. MOTTIER,

Professor of Botany in Indiana University.

With Plate XXIII.

IN a recent paper, dealing with the development of the heterotypic chromosomes in the pollen mother-cells of *Lilium* and in certain other plants, attention was directed to the fact that the chromatin in the stage just preceding the synaptic contraction appeared as larger and coarser lumps in some cells of the same loculus than in others. On the one hand the chromatin was found to be distributed upon the linin reticulum in the form of relatively large lumps (Mottier, '07, Figs. 17, 22), in which condition the nucleus passed into synapsis; and on the other hand, a readily distinguishable and distinct thread with small and uniform chromatin granules represented the contracting mass (l. c., Figs. 19, 20, 21). It was suggested as a probable explanation that the action of the fixing fluid might have been in a measure responsible for this behaviour of the chromatin, and it was with the view of determining more definitely the effect of the reagents, as well as ascertaining more fully the early development of the heterotypic spirem in the megaspore mother-cell of *Lilium* as compared with that in the pollen mother-cell, that I undertook this investigation. With these two objects chiefly in view, a careful study of the prophases of the heterotypic mitosis was made in the developing megaspore mother-cell of *Lilium*, *L. Martagon*, and *L. candidum* being used.

Methods.

The same methods of fixing and staining were used as in the study referred to in the preceding. However, before the ovaries were placed in the killing fluid (chrom-osmic acetic acid of the writer's formula), the walls of the ovaries were carefully cut away with the razor on the sides opposite the loculi, so that in some cases only a very thin layer of tissue was left to enclose the cavity; in others the wall was cut away, laying

[Annals of Botany, Vol. XXIII. No. XCI. July, 1909.]

bare the ovules to the direct contact with the fixing fluid, while in still others, not only were the ovules exposed but a part of their chalazal ends were cut away, as will be seen, for example, from Figs. 1*a* and 2*a*. From this method of preparation, it will be seen that in case the ovule is exposed, or if a portion be cut away from the chalazal region, the fixing fluid will more readily penetrate the large megaspore mother-cell. Long experience with the tissues of higher plants in the use of the three-acid mixture, as well as of other fixing reagents, has shown that cells thus treated present slightly different appearances in certain structural details of cytoplasm and nucleus, and in the readiness with which stains are retained by these structures. If the fixing fluid must first pass through a layer of tissue of considerable thickness before reaching a given cell, the effect of the osmic acid upon that cell, as shown by structure and staining qualities, will be somewhat different than had the fluid a more direct contact with that cell. Experience has demonstrated also that, whenever cells to be studied are embedded any considerable depth in a tissue, there is likewise danger in cutting away too much, assuming that one operates with a fixed strength of the killing fluid. It is always to be understood that when cells show poor or faulty fixation, such preparations are not used as evidence in support of a conclusion. What is good, and what constitutes poor or faulty fixation, are matters to be determined by experience. Practice must also determine how material is to be prepared for any given reagent of known or unknown merit as tested for a similar tissue.

In order that any effect of preconceived ideas of the author may be eliminated in the preparation of the illustrations, he has had the drawings of the nuclei made by an artist who has been thoroughly trained in microscopic observation, and a careful comparison of the preparations and the respective drawings shows that each illustration is a very faithful representation of the object. In this connexion it gives me pleasure to acknowledge my indebtedness to Miss Carolyn A. Black for making the drawings of the nuclei.

Observations.

With the foregoing remarks on methods, we are now ready to present the results of observations. As mentioned in a foregoing paragraph, *Lilium Martagon* and *L. candidum* furnished the material for study. Even before the inner integument of the ovule is apparent, the megaspore mother-cell is conspicuous because of its large size and the density of its contents.

Both cell and nucleus now begin their period of rapid growth. At first the rate of growth of cell and nucleus seems to be equal. When the inner integument has begun to grow up about the nucellus (Pl. XXIII,

Fig. 1), the nucleus contains one to three large nucleoli and sometimes several additional smaller ones. The linin is disposed in a very delicate network, with many delicate threads extending radially from the larger nucleoli. The chromatin is distributed upon the linin threads in small granules. Here and there are to be seen larger aggregations of chromatin granules at the angles of the linin meshes (Fig. 1). This is the structure of the typical resting nucleus.

Each figure of the nucleus is accompanied by an outline drawing of the ovule in which the nucleus is found. Certain stages in the development of the ovule correspond rather closely with certain steps in the prophase of mitosis, so that the form of the ovule is helpful in enabling one to determine the correct stage in karyokinesis, especially when there is some variation in the form of the chromatin, due to the reagents or other causes. As will be seen from Fig. 1*a*, the ovule from which Fig. 1 was taken was not only exposed to the direct contact of the fixing fluid, but a little of the ovule itself (only the epidermis in this case) was cut away along the line *x*–*x*. In this and in neighbouring ovules in the same section, the beginning of the outer integument was just perceptible as a slight bulging out of a few epidermal cells.

In Fig. 2 is shown a nucleus which, judging from the stage of development of the ovule to which it belonged, and from the size and form of the cell, seems to represent the same stage in mitosis as Fig. 1. One very large and two or three small nucleoli are present. The chromatin, however, presents a striking contrast from that of Fig. 1 or of a later stage. Here we have rather large angular lumps of chromatin and a sparse linin reticulum, a condition which, it seems to the writer, may be due to the action of the fixing fluid. In this case the wall of the ovary was only partly cut away, so that the fixing fluid must first pass through a layer of tissue of six or seven cells in thickness before reaching the ovule. In nearly all preparations of this kind, the nucleus reveals a similar disposition of the chromatin, and the writer is of the opinion that the lumpy nature of the chromatin in this nucleus is due partly, if not wholly, to the action of the fixing fluid. Fig. 2*a* is an outline of the section of the ovule containing Fig. 2. Owing to the shape of the ovarian cavities, it sometimes happens that the nucellus of the developing ovules is pressed against the inner wall of the loculus, especially in the ends of the ovary, thereby becoming somewhat truncated as in Fig. 2*a*. In other parts of the loculus the nucellus has more room to elongate and round out at this stage in the development (Fig. 3*a*). With further growth the nucleus increases in size, and the chromatin granules (not the lumps of Fig. 2) not only become larger, but they are more regularly distributed upon the linin framework, whose meshes are now larger (Fig. 3). The change in the nuclear net from that of Fig. 1 to Fig. 3 foreshadows the transformation into a con-

tinuous thread, which is to appear later. In fact one sees now, in Fig. 3, the beginning of such a pre-synaptic thread, although there is not yet a continuous spirem. At this stage one large nucleolus is usually present, with sometimes one or more small ones. However, there may be considerable variation in regard to the number of smaller nucleoli. In preparing the ovary from which Fig. 3 was taken, not only was the outer wall of the loculus but a considerable slice of the chalazal end of the ovule cut away (Fig. 3*a*), so that the killing fluid undoubtedly had a more direct access to the megaspore mother-cell than in the case of Fig. 2. In this ovule the outer integument had just become evident. Both cell and nucleus are elliptical in longitudinal section. Following close upon the stage of Fig. 3 is the beginning of the synaptic contraction (Figs. 4, 5). Sections of the ovules (Figs. 4*a*, 5*a*) to which these nuclei belong show that the stage of mitosis is only slightly in advance of that in Fig. 3. The nucleolus has the form and position frequently seen in this stage of synapsis. That there is a single thread with a single row of chromatin granules cannot be questioned, and that there is no union side by side of two spirems is equally evident. Figures 4 and 5 represent rather thick sections of the nuclei, but they do not include the whole of their respective nuclei. In Fig. 5 the contraction of the thread has progressed further than in Fig. 4. The spirem seems thicker and more definite, whilst the chromomeres or granules are perceptibly larger. In the case of these two ovules, the wall of the ovary was cut away, giving the fixing fluid direct contact with the ovules from the instant the ovaries were thrown into the reagent. In Fig. 6 is shown the compactly balled-up synaptic mass, the stage so frequently observed, and the one almost invariably figured in the literature dealing with the heterotypic mitosis. Here the mass is so dense that little in detail can be made out. It seems, however, that the chromatin granules in this nucleus were much larger and less uniform in size than would result from a compact balling-up of the structure shown in Fig. 5. In preparing the ovary from which Fig. 6 was obtained, the outer wall of the loculus was not entirely cut away; a layer of tissue five cells in thickness remained to be penetrated by the fixing fluid before it reached the ovule. To what extent the fixing fluid was responsible for the denseness of the synaptic mass the writer is unable to say, but that the size and form of the chromatin masses observable in the contracted ball are due in some measure to the reagents is highly probable. The author called attention to precisely similar conditions observed in the pollen mother-cell of *Lilium* (l. c., Figs. 19, 20, 21, 22, and 23). Whether the chromatin thread presented by Fig. 5 normally contracts into a mass as dense as that in Fig. 6 cannot be stated with certainty, but subsequent events indicate the possibility that it may not always do so. The nature of the chromatin thread fol-

lowing close upon synapsis is shown in Fig. 7. The spirem has not become completely distributed within the nuclear cavity. It is, moreover, shorter and thicker. A longitudinal fission cannot be made out, although here and there a few chromomeres are seen to be double. The developmental stage of the ovule (Fig. 7*a*) shows that we have a stage in mitosis only a little later than Figs. 5 and 6, since the inner integument has just attained the height of the nucellus. Fig. 8 is a stage slightly older than Fig. 7. In a number of places the longitudinal fission of the chromomeres can be seen, and the whole thread is rapidly approaching the stage of the hollow spirem. The nucleolus, which lies in a neighbouring section, presents the same appearance as in the preceding figure.

The somewhat closely entangled spirem of Fig. 8 soon passes over into the typical hollow spirem. At this stage there enters a phenomenon which has generally led to confusion in the interpretation of subsequent events. Generally speaking, the spirem in this stage in both micro- and megaspore mother-cells is a rather uniform cord more or less regularly and loosely disposed in the nuclear cavity. It may, or may not, reveal the presence of a longitudinal split. Occasionally this longitudinal fission is seen here and there in the cord, where the halves show a tendency to spread apart (Fig. 9), but more frequently no evidence of a fission is apparent. In Fig. 9 only a portion of the chromatin cord is shown. Comparing the ovule containing this nucleus (Fig. 9*a*) with that of the preceding figure (Fig. 8*a*), it will be seen from the height of the integuments that the two nuclei represent very closely related stages in mitosis. It may be well to bear in mind, however, that the author does not regard the form of the ovule as the sole guide in determining the mitotic stage, but it has been found all along that certain steps in the growth of the ovule correspond closely to definite mitotic stages. While the rather uniform chromatin cord, with or without indications of a longitudinal fission, is the rule in the stage of the hollow spirem, yet there are found nuclei in which the spirem is more slender and the longitudinal halves more widely divergent than in Fig. 9,—the phenomenon referred to in the foregoing. A part of such a nucleus is shown in Fig. 10. Here the longitudinal segments diverge, becoming widely separated for relatively long stretches. Where short turns of the divergent halves are cut by the knife, the two pieces appear as two isolated portions lying somewhat parallel. Attention was called by the writer to a similar condition in the pollen mother-cells (Mottier, '07), except in the latter this phenomenon seemed to appear more frequently at a later stage,—that of the looping or 'second contraction'. Whether this divergence of the longitudinal halves occurs as frequently here as in the pollen mother-cell is not known, for in any study it is not possible to observe as many megaspore as pollen mother-cells. That Figs. 9 and 10 represent about the same or

very closely related, stages is evidenced by the size of the cells and by the developmental stages of the ovules (Figs. 9*a* and 10*a*). There remains to be considered the probable effect of the reagents as being responsible, at least in a measure, for the more slender spirem and the wider divergence of its longitudinal segments,—a suggestion expressed in a preceding paragraph and in the writer's former publication. From the ovary in which Fig. 10 is found, a part of the ovule as well as the wall of the loculus was cut away; consequently the osmic acid of the fixing fluid had freer access to the cell (Fig. 10*a*). While the author is strongly of the opinion that the fineness or coarseness presented by the chromatin granules in the case of the resting nucleus in question (Figs. 1 and 2) is probably due to the fixing fluid, yet he feels that he cannot speak with positive assurance until experiments now in progress are completed. However, this is certain: no matter how widely, or for whatever length, or lengths, the longitudinal halves of the chromatin spirem may separate and diverge from each other at this stage, they soon come together again and adhere or unite so closely that, apart from rare exceptions, the double nature of the cord is quite obscured at the time of the looping or approximation of parallel parts to form the bivalent chromosomes.

Fig. 11 represents the stage of looping and the approximation of parts of the spirem to form the bivalents just before the cross segmentation. The number of loops is not equal to the number of chromosomes, and it has never been contended by the writer that such is the case. The fact that loops frequently occur may have little or no importance theoretically, but that the two members of a bivalent chromosome are brought together side by side by such a looping is conclusive evidence that these two members were placed end to end in the spirem, no matter whether the spirem was continuous, or interrupted, or 'heterogeneous'. In the case of Fig. 11 *a*, the inner integument of the ovule has grown up beyond the end of the nucellus to form the micropyle. In this respect there is some variation, as the nucleus may reach the stage in question while the integuments are shorter. From this time on the segmentation of the spirem and the further behaviour of the bivalents are quite similar to those processes in the pollen mother-cell as described in my former paper, so that a further description and a duplication of illustrations seem unnecessary.

Summary and Conclusions.

From the foregoing it will be seen that the heterotypic mitosis in the megaspore mother-cell of *Lilium* agrees essentially with that mitosis in the pollen mother-cell as described by the author in his former publication (Mottier, '07). In the micro-, as well as in the megaspore mother-cell, the chromatin in the resting nucleus, or in an early pre-synaptic stage, was either in the form of fine granules or larger and coarser lumps. It was

suggested that this difference in form presented by the chromatin might be due in part to the effect of the osmic acid in the fixing fluid, or to the action of the fluid as a whole. If the fluid penetrated readily and quickly, a coagulation in finer granules would result, whilst a slower penetration, or one such that the strength of the osmic acid is made weaker by virtue of its having to pass through a thicker layer of tissue, would lead to the coarser lumps of the chromatin. The results obtained by the method of preparing the ovaries for this study seem to favour this view. The writer does not advance this explanation as a theory to explain all similar phenomena, but merely offers it as a suggestion which seems to be in harmony with certain facts. Nor should it be inferred that he regards synapsis as an artifact, when it is suggested that the reagents may be responsible for the greater compactness of the synaptic ball, for it is generally true that the reagents used in this and similar indirect methods of study cause a certain amount of contraction of the material, although this may be very slight.

In both micro- and megaspore mother-cells, there is a *single* spirem developed which passes into synapsis, assuming, of course, that a complete spirem is formed prior to synapsis. This seems nowhere else so beautifully shown as in the megaspore mother-cell of *Lilium*. There is, therefore, no pairing or fusion of spirems in synapsis. The hollow spirem appearing after synapsis is seen to be double, and all facts point to the conclusion that this is due to a real longitudinal fission—a fission as real as in the ordinary, or ' allotypic ', mitosis. Occasionally the halves may separate or diverge for considerable lengths, but this divergence or separation of the halves is only temporary, the segments coming together and uniting or adhering closely before complete cross-segmentation of the chromatin cord. It is suggested that this divergence of the halves of the longitudinally split cord may be due in part to the action of the reagents, but this statement is not made with positiveness.

The somatic chromosomes (assuming the individuality of the chromosomes) are arranged end to end in the spirem, and two members of each bivalent chromosome, whenever they lie side by side in their final position, are brought to that position by a looping or other manner of approximation. A very striking proof of the end-to-end arrangement of the somatic chromosomes in the spirem is seen in *Oenothera*, according to the results of Gates ('08). Here, in nuclei uncut by the knife, the majority of chromosomes (l. c., Figs. 26, 27, and 28) can be seen adhering end to end in the segmenting spirem. In *Oenothera* there is likewise no union of spirems side by side in synapsis, as only a single thread is present prior to synapsis. Of the species studied by me, *Tradescantia* more closely resembles *Oenothera*. In *Peperomia* the recent investigations of Brown ('08) have shown that a single spirem passes into synapsis, and that the chromosomes are arranged end to end in the spirem, the members of the bivalents being

brought side by side by a looping or other form of approximation. Substantially the same history of the origin of the bivalent chromosomes has been observed by Lewis ('08) for *Pinus* and *Thuja*.

In a recent number of this journal, Overton ('09) has published the results of his latest observations on the heterotypic mitosis in the pollen mother-cells of *Thalictrum purpurescens*, *Calycanthus floridus*, and *Richardia africana*. All of his data are interpreted in the light of the prochromosome theory and of the lateral union of two spirems prior to or during synapsis. In the pre-synaptic as well as post-synaptic stages he sees bivalent 'heterogeneous' spirems, and the line of longitudinal fission of the post-synaptic spirem is regarded as the line of approximation of two spirems. I shall not discuss his data nor his deductions at this writing, except in so far as they pertain to *Helleborus foetidus* and *Podophyllum peltatum*. In his last, as in his preceding paper ('05), he cites *Helleborus foetidus* as revealing prochromosomes and a bivalent spirem in pre-synaptic nuclei (see his Figs. 39 to 43 for *Helleborus*, and Figs. 53 and 54 for *Podophyllum* (l.c., '05); also pp. 39 and 47 (l.c., '09). From a study of my numerous preparations of *Helleborus*, made from material fixed in several of the well-known fixing fluids, I may say with positive assurance that the pre-synaptic stages in the pollen mother-cells of this plant are quite similar to those described and figured by me for the pollen mother-cells of both *Podophyllum* and *Lilium* (Mottier, '07). In fact, my Figs. 1, 2, and 4 of *Podophyllum*, and Figs. 16, 17, 34, 19, and 20 of *Lilium*, are precisely like the corresponding stages in *Helleborus*, and might be readily substituted as illustrating the same conditions in the latter plant. In the very early stages of *Helleborus*, as in *Podophyllum* and in *Lilium*, there is no definite spirem, but a network or reticulum, consisting of chromatin lumps or granules of varying sizes and shapes connected by delicate linin strands. The number of these lumps always exceeds the number of somatic chromosomes. Some of these lumps are paired, as well as some of the linin strands; but there are also clusters of three or more lumps, as well as three or more parallel linin strands connecting different lumps. There are also linin strands radiating or extending in several directions from some of the lumps. Just prior to synapsis in *Helleborus*, a definite slender spirem may be developed precisely as figured for *Lilium Martagon* (l.c., Figs. 19 and 20). As already stated for *Podophyllum*, in one end of the anther of *Helleborus* nuclei may show the structure of my Figs. 1 and 2 (l.c., '07), while in the other end of the same loculus the chromatin is much more finely divided, as in Fig. 4 (l.c., '07).

Judging from Overton's figures of the pre-synaptic stages which he gives in his recent paper (l.c. '09), it seems to me that he has failed to distinguish between a network and a spirem. The structure shown in his Figs. 1, 2, 3, 4, 5, 6, and 7 of Pl. I, and Figs. 1, 2, 3, and 8 of Pl. II, for

example, would not be regarded by the writer as spirems, much less as bivalent spirems, but merely as a network—a number of scattered chromatin lumps connected by linin threads. It seems to me also that one may just as well interpret the cytoplasm of Overton's Fig. 12, Pl. II, as consisting of bivalent 'heterogeneous' spirems as to apply such a designation to the nuclear structures just mentioned.

The foregoing results of observations upon the prophases of the heterotypic mitosis in the megaspore mother-cell of *Lilium* may, therefore, be briefly summarized as follows :—

1. Previous to synapsis a single nuclear thread is developed, which, in many cases, can be demonstrated clearly as a definite spirem with somewhat regular and uniform chromatin granules.

2. There is no union side by side of two distinct chromatin spirems prior to or during synapsis. Synapsis is regarded as a normal process, but the greater compactness of the balled-up mass may be due partly to the reagents.

3. The hollow spirem following synapsis is double, due to a longitudinal fission, which, as a rule, becomes completely obscured before the cross segmentation.

4. The segments arranging themselves in pairs to form the bivalent chromosomes were placed end to end in the spirem.

5. The separation of the members of each bivalent in the first mitosis is, therefore, a cross segmentation. The heterotypic mitosis is thus reductional, and, if one chromosome differs from another potentially or otherwise, it is also qualitative.

6. In the pre-synaptic phase, that the chromatin may appear as larger lumps instead of smaller and more uniform granules, has been suggested as being due in part to the fixing fluids, the finer more uniform granules being nearer normal. The wide divergence of the halves of the chromatin thread appearing occasionally in the stage of the hollow spirem may also be due in part to the reagents.

February 15, 1909.

Publications referred to.

Brown, William H. ('08): The nature of the embryo-sac of *Peperomia*. Bot. Gaz., xlvi, 445–60, 1908.

Gates, Reginald R. ('08): A study of reduction in *Oenothera rubrinervis*. Bot. Gaz., xlvi, 1–34, 1908.

Lewis, Isaac M. ('08): The behaviour of the chromosomes in *Pinus* and *Thuja*. Ann. of Bot., xxii, 529–56, 1908.

Mottier, D. M. ('07): The development of the heterotype chromosomes in pollen mother-cells. Ann. of Bot., xxi, 309–47, 1907.

Overton, James B. ('05): Ueber Reduktionsteilung in den Pollenmutterzellen einiger Dikotylen. Jahrb. f. wiss. Bot., xlii, 121–53, 1905.

——————— ('09): On the organization of the nuclei in the pollen mother-cells of certain plants, with especial reference to the permanence of the chromosomes. Ann. of Bot., xxiii, 19–60, 1909.

EXPLANATION OF PLATE XXIII.

Illustrating Professor Mottier's paper on the prophase of the heterotypic mitosis.

All figures were drawn from sections with the aid of the Abbé camera lucida, and with Zeiss apochromatic immersion 2 mm., apert. I. 40, and compensating ocular 12, excepting those showing outlines of ovules, 1 *a*, 2 *a*, &c., which were drawn with low powers. Magnification of figures of nuclei about × 1800.

Figs. 1 *a*, 2 *a*, &c., represent outline drawings of the ovules in which the nuclei, Figs. 1, 2, &c., respectively occur.

Fig. 1. The nucleus of a megaspore mother-cell at an early stage in the period of growth prior to synapsis. In Fig. 1 *a*, a small piece of the ovule was cut away along the line *x*—*x* before fixing.

Fig. 2. About the same stage in mitosis as Fig. 1. The chromatin is in form of irregular lumps of varying size. One large and two or more smaller nucleoli are present. In this case the wall of the ovary was not cut away to allow the fixing fluid direct access to the ovule. A layer of tissue six or seven cells in thickness must have been traversed before the fluid reached the ovule. The lumpy condition of the chromatin is supposed to be due in a measure to the action of the reagents.

Fig. 3. A later stage than Fig. 1. The nuclear structure consists of a delicate network holding the small chromatin granules uniformly distributed. A large and two small nucleoli are present. Fig. 3 *a* shows the part of the ovule cut away before fixing.

Fig. 4. A nuclear thread or spirem has been formed which is undergoing synaptic contraction.

Fig. 5. Synapsis has progressed further. The spirem is a single thread, and the chromomeres are somewhat larger than in the preceding figure.

Fig. 6. The stage of the dense synaptic ball.

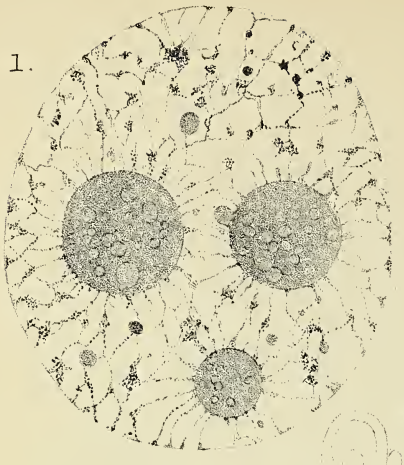
Fig. 7. The spirem loosening up after synapsis. The longitudinal fission, if present, is not visible.

Fig. 8. The spirem is becoming distributed in the nuclear cavity. Only here and there can any evidence of a longitudinal fission be seen (at the right near a cut end of the thread).

Fig. 9. The stage of the hollow spirem; the section includes a part of the spirem only. The longitudinal fission is quite distinct, the segments diverging somewhat in places.

Fig. 10. The same stage in mitosis as Fig. 9. The spirem is more slender, and the longitudinal segments separate for longer stretches. It is probable that the slender nature of the spirem and the greater divergence of the halves are due partly to the reagents. Fig. 10 *a* (*x*—*x*) shows the part of the ovule cut away before fixing.

Fig. 11. A later stage, at the time of the second contraction and of the looping and approximation of different parts of the spirem to form the bivalents prior to complete cross segmentation. The longitudinal halves of the spirem, which frequently show a tendency to diverge more or less at an earlier stage, have unmistakably and beyond all question united, so that there is now a single and rather thick and smooth cord. At this stage, both here and in the pollen mother-cell, indications of the longitudinal split may occasionally be made out. Fig. 11 *a* will indicate the structure of the ovule at this stage of mitosis.



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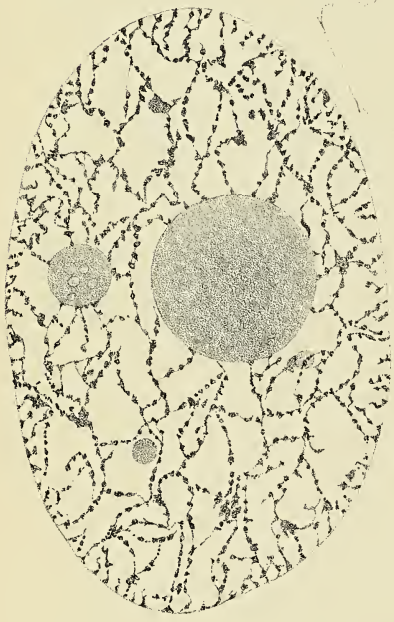

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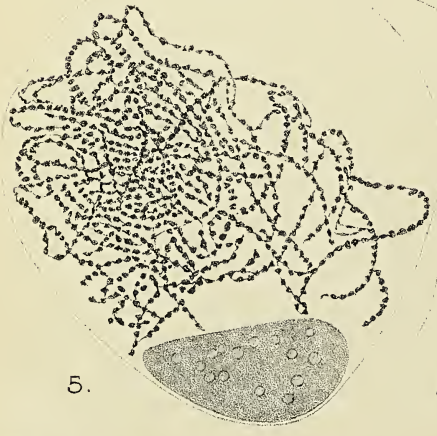

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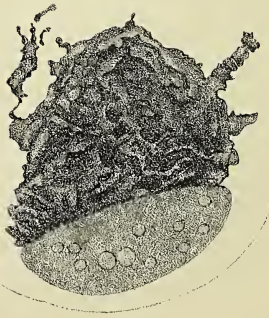
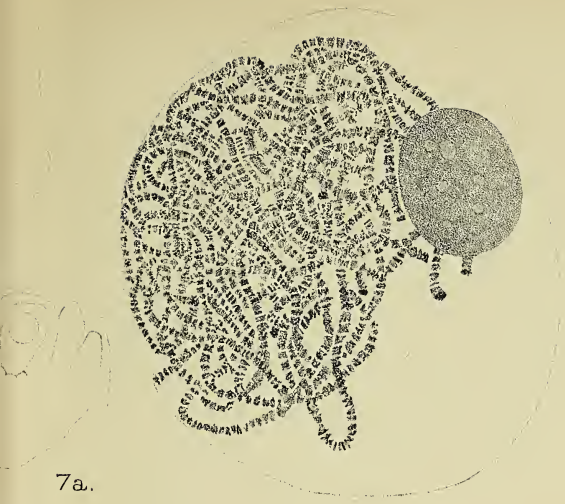


Mottier and Black del.

MOTTIER — HETEROTYPIC MITOSIS.



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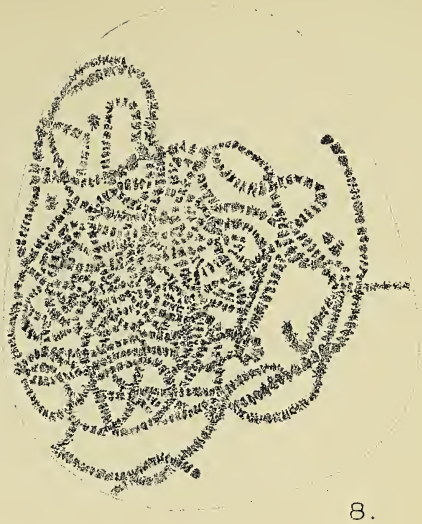

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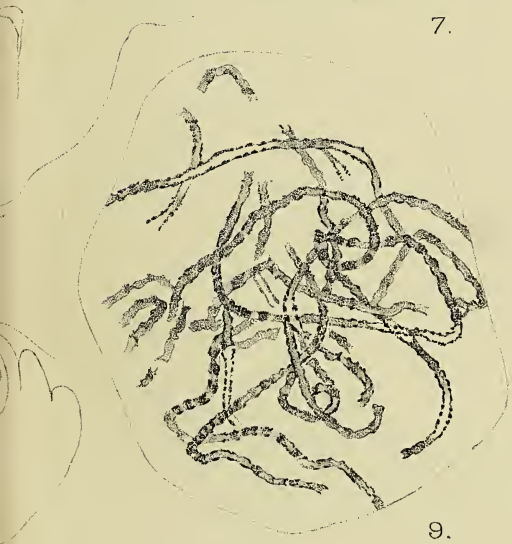

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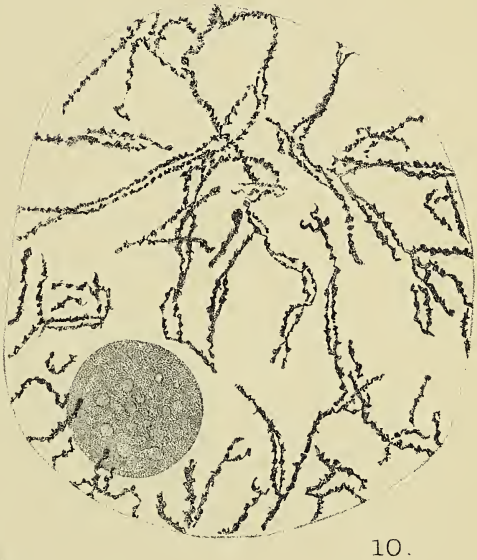

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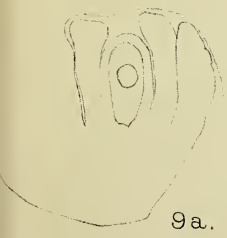

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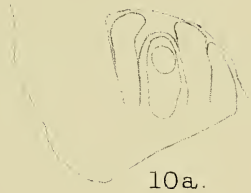

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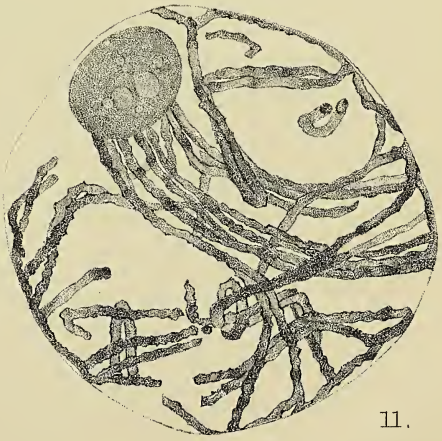

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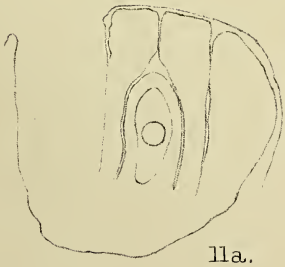

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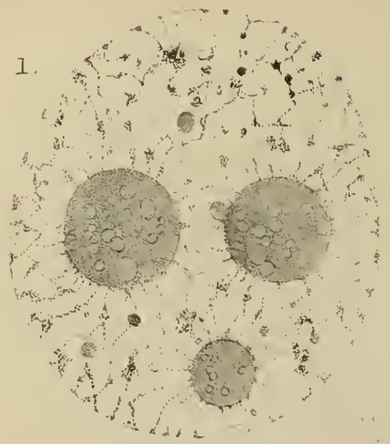

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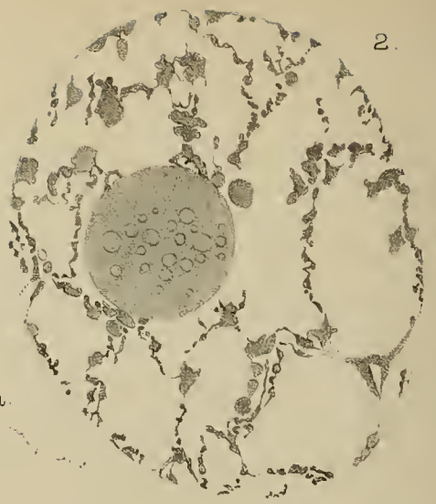

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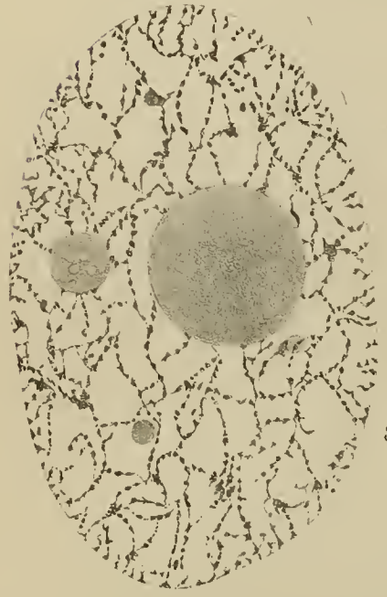

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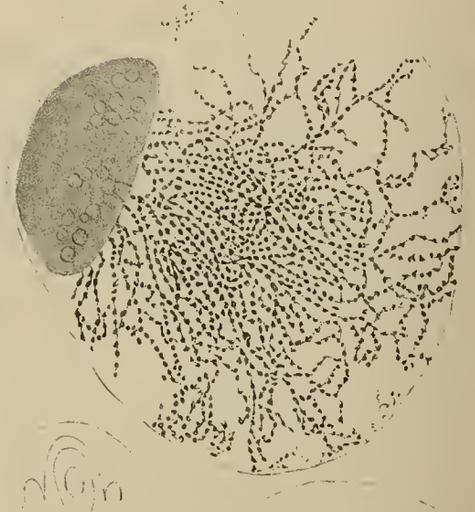

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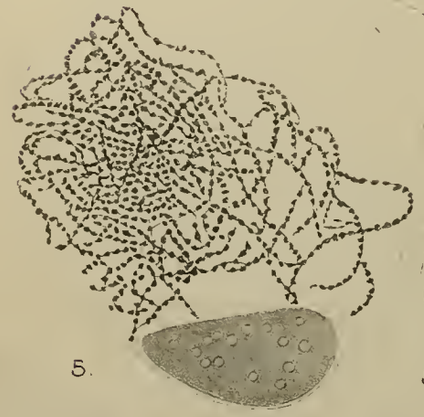

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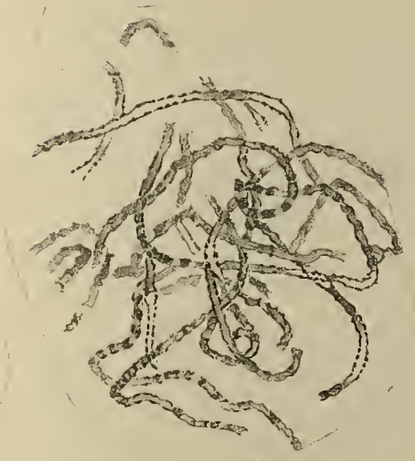

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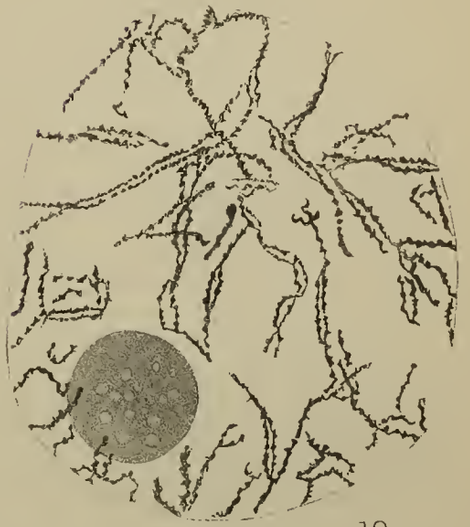

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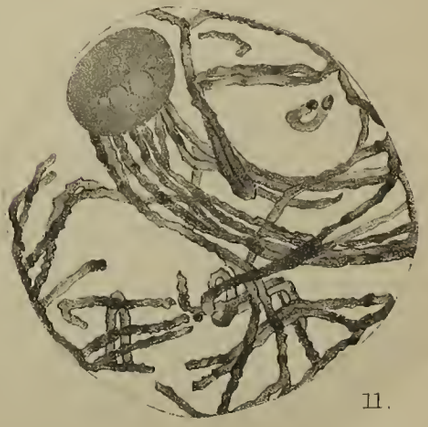

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Mottier and Black del

Huth, lith. et imp

MOTTIER — HETEROTYPIC MITOSIS.

The Structure and Development of the Ovule of Myrica Gale.

BY

EDITH MAY KERSHAW, M.Sc.,

Graduate Scholar in Botany in the University of Manchester.

With Plate XXIV, and two Figures in the Text.

IN view of the important peculiarities noted in the structure and development of the ovule of *Casuarina* and the Amentiferae, and of our rapidly extending knowledge of the seeds of the older Gymnosperms, it seems important that the ovules of allied orders such as the Myricaceae should be carefully examined, as so far only *Myrica Lobbii* had been examined in detail by Treub.

When Treub[1] published in 1891 the account of his very thorough investigation of the Casuarinaceae which had led to interesting discoveries, he suggested that this group should be placed in a separate subdivision of Angiosperms. In summing up his conclusion (p. 209), he says, 'Les Casuarinées occupent très probablement par les phénomènes qui se passent dans leur nucelle avant la fécondation une place tout à fait exceptionnelle parmi les Angiospermes.' These exceptional phenomena in the nucellus are—the considerable development of sporogenous tissue, composed of hundreds of cells—the great number of macrospores which develop, sometimes twenty or more,—the entrance of the pollen-tube into the nucellus by the chalaza. 'Ce sont là autant de points cardinaux qui distinguent les *Casuarina* des autres Angiospermes' (p. 215).

From a comparative study of the Amentiferae, Juglandaceae, and Myricaceae which were considered as groups closely allied to the Casuarinaceae, Treub concluded that Casuarinaceae were the only group which showed these exceptional characters, and so he suggested a division of the Angiosperms into two classes:—

Chalazogames—containing the family Casuarinaceae, and *Porogames*—containing the rest of the Angiosperms, the names being derived from the mode of entrance of the pollen-tube into the nucellus.

[1] Treub: Ann. du Jard. Bot. de Buitenzorg, vol. x, pp. 145–231.

[Annals of Botany, Vol. XXIII. No. XCI. July, 1909.]

Miss Benson's [1] work on the Amentiferae showed that this classification could not possibly be adopted. Treub was evidently misled by Hofmeister's account in 1858 of the embryology of the Amentiferae, for Miss Benson very clearly shows that *Betula*, *Alnus*, *Corylus*, and *Carpinus* are also Chalazogams. In many other characters also the Amentiferae very closely resemble *Casuarina.*

There is a considerable amount of sporogenous tissue, which although differing in detail from that of *Casuarina*, is thought by Miss Benson to constitute an important point of resemblance; the pollen-tubes are of the branching type, and in many genera enter the nucellus through the chalaza. Such characters as the large amount of sporogenous tissue in the ovule and the great number of macrospores which develop, may be regarded as primitive, and have been considered to support the position assigned to the Amentiferae and Casuarinaceae by Engler in his scheme of classification of the Angiosperms.

The investigations of Nawaschin [2] on *Juglans regia*, and those of Karsten [3] which extended to other members of the Juglandaceae, show that chalazogamy prevails also in this family.

It is interesting to note Nawaschin's views on chalazogamy. He states: 'die Chalazogamie stellt eines von den Uebergangsstadien dar bei der Umwandlung des intercellularen Wachsthums des Pollenschlauches im gymnospermen Fruchtknoten zum freien Wachsthum durch die Fruchtknotenhöhle der Angiospermen.'

A more detailed account of the structure and development of the ovule of *Juglans regia* was given by Nicoloff [4] in 1904, details of which will be referred to later.

As regards the Myricaceae, Treub [5] had already in his paper on *Casuarina* shown that in *Myrica Lobbii* the course of the pollen-tube was not chalazogamous, but that development and fertilization were of the normal Angiospermous type.

As this was based on one species only, it was suggested that an investigation of the ovules of other species might prove interesting as a further comparison.

For this purpose *Myrica Gale*, which grows fairly abundantly round some of the Cheshire meres,—particularly Hatchmere in Delamere Forest—was chosen.

I found in this British species, just as Treub found in *Myrica Lobbii*, that

[1] Benson: Trans. Linnean Soc., London, vol. iii, part 10, 1894.

[2] Nawaschin: Ein neues Beispiel der Chalazogamie. Bot. Centr., 1895, 63.

[3] Karsten: Ueber die Entwickelung der weiblichen Blüthen bei einigen Juglandaceen. Flora, 90. Band, 1902.

[4] Nicoloff: Sur le type floral et le développement du fruit des Juglandées. Journal de Botanique, t. xxviii–xxix.

[5] Treub: loc. cit.

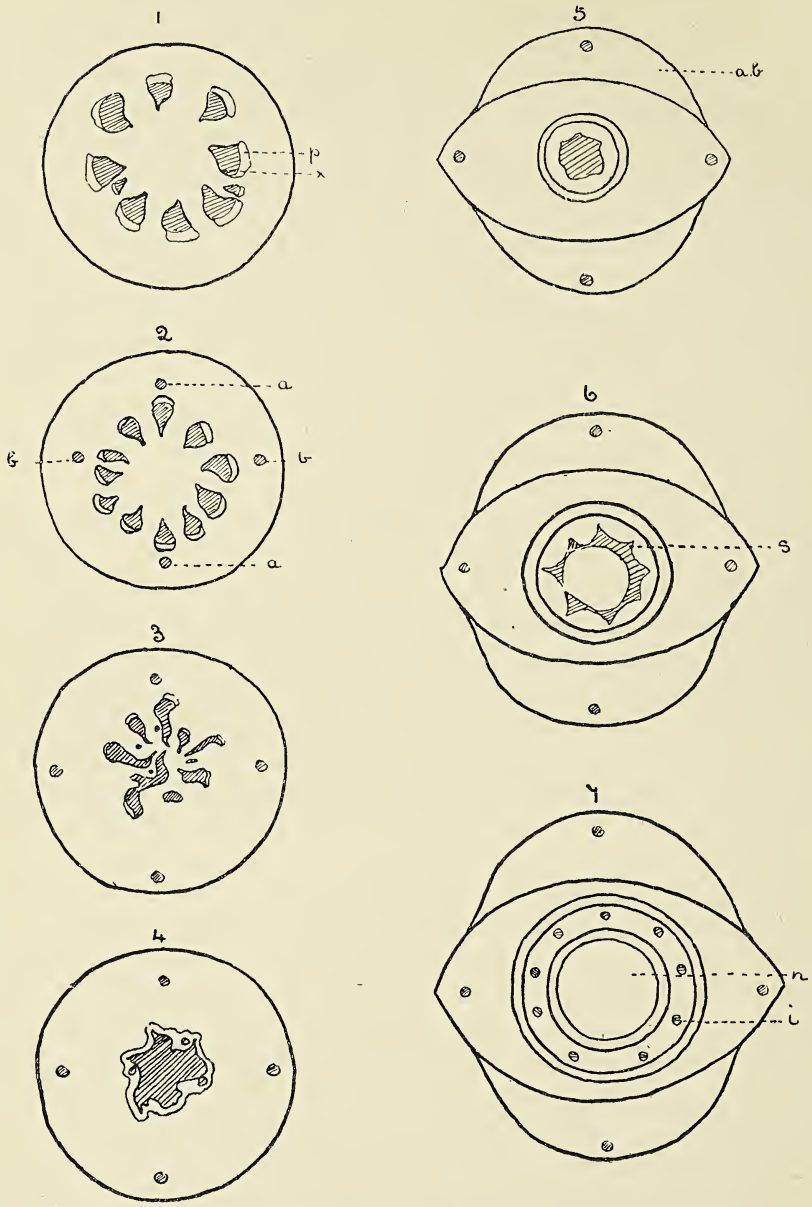
the development and fertilization of the ovule is quite of the normal Angiosperm type. There is only a single embryo-sac mother-cell which divides to form several superposed cells, the lowest of which absorbs the rest and forms the embryo-sac. The nucleus of the embryo-sac divides, eight nuclei eventually being formed as in the typical Angiosperm. The pollen-tube bearing two male cells enters the micropyle (Pl. XXIV, Fig. 1), penetrates the apex of the nucellus and pushes its way into the embryo-sac, at the top of which is the sexual apparatus, consisting of the egg-cell and two synergids. Fusion takes place resulting in the formation of a normal dicotyledonous embryo and a development of endosperm.

In the development of the ovule then, *Myrica Gale* does not show any of those characteristics, e. g. great development of sporogenous tissue, numerous macrospores, chalazogamy, &c., which are found in nearly related families, and which are generally regarded as primitive characters. Certain anatomical features of the ovule, however, seem to be of sufficient interest to be recorded in some detail.

As is well known, the flowers which are borne in axillary catkins are either monoecious or dioecious. The female flower is devoid of perianth. The ovary, sessile in the axil of a bract, is furnished with two sterile scales, which become fused with the ovary wall. The ovary contains a single ovule which is orthotropous and quite sessile (Fig. 2). The nucellus, which is long and oval in shape (*n*), tapering to form a distinct stalk at the base, is surrounded by a single integument (*i*). A peculiar feature is that the nucellus stands up quite freely within this integument, from the stalk-like portion at the base to the apex.

Another very interesting feature is found in the vascular supply to the ovule (Text-fig. I).

A transverse section below the base of the ovule shows a ring of vascular bundles, usually eight or nine in number, with phloem on the outer side (Text-fig. I, 1). As the ring of bundles traverses the stalk of the ovule, four small branches are given off and run outwards—two to supply the two bracts which are fused to the ovary (Text-fig. I, 2 *a* and Fig. 2 *b*), and the remaining two to supply the ovary wall (Text-fig. I, 2 *b*). These four vascular bundles traverse their courses without branching, the ones to the bracts running up the centre of the bract to the tip, those to the ovary wall running up into the stigmas (Fig. 2, *st.*). Just after these four bundles have branched off the ring of bundles in the stalk of the ovule begin to curve inwards (Text-fig. I, 3 and Fig. 2 *m.b.*), and gradually unite by fusing together, so that a transverse section immediately at the base of the ovule shows a solid strand of wood surrounded by a ring of phloem (Text-fig. I, 4). When this solid strand reaches the base of the nucellus it expands to form a shallow cup-like mass of tracheids. Text-fig. I, 5 shows the centre of the cup, and Text-fig. I, 6,



TEXT-FIG. I. Diagram of a series of transverse sections from base of ovary to upper part. 1, 2, 3, 4, through base of ovary; 5 and 6, through stalk of ovule; 7, through upper part of ovule. *a* = vascular bundle supplying bract; *b* = vascular bundle supplying ovary wall; *i* = integumentary bundle; *n* = nucellus; *s* = vascular strand about to enter integument; *a.b.* = adherent bract; *p* = phloem; *x* = xylem.

a section a little higher in the series shows the edges. This is shown in longitudinal section in Fig. 2.

Generally in the ovules of recent plants, the vascular supply terminates here, i. e. in a flat plate of tracheids at the base of the nucellus, but in *Myrica Gale*, the edges of this cup of tracheids run out into eight or nine slender vascular strands, each composed of a few tracheids and a small quantity of phloem, which traverse the single integument (Text-fig. I, 7 *i*, and Fig. 2 *i.b.*).

A tangential section of the integument showing these vascular strands is represented in Fig. 3. The strands pass up the integument without branching, getting smaller as they get higher, until, almost at the apex of the ovule, the strand which has diminished to a single tracheid dies out (Fig. 2 *i.b.*).

In a transverse section of the ovule, therefore, one finds a ring of eight to nine small vascular bundles as seen in Text-fig. I, 7 *i*.

Running up the centre of the nucellus from its base to the base of the embryo-sac is a strand of tissue, consisting of parenchymatous cells considerably elongated in the vertical direction (Fig. 2 *c.s.*). This strand of tissue probably facilitates the conduction of food material to the embryo, since it connects the cells at the base of the nucellus which have very dense contents, with the embryo-sac.

These characters, i. e. the free nucellus and the vascular supply running up into the integument are remarkable—occurring as they do in a family of recent plants, and may be regarded as being of phylogenetic interest.

In living Angiosperms the nucellus and integument are generally fused together almost to the apex, and the integument is devoid of vascular tissue.

It will be remembered, however, that a free nucellus and vascular nteguments were prevalent in many of the fossil plants.

The ovules of some of the Pteridosperms and many of the fossil Gymnosperms have been described as having a free nucellus. Almost all the fossil seeds described by Brongniart[1] in his 'Recherches sur les graines fossiles silicifiées' are represented as having a nucellus quite free from the surrounding integument.

Trigonocarpus, probably the seed of *Medullosa*, recently described by Scott and Maslen,[2] and the ovule of *Myrica Gale* show a great similarity of structure (Text-fig. II). Both of them possess a single integument, and in each case the nucellus stands up freely within it.

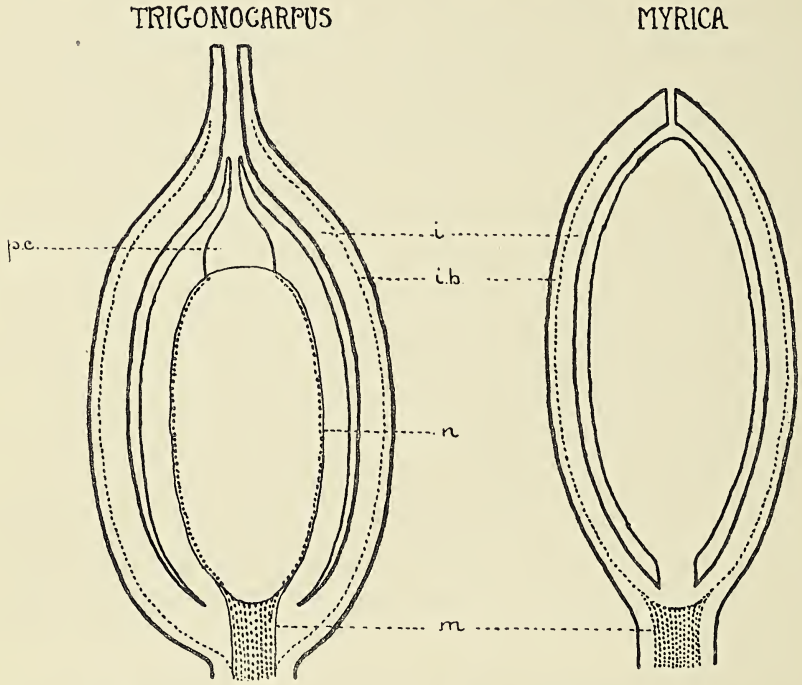
This character of a free nucellus in these older fossil seeds may indicate that the integument had not as yet become an integral part of the seed.

[1] Brongniart ('81): Recherches sur les graines fossiles silicifiées. Paris.

[2] Scott and Maslen: The Structure of the Palaeozoic Seeds, *Trigonocarpus Parkinsoni*, Brongniart, and *Trig. Oliveri*, sp. nov. Ann. of Bot., vol. xxi, Jan., 1907.

A free nucellus, therefore, may be regarded as a primitive character which has been lost in the greater number of Angiosperms, where the integument and nucellus are fused together almost to the apex of the ovule.

Oliver,[1] in his paper on 'The Ovules of the Older Gymnosperms', suggests that in the seeds of Cycads, &c., where the nucellus is not free to the base, 'the whole body of the ovule, below the level at which the



TEXT-FIG. II. Diagrammatic representation of median sections through ovules of *Trigonocarpus* and *Myrica Gale* to show similar construction of the seed and vascular supply to the integument. *i* = integument; *i.b.* = integumentary vascular bundle; *m* = main supply bundle; *n* = nucellar vascular supply; *p.c.* = pollen chamber.

nucellus becomes free, is phylogenetically younger than its apical parts,' and 'between the original ovule and its insertion a new region has been intercalated'.

Myrica, therefore, seems to have retained an ancestral character or to have reverted to the former state such as obtained in the fossil seeds with a free nucellus, and there is no indication of an intercalated portion such as has been suggested for those seeds where the nucellus and surrounding integument are fused together.

Whether this character is really the ancestral one retained or a secondary character almost identical with the ancestral one, can hardly be

[1] Oliver ('03): The Ovules of the older Gymnosperms. Ann. of Bot., vol. xvii.

determined; but it is interesting to remember that it occurs in a group of recent plants which is regarded by many botanists as primitive.

Turning now to the other character of the ovule of *Myrica Gale* which has been specially noted—the continuation of the vascular supply into the integument—it does not seem very easy to explain the presence of this integumentary vascular system by any special requirements of the integument, as it is neither of great thickness, nor does it become succulent when ripe. Probably it is correlated with the complete separation of the nucellus from the integument, which renders it impossible for the integument to receive its nutriment through the nucellus, as is possible in most seeds where only the apical portion of the nucellus is free.

Comparing the ovule of *Myrica Gale* with the seed of *Trigonocarpus* in respect to the vascular system, we find again a certain resemblance (Text-fig. II). In *Trigonocarpus* the main supply bundle to the ovule gave off at a short distance below the chalaza a number of branch bundles, usually six to nine, which, bending outwards, traversed the sarcotesta of the integument. In *Myrica* we find a similar arrangement of integumentary bundles given off from the main supply bundle.

The existence of such an integumentary vascular supply in fossil seeds such as *Trigonocarpus*, where we have an integument free from the nucellus, is interesting in comparison, and supports the view of a correlation of these two characters.

In *Trigonocarpus* the main supply bundle, after giving off the integumental bundles, continued unbranched through the stalk of the seed until it reached the nucellus. There it expanded to form an apparently continuous mantle of tracheids investing the macrospore and terminating as anastomosing strands just below the pollen-chamber.

In *Myrica*, of course, there is no necessity for such a system of nucellar tracheids as was required by an ovule with a large pollen-chamber, filled at the time of fertilization with water; but there is, as described above, the distinct strand of elongated cells running up the centre of the nucellus which evidently serves the purpose of nutrition of the embryo-sac (Fig. 2, *c.s.*).

This central strand of elongated parenchymatous cells in the nucellus of *Myrica Gale* may possibly represent the remains of an ancient nucellar vascular system, or it may be a new structure developed to facilitate the conduction of nutritive material to the embryo-sac. When one remembers the tracheids found by Miss Benson[1] in the nucellus of *Castanea*, and by Treub[2] in that of *Casuarina*, regarded by Miss Benson as 'a vestige of some long lost structure', one is inclined to regard this

[1] Benson: loc. cit.

[2] Treub: loc. cit.

central strand in the nucellus of *Myrica Gale*, which is a comparable structure to the tracheids in *Castanea* as also 'a vestige of some long lost structure'.

It is interesting to find that in two families generally regarded as closely related to the Myricaceae, the structure of the ovule resembles very closely that of *Myrica Gale*.

Nicoloff[1] in his treatise on the Juglandaceae produces several figures of the ovule of *Juglans regia* which show that very similar features are found to those which have been described for *Myrica Gale*. In a longitudinal section of the ovule which is orthotropous, he figures the nucellus as being quite free to the base from the integument (Nicoloff, l. c., Fig. 18). In a transverse section he represents the integument as having a ring of vascular bundles, ten in number (Nicoloff, l. c., Fig. 31). Although these characters are described by Nicoloff he does not suggest in any way their probable phylogenetic value.

In the recently investigated allied order of Julianiaceae,[2] the ovule of *Juliania* is described as having an integument containing a branching system of vascular bundles. The nucellus is figured as being fused to the single integument however. This may be accounted for by the fact that the ovule is hemi-anatropous, not orthotropous as in the cases described where the nucellus is free.

The absence of these two apparently primitive characters of the ovule in the allied groups of Amentiferae and Casuarinaceae may be connected with the change from the orthotropous to the anatropous type of ovule.

A comparison of the structure of the ovule in the families Juglandaceae, Julianiaceae, and Myricaceae may throw some light on the position of these families in the Natural Classification, a point which has given rise to some discussion.

Hallier[3] regards the Amentiferae including Myricaceae as a degenerate group derived probably from the Terebinthaceae in which are included Anacardiaceae, Juglandaceae, and Julianiaceae.

Hemsley,[4] on the other hand, regards the groups Juglandaceae, Julianiaceae, and Amentiferae as very closely allied, the Julianiaceae probably occupying a position immediately between Juglandaceae and Amentiferae.

The evidence derived from the structure of the ovule undoubtedly

[1] Nicoloff: Sur le type floral et le développement du fruit des Juglandées. Journal de Botanique, t. xxviii–xxix.

[2] W. Botting Hemsley: On the Julianiaceae. A New Natural Order of Plants. Phil. Trans. Roy. Soc., vol. cxcix, 1908.

[3] Hallier: 'Über *Juliania*, eine Terebinthaceen-Gattung mit Cupula, und die wahren Stammeltern der Kätzchenblütler: Dresden, 1908.

[4] Hemsley: loc. cit.

supports the latter view. The ovules of the orders Julianiaceae, Myricaceae, and Juglandaceae—especially of the two latter—are very similar, retaining, if the foregoing is correct, ancestral characters. The Julianiaceae show one step in advance of the other two orders in the change from the orthotropous to hemi-anatropous type of ovule, thus obliterating one of the 'ancestral characters' described in the other two orders. The Amentiferae show a further advance in this respect, the ovule being truly anatropous. As a result of this change in the position of the ovule, probably more pressure came to bear on the parts of the ovule than they would be subjected to in the orthotropous condition, the integument and nucellus therefore came into closer contact, and since the integument was not large, and did not become succulent, there was no longer any need for an integumental vascular system.

Although the Amentiferae have advanced further than the Myricaceae and Juglandaceae in respect to the structure of the ovule, they show a more primitive state of affairs than do the latter in the development of the ovule, examples of chalazogamy, numerous macrospores, &c., being found as described above.

Thus in the structure and development of the ovule these closely allied orders show, as is shown in the evolution of all groups of plants, an unequal development of parts. The Amentiferae which have advanced farthest in the specialization of the ovule, losing such ancestral characters as the free nucellus and integumentary vascular supply, still retain a primitive mode of development of the embryo-sac; while others such as the Myricaceae which show these primitive characters in the structure of the ovule, have advanced to the normal Angiospermous type of development of the embryo-sac.

The evidence derived from the structure and development of the ovule in the series of plants including Juglandaceae, Myricaceae, Amentiferae, Casuarinaceae, and Julianiaceae, seems to me to support the view that they are a primitive group of Angiosperms, and should therefore retain the position assigned to them by Engler in his scheme of classification.

The alternative suggestion made by Arber and Parkin [1] and by Hallier [2] that the Amentiferae are degenerate orders descended from ancestors of the Ranales type is not supported by the facts obtained in my investigation of *Myrica Gale*, for the development of vascular supply in the integument can hardly be regarded as a step in the degeneration of an ovule, nor is the development of a large amount of sporogenous tissue in the ovule observed by Miss Benson in some of the Amentiferae in accordance with our views of degeneration.

[1] Arber and Parkin ('07): On the Origin of Angiosperms. Linn. Soc. Journal of Botany, vol. xxxviii.

[2] Hallier ('08): Über *Juliania*, eine Terebinthaceen-Gattung mit Cupula. Dresden, 1908.

I take this opportunity of thanking Professor Weiss for the help he has given me in this work, and the many suggestions he has made from time to time; also my thanks are due to Miss K. H. Coward, M.Sc., for preparing many of the necessary microtome sections.

University of Manchester,
March, 1909.

EXPLANATION OF PLATE XXIV.

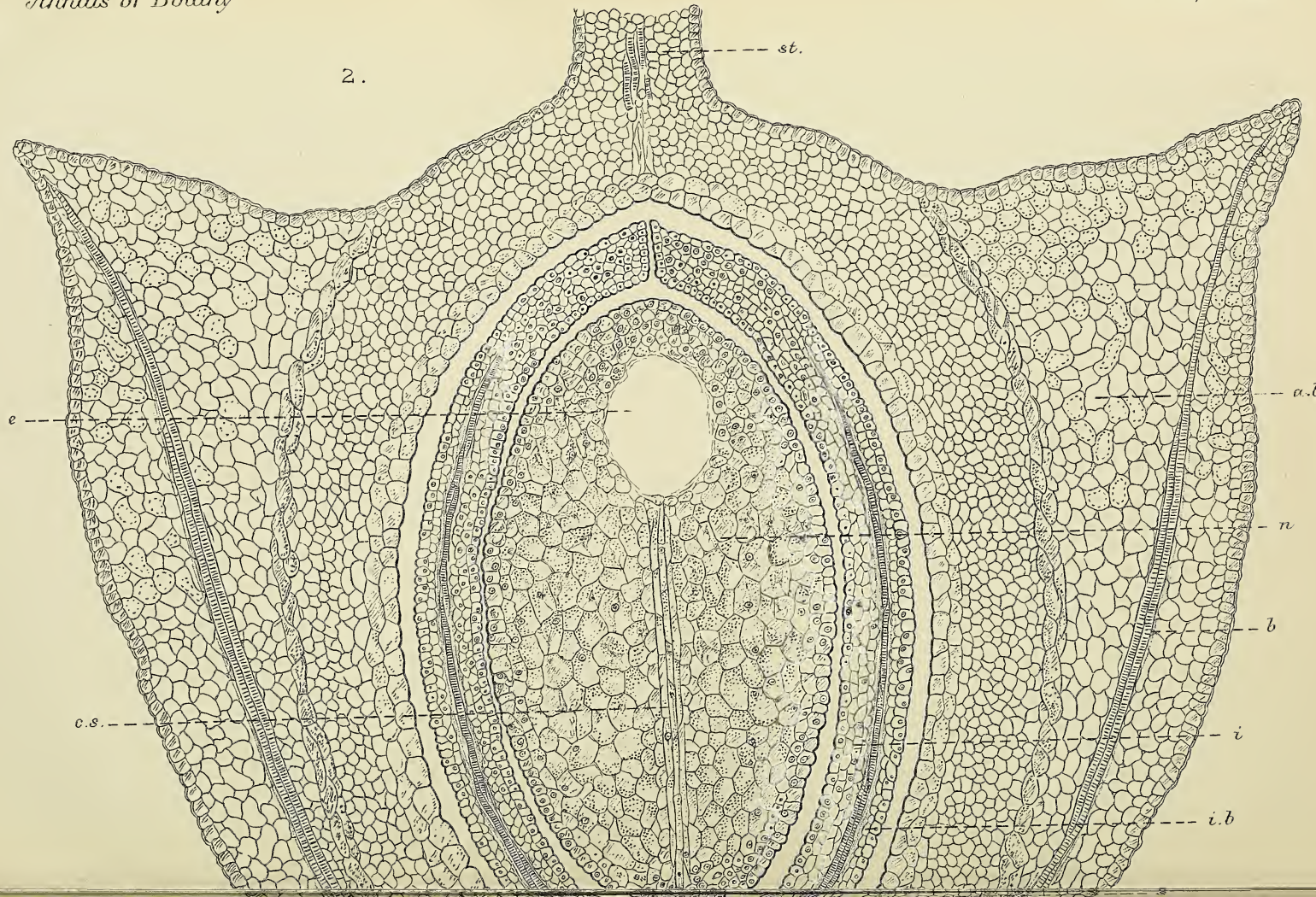
Illustrating Miss Kershaw's paper on *Myrica Gale*.

Fig. 1. Long. sect. through apex of ovule of *Myrica Gale* showing pollen-tube with two male cells entering through the micropyle.

Fig. 2. Long. sect. through ovary at end of July. *n.* nucellus; *s.* stalk of nucellus; *i.* integument; *b.* vascular bundle of bract; *st.* vascular bundles of ovary wall, terminating in stigma; *m.b.* main supply bundle to ovule; *i.b.* vascular bundle supplying integument; *e.s.* central strand of elongated parenchymatous cells running from base of nucellus to embryo-sac; *e.* embryo-sac; *a.b.* adherent bract.

Fig. 3. Tangential section through integument of ovule showing vascular strands.

2.



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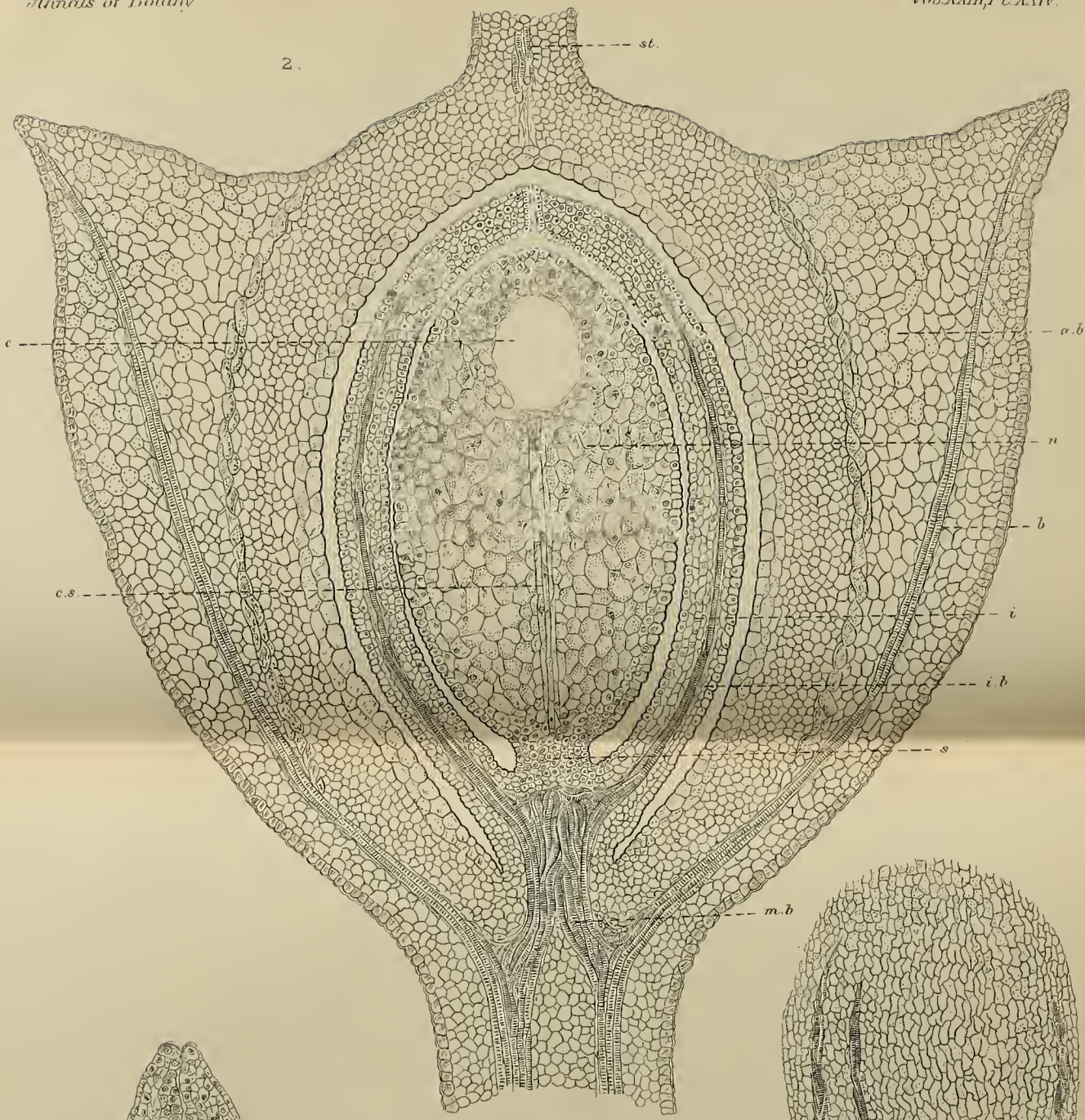

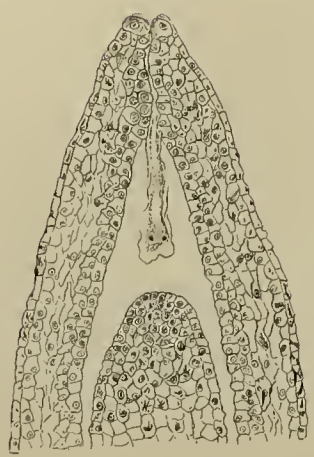

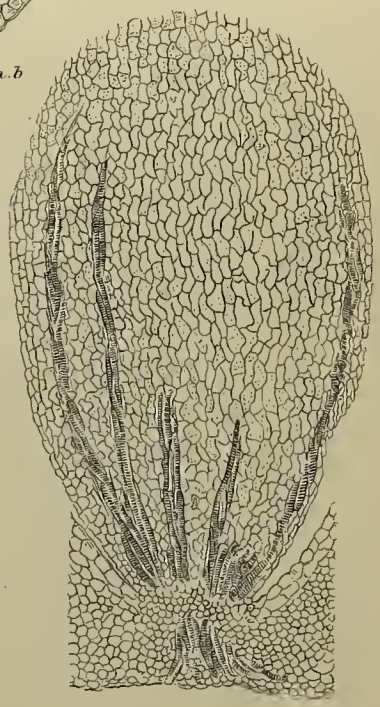

E M K ad nat. del

Huth lith et imp.

KERSHAW. — MYRICA GALE.

The Embryo-Sac and Embryo of certain Penaeaceae.

BY

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With Plates XXV and XXVI.

THE Penaeaceae are a small family of shrubby Dicotyledons, of an ericoid habit, entirely confined to the south-west region of Cape Colony. The order has yet to be studied from a systematic standpoint, but five genera, with twenty-two species, are recognized by Gilg[1]—*Sarcocolla*, *Penaea*, *Brachysiphon*, *Endonema*, *Glischrocolla*. Of these, I have investigated the following species, representing three of the genera—*Sarcocolla squamosa*, *S. fucata*, *S. formosa*, *Penaea mucronata*, *P. ovata*, *Brachysiphon imbricatus*. Of *Endonema* and *Glischrocolla* I have not yet been able to obtain material suitable for embryological work. All the species investigated show exactly the same life-history, and the account that follows may be taken as applying to them all. Material was collected for the most part on the slopes of the mountains of the Cape Peninsula during 1907, and again during a visit to S. Africa from July to September, 1908. Material of *Geissoloma marginata*, belonging to the allied order Geissolomaceae, was collected for comparison at Garcia's Pass in the Langeberge Mountains in September, 1908, during an excursion, kindly arranged by Prof. Pearson for that object; information as to the exact locality of this very rare plant was obtained through the kind agency of Dr. Marloth. The material examined was fixed in acetic-alcohol (two parts of absolute alcohol to one of acetic acid), mercuric chloride (solution in one per cent. acetic acid), various chromo-acetic mixtures, or Fleming's solution. The best results for nuclei and for mature embryo-sacs at about the fertilization period were given by the acetic-alcohol mixture; in the free nuclear stages, however, this fixative caused contraction (cf. Pl. XXV, Fig. 14); for these and for the developing embryo, the chromo-acetic and Fleming's solution

[1] Gilg, in Engler and Prantl, 1894, p. 208.

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were more satisfactory. Combinations of Licht-Grün with Diamant Fuchsin (a rapid and clear stain for embryological work), and of the haematoxylins with eosin were the chief stains used.

Ovule and Embryo-sac.

Throughout the order a four-carpellary, four-locular ovary is found. In *Sarcocolla*, *Penaea*, and *Brachysiphon* each loculus contains two to four erect anatropous ovules inserted in axile placentation near the base; in *Endonema* and *Glischrocolla* the four ovules in each loculus are inserted on an axile placenta, two erect and two hanging. Each ovule is surrounded in its earlier stages by two free integuments, each two cells thick (Pl. XXV, Figs. 1, 2); these, however, soon coalesce with one another, and with the nucellus, and are then recognizable, below the level of the micropyle, only as four regular cell layers at the periphery of the ovule (Pl. XXVI, Fig. 24). In the later stages of their development the outer integument projects beyond the inner. Before they coalesce, the cells at the lip of each integument have begun to divide (Fig. 3), and their further divisions cause each lip to swell out into a cushion-like ring, which, meeting at the centre, blocks the micropyle (Fig. 24).

The archesporium consists of a single sporogenous cell, which at its earliest recognizable stage is found sunk one layer deep beneath the epidermis (Fig. 1). The arrangement of the cell or cells in the layer immediately above it indicates that they and the sporogenous cell were probably derived from a single hypodermal cell by periclinal division. In one case only was there any sign of another sporogenous cell; this is shown in Fig. 2, where the large cell above the embryo-sac is clearly differentiated from the rest of the nucellus by its size and staining properties, and may possibly represent a second potential megaspore mother-cell which has not functioned. The sporogenous cell of Fig. 1 enlarges considerably, becoming meanwhile sunk four to five layers deep by the periclinal divisions of the overlying cells, until it attains the size shown in Fig. 3, when it passes into the megaspore mother-cell condition; the nucleus of the cell there figured is in an early stage of synapsis. In a preliminary note,[1] it was stated that this cell 'appears to form a row of three (?) macrospores'. Further examination leaves no doubt that the reduction division takes place in the embryo-sac itself, and that a preparation formerly interpreted as a row of three megaspores, the upper two disintegrating, must really represent an enlarging mother-cell capped by crushed nucellar cells. Characteristic stages of the reduction division are shown in Figs. 4–8. These are apparently passed through with some rapidity, as ovules in the same ovary may show all stages from the mother-cell nucleus before synapsis to the four-nucleate sac of Figs. 9–11. Material was fixed at different times of day

[1] Stephens, 1908, p. 329.

with the object of securing these divisions, and all the stages here figured, and also the dividing endosperm nucleus of Fig. 25, were found in material fixed on several occasions within a few minutes of mid-day. Other nuclear divisions showed no such relation to any particular time of day. The metaphase of the heterotype division was only seen in one case (Fig. 6), the spindle being here arranged obliquely to the axis of the ovule. The gametophytic number of chromosomes is eleven or twelve. The metaphase of the homotype division is shown in Fig. 7, and the four daughter-nuclei derived from this division in Figs. 8–12. These nuclei usually lie tetrahedrally or crosswise, but they show some variation in this respect; thus in Fig. 10 three are grouped at the bottom of the sac, and the fourth lies towards the top.

As the embryo-sac enlarges a central vacuole is formed, and these nuclei are relegated to the parietal layer of protoplasm (Figs. 11, 12). Each nucleus now divides (Fig. 12), so that four pairs of nuclei are formed (Fig. 13). In one case five groups of nuclei, consisting of four pairs and one single nucleus, were present at this stage (Fig. 14). Possibly this may mean that one of the four original nuclei has divided in advance of the others, and its daughter-nuclei have divided again after becoming separated as the sac enlarged. This preparation was fixed in acetic-alcohol, and is so much contracted that the wall of the embryo-sac has become drawn in, and the nuclei of the nucellar cells which have been crushed by the growth of the sac can be seen imbedded in it. This was the only case observed, at this or at any later stage, where more than the normal number of nuclear or cell groups were present. The four pairs of nuclei normally formed are found usually lying crosswise, one at each end of the sac, and the two at the sides; and as the embryo-sac shows much less variation in the arrangement of its nuclei at this than at the four-nucleate stage, it is probable that the nuclei usually take up this position as the sac elongates. This also is the arrangement most commonly found in the mature embryo-sac (cf. Figs. 17, 18, 25, 26), but it should be noted that both the free-nuclear groups, and the cells that are later formed from them, may take up any position around the periphery of the sac (cf. Figs. 15, 16, 19, 21).

Each of the nuclei composing these four pairs now divides again (Fig. 15), so that four groups, each consisting of four nuclei, are formed (Fig. 16), one group from each of the four original nuclei. The two successive divisions to form these tetrads are usually simultaneous, but occasionally one nucleus divides in advance of the others. Protoplasm now aggregates around three of the nuclei in each group, and a definite limiting membrane appears round each of the cells thus formed,[1] while the fourth nucleus remains free (Fig. 17). While these membranes are appearing, or even after they have been formed, the fourth free nucleus of each group migrates to the centre of the embryo-sac (Fig. 18). There these four meet (Fig. 19), and gradually

[1] Exceptionally, two nuclei may be enclosed in a single cell (cf. Fig. 25).

fuse to form the primary endosperm nucleus. A series of stages of this fusion is shown in Fig. 20. The process of fusion seems to occupy a comparatively long time. The nuclei can usually be seen in contact in the centre of the sac in the ripe flower bud, but the mature definitive nucleus (Fig. 20 *d*) has only been seen in a couple of cases where some of the other ovules in the same ovary had already been fertilized. While these nuclei are fusing, the three cells that remain in each of the four peripheral groups have taken on more or less of the appearance and arrangement of an egg-apparatus. In Fig. 19 *a*, the group at the bottom of the sac already shows this form, and the other groups are assuming it. It will be noted that the cells in the group indicated are rather more equal in size and form than is quite typical for an egg-apparatus, and this equality is often much more marked (cf. Pl. XXVI, Fig. 25). Another variation in form occasionally seen is that of the uppermost group of Fig. 17, where one of the cells is small and the two others are elongated, with their nuclei lying at their free ends—the whole group appearing to consist of two eggs and one synergid. This is exactly the appearance described by Modilewski[1] as typical for the two lateral cell-triads of the sac of *Euphorbia procera*, which is constructed similarly to that of the Penaeaceae. In the case of the Penaeaceae, however, this or any other deviation from the normal form of an egg-apparatus is shown indifferently by any of the groups, and examination of several hundred embryo-sacs about the fertilization period has made it clear that these groups are as alike in structure as they are in development. Indeed, when (as quite often occurs) two groups are equidistant from the apex of the sac (Fig. 21 *a*), or, as has been several times observed, three groups are thus placed (Fig. 21 *b*), it is impossible to say before fertilization which is to function as the egg-apparatus. For this reason I have preferred to use the non-committal term ' peripheral group ' for all of these cell-triads.

In the usual arrangement of these groups (that of Figs. 17–19), naturally the one at the apex of the sac has the best chance of being fertilized, and from it the embryo therefore usually arises. In several cases, however, embryos have been observed developing at the sides of the sac in positions so far removed from its apex as to suggest that, if the sac possessed before pollination the structure shown in Figs. 17–19, fertilization must have taken place in one of the lateral groups instead of that nearest to the apex. The clearest case of this—one in which the remains of the apical group can still be seen—is shown in Fig. 29. This also illustrates the only certain case of polyembryony noted. As there is no indication of parthenogenesis or apogamy, but on the contrary pollen-tubes are freely formed and fertilization has been several times observed (though in the apical group only), this might perhaps be provisionally interpreted as a case where normal fertilization has occurred in two of the other peripheral groups instead of in the apical one. This suggestion, however, is only tentative.

[1] Modilewski, 1909, p. 22.

The mature embryo-sac, therefore, in all the species studied contains four peripheral groups of cells, each group more or less resembling an egg-apparatus, and four nuclei fusing to form the primary endosperm nucleus. Some interesting exceptions to this normal type may now be noted. In six cases it was found that more than four nuclei were fusing to form the primary endosperm nucleus. In two of these cases, the total number of nuclei in the sac could not be accurately computed; one of the two showed either seven or eight nuclei thus fusing, and only three peripheral groups present, two of which were normal, while the composition of the third was uncertain. In the remaining four, sixteen nuclei altogether were present in each case, and it would seem probable that the sixteen-nucleate stage of Fig. 16 had been reached as usual, but that then more than the customary four nuclei had been contributed to form the primary endosperm nucleus. Two of these cases showed five nuclei thus fusing (cf. Fig. 20 *b*), one of the peripheral groups being correspondingly two-celled; in the remaining two there was seen a fusion of six nuclei, an extra nucleus having been contributed from two of the groups, each of which consisted of only two cells (cf. Fig. 23). Two cases should also be noted in which it seemed as if less than sixteen nuclei had been formed in the embryo-sac. One of these is shown in Fig. 22. Here the large nucleus at the apex of the sac may perhaps be interpreted as one of the four original daughter-nuclei which has not undergone division (becoming directly converted into an egg) or has only divided once. The latter is the more probable interpretation, as while the fusion nucleus seems here to be made up of only three nuclei, the other peripheral group figured is not connected with it by the usual protoplasmic strands, and does not seem to have contributed to it. If this interpretation be correct, the second mitosis has been omitted in this group also, as only two cells are present in it. In the other similar case, one peripheral group is again represented by a single cell, and probably here also the original nucleus of this group has only undergone one division, for only the normal four nuclei are fusing to form the definitive nucleus. The remaining groups in all these cases are quite normal.

Endosperm.

With the few exceptions above recorded, the primary endosperm nucleus is always formed by a fusion of four nuclei. Whether a male nucleus usually enters into its composition is still uncertain, owing to the difficulty of interpreting the fertilization processes in these plants—the embryo-sac being small, and the male nuclei showing no differentiation in size, form, or staining properties to distinguish them from the other nuclei present in it. Fertilization, apparently normal, has been observed in the apical group of cells; figures of it have been reserved till the point mentioned has been settled. In *Euphorbia procera*, where the male nuclei are easily

distinguishable, such a fusion of the second male nucleus with the primary endosperm nucleus is seen,[1] and there is no reason why it should not also take place in the Penaeaceae.

If fertilization does not take place, the endosperm nucleus ordinarily disorganizes and disappears with the other contents of the sac. In one case, however, it was seen to divide before fertilization had occurred (Fig. 25). A similar formation of endosperm before fertilization (apparently the only clearly proven case among plants where fertilization normally occurs[2]) has been reported as being occasionally seen in Ranunculus.[3] Coulter suggests that the endosperm nucleus might here have been stimulated to divide through the influence exercised by the presence of the pollen-tube in the style being felt in the embryo-sac. The same explanation is possible in the case here figured, as pollen-tubes had already entered several other ovules in the same ovary. A like stimulus of the endosperm nucleus to division is seen in the Caprifig,[4] the exciting cause here being the presence of the egg of the Blastophagus wasp. Such cases of division of the primary endosperm nucleus before fertilization furnish a suggestive comparison with the behaviour of the same nuclei in *Welwitschia*.[5]

Immediately after fertilization, the embryo-sac begins to enlarge rapidly at the expense of the surrounding nucellar tissue, until by the time the seed is ripe the nucellus has entirely disappeared. Even before fertilization the cells in the mid-line of the nucellus seem to part with some of their contents, losing in consequence their rounded outlines, and fitting together more compactly than the surrounding tissue (Fig. 24). If the ovule is not fertilized these axial cells simply collapse together under the pressure caused by fertilized ovules developing in the same loculus; but if fertilization does take place, they rapidly disorganize (Fig. 26), and the embryo-sac, which is remarkably long and narrow, grows down into the cavity thus formed (cf. Figs. 27, 28 *a*, 30 *a*, 35). The unfertilized cell-groups usually break down and disappear as soon as the embryo-sac begins to enlarge, but sometimes (very rarely) one or more may persist through the first few divisions of the embryo. As a rule, the endosperm nucleus begins to divide immediately after fertilization, or while fertilization is proceeding, and before the fertilized egg, 8–12 nuclei being present by the time the embryo is two-celled (cf. Fig. 27). (Fig. 29, however, if correctly interpreted, shows a case in which fusion of the primary endosperm nucleus is not yet complete, though fertilization has apparently taken place in the apical group and the other peripheral groups are already disorganizing.) I have not observed the stages of its first divisions, but later the endosperm nuclei have often been seen actively, and for the most part simultaneously, dividing (cf. Fig. 32). As might be expected from their multinuclear origin, they have a large number of chro-

[1] Modilewski, 1909, p. 23. [2] Coulter and Chamberlain, 1904, pp. 167, 170.
[3] Coulter, 1898, p. 83. [4] Du Sablon, 1908. [5] Pearson, 1909.

mosomes, and usually several nucleoli (cf. Figs. 31–3). The endosperm exists at first only as a number of nuclei, scattered through the parietal layer of cytoplasm which surrounds the large central vacuole that is formed as a result of the growth of the embryo-sac (cf. Figs. 27, 28 *a*, 30 *a*). As may be seen in Figs. 28, 31–3, at the micropylar end of the sac, this layer closely invests the embryo. In the thicker protoplasm found at the lower end of the sac as it extends downwards into the nucellus, from three to eight of the endosperm nuclei become associated in a deeply-staining group, their nucleoli at the same time fusing to form one large and deep-staining nucleolus in each. This group is doubtless haustorial in function, passing on to the rest of the embryo-sac food obtained from the disorganization of the axial cells of the nucellus. After these cells have been absorbed the group is no longer recognizable. It is not until the embryo has attained to the size shown in Fig. 33 that any cell-walls are formed in the endosperm. Cell-wall-formation continues until by the time the cotyledons have appeared (Fig. 35), the greater part of the embryo-sac is filled with a delicate endosperm tissue. This is all used up by the developing embryo, which grows down into it.

Embryo.

The most striking fact in connexion with the development of the embryo is the absence of any form of suspensor in even the youngest stages. The first wall formed is transverse (Fig. 27) ; a longitudinal wall is then formed in each cell (Figs. 28, 29 *b*), and the whole of the spherical proembryo thus formed enters into the formation of the embryo, which preserves this spherical outline until the formation of the plerome (cf. Fig. 32). The dermatogen early becomes differentiated (Figs. 30, 31), and the plerome, is marked out at a slightly later stage (Figs. 32, 33). The embryo begins to elongate (Figs. 33, 34), and the cotyledons are formed (Fig. 35). They are very slightly developed (Fig. 36), the storage function, which is their normal one in an exalbuminous seed, being here performed by the unusually massive hypocotyl, whose cells are closely packed with starch. This great development of the hypocotyl, and its adaptation thus early in life to the function of food storage, may very probably be considered as an adaptation to a xerophilous habit. The seedlings of geophilous xerophytes have been shown to exhibit two marked characteristics[1]—a tendency towards reduction in the size of the cotyledons (usually by a partial fusion, or by the abortion of one), and an adaptation of the hypocotyl for the storage of food after the seed has germinated. In this strongly xerophilous and probably ancient order it is then not surprising to find similar characteristics so marked even in the seed.

The root-tip of the embryo in the mature seed (Fig. 37) has some peculiar features. It is of unusual breadth, and the growing point is situated at the bottom of a slight depression. There is no root-cap, and the

[1] Sargent, 1903, pp. 78–81.

dermatogen is a single external layer. It may be anticipated that a root-cap is formed, when germination begins, by the periclinal division of the dermatogen overlying the tip, but as uniform failure has attended all attempts to germinate the seeds, this point is still in doubt.

The chief peculiarities of the embryo are thus :—

(1) Absence of a suspensor.

(2) The great development of the hypocotyl and its adaptation thus early in life to the function of food storage.

(3) The reduction in size of the cotyledons.

(4) The absence of a root-cap at the stage found in the mature seed.

Discussion.

Before discussing the nature of this type of embryo-sac it may be worth while to note a few facts regarding the affinities and distribution of the little-known order in which it occurs. The Penaeaceae show no very close relationships to other orders, except to the monotypic Geissolomaceae, which is united to them by Bentham and Hooker. Both Engler and Bentham and Hooker have placed the order near the Thymeleaceae (Engler among the Thymeleales, Bentham and Hooker among the Daphnales), and this seems to be its natural position; Van Tieghem, however, mainly on the strength of its anatomical characters, places it next to the Melastomaceae.[1] It was hoped that this investigation might throw some light upon its true systematic position, but the whole life-history here disclosed is so abnormal that it merely serves to isolate the order still further. It is true that Modilewski has recently described in *Euphorbia procera*[2] an embryo-sac which, so far as his investigations have been carried (from the four-nucleate stage to the first divisions of the embryo), shows a structure almost exactly the same as that here described. But as the embryo-sac in other species of *Euphorbia* is of the normal type, and as the two orders otherwise show no points of resemblance sufficient to warrant a close relationship, there appears to be no doubt that this is a case of parallel development. As regards the other orders with which the Penaeaceae are grouped, the Thymeleaceae is the only one of whose life-history any account has been published, Strasburger[3] having recently investigated species of *Wiksstroemia*, *Daphne*, and *Gnidia*, none of which show a similar departure from the normal. The same may be said for *Cryptadenia uniflora*,[4] of which I have made a preliminary examination. *Geissoloma marginata*, the sole representative of the Geissolomaceae, appears to show other peculiarities, which will be described later.

The peculiarly limited distribution of this order may be of interest in connexion with its rather isolated systematic position. It is entirely

[1] Van Tieghem, 1893, p. 291. [2] Modilewski, 1909. [3] Strasburger, 1909.

[4] Mr. A. J. Ballantine kindly preserved material of this for me.

confined to the south-western region of South Africa, a region in which 'an extraordinary number of species, many of them belonging to a few genera and orders elsewhere rare, are massed . . . between the sea and a desert interior'.[1] Hooker has pointed out[2] the striking affinities that this flora shows with that of Australia, especially South-West Australia, and the less marked relationships of these two floras to that of the Antarctic regions. This connexion between these three southern floras, combined with their separation from the tropical floras which succeed them to the north, seems to him to indicate that they may have been 'members of one great vegetation, which may once have covered as large a southern area as the European now does a northern'.[3] Schönland, recently re-examining the evidence derived from the distribution of the plants composing these floras,[4] comes to the conclusion that there was a direct land-connexion between Australia and South Africa at the time when the types common to the two developed, and a later one between Australia and South America. The isolated groups of this now dismembered flora are being encroached upon everywhere by northern forms, through whose usurping tendencies the small local genera are beginning to disappear.[5] Bolus says:—'Few botanists who, like the present writer, have spent many years in South Africa, and especially in the south-western districts, have not been penetrated by a gloomy impression that the south-western flora is dying out and is doomed to extinction. . . . In general, species of the Bruniaceae, Penaeaceae, and Proteaceae, so peculiar to this region, seem to have become much more rare.'[6] He adds:—'No weight can be attached to this, for it is wanting in adequate evidence'; but as regards the Proteaceae, Phillips's recent investigations[7] show that the power of setting seed is in many species on the wane, and in the case of the Penaeaceae, it may be significant in this connexion to note that although they set seed freely, all attempts to germinate these seeds have failed, and in the genera examined careful search on many occasions has failed to discover any seedling plants. Moreover, even young plants less than about a dozen years old seem extremely rare; I have not found above five such in all the species examined.

This order then may probably be regarded as belonging to an ancient flora, and its restriction to the one region within that flora would seem to indicate either that it is a 'survival' which has lingered on in this south-western region, while dying out in other regions where the flora to which it belonged still flourishes; or else that it is a new type developed since the isolation of this region. Two considerations would seem to point to its being an original member of the flora rather than a lately-evolved

[1] Hooker, 1859, p. xcii.
[2] loc. cit.
[3] Hooker, 1859, p. civ.
[4] Schönland, 1909.
[5] Hooker, 1859, p. cv.
[6] Bolus, 1905, p. 235.
[7] Ballantine, 1909, p. 161.

type, namely, the absence of close affinities to other types in the same region, and the pronounced adaptation it shows to xerophilous conditions—an adaptation which extends even to details of the embryonic structure, and must have been the result of a prolonged exposure to such conditions. Additional weight is lent to this view by the fact that in all three genera investigated the embryo-sac shows the peculiar structure that has been described ; these genera then would appear to have been evolved since the establishment of this type of sac. In considering the morphological bearing of any deviation from the normal angiosperm life-history shown by such a group there are two possibilities to be kept in view ; they may be relatively primitive features retained by it, or recent developments of no phylogenetic significance. The former view would seem more probable on the preceding facts, and it would be quite legitimate to regard this sixteen-nucleate embryo-sac as more primitive than the usual eight-nucleate type if it were formed, as is usually the case in the latter, from one of four megaspores. Indeed, although this is not the case, it is still quite possible to consider that the reduction division by which these megaspores are formed has here been shifted, and takes place at the germination of the spore—that is, the megaspore mother-cell functions directly as the embryo-sac, and all the nuclei formed in it belong to the gametophyte. This view is held by Ernst, who has discussed [1] this point for the sixteen-nucleate embryo-sacs of *Gunnera*, *Peperomia*, and the Penaeaceae. In all of these the formation of walled megaspores is omitted, and this is a strong argument in favour of regarding them as derived rather than primitive forms. If they were really primitive in respect of their embryo-sac structure, the normal row of four megaspores might be expected. Ernst, however, arguing from such cases as that of the Liliaceae, where tetrad-formation may be partly or entirely suppressed within the limits of a single order without influencing the development of the embryo-sac, considers that these processes of development that go on within the embryo-sac are quite independent of its origin, and can be considered apart from it. He points out that the divisions within the macrospore mother-cell are the last divisions within a macrosporangium, while those within the embryo-sac represent the germination of one of its derived spores, and concludes that these two processes can undergo various grades of reduction quite independently of one another; thus if the two divisions forming the spore-tetrad are omitted, chromosome reduction will be shifted so as to take place within the spore at its germination.[2] This he considers has happened in the cases under discussion.

But on the other hand, sporogenesis is a normal phase in the life-history—the culminating point of the sporophyte generation—and some

[1] Ernst, 1908 (B), pp. 26–9. As regards the Penaeaceae, Ernst was misled by my former statement (see ante, p. 364) that three megaspores appeared to be formed.

[2] See also Farmer, 1907, pp. 192, 196.

writers even consider that the four nuclei formed by the reduction division should be always regarded as the nuclei of four spores, whatever may be their subsequent history.[1] The gametophyte of plants, normally a post-meiotic phase,[2] is to be regarded on this view as always post-meiotic, the number of cell-generations in it corresponding to the number of post-meiotic divisions of these four nuclei.

This view has lately been elaborated for the embryo-sac of the angiosperms by Coulter,[3] who applies it to all cases in which the mother-cell divides only once, or else functions directly as the embryo-sac ; and therefore in which, according to him, two spores in the first case, and four in the second have been involved in forming the embryo-sac. That this is probably the case in *Peperomia* has been shown by Brown,[4] who describes the formation of evanescent walls or cell-plates between the first four nuclei of the embryo-sac in the species he has studied. Though careful search has discovered no signs of such walls or cell-plates in the first divisions of the macrospore mother-cell in the Penaeaceae, the absence of a row of megaspores, combined with the tetrahedral arrangement of the first four nuclei formed, makes it seem possible to the present writer that the peculiarities of the embryo-sac may here also be best explained by regarding it as a specialized type, in the development of which all four megaspores have become included, the germination of each ceasing at the four-nucleate stage. Some similar cases of four potential megaspore nuclei being included in a single cell (only one, however, germinating) are cited by Brown.[5] Evidence on this point is unfortunately wanting in *Euphorbia procera*, since Modilewski's material showed nothing earlier than the four-nucleate stage. If the early stages of development in this form should prove to be the same as in the Penaeaceae, it is probable that the two may be regarded as cases where specialization has proceeded on parallel lines.

Whether the embryo-sac of these Penaeaceae is to be regarded as a relatively primitive one, or as one which has been reduced and specialized in the manner suggested, two explanations of its structure will still both be applicable. One of these has been advanced by Ernst, who applies to the Penaeaceae[6] the homologies suggested by Porsch[7] as an explanation of the embryo-sac and double fertilization in the angiosperms. Porsch interprets the egg-apparatus as being homologous with a Gymnosperm archegonium, the synergids representing the neck cells,[8] and the antipodal cells as being equivalent to another 'sexually-degenerate' archegonium.

[1] Davis, 1905, pp. 471–2.
[2] Farmer and Moore, 1905, pp. 548–9.
[3] Coulter, 1908.
[4] Brown, 1908.
[5] ibid., pp. 453–4.
[6] Ernst, 1908 (A), p. 434 ; 1908 (B), p. 33.
[7] Porsch, 1907, pp. 19, 20.
[8] Chamberlain (Bot. Gaz., xlvi, p. 155, 1908) and Went (1909, p. 13) interpret Porsch as holding the synergids homologous with neck *canal* cells, but this seems a misinterpretation of Porsch's view.

The polar nuclei are homologized with ventral canal cells, and as the ventral canal cell has been shown capable of fertilization, the fusion of the second male nucleus with them can be regarded as a kind of fertilization, and the endosperm as of the nature of a modified embryo. Ernst, applying this 'Archegontheorie' to the Penaeaceae, considers then that each of the four peripheral groups represents an archegonium, and that the four nuclei fusing to form the primary endosperm nucleus are the four ventral canal cells of these archegonia. This would hold equally well if each group were regarded as formed from a macrospore, only in this case the sac would be reduced rather than primitive, as Ernst interprets it. But it is difficult to imagine that a gametophyte so greatly reduced as that of the Angiosperms could continue to form the rudiments of such specialized structures —structures which in the Gymnosperms appear only at a much later stage in the life-cycle, and moreover have disappeared in the highest group of Gymnosperms, so that in *Welwitschia* and *Gnetum* the naked cells of the prothallus function as eggs. Moreover, a view that regards the synergids as homologous with neck-cells affords no explanation of such a case as that described for *Naias major*,[1] where a synergid is often fertilized and gives rise to an embryo. Porsch, it is true, regards such 'Synergidenbefruchtung' as of no more significance than the fact that embryos can arise from nucellus or even integuments,[2] but the cases are surely very different, the latter being merely sporophytic budding. For these reasons, the present writer is inclined to look for a more probable explanation for the peculiarities of this embryo-sac.

A suggestion which seems of service here is that offered by Pearson regarding possible homologies in the embryo-sac of the primitive angiosperm. In an abstract of some recent observations on *Welwitschia*,[3] he describes all the nuclei found in the embryo-sac at the end of free-nuclear division as being potential gametes. Some of these remain free and can function as gametes; the remainder fuse to form the primary endosperm nuclei. He suggests that the endosperm of the primitive angiosperms was homologous with that of *Welwitschia*. If the sixteen nuclei formed in the free-nuclear stage in the Penaeaceae can be similarly regarded as potential or reduced gametes, and the endosperm as physiologically homologous with that formed by the fusion-nuclei in *Welwitschia*, the chief peculiarities noted—the apparent formation of an egg-apparatus in each peripheral group; the equality in development and structure of these groups and their component cells; the formation of a normal endosperm by a fusion of four (exceptionally more) nuclei; the exceptional case of division of the primary endosperm nucleus before fertilization—all find an explanation. This suggestion thus seems to the writer to meet the facts of the present case. If this embryo-sac can be regarded as more primitive than the normal type,

[1] Guignard, 1901. [2] Porsch, 1907, p. 25. [3] Pearson, 1909.

it forms an interesting transition stage between that type and a possible ancestor possessing a larger number of free nuclei, sufficient to form several primary endosperm nuclei where here only one is present. On the other hand, if, as seems more probable, it is a derived form, the same hypothesis is equally helpful, especially in explaining how it is possible for a normal endosperm to arise from a four-nucleate instead of the usual bi-nucleate fusion.

Summary.

Species of *Sarcocolla*, *Penaea*, and *Brachysiphon*, three of the five genera of the rather isolated order Penaeaceae, have been examined. The development and structure of the embryo-sac and embryo in all three genera is the same. The megaspore mother-cell forms four nuclei by a reduction division. These are usually tetrahedrally arranged. They migrate to the periphery of the embryo-sac, and there as a rule lie cross-wise, one at each end and the other two at the sides; their position, however, often varies. Each gives rise to a group of four nuclei. Cells are formed round three of these nuclei in each group, and these cells in each case assume more or less of the appearance of an egg-apparatus; whether they can all function thus is uncertain. The four remaining free nuclei fuse in the centre of the sac to form the primary endosperm nucleus. After fertilization this forms a parietal layer of endosperm nuclei, cell-walls appearing only at a late stage.

The embryo has no suspensor. It divides to form a sphere of cells, which elongates as the plerome is formed. It has a broad root-tip, no root-cap, a massive hypocotyl, and rudimentary cotyledons.

This embryo-sac is probably to be regarded as a derived form, with all four megaspores included in its development, rather than as a primitive one; but in our present state of knowledge of the embryo-sac of the Angiosperms, this cannot be considered as definitely proved. It is suggested that in either case its endosperm is formed, like that of *Welwitschia*, by a fusion of potential gametes, all the nuclei in the sac being looked upon as potential or reduced gametes.

This investigation was begun in the Botanical Laboratory of the South African College, at the suggestion of Professor Pearson, to whom I am indebted for much help and advice in its early stages, and for assistance in procuring material. I wish also to thank Professor Seward for kindly allowing me to continue it in the Cambridge Botany School.

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EXPLANATION OF FIGURES IN PLATES XXV AND XXVI.

Illustrating Miss Stephens's paper on the embryo-sac and embryo of certain Penaeaceae.

Abbreviations used: *end.* endosperm; *em.* embryo; *e.s.* embryo-sac; *e.s.w.* embryo-sac wall; *int.* integument; *m.m.c.* megaspore mother-cell; *nuc.* nucellus; *o.* oospore; *peri.* periblem; *pl.* plerome; *p.g.* peripheral group; *p.n.* polar nucleus; *p.t.* pollen-tube.

PLATE XXV.

Fig. 1. Longitudinal section through young ovule, showing megaspore mother-cell, a periclinal division in the cell-layer above it, and developing integuments. × 390. *Sarcocolla squamosa.*

Fig. 2. Longitudinal section through upper part of nucellus and embryo-sac (at stage of Fig. 13), showing a second undivided megaspore mother-cell (?) above the embryo-sac. × 390. *Sarcocolla squamosa.*

Fig. 3. Longitudinal section through ovule just prior to division of the megaspore mother-cell, in the nucleus of which synapsis is beginning. × 390. *Sarcocolla minor.*

Fig. 4. Nucleus of megaspore mother-cell, showing synapsis. × 1300. *Penaea mucronata.*

Fig. 5. The same, showing chromosomes. × 1300. *Penaea mucronata.*

Fig. 6. Longitudinal section of metaphase of first (heterotype) division of megaspore mother-cell. × 1300. *Sarcocolla minor.*

Fig. 7. Optical section of embryo-sac, made up of two successive transverse sections, showing metaphase of second (homotype) division. × 1300. *Penaea mucronata.*

Fig. 8. Optical section, made up of two successive transverse sections, showing telophase of the same. × 1300. *Sarcocolla minor.*

Fig. 9. Four-nucleate embryo-sac, slightly older than that of Fig. 8, showing tetrahedral arrangement of nuclei. The nucleus marked *a* really lies at the same distance from the one that overlies it in the figure as it does from the two others. × 630. *Sarcocolla minor.*

Fig. 10. The same, showing different arrangement of nuclei. × 600. *Sarcocolla formosa.*

Fig. 11. Later stage, showing nuclei separating. The nuclei marked *α* and *β* occur at the beginning and end respectively of the series of longitudinal sections composing this embryo-sac; the other two lie about its middle. × 630. *Sarcocolla minor.*

Fig. 12. Later stage; nuclei in prophase of division. × 420. *Sarcocolla minor.*

Fig. 13. Eight-nucleate embryo-sac. × 420. *Sarcocolla formosa.*

Fig. 14, *a* and *b*. Same stage but with five groups of nuclei; the fifth group is shown in 14 *b*. (Fixed in acetic alcohol and much contracted, so that the embryo-sac wall can be seen; imbedded in it are the nuclei of disorganized nucellar cells). × 420. *Sarcocolla squamosa.*

Fig. 15. Later stage; nuclei in prophase of division. × 333. *Sarcocolla squamosa.*

Fig. 16. Sixteen-nucleate embryo-sac. × 420. *Sarcocolla squamosa.*

Fig. 17. Later stage; cell-walls are being formed in the peripheral groups; one nucleus in each remains free (*p.n.*). × 630. *Penaea mucronata.*

Fig. 18. Later stage; the free nuclei are moving to the centre of the sac. × 420. *Penaea mucronata.*

Fig. 19, *a*, *b*. Later stage; the free nuclei are meeting in the centre of the sac. The fourth peripheral group lies in a plane perpendicular to the other three, and is seen in 19 *b* in transverse section. × 420. *Sarcocolla squamosa.*

Fig. 20, *a–d*. Successive stages of nuclear fusion; in *b*, a fusion of five nuclei. All × 420. *a*, *b*, *c*, *Sarcocolla squamosa*; *d*, *Penaea mucronata.*

Fig. 21, *a–e*. Diagrammatic outline of five embryo-sacs, showing varying positions of the peripheral groups. The upper half of *d* is shown in Fig. 22, and of *e* in Fig. 23.

Fig. 22. Upper half of embryo-sac of Fig. 21 *d*. with three nuclei fusing to form the primary endosperm nucleus. The two peripheral groups shown contain only one and two cells respectively, the remaining two groups are normal. × 630. *Sarcocolla squamosa.*

Fig. 23. Upper half of embryo-sac of Fig. 21 *e*, with six nuclei fusing to form the primary endosperm nucleus. The two peripheral groups shown contain only two cells; the remaining two groups are normal. × 420. *Sarcocolla squamosa.*

PLATE XXVI.

Fig. 24. Outline of longitudinal section through ovule at the time of fertilization, showing axial row of cells in nucellus. × 28.

Fig. 25. Mature embryo-sac, showing exceptional case of division of the primary endosperm nucleus before fertilization. × 630. *Penaea mucronata.*

Fig. 26. Embryo-sac after fertilization; the unfertilized peripheral groups are disappearing and the cells of the axial row of the nucellus are breaking down. × 630. *Penaea mucronata.*

Fig. 27. Embryo-sac with two-celled embryo. × 420. *Penaea mucronata.*

Fig. 28. Upper part of embryo-sac with four-celled embryo, seen in optical section. The wall dividing the upper two cells is in the plane of the drawing: that dividing the lower two is cut obliquely. An empty pollen-tube is seen in the nucellus. × 390. *Sarcocolla squamosa.*

Fig. 28 *a*. Outline of embryo-sac of Fig. 28, showing endosperm. × 28.

Fig. 29 *a, b*. Sections through the same embryo-sac showing two embryos of eight cells (29 *a*) and four cells (29 *b*) respectively. The latter is seen in obliquely transverse optical section, being in a plane almost perpendicular to that of the drawing. × 333. *Penaea mucronata.*

Fig. 30. Longitudinal section through embryo, showing (probably) the beginning of the dermatogen. × 390. *Sarcocolla squamosa.*

Fig. 30 *a*. Outline of ovule containing embryo of Fig. 30. × 15.

Fig. 31. Longitudinal section of embryo older than Fig. 30, with investing layer of cytoplasm containing endosperm nuclei. × 300. *Sarcocolla squamosa.*

Fig. 32. Longitudinal section through top of embryo-sac at a later stage, showing embryo in median section, and dividing endosperm nuclei. The shaded cell in the centre of the embryo probably represents the beginning of the plerome. × 300. *Sarcocolla squamosa.*

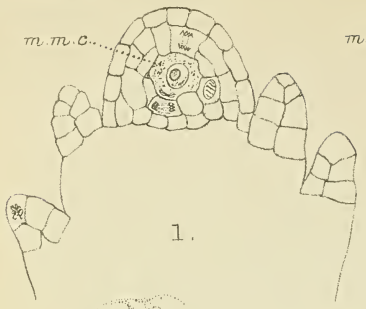
Fig. 33. Longitudinal section through top of embryo-sac at a later stage, showing embryo in median section (with longitudinal division of two cells in the plerome region) and formation of cell-walls in the endosperm. × 196. *Sarcocolla formosa.*

Fig. 34. Longitudinal median section through embryo at a later stage. × 300. *Sarcocolla squamosa.*

Fig. 35. Longitudinal section through ovule at a later stage, showing endosperm tissue and embryo with developing cotyledons. × 15. *Sarcocolla squamosa.*

Fig. 36. Mature embryo. × 15.

Fig. 37. Root-tip of mature embryo. × 125. *Sarcocolla squamosa.*



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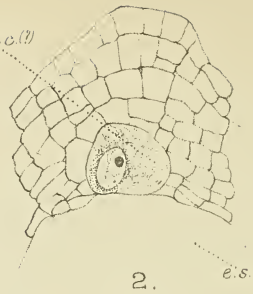

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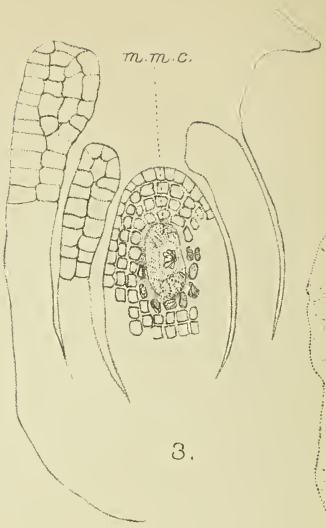

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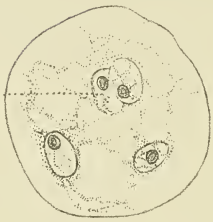

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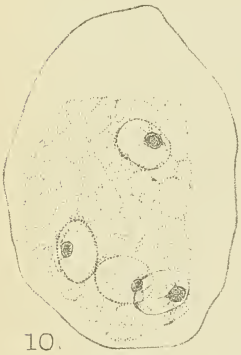

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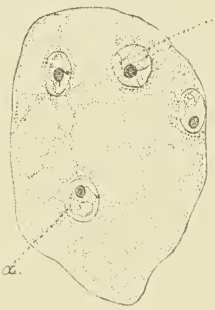

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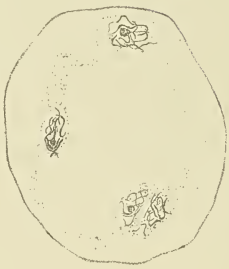

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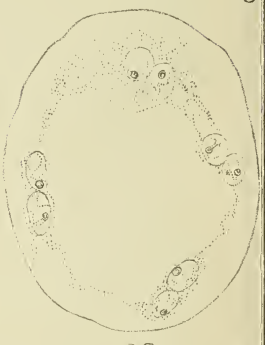

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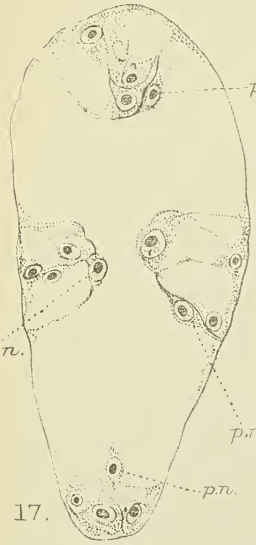

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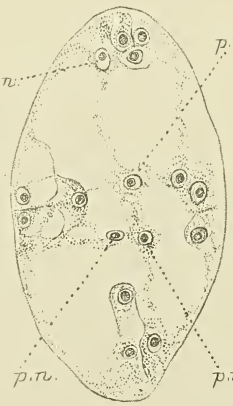

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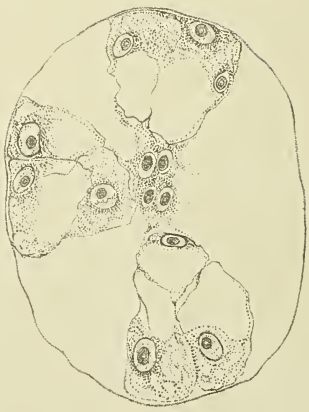

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20a.

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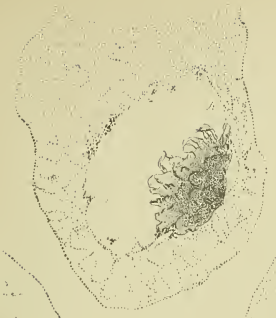
E.I.S. del.

STEPHENS — PENAEACEÆ.



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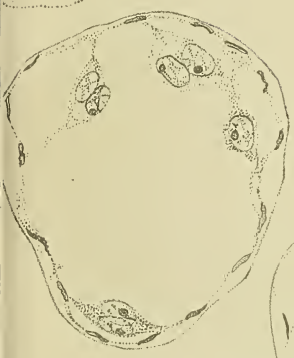

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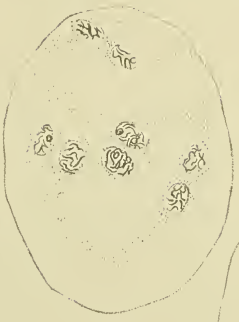

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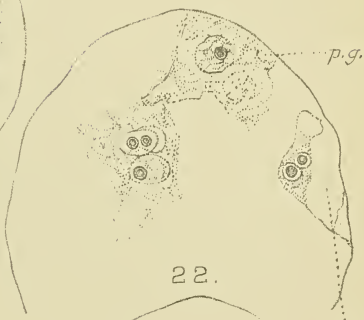

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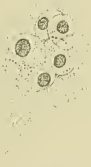

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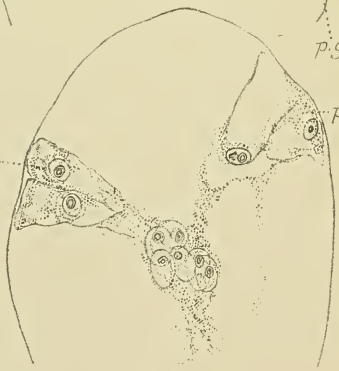

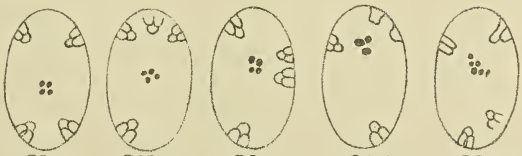

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21b.

21c.

21d.

21e.

23.

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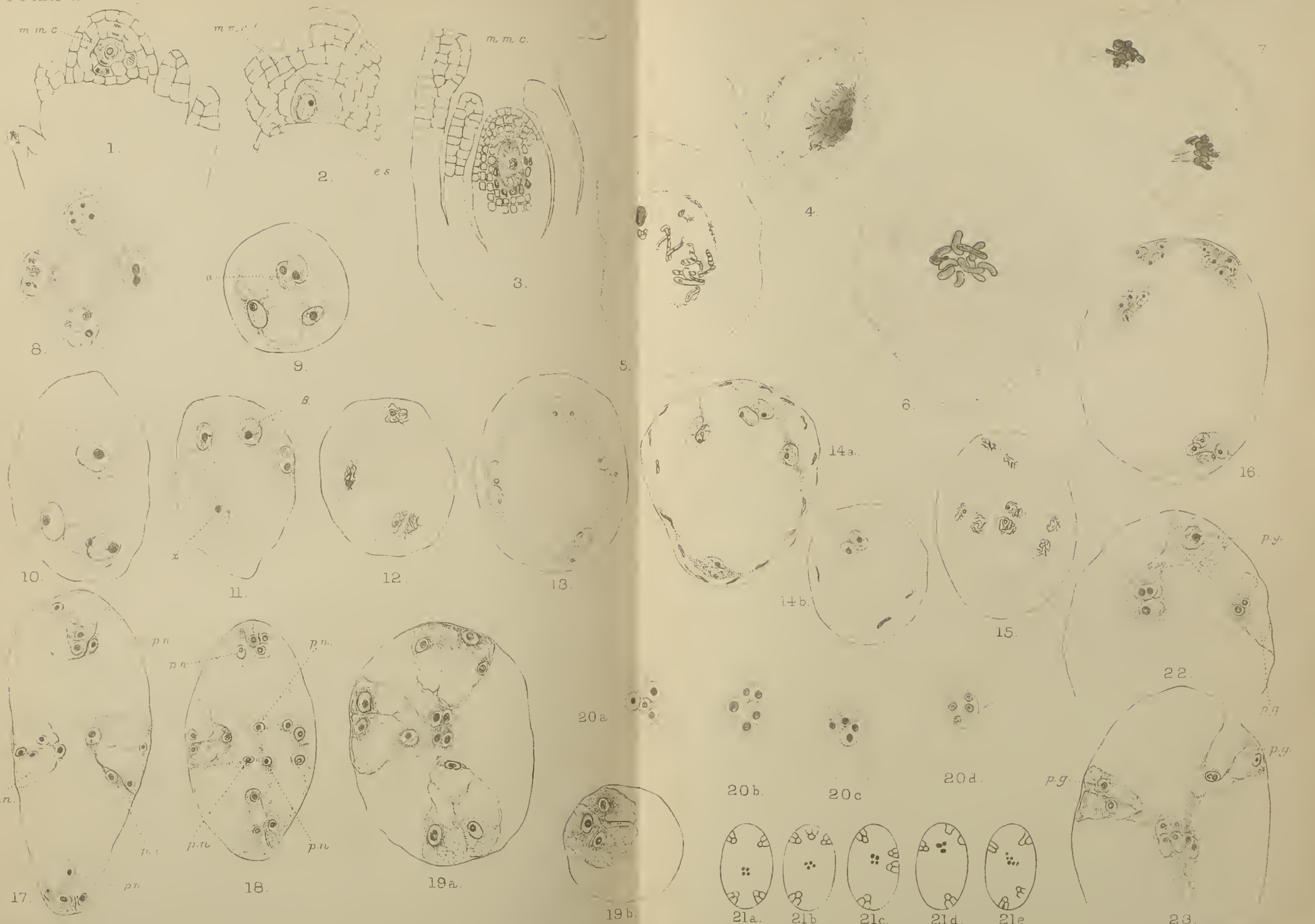

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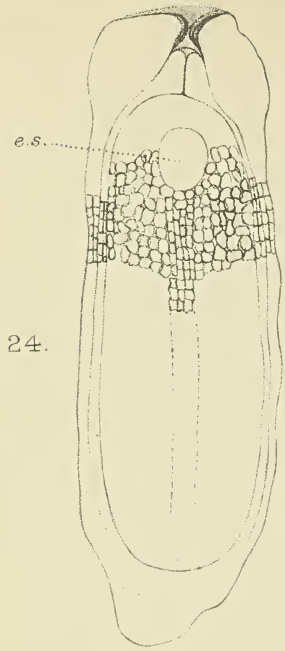

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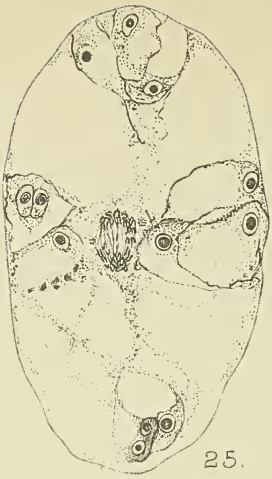

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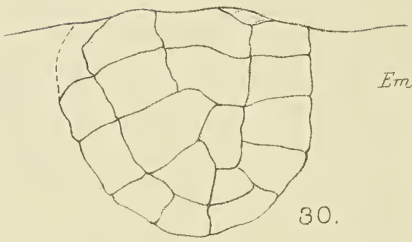

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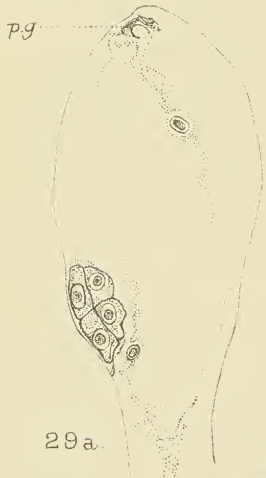

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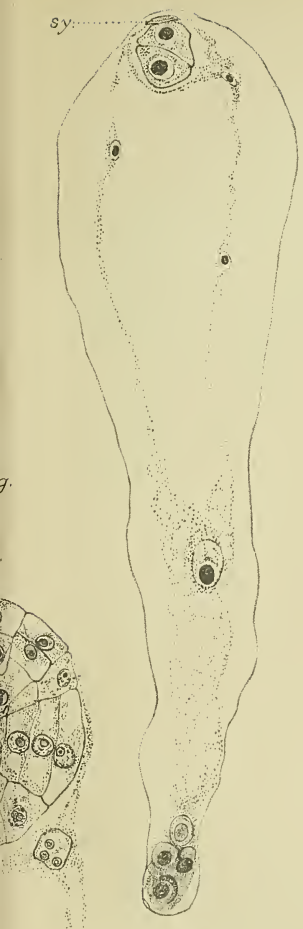
STEPHENS — PENAEACEÆ.



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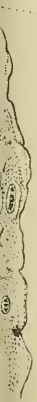

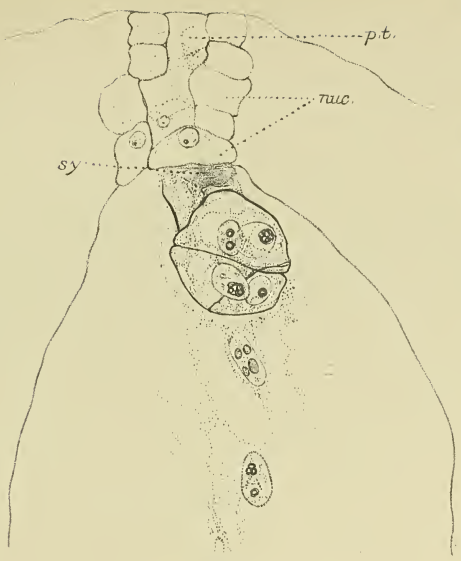

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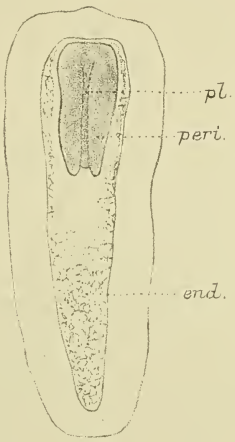

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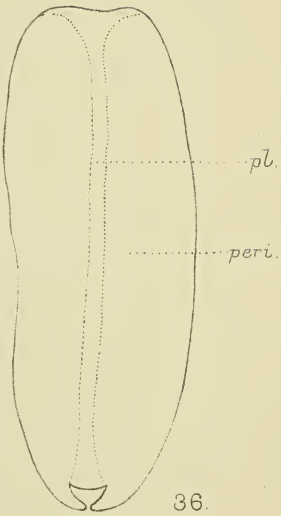

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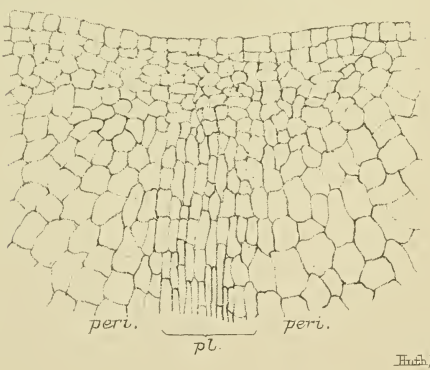

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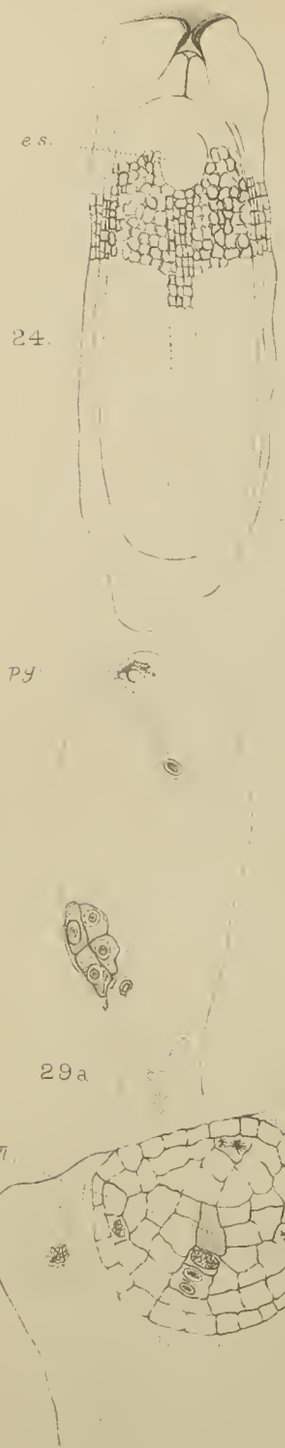
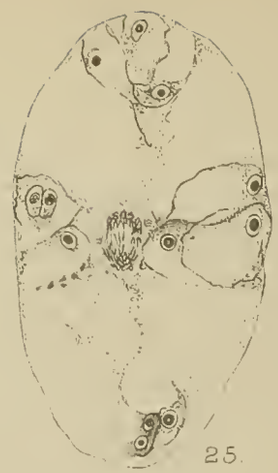
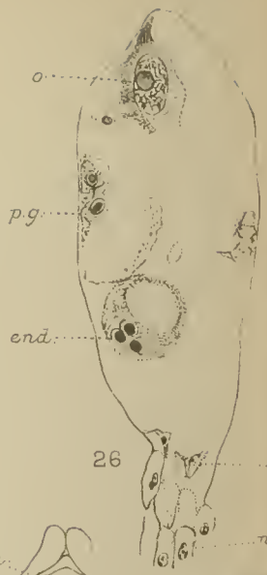
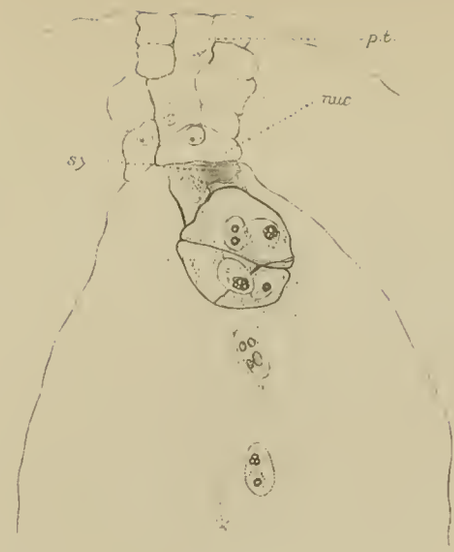
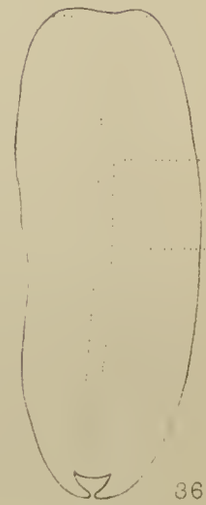
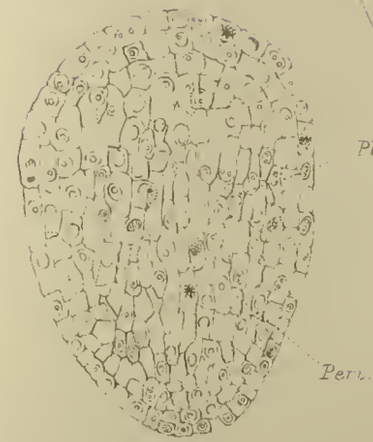


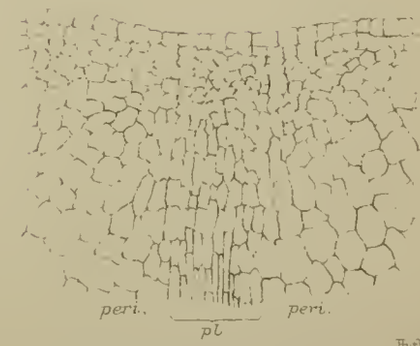












STEPHENS — PENAEACEÆ.

On Mesostrobus, a New Genus of Lycopodiaceous Cones from the Lower Coal Measures, with a Note on the Systematic Position of Spencerites.

BY

D. M. S. WATSON, B.Sc.,

Beyer Fellow of the Geological Department of the Victoria University of Manchester.

With Plate XXVII, and six Figures in the Text.

IN 1907 the Manchester Museum received from Mr. Lomax, of Bolton, a series of four transverse sections of a cone from the Mountain 4 ft. Mine of Cloughfoot, Dulesgate. Mr. Lomax had recognized the specific distinction of the cone from all yet described, and has published a photograph of a section in a work on Coal, by Mr. J. Tonge. Prof. Weiss very kindly handed the sections over to me, and I now wish to offer some description of them.

Of the four sections, the uppermost only shows the sterile portion at the top of the cone, and the lowest is through a part of the cone that had been badly damaged before fossilization; only the middle two are therefore of much use in gaining an idea of the arrangement of the parts of the cone, and one of these two has somewhat broken up during the process of grinding the section. The sections are, however, enough to enable one to give a fairly complete account of the whole structure of the cone. The axis of the cone is 2·6 mm. in diameter (Pl. XXVII, Phot. 1).

The wood is small, only 0·34 mm. in diameter, and is solid, there being no pith (Phot. 3 and 4 *xy*). The protoxylems apparently do not project much, and are not very well preserved. The main mass of the wood is composed of tracheids of nearly equal lumen, which do not seem to pass by any easy transition to the smaller tracheids of the protoxylem, which appear to form small groups on the outside edge of the wood. The wood is succeeded by an ill-preserved belt of tissue which includes, no doubt, the phloem and (?) pericycle (Phot. 4). This layer shows that the phloem was broken up by patches of parenchyma on the inside of the leaf traces in the way common in the Lepidodendra.

[Annals of Botany, Vol. XXIII. No. XCI. July, 1909.

It is succeeded by a zone of parenchyma, seven or eight cells wide, which is composed of isodiametric cells about 0·015 mm. in diameter; this belt is the zone, called by Bower, Weiss, and myself the inner cortex; it has a definite termination outwardly, and is succeeded by the middle cortex. This forms a zone about 0·16 mm. in breadth (Phot. 3 and 4). It is composed of isodiametric cells about 0·05 mm. in diameter, which have large air spaces between them. It is not very well preserved, but this is, I believe, the first Lepidodendroid cone, except *Lepidostrobus Brownii*, in which it has been at all fit for description. It resembles exactly the middle cortex of any small Lepidodendroid twig, and bears a particularly close resemblance to that of an undescribed *Lepidostrobus*, of which I possess sections.

The middle cortex changes suddenly into the outer cortex (Phot. 3). This latter consists of a parenchymatous tissue which is strengthened by a sclerized[1] skeleton. The parenchymatous foundation is composed of cells which are isodiametric in transverse section, and about 0·04 mm. in diameter; so far as can be seen from transverse sections, the sclerized portions are composed of cells of exactly similar size and shape.

The sclerized skeleton is associated with the insertion of the sporophylls, and is arranged roughly as follows (Phot. 1):—

At the lowest point, where the sporophyll-trace is just emerging from the inner cortex, it forms, as seen in transverse section, a small patch on the inner side of the outer cortex. Followed upwards this patch enlarges radially until, where the leaf-trace begins to turn out from the middle cortex to the outer cortex, it forms a somewhat narrow plate extending radially across the entire outer cortex. This gradually broadens tangentially until it is the entire width of the base of the sporophyll. In this region the trace passes out through a non-sclerized passage in the sclerized tissue. Above the insertion of the sporophyll the sclerized patch gradually contracts, appearing last at the outer edge of the cortex. It is very hard to be sure of the exact details of the arrangement of this skeleton owing to lack of sufficient sections, and to the fact that it is probably liable to considerable individual variation in detail. The foregoing account is, I believe, in the main correct. These patches obviously serve to afford a firm attachment for the sporophyll.

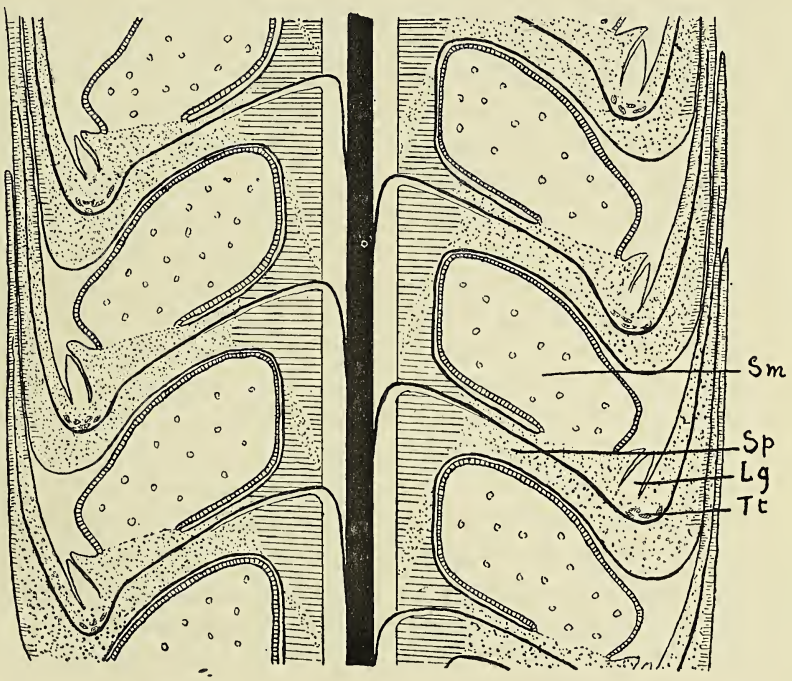
The sporophylls are probably inserted on the stem in a series of spirals, they are at any rate not in whorls.

They are composed of a more or less horizontal portion, and a vertical lamina on the surface of the cone. The horizontal portion is triangular in tangential section (Phot. 5), the upper surface being flat and the under side provided with a fairly sharp keel. The upper portion is of considerable breadth, measuring at its attachment to the axis as much as 1·2 mm. across,

[1] This word is used throughout in a purely descriptive sense.

it retains this width until it expands into the base of the lamina (Phot. 1 and 4).

The horizontal portion is composed of somewhat thick-walled cells which are slightly elongated radially. It is traversed throughout its entire length by the vascular bundle, which passes straight through it. The wood of this bundle arises from the wood of the main axis and ascends steeply through the phloem and inner cortex, as is shown by the number of traces met with in this region in transverse section. It passes quickly through the



TEXT-FIG. 1. Diagrammatic median longitudinal section of a cone of *Mesostrobus Scottii*. ×12 approx. The sporophylls should not be superposed, but there is evidence for all other characters shown. Black = vascular tissue; White = middle cortex and parichnos; Horizontal lines = sclerized areas; Dotting = parenchyma. *Lg* = Ligule; *Tt* = Transfusion tracheids at its base; *Sm* = Sporangium; *Sp* = Sporophyll.

middle cortex and into the outer cortex, into which it is accompanied by only a very small strand of middle cortex as a parichnos. In its course through the outer cortex it is surrounded by a sheath of parenchyma which fills up the hole in the plate of sclerized tissue which supports the sporophyll. In the mid-region of the horizontal portion I have not been able to see any definite parichnos, but I believe it occurs further out, as will be shown later.

The horizontal portion of the sporophyll at about 3 or 4 mm. from the axis broadens out tangentially and vertically to form a solid mass, which is the base of the lamina (Phot. 6 and 7); in this region the bundle takes

a curve downwards, forming a sort of pocket, the upper surface of which is richly lined with transfusion tracheids. This pocket surrounds the base of the ligule, which is set in a deep pit, over the edge of which its tip probably did not rise.

I believe that in this region the parichnos bifurcates, at any rate there are two patches of tissue, one on each side of the vascular bundle, which are often defective, and when present resemble in appearance some examples of the parichnos of *Lepidodendron* leaf-bases; these areas are, however, less well defined than the parichnos of ordinary leaf-bases.

The lamina takes its rise from the upper surface of this mass, and has on its inner surface a groove, which is the continuation of the ligular pit (Phot. 7 *gr*). This groove is exactly similar to one which is a very characteristic feature in the sporophylls of *Bothrodendron mundum*.

The vertical part of the lamina is of very simple structure, being regularly lenticular in transverse section, and having a single vascular strand up the middle (Phot. 7). The main mass of the leaf is composed of ordinary parenchyma limited on the outside by a single layer of cells which forms the epidermis. The vascular bundle, which is central in position, is surrounded by a small amount of transfusion tissue consisting of the familiar short tracheids, with the dimensions of an ordinary parenchymatous cell and spiral thickening. The structure of the bundle is apparently quite simple, but cannot be made out in detail. Just to the outside of the bundle is a rib of sclerized tissue of quite small dimensions, running straight up the lamina, which, as a whole, tends to become sclerized towards the tip. The cells to the sides of the transfusion tissue sometimes have dark walls; they may have been secretory cells of some sort.

The sporangia are inserted on the upper surface of the horizontal portion of the sporophyll, and their attachment extends from just inside the ligule to a spot rather more than half the length of the horizontal portion from the axis (Phot. 1, 4, and 5). They are thus only attached to the distal half of the sporophyll. Their attachment is narrow tangentially, exactly resembling in this respect that of *Lepidostrobus* (Phot. 5). Diagram 1 will convey a much clearer idea of this arrangement than any amount of description.

In the actual cone under consideration the sporophylls incline downwards, and I think that this character is normal, and not the result of post-mortem crushing; at any rate this inclination enables one to settle the question of the length of the attachment of the sporangium quite definitely, for it gives a series of sections across the upper surface of the sporophyll at measurable distances from the axis.

The sporangia are not well preserved, but their wall appears to be only one cell thick, and of the normal Lepidostrobus type. In section parallel to the surface, however, the cells (although isodiametric) are seen to be

arranged in rows, somewhat recalling the arrangement of the cells of the prosenchymatous sporangial wall of *Spencerites*.

The spores themselves are not definitely preserved in any sporangium but two brown masses of ill-preserved material in the lowest section of the series may represent decayed masses of microspores.

The cone which has been described above differs from all other described Lycopodiaceous cones in the manner of attachment of the sporangia. In its general characters it much resembles *Lepidostrobus*, and I think is undoubtedly a member of the Lepidodendraceae; at the same time the distinctly different insertion of the sporangium entitles it to generic distinction, and I beg to propose for it the name of *Mesostrobus Scottii*, in commemoration of Dr. D. H. Scott's connexion with the three most interesting known Lycopod cones, *Spencerites, Lepidocarpon*, and *Miadesmia*.

The genus *Mesostrobus* may be defined as follows:—

Lycopodiaceous cones resembling *Lepidostrobus* in all characters except in having the sporangium only attached to the distal portion of the horizontal portion of the sporophyll.

This diagnosis is to be regarded as provisional; it is possible, for example, that the insertion of the ligule in a deep pit may turn out to be of generic value if further species are found.

Any general discussion of the inter-relationships of the Lycopodiaceous cones has to consider especially the following types:—*Bothrodendron mundum*, *Lepidostrobus*, *Lepidocarpon*, *Miadesmia*, *Lycopodium*, *Selaginella*, *Spencerites*, and the cone just described.

I propose to discuss primarily the relationships of *Lepidostrobus*. This shows itself a highly specialized genus in the great radial extension of the sporophylls. It appears highly unlikely that the primitive Lepidodendroid had this character, and I believe its cone to be nearly represented morphologically by that of *Bothrodendron mundum* and the male *Miadesmia*.

Two cones have now been attributed to *Bothrodendron*; the small cone of *B. mundum* = (?)*B. punctatum*, and *Bothrostrobus Olryi*, Zeiller, which was found by Mr. Kidston, attached to a twig of *Bothrodendron minutifolium*, Boulay. This latter cone is exactly like *Lepidostrobus*, except that its sporophylls are in verticils, and are relatively short.

Bothrodendron is one of the most ancient genera of the Lycopodiales. In the upper Old Red Sandstone of Kiltorkan two species occur. *Bothrodendron kiltorkense* and the plant described as *Knorria* or *Sagenaria Bailyana*; this last plant, as I have determined from the examination of a specimen in the Manchester Museum, is undoubtedly a *Bothrodendron*, although of a rather curious type.

The same plants occur in the Ursa flora of Bear Island, with *B. Wilkianum* in addition.

The only Lycopodiaceous fructification known from Kiltorkan is

Lepidostrobus Bailyanus Sch., which is of the ordinary Lepidostroboid type, and is heterosporous, but appears to be verticillate.

The very close general resemblance between the sporophylls of *Bothrodendron mundum*, *Miadesmia*, and *Selaginella* is an interesting feature. It is fairly certain that *Selaginella* and *Bothrodendron* are not very closely related, and I do not think that there is much more connexion between *Miadesmia* and *Selaginella*.

The evidence connecting *Miadesmia* and *Selaginella* is only that they are both herbaceous forms of ligulate Lycopods, with heterosporous cones and short sporophylls. Miss Benson ('08) in addition points out that the *Miadesmia* stem resembles that of some *Selaginellae* in its wood, and in the presence of trabeculae, probably representing the endodermis.

The wood certainly resembles fairly closely that of some vertical Selaginellas, but it resembles equally closely that of many small Lepidodendroid twigs.

The layer of trabeculae does not show up very well in any of the fairly numerous sections of *Miadesmia* stems that I have now examined, and it might apparently represent the middle cortex of the Lepidodendraceae, some types of which it considerably resembles. Heterospory combined with a ligule and a short sporophyll is met with in *Bothrodendron mundum* in a form which greatly resembles that of *Miadesmia*.

There is, I think, no doubt that the Lepidodendraceae were a dominant group in the Carboniferous period, the very great specialization reached by *Lepidocarpon*, and shown generally by the Halonial branches and Ulodendroid scars is, I think, proof of this.

It is a general rule that a dominant group contains members of all sizes and fitted for many different conditions, for example, the Deinosaurs, the dominant group of land animals during the mesozoic, are represented equally by the giant Sauropods, *Diplodocus* and *Atlantosaurus*, 80–100 ft. long, and by the little *Compsognathus* about as big as a rook and quite as lightly built.

In just the same manner one would, *a priori*, expect the Lepidodendraceae to be represented by herbaceous forms as well as by forest trees, and it is possible that in *Miadesmia* we have one of the herbaceous forms we should thus theoretically expect. So far as I know there is no evidence to prohibit this view, although there is little in its favour; at any rate the evidence is as strong as for its relationship to *Selaginella*.

If these three forms are not closely allied then the striking resemblances between their cones must be due to one of three causes:—

1. That their distant common ancestor had a cone of this type, and they have descended from it without modification.

2. Heterogenetic homoeomorphy, i. e. that their cones were once distinct, and have reached their present resemblances by convergence during their evolution.

3. That their cones were originally similar and have reached their present state after passing through similar series of stages; this is, I believe, expressed by Osborn's term Rectigradation.

The fact that the cones of *Lycopodium* and *Phylloglossum* agree very closely with this type, except in the absence of the ligule, I think lessens very greatly the probability of the second explanation being correct. The assumption here is that, as the ligule is an obviously important organ in the heterosporous Lycopods, it is rather unlikely that the homosporous forms should have reached the same result in its absence, starting from different material.

We are, therefore, left with two theories (the first and third above) to account for the resemblances between these cones; these theories do not differ very much, and I propose to take the first as a working hypothesis and discuss the third later.

It seems pretty safe to say that *Lycopodium*, *Selaginella*, *Miadesmia*, and *Bothrodendron* parted company a considerable time ago, and hence, assuming the first theory to be true, we may suppose that the cone of *Bothrodendron mundum* affords a pretty accurate representation, morphologically, of the primitive Lycopod cone, which would of course be homosporous.

Assuming this, let us investigate what would be the effect of the development of an arboreal habit by the Lepidodendraceae.

There is no doubt that it would lead to an increase in the number of spores required. This increase might be supplied in two ways.

1. By increasing the number of sporophylls, either by increasing the number of cones or adding to their length.

2. By increasing the size of the sporangium.

Increase in the number of component parts is a very clumsy method of achieving the desired result, for a cone with small sporangia has a much smaller ratio of sporogenous to sterile tissue than has a cone with larger sporangia; compare, for example, *Bothrodendron mundum* with *Lepidostrobus*.

Similar considerations show that the most efficient method of adding to the volume of a sporangium is by increasing its radial extension, for a greater height would not only increase the length of axis per sporophyll, but also the size of the lamina required for adequate protection of the spores.

Hence, simple geometrical considerations show that it is likely that the adoption of an arboreal habit would lead to a radial extension of the sporangium, assuming that such habit necessitated increased spore production.

If we suppose the stalk of the *Bothrodendron mundum* sporophyll to be elongated, we get an arrangement by which the radial extension of the sporangium is increased.

If we imagine this to have taken place, we are left with an arrangement recalling that of *Spencerites*.

This arrangement has certain obvious disadvantages :—

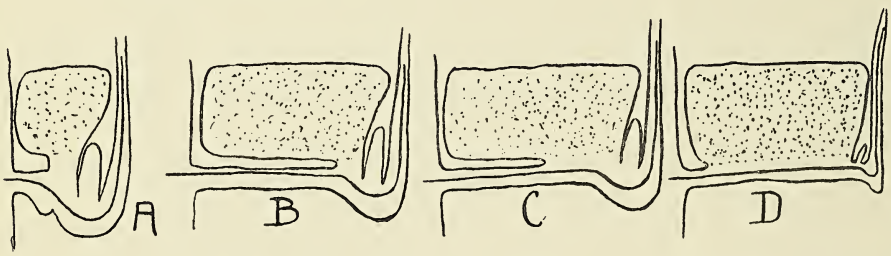
1. The attachment-area of the sporangium is so limited that provision of sufficient food material to the developing spores would be a difficulty.

2. It is weak mechanically.

Both these disadvantages will be removed if we increase sufficiently the attachment area of the sporangium. This increase can only be made by extending the attachment of the sporangium down the horizontal portion of the sporophyll towards the axis.

In this way we reach a condition which is preserved for us in *Mesostrobus* ; here we have considerable radial extension of the sporophyll combined with attachment of the sporangium to it only along its distal half. It is noteworthy that the *Mesostrobus* sporophyll strongly recalls that of *Bothrodendron mundum.*

Further continuance of this process leads directly to the ordinary *Lepidostrobus* (cp. Text-fig. 2).



Text-fig. 2. The rise of the *Lepidostrobus* condition. *A* = *Bothrodendron mundum* ; *B* = Hypothetical ancestry corresponding to *Spencerites* ; *C* = *Mesostrobus* ; *D* = *Lepidostrobus.*

Spencerites does not appear to me at all the sort of thing one would expect, if it were a cone retaining very archaic features.

Certain specimens, at any rate, have their sporophylls arranged very regularly in alternating verticils (cp. Text-fig. 3). Now the vast majority of Lycopods have not a trace of verticillate arrangement, and where such an arrangement does occur it is usually in the case of a cone.

In a cone it is easily seen that a verticillate arrangement with alternating verticils is that which will secure adequate protection for the sporangia with the least possible area of lamina.

The importance of this saving is well illustrated by the fact that in the cone *Calamostachys Binneyana*, whilst the sporangiophores are superposed, the bracts are in alternating verticils ; the whole leading to a very curious arrangement of leaf-traces, which is not yet understood.

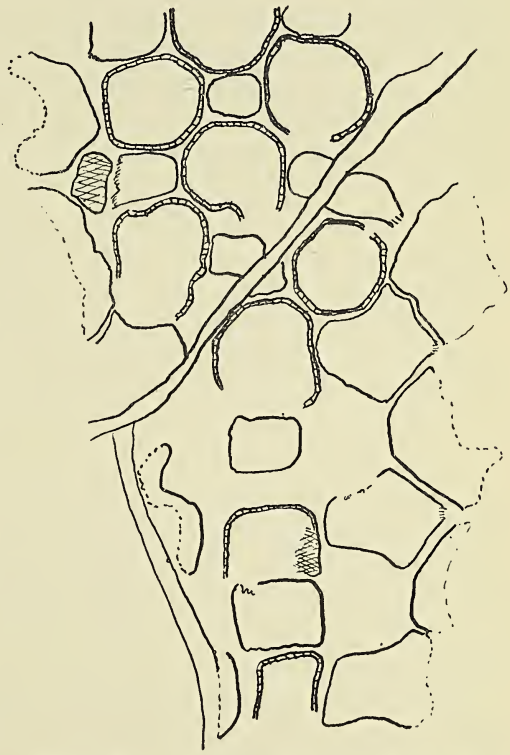
A far more striking case, however, is that of *Sphenophyllum Dawsoni*, where, although the facts are not absolutely conclusive, it seems that the bracts alternate, although such an arrangement is hard to correlate with the vertical course of the protoxylems, and with the fact that the superposition

of appendages is one of the most characteristic of the vegetative characters of the group.

There is hence a considerable probability that the acquirement of an alternating verticillate arrangement of appendages in the cones of a Lycopod is a specialization.

The modern stock description of *Spencerites* as having the sporangium attached distally to a 'ventral hump' on the sporophyll, gives only a very misleading idea of the real arrangement of the cone, which was so accurately described by Dr. Scott.

The 'ventral hump' when cut tangentially is seen to be a rhomboidal peltate head which fits very tightly on to its neighbours, forming a magnificent protection to the sporangia, compared to which that given by the weak and obviously easily damaged lamina described by Miss Berridge is negligible (cp. Text-fig. 3). It is to be noticed that the arrangement of the sporophylls in alternating verticils makes this much neater that it otherwise would be. There is thus an obvious utilitarian purpose in the 'ventral hump'.



TEXT-FIG. 3. Tangential section of a cone of *Spencerites insignis* (Q. 489). × 14. Dotted lines indicate that the present edge of an organ is manifestly incomplete. The sporophylls are unshaded. The figure serves to show the way in which the sporangia fill up the entire space between the pedicels of the sporophylls, and the mechanical protection afforded to the sporangia by the peltate expansions, which are suitably cut on the right hand side in the lower part of the figure.

The fact which probably impresses most strongly the student of palaeozoology is that two branches of a race which separate at an early period often pursue similar courses and add similar organs, as though there was some tendency in their original stock to produce these particular characters: this is, I believe, Osborn's 'Doctrine of Rectigradations'.

I have shown that it is possible to provide a plausible explanation of the rise of a radially elongated sporophyll in the heterosporous arboreal Lycopods, and that this sporophyll probably passed through a condition reminding one of *Spencerites*.

It is quite safe to assume that the early homosporous cone did not differ much from the heterosporous cone which had just branched off from it. The causes which led to the production of *Lepidostrobus*, on the increase in size of the heterosporous Lycopods, would in a precisely similar manner tend to produce a similar effect on the cones of any homosporous Lycopod which similarly increased in size.

The whole would be an illustration of the Doctrine of Rectigradation. Hence, it seems possible to explain *Spencerites* as a stage in the production of a *Lepidostrobus* on the homosporous side, which has been prevented from going on, by the development of a new method of protection, by an outgrowth of the sporophyll just to the outside of the sporangium. This protection would be needed to make amends for the weak mechanical attachment of the sporangium, and might begin as a small lump and carry the attachment of the sporangium up with it.

If *Spencerites* is secondarily derived from a homosporous cone resembling that of *Bothrodendron mundum*, we should expect to see vestiges of its ancestry. Two characters can, I think, be interpreted as such.

On reference to Scott's original description (Scott, '97), Pl. XIV, Fig. 11, a figure of a radial section of two sporophylls of *Spencerites insignis* shows that the course of the trace is not straight through the sporophyll, but that in the region of the proximal part of the peltate head, the trace takes a little loop downwards and then comes up again.

Miss Berridge's restoration ('05) does not show this, but apparently the trace is not shown in this region in her radial section.

I have been able to see this arrangement (only very badly) in tangential section Q. 489 in the Cash Collection of the Manchester Museum, from Halifax, and it seems to me to be natural, although it may not be always present.

This loop is, it seems to me, quite comparable, and in fact homologous with the loop round the base of the ligule in *Bothrodendron mundum* and *Mesostrobus*. It may be objected that the loop is rather nearer the axis than would be expected on this view; in answer to this I would point out that in *Lepidostrobus* it is further out, and no longer embraces the base of the ligule.

This loop suggests that the ligule is possibly included in the 'ventral hump', a suggestion which has, I believe, been already made by Miss Berridge. In the Lycopods generally, the ligule is a very definite organ well marked off from its base, so that not very much weight should be laid on this suggestion.

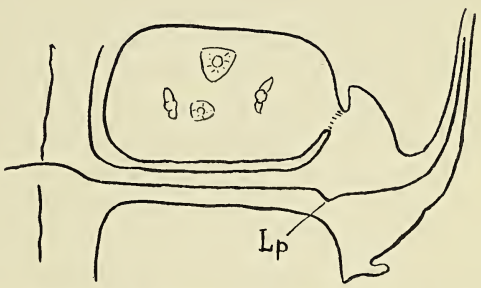
Miss Berridge in her paper on *Spencerites* shows that the trace in passing from the outer cortex to the sporophyll rises slightly above the base and then comes down to enter it. This arrangement is exactly similar to that which occurs in *Mesostrobus* and probably in *Bothrodendron mundum*.

That it is unlikely that *Spencerites* is at all a primitive cone is also shown by the fact that a prosenchymatous sporangial wall is known in no other strobiloid Pteridophyte. If it were a primitive condition we should expect to see at least traces in other Lycopods; such traces we do not find.

The fact that both species of *Spencerites* have elaborated spores is also against their primitive nature.

It may be objected that the sporophylls of *Spencerites* are really quite short. This is certainly true, but reference to Miss Berridge's paper (or Text-fig. 4) will show that if measured to the lamina they are long compared with such sporophylls as those of *Bothrodendron mundum*.

It is unfortunate that we know so little of the morphology of *Spencerites majusculus*; the analogy of *Spencerites insignis* should teach caution in drawing arguments from the other and rarer species.



TEXT-FIG. 4. *Spencerites insignis*, Scott. Diagrammatic radial longitudinal section of a single sporophyll, to show general morphology, the considerable length of the limb, and the loop at *Lp* reminiscent of the loop which surrounds the ligule in *Bothrodendron* and *Mesostrobus*. Modified from Miss Berridge's figure.

It is at any rate interesting that *Spencerites* is the only homosporous Lycopod which is known to have attained to even moderate dimensions. I possess a section of a branch of *Spencerites* (A. 31), duplicates of which are in the Manchester Museum Collection, over 30 mm. in diameter. This is, I believe, by far the largest known branch of a homosporous Lycopod.

Throughout this discussion I have assumed that *Spencerites* is homosporous; there is so far as I know no evidence against this, and although it is of course impossible to prove that it did not have microspores, the evidence in favour of its being homosporous seems to be considerable.

The theory that *Spencerites* is a cone retaining primitive characters really rests entirely on the assumption that the Lycopod cones are derived from a condition resembling that of *Sphenophyllum Dawsoni*, and that the peculiar features of the *Spencerites* cone can be made to square with such ancestry.

The evidence for any connexion between the Sphenophyllales and the Lycopodiales does not seem to be of very great weight. It is entirely comprised under two heads:—

1. The general resemblance between the wood of *Cheirostrobus* and that of some of the Lepidodendraceae.

2. The resemblance between the pair of sporangia and the organ

which bears them in *Tmesipteris*, and the sporophyll of the Sphenophyllales, particularly *S. majus*. This involves the assumption that *Tmesipteris* is related to the Lycopodiales.

The resemblance between the wood of *Cheirostrobus* and that of *Lepidodendron* is real, but does not extend to details. It seems to me that these resemblances are just what one would expect.

The resemblance really depends on the fact that exarch protoxylem is primitive, at any rate, in the case of microphyllous plants. It has been shown by Dr. Scott that this is the case in the Cordaiteae, the Cycadofilices, and the Calamariales, a very varied set of plants.

A medullated monostele is a very simple type of wood, and the fact that both *Cheirostrobus* and *Lepidodendron* have leaf-traces so small that they only take away a few tracheids from the edge of the wood, explains the fact that neither plant has the wood broken up into bundles.

The second reason for regarding the Lycopodiales as allied to the Sphenophyllales is dependent on the assumption that the Psilotales are connected with the Lycopods; so far as I know the evidence for the latter connexion is not much stronger than the first.

Even if the Sphenophyllales are at all closely connected with the Lycopodiales (and that they are connected in some degree I do not wish to deny) it has yet to be shown that the primitive ancestor had a sporophyll of the type of *S. Dawsoni* or *S. trichomatosum*. As Dr. Scott pointed out in his original description of *Cheirostrobus*, that cone seems to be in many ways the most primitive of the known Sphenophyllaceous cones. I do not see how the *Spencerites* cone could be derived from a cone at all resembling that of *Cheirostrobus*.

Finally, the idea advocated by Miss Sykes that the 'ventral hump' of *Spencerites*, the sporangiophore of *Palaeostachya*, and other similar structures are of axial origin and homologous seems to me to rest on a misconception of the nature of the evidence.

The idea seems to one, who, like myself, was not trained in the ideas of rigid morphology, derived from the higher Angiosperms, rather forced when applied to *Tmesipteris*, but when extended to the cones of the Lycopodiales, none of which show the slightest trace of such derivation, it seems quite inconceivable.

Summary.

The new genus *Mesostrobus* is founded for a small Lepidodendroid cone (*M. Scottii* sp. nov.) from the Mountain 4 ft. Mine of the Lancashire Lower Coal Measures.

The cone strongly resembles that of *Lepidostrobus*, but differs in the fact that the sporangium is only attached to the distal half of the horizontal

portion of the sporophyll. The ligule is set in a deep ligular pit, and is somewhat large when compared with its condition in *Lepidostrobus.*

It is suggested that the type of sporophyll represented by *Bothrodendron mundum*, the male *Miadesmia*, and *Selaginella* is a close copy of a very early Lycopodiaceous type. *Lepidostrobus* would be derived from a cone having sporophylls of this type, on the adoption of an arboreal habit by the heterosporous Lycopods, because radial elongation of the sporangium is the most economical way of increasing the number of spores produced, a necessity for a large tree.

If this elongation takes place in the part of the sporophyll between the axis and the insertion of the sporangium, we arrive at a condition much like that of *Spencerites*, and from that condition we can pass through *Mesostrobus* to *Lepidostrobus.*

The fact that two branches of one stock, differing in important characters, tends to follow similar courses during their evolution is one very strongly borne in on students of Palaeozoology, and it is suggested that a similar parallelism is to be found between the homosporous and the heterosporous Lycopods; and that *Spencerites*, which is probably homosporous, has been derived from a cone resembling that of *Bothrodendron mundum*, in consequence of that increase in size of the homosporous stock which we see in the genus.

The peculiar feature of the *Spencerites* cone, the peltate expansion of the horizontal portion of the sporophyll between the sporangium and the lamina, is to be regarded as having a utilitarian purpose, that of affording protection to the very weakly attached sporangium. It is further pointed out that the idea that *Spencerites* is an ancient and archaic type rests entirely on the assumption that the 'ventral hump' is derived from a sporangiophore homologous with that of *Sphenophyllum Dawsoni*, and on the further assumption that the Lycopods in general are derived from a Sphenophyllaceous or proto-Sphenophyllaceous ancestor. It is pointed out that the evidence in favour of this view is really extremely slight, although there is also not much against it.

I wish to express my thanks to Prof. F. E. Weiss and Dr. W. E. Hoyle for allowing me to describe the unique series of sections of *Mesostrobus* now in the Manchester Museum.

I hear from my friend Mr. W. T. Gordon that he has a cone showing similar morphological characters from the petrified plant material of lower carboniferous age at Pettycur. I look forward with interest to his description of this cone.

Appendix, written February 19, 1909.

Since the above account of the affinities of *Spencerites* was written, I have seen Dr. Lang's paper dealing with the same subject published in the Proceedings of the Royal Society of Edinburgh, vol. xxviii, p. 356.

Dr. Lang discusses the morphology of the cone of *Lycopodium cernuum*, and points out that, after the disappearance of certain mucilaginous areas, the radial section of a sporophyll resembles that of *Spencerites insignis*: he therefore concludes that the *Spencerites* sporophyll, as we know it, is only the remnant of a much larger mass of tissue, much of which has broken down into mucilage, and that there is some genetic connexion between the two species.

Dr. Lang shows that in the cone of *Lycopodium cernuum* the sporophylls, which are arranged in alternating verticils, are connected with one another, and brings forward certain evidence to show that in some cases the distal parts of the sporophylls of *Spencerites insignis* may be confluent, and from this draws the conclusion that their proximal parts must have formerly had a similar connexion.

Lang concludes that the outgrowth which actually bears the sporangium in *Spencerites* is of no morphological importance, a view which it will be noticed is in agreement with my own.

He also denies any justification for the view that the portion of the sporophyll between the insertion of the sporangium and the axis is of axial nature.

He states that in *Lycopodium cernuum* this region appears late in ontogeny. The theory of the development of *Lepidostrobus* and *Spencerites*, outlined above, requires this very region to elongate comparatively late in the phylogeny of those genera. The comparison between the observed fact of its late appearance in ontogeny in *Lycopodium cernuum*, of which I was formerly unaware, and the assumed late appearance in phylogeny of the same region in *Lepidostrobus*, seems to add considerably to the probability of my explanation of the origin of that genus.

1. The whole weight of Lang's conclusion with regard to the affinities of *Spencerites* rests entirely on a detailed comparison of certain points in the anatomy of one recent species with a Carboniferous species of a very distinct genus.

It is, I think, obvious that comparison of two more or less promiscuously picked species of different genera is far from satisfactory evidence from which to draw important conclusions, even if the species are of the same age ; if, as in this case, their relative ages are immensely different the method becomes even more risky. It is unfortunate that the use of this method appears to be on the increase amongst botanists.

2. Comparison of Fig. 1 of Dr. Lang's paper with my Text-figures

1 and 4 will show that *Lycopodium cernuum* far more resembles *Mesostrobus* in the morphology of its sporophyll than it does *Spencerites*. The resemblance in the mode of attachment of the sporangia is apparently exact, the shape of the sporophyll of *Lycopodium cernuum* after the removal of the mucilaginous areas is extremely like that of *Mesostrobus*, there is in *Mesostrobus* the same 'dorsal lobe' at the distal end of the sporophyll, and the same more or less peltate form. In fact, if Lang's explanation applies to *Spencerites* it must also apply to *Mesostrobus*; that is, the known part of the sporophyll of *Mesostrobus* should have been surrounded on the lower side by a mass of tissue which has broken down into mucilage. Now the stalk of the sporophyll of *Mesostrobus* is a neatly finished affair of triangular section. The actual preservation of the sections is not very good, but it appears to be certain that the under surface of the stalk is completely covered by an epidermis, in which I have been able to see what I believe are stomates.

If Lang's explanation applies to *Mesostrobus* it must also apply to *Bothrodendron mundum*, the sporophyll of which only differs in the shortness of its stalk from *Mesostrobus*, and to *Lepidostrobus*, in which this region is elongated. It is, I think, unnecessary to point out the improbabilities of this position.

3. There is not the slightest direct evidence that the pedicel of the *Spencerites* sporophyll is not a complete organ. It is apparently surrounded by one of the most definite epidermises known in fossil plants, as may be seen, for example, in Dr. Scott's Fig. 11 and Miss Berridge's Phot. 7.

4. The sporangia are closely packed in the case of *Spencerites*, and fill up the whole space available with the exception of that occupied by the pedicels (cp. Dr. Scott's Fig. 11, Williamson's Fig. 55, and Text-fig. 3 of this paper).

It is possible that this might be explained on Dr. Lang's theory by assuming that the mucilaginous portion disappeared before the growth of the sporangia ceased.

5. Dr. Lang's theory provides not the slightest explanation of the origin of the ventral process, and takes no account of the utilitarian purpose of the whole arrangement of the peltate ends of the sporophylls.

This utilitarian purpose is fact, not theory; see, for example, the lower left-hand corner of Dr. Scott's Fig. 3 and Text-fig. 3 of this paper; here the peltate expansions form a close-fitting mosaic, affording magnificent protection to the sporangia.

6. With regard to the possible coalescence of the distal ends of the sporophyll it is impossible to express an opinion without seeing the original preparations on which that opinion is based.

Williamson's ('78), Fig. 53, is a particularly unconvincing one, and the section appears to be very abnormal: it is according to Lang cut near the

tip of the cone. The sporophylls are far more closely set than usual, and from the drawing it appears that their peltate heads have been destroyed.

I have carefully examined some twenty sections of cones of *Spencerites*, and have seen no trace of this coalescence.

Assuming that the distal ends of the sporophylls are in some cases confluent, I cannot see the logical necessity of assuming that the pedicels must therefore have been coherent at some period. It is quite probable that the adherence in the Williamson section (if really present) is teratological, and I believe that botanists are now becoming chary of using such evidence.

I might point out here that the enclosure of each sporangium in a cell by outgrowths from the sporophylls, which Dr. Lang has shown to take place in *Lycopodium cernuum*, is paralleled by the condition of the Calamitean cone *Calamostachys Grand'Euryi*, Ren., where the sporangia are isolated in groups of four by outgrowths from the bracts above and below.

I would finally point out that Dr. Lang's theories of *Spencerites* must be accepted in their entirety or not at all.

A further Note on Dr. Lang's Paper, added April 28, 1909.

I have now examined the section in the Williamson collection on which Dr. Lang relies for his opinion that the sporophylls of *Spencerites insignis* were united distally.

The section is somewhat thick, and the cone had certainly been much macerated before petrifaction.

Those palaeobotanists who have worked much with sections of coal-balls will know the great difficulty of determining whether two adjacent organs are in continuity or not under such conditions.

From a careful examination of the section I came to the conclusion that there was no evidence to show that the sporophylls in question were really organically connected. The tissue of the peltate expansion is seldom well preserved, and in the section under discussion is very much crushed; under these circumstances the evidence is certain to be of the weakest.

Text-figure 5 of this paper is a camera lucida drawing of a portion of a tangential section of *Spencerites insignis* of which another portion is shown in Fig. 3.

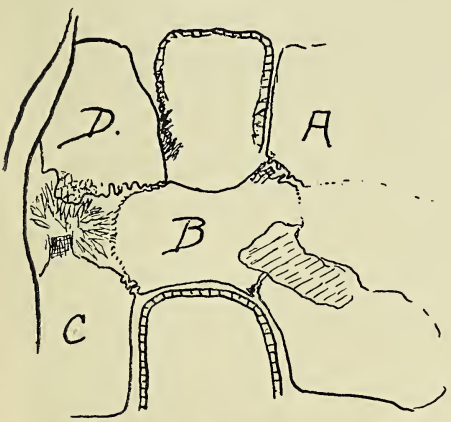
In this section the three sporophylls *B*, *C*, and *D* appear at first sight to be organically connected.

With an 8-mm. Zeiss apochromatic objective, however, it is quite evident that this appearance is completely deceptive; sporophyll *C* is cut through the attachment of the sporangium, and the section also cuts the sporangium wall parallel to its surface, this wall passes under the edges of

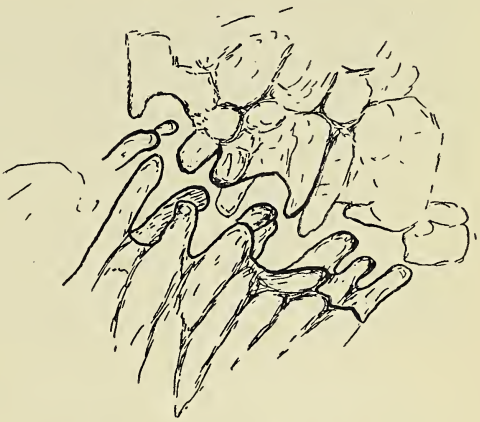
the peltate expansions of sporophylls *B* and *D*, which are also cut very nearly parallel to their surfaces.

It would have been impossible to have determined this point with an inferior lens.

I do not publish an enlarged drawing of this area because it is not



TEXT-FIG. 5. Another portion of the same cone. × 20. To show the way in which the sporophyll *B* fits on to *A*, *C*, and *D*. Shows also the attachment of a sporangium to *C*. Further described in the text.



TEXT-FIG. 6. A portion of Fig. 3 enlarged. × 160 approx. This shows the area where sporophylls *A* and *B* approach closely. Further described in the text.

essential for my purpose, and my artistic ability is not sufficient to represent it adequately in two dimensions.

Sporophylls *A* and *B* very nearly meet, and the spot where they do so corresponds with the place in the Williamson section, where Dr. Lang believes them to be confluent. Text-figure 6 is an enlarged drawing of portions of the two sporophylls where nearest together; their edges are provided with a forest of hairs which interlock.

Such a region in a crushed and macerated specimen might very easily be taken to show actual continuity, as I believe Dr. Lang has done in the case of the Williamson section.

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EXPLANATION OF PLATE XXVII.

Illustrating Mr. Watson's paper on *Mesostrobus.*

All the figures are untouched photographs. Phot. 1 by Flatters and Garnet, Manchester, the rest by the Author.

Phot. 1. A complete transverse section of the cone of *Mesostrobus Scottii,* sp. nov. This gives a good general idea of the build of the cone. At *a* are seen two sporophylls cut so near to the axis that the sporangia are not yet attached. This section also indicates the distribution of the sclerized patches in the outer cortex. R. 1122. 3. Manchester Museum Collection. × 6·1.

Phot. 2. Transverse section of the 'bud' at the tip of the cone. At *par* will be seen the two patches of parichnos in a leaf-base. R. 1122. 1. Manchester Museum Collection. × 15.

Phot. 3. The axis of the section represented in Phot. 2 enlarged. Shows the wood *xy.*, the concentration of the leaf-traces *l.t.* in the zone of the inner cortex and Phloem, which are in this section defective, some remains of the middle cortex, and the outer cortex with the leaf-trace, surrounded by its parenchymatous sheath passing out through a hole in a sclerized patch of the outer cortex. R. 1122. 1. Manchester Museum Collection. × 45.

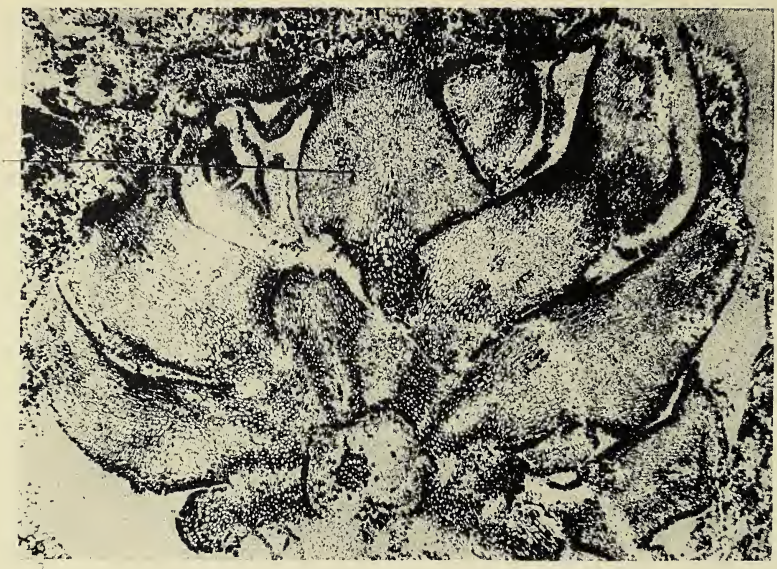
Phot. 4. A portion of the original of Phot. 1 enlarged. Shows the wood *xy.*, phloem *ph.*, and inner cortex *i.c.*, and the middle cortex *m.c.*, quite well preserved. The photograph shows also the insertion of three sporophylls, to none of which is a sporangium attached; the one shown completely in the photograph is the section cut furthest from the centre to which a sporangium is not attached, and I think from the form of the upper surface, which it must be remembered is cut obliquely, so that all projections appear much magnified, is very near the point where the sporangium becomes attached to the sporophyll. R. 1122. 3. Manchester Museum collection. × 26.

Phot. 5. A transverse section showing a sporophyll with a sporangium attached. R. 1122. 2. Manchester Museum Collection. × 18·3.

Phot. 6. A portion of a transverse section showing the distal ends of two sporophylls *sp'* and *sp''*. In *sp'* the vascular bundle is cut twice at *vb'* and *vb''*, first at *vb'* before dropping below the ligule, and at *vb''* when rising to go into the lamina; at *lg* is seen the ligular pit which lies between the two points of section of the vascular bundle; with a lens it may be seen that the tissue on each side of *vb'* is reminiscent of parichnos.

The sporophyll *sp''* is cut lower down than the one just described; it shows a longitudinal section of the vascular bundle at the bottom of the loop below the ligule. On each side of it is a defective patch probably representing the parichnos. R. 1122. 3. Manchester Museum Collection. × 15·6.

Phot. 7. Portion of a transverse section showing the distal portion of a sporophyll just at the point of origin of the lamina. At *gr* is seen the grove which continues the ligular pit. Notice the defective parichnos on the left-hand side of the vascular bundle. R. 1122. 4. Manchester Museum Collection. × 14.



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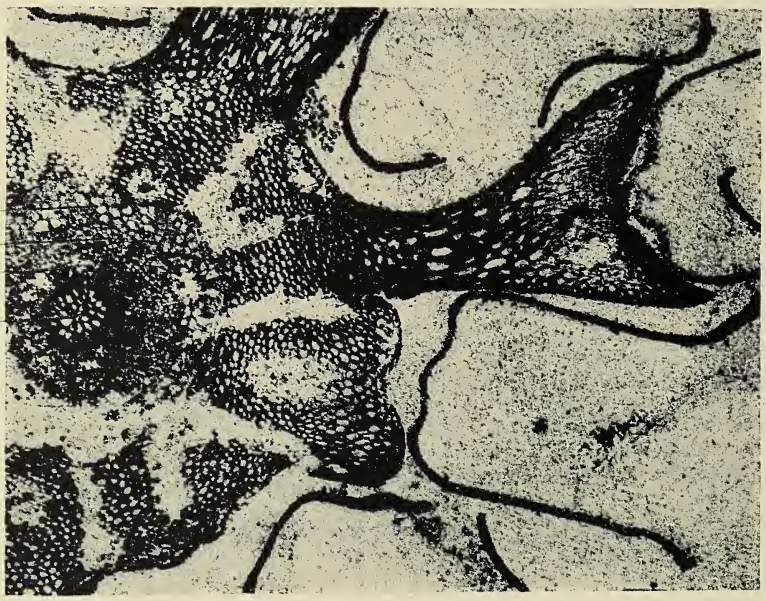

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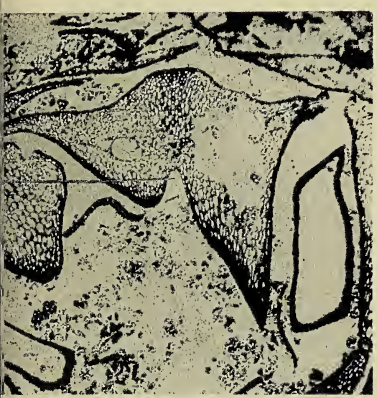

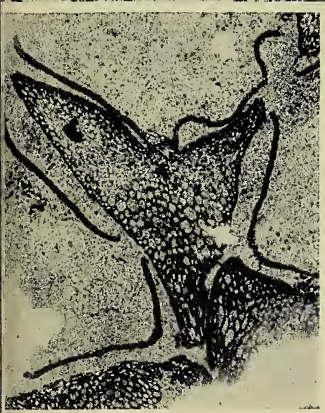

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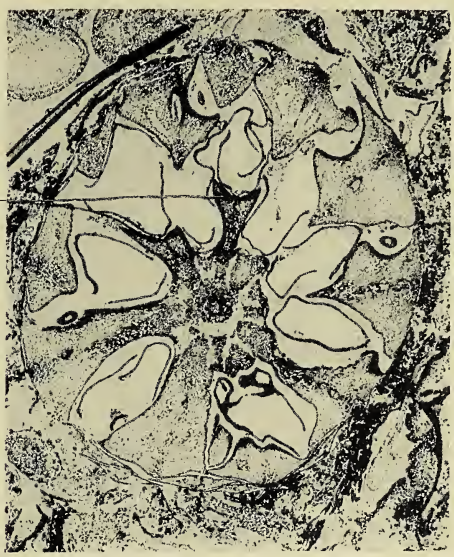

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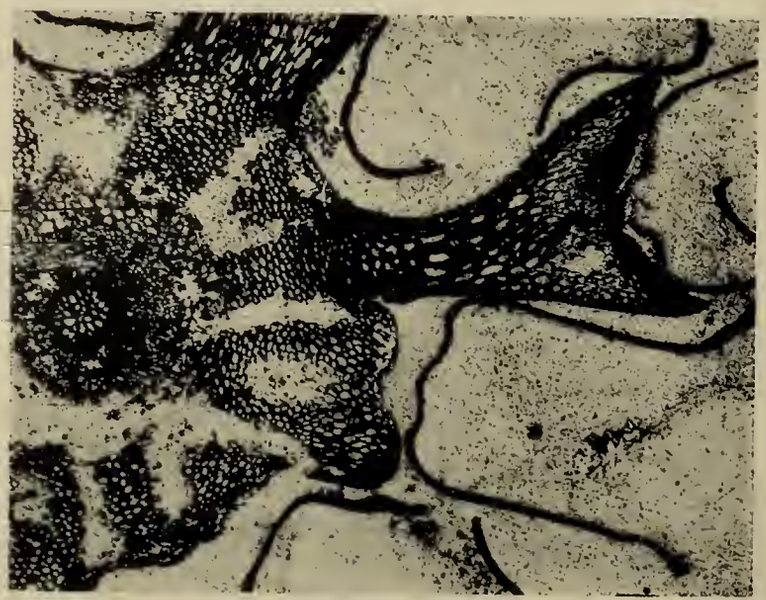

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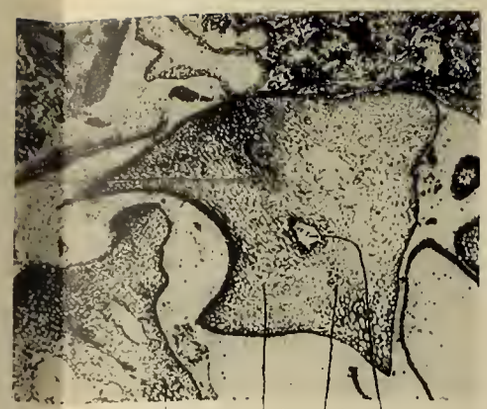

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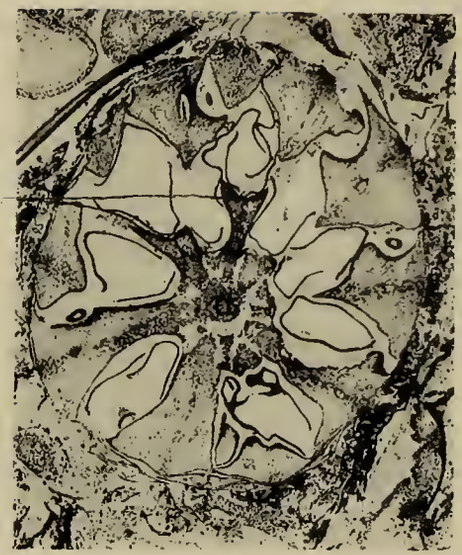

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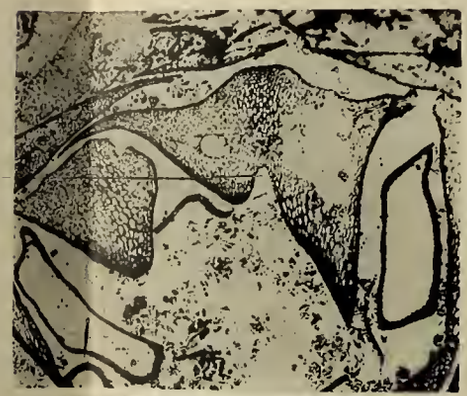

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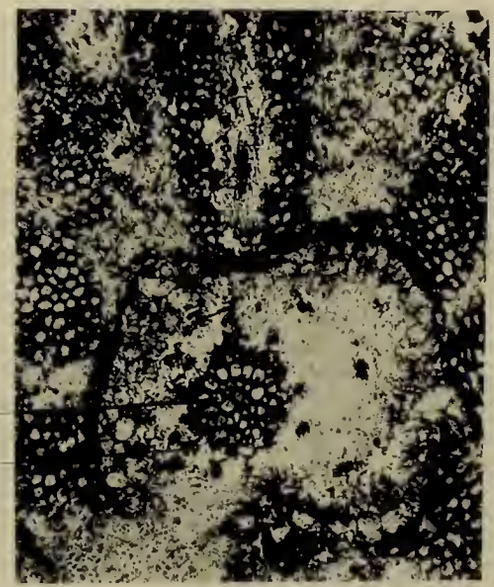

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WATSON – MESOSTROBUS SCOTTII.

On the Sexuality and Development of the Ascocarp in Ascophanus carneus, Pers.

BY

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With Plate XXVIII.

IN 1906, at Prof. V. H. Blackman's suggestion, I began a search amongst the coprophilous Fungi for a form possessing an ascogonium favourable for working out the developmental features from its inception up to the formation of ascogenous hyphae and asci. About this time Prof. Blackman and Miss Fraser (7) had investigated the Ascomycete *Humaria granulata*, and had found that the female nuclei of its unicellular ascogonium fused in pairs, and they expressed the opinion that, in this form, this process takes the place of the ordinary sexual process which had been described by Harper in *Sphaerotheca* (18, 19) and *Pyronema* (20). In their paper the authors suggested that a similar reduced fertilization might be found to take place in the ascogonium of *Ascobolus furfuraceus* and other forms.

On the discovery of a well-marked ascogonium, of the type described by Woronin (28) as a scolecite, in an ascocarp occurring abundantly, and in colonial growths on rabbit's dung, it was decided to work out the nuclear features of the life-history of this species.

The colour of this fungus, although varying within somewhat wide limits, is of a quite characteristic fleshy hue. Occurring abundantly on most rabbit's dung, it is not confined to this medium; I have found it growing on earth where there had recently been a fire and forming a patch of so large a size and so densely packed as to give the ground a perceptibly fleshy colour even from a distance of several yards.

From its characters it very obviously belonged to the genus *Ascophanus*, but some little difficulty was experienced in determining whether the species was *carneus* or *furfuraceus*. These two species would seem to be very closely allied, and indeed, Massee, in his British Fungus Flora (23), makes *furfuraceus* a variety of *carneus*; moreover, the description of the two species given in different systematic works cannot be quite reconciled. In this

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paper I am following the description given in Rabenhorst's Kryptogamen Flora (25), which undoubtedly indicates that the fungus is *A. carneus*.[1]

Miss Welsford (27) has so recently given a summary of our knowledge of the development of the fruits of the Ascobolaceae as to render it unnecessary for me to give a further account here. I cannot find, however, that the fungus *Ascobolus pulcherrimus*, Cr., the ascogonium of which Woronin (28) described, was ever named *Ascophanus pulcherrimus* by Crouan. This systematist placed the fungus in the genus *Ascobolus*, and since then it has been placed in the genus *Lasiobolus* by Schröter. I cannot find, in any of the systematic works that I have consulted, any trace of its ever being placed in the genus *Ascophanus*, although Massee (23), in his British Fungus Flora, has placed *Lasiobolus equinus*, an English species, in the latter genus.

When nearly all the observations recorded in this paper were made, I found a reference to a paper on *Ascophanus carneus*, Pers., by Miss Ternetz (26). This paper is mostly concerned with the protoplasmic streaming found in the species, but it also shortly describes the fruit development. The account there given agrees very well indeed with what I have found.

Miss Ternetz describes the archicarp as a 'scolecite', and says that it is very variable, both in the number and dimensions of the cells composing it, and also in the amount of curling of the organ. She finds no central cell as in *Ascobolus furfuraceus* and says that, in some cases, the tip of the ascogonium grows on so that the ascogonium becomes intercalary, and that it is possible that at times the portion that has grown on may form another ascogonium.

She observed no copulation, and says that the wall of the young ascogonium was unbroken. The cells are at first full of dense protoplasm, but after a time are emptied, and could not be found in full-grown apothecia.

Methods.

Thin slices were cut from the surface of bits of rabbit's dung on which the fungus was growing. These were fixed in Flemming's weak fluid, which was allowed to act for twenty-four hours. The sections were cut 4 μ or 5 μ in thickness and were stained either with the triple stain or with Heidenhain's iron haematoxylin, and, in the latter case, sometimes counter-stained with erythrosin and mounted in Damar Lac.

Germination of the Spores.

Many attempts were made at an early period in this work to germinate the spores of this fungus, but these were all in vain. Agar-agar dissolved in rabbit's-dung extract and acidified with orange juice was used in these

[1] I have to thank Miss A. Lorrain Smith, of the Natural History Museum, South Kensington, for advice on this and other systematic points.

cultures. On the appearance of Miss Fraser's paper on *Lachnea stercorea* (14), in which she describes the germination of the spores of this fungus after treatment with alkaline solutions, it was determined to make more experiments and under divers conditions. De Bary (13) had observed that both acid and alkaline media were sometimes useful in inducing the germination of spores which, under ordinary conditions, could not be made to germinate, so in making solutions, alkaline, acid, and neutral fluids were employed, and the effect of different temperatures was noted as well.

Extracts of rabbit's dung and of prunes were used, and also water. In only one case was a germination observed in an acid medium, although both in acid and neutral solutions the spores were often observed to become more transparent, and a number of vacuoles were noticed inside them, these phenomena being the preliminaries to germination. These changes were observed in spores which had been subjected to a fairly high temperature, 38° or 40° C., for a few days. The processes of germination, however, got no further except in the one case mentioned above.

In the alkaline solutions, however, the spores easily germinated over-night, and this process took place more quickly at higher than at lower temperatures, so that a greater percentage of spores would germinate over-night in a high-temperature culture, 38° C. or 40° C., than in a lower temperature culture (30° C.).

No very exact experiments were tried, the only object being to obtain pure cultures of the fungus; but the results obtained seem to show that the germination of the spores depends on the softening process of the alkaline media on the wall, and that this latter process was hastened by an increase in temperature.

The attempt to obtain pure cultures was not successful. In the alkaline media the mycelium only grew to about $\frac{1}{2}$ inch long, and then died away, probably overcome by the bacteria which could not be kept out of these cultures. The germinating spores were also removed to acid media, or acid agar-agar extract of rabbit's dung, but in all cases the mycelium soon perished.

The spores usually put out two germ tubes, one at each end of the spore, and in some cases a transverse wall was formed in the middle of the spore, so that the two germ tubes were to some extent independent of each other. This wall-formation is comparable to the formation of transverse walls such as are found in the ascospores of many ascomycetes, such as *Sphaerulina*, *Coryne*, &c., but here the wall-formation does not take place in the ascus, but is delayed until after the earlier stages of germination are passed.

It was observed in several cases that as a preliminary to the stopping of germination by adverse causes, the protoplasm from the different cells of the mycelium collected either in one cell of the hypha or in the spore itself,

probably passing through the pore in the transverse wall, which will be described at the same time as the mycelium, and here it was cut off from the adjoining empty cells by pads of material which stopped up the pores in the transverse walls.

Mycelium and Chlamydospores.

The cells of the vegetative mycelium contain a varying quantity of protoplasm and numerous nuclei (Figs. 6 and 7). The side walls bear a number of spherical granules which Miss Ternetz has already seen and described. These granules seem to be similar to those described in *Ascobolus* (Harper, 19, Welsford, 27), *Pyronema* (Harper, 20), *Humaria granulata* (Blackman and Fraser, 7), and other Ascomycetes.

Harper (19) has suggested that these bodies are concerned with a transmission of materials from cell to cell, and that they are probably connected in some way with a pore. He does not, however, figure or describe any such pores in *Pyronema.*

In *Ascophanus carneus* these granules, which vary very much in number, are situated on either side of a small pore in the transverse walls of the hyphae (Fig. 7). When the sections were stained with the triple stain I was at first unable to be absolutely certain of the presence of this pore, but after staining with Heidenhain's haematoxylin and counter-staining with erythrosin, there was no doubt that the pore was always present in the vegetative hyphae, and I have since seen many well-marked pores in sections stained with the triple stain.

Miss Ternetz (26) recorded that the protoplasm and vacuoles pass from cell to cell in this species, and suggested that there is a pore in the transverse wall. Woronin (28) has described a similar protoplasmic streaming in an allied form, *Lasiobolus* (*Ascobolus*) *pulcherrimus.* This latter species is also provided with granules. Neither of these authors, however, actually saw the pore.

The composition of the granules and the part that they play in the life-history of this fungus is somewhat difficult to determine. Miss Ternetz examined them with microchemical tests, but was unable to come to any definite conclusions as to their chemical nature. She found that neither methylene blue nor iodine stained them, but that nuclear dyes were taken up with great avidity.

I found that, although they were often stained with the safranin of the triple stain, they more commonly took up the gentian violet; they had also the very greatest avidity for Heidenhain's haematoxylin. I tried several microchemical tests as well, but was unable to form any conclusion as to their chemical nature.

As to their function I can only say that under certain conditions

(usually lack of food material, or presence of sugar [1]) certain rows of cells in the vegetative mycelium are differentiated as Chlamydospore-rows, and that in these cases the pores are closed by the granules, which then become surrounded by a common envelope of lightly-staining material closely pressed to the pore.

The granules form a similar pad in certain unfavourable germinations in which the protoplasm retreats from the germ-tube into the spore, and the contents are closed off from the empty cells.

A similar phenomenon is to be seen in the ascogonium; this will be considered more in detail when that organ is being described.

The nuclei in the vegetative hyphae are very small, and are differentiated with great difficulty. They showed signs of a chromatic network and appear to divide karyokinetically, but no details of the process were made out owing to the minuteness of the objects.

Development of the Apothecium.

Grosser Morphology. The archicarp (Fig. 1) arises as a branch of a vegetative hypha. It resembles in shape the organ described by Woronin as a scolecite, and in its young condition the cells composing it are very similar in appearance. As it develops further, however, three fairly definitely marked regions can be distinguished—a basal vegetative part, a central ascogonial, and a terminal vegetative. In some cases this last seems to be absent, but, as this point was determined in the case of ascogonia which were already covered in, it may be that this portion was present in all cases, though at this period unrecognizable in some fruits. The basal vegetative part of the archicarp and the surrounding mycelium send up numerous hyphal branches which quickly envelop the young ascogonium. Each fruit contains, therefore, only one ascogonium.

No trace of an antheridium or of any other kind of male organ was found.

Sometimes the archicarp seems to arise in a dense tangle of 'chlamydospore-rows', suggesting a comparison with Miss Welsford's description of the beginnings of the archicarp of *Ascobolus* (27).

In *Ascophanus carneus* the number of cells in each of the three above-mentioned portions of the archicarp varies greatly. The basal part may be composed of from three to about a dozen cells; the precise number, however, can seldom be determined owing to the early degeneration of the cells nearest the parent hypha. These cells are used in contributing towards the formation of enveloping branches for the fruit. Another point which sometimes makes the determination difficult, is the fact that in many cases the ascogonial portion is not marked off from the basal portion until late in the development.

[1] See Miss Ternetz's paper (26).

The ascogonium or part concerned in the formation of the ascogenous hyphae is also composed of a varying number of cells from about three to seven. All these cells are very nearly equal in size, and there is no sign of the presence of any specially differentiated 'central cells' as in *Ascobolus furfuraceus*.

The terminal portion is sometimes composed of about three or five cells, but at other times it seems to grow on, and Miss Ternetz says that it is capable of growing on and forming an ascogonium, thus giving rise to another fruit. I have very carefully searched my preparations to see if I could find any trace of such a process, but have not succeeded in doing so. It would, however, be difficult to make sure of such a point in sections.

The fruits often occur quite close together, and such cases were carefully examined ; but neither in these, nor even in those rare cases in which two ascogonia are seen enclosed in one spherical pseudo-fruit-mass, was there any sign of continuity between two archicarps.

It was found that a fairly large branch was in some few cases given off from about the middle of the ascogonium. This branch agrees in structure with the ascogonial region of the archicarp, and I am inclined to regard it merely as an ordinary branch of that region, and not as a trichogyne or an ascogenous hypha.

Cytological Features.

The cells of the archicarp, even when in an uncovered condition, are much larger than those of the ordinary mycelium, indeed, they are of about the same size as the cells of the chlamydospore-rows. The protoplasm of these cells is very dense and contains a few large, more or less centrally situated, vacuoles. These contain a number of spherical, highly refringent bodies with no affinity for stains. The cross-walls are perforated like those of the ordinary vegetative cells, and the pores are provided with the characteristic granules (Fig. 4). These cells are multinucleate, and contain many more nuclei than the ordinary hyphal cells (Figs. 2, 3).

Special care was taken in the determination of the number of nuclei present in the cells of the young archicarp because of the claim of Harper (19), confirmed by Miss Welsford (27), but denied by Dangeard (10), that the cells of the archicarp of *Ascobolus* are uninucleate when first formed. No evidence of such a condition was obtained in *Ascophanus*. Very numerous nuclei were found in the ascogonial cells, even in the youngest of the uncovered ascogonia which I have seen. This question will be more fully discussed later.

As the archicarp gets covered in, the cells increase in size, and the number of vacuoles increases. The protoplasm gets less dense and the nuclei become more distinct than before. Also about this time a yellow, irregularly spherical body appears in the middle of the ascogonial cells and

also at times in the cells of the terminal vegetative portion of the archicarp. It is evidently of the nature of food-reserve and is not found in very old fruits (Fig. 5).

The nuclei in the ascogonial region show at this time a well-marked nucleolus surrounded by a homogeneous space and bounded by a nuclear membrane. The nuclei in the basal and terminal parts are in general intermediate in structure between the ascogonial and the ordinary vegetative nuclei. In slightly different stages, however, the nuclei vary in minute details as, for example, in the relative sizes of the nucleolus and the nuclear cavity.

When the archicarp is as yet but slightly covered, the characteristic granules on the transverse walls fuse and form pads which close the pores (Fig. 5).

The apical portion of the archicarp as a rule degenerates early, and with the loss of contents all of the pads disappear, except the one immediately adjoining the transverse wall which separates this region from the ascogonial. This pad is very well marked, and the wall bearing it is usually much thickened (Fig. 14).

In the basal portion of the ascogonium the pads are more persistent and very well marked; so much so as to be often of great service in the identification of this region, especially when the individual cells have to be followed through a long series of sections (Fig. 14).

The pads in the ascogonium soon, and, as far as could be ascertained, suddenly disappear, and with them that portion of the wall around the pore which was covered by them. In this way a very wide secondary pore, about a third of the width of the transverse wall, is formed (Fig. 11). The ascogonial transverse walls do not lose their pads simultaneously nor, as far as could be ascertained, in any definite order, but quite irregularly (Fig. 5).

When the whole series of pores is formed, the ascogonium is practically one large cell (Fig. 22) with a varying number of ring-like ingrowths, and it presents a very similar appearance to the adult ascogonium of *Ascobolus furfuraceus* as described by Harper (19) and by Miss Welsford (27).

The exact manner in which these secondary pores are formed has not been observed. No evidence was obtained for their formation by the gradual widening of the original pores, on the contrary the dissolution of the pads and the formation of the secondary pores seem to have always taken place simultaneously, and this suggests that the two processes are intimately connected. Pads were often found lying free in the cells, and showing indications that they contained the portion of the wall on which they were originally deposited. It is possible that some of these may have been torn away in the process of cutting, but in some cases the transverse wall was at right angles to the razor during the process of sectioning, and this explanation would hardly count here. Often traces of what seemed

to be a mucilagenous degeneration were seen in the lighter portions of the pad, and it was thought that perhaps the wall underwent some sort of mucilagenous change brought on perhaps by the action of some substance or substances originating in the granule, but as a similar appearance is seen in the basal vegetative cells, and here no secondary pores are formed, it seems likely that this appearance has a different meaning.

The observed facts seem to me to suggest that the pads act, in some way, on the transverse wall, resulting in that portion of the wall on which they are seated coming away with them. This may perhaps be due to some weakening action on the periphery of this portion of the wall.

Fertilization.

Nuclear fusions were found to take place in the ascogonium, and it was in old ascogonia, with secondary pores, that the process was first studied. At this period, and also at the time of formation of the ascogenous hyphae, the protoplasm is not nearly as dense as in the earlier stages of development, and the nuclei are more easily differentiated.

The fusion stages are as follows:—A pair of nuclei approach each other and touch; the nuclear membranes, at the point of contact, disappear, giving rise to the characteristic dumb-bell-like structure. After this the nuclear membrane takes on a spherical shape, and inside it are to be seen the two nucleoli of the original fusing nuclei (Figs. 8, 9, 10, 11, 12, 13). These nucleoli fuse later, going through a dumb-bell stage just as the fusing nuclei themselves have done. The fusion nucleus is easily recognized, both by its size and by the size of its nucleolus.

Nuclei in the first 'contact' stages of fusion are very difficult indeed to distinguish from nuclei in accidental contact, and such stages are comparatively rarely met with. It is probable that the actual fusion-process takes a very short time indeed, owing, partly at least, to the large amount of nuclear sap.

In his paper on *Pyronema* (20) Harper says that all the fusions take place at about the same time, while Blackman and Fraser (7) report that in *Humaria granulata* the fusions are spread over a long period of time. In *Ascophanus carneus* there are epidemics of fusions, that is to say, the fusions seem to last over a long developmental period, and yet when one discovers a fusion stage in an ascogonium many others are almost sure to be found at the same time.

It has already been pointed out that no cell in the ascogonium of this fungus is specially marked out from the others, either by its size, as in *Ascobolus*, or by any other feature. Corresponding to this, it was found that the fusions were not limited to one cell, as has been described in the latter genus.

Great difficulty, however, was encountered when an attempt was made to make out whether fusions took place in all of the ascogonial cells. It was found, for example, that fusions might be taking place fairly freely in one or two adjacent cells of the ascogonium, and no signs of fusions in the neighbouring cells. This point could not, therefore, be determined by working at any one ascogonium. It may seem that this difficulty might be got rid of by comparing cells in which fusions were taking place in one ascogonium with those of another ascogonium with fusions, and so obtain a complete series: but the number of cells in different ascogonia, as has already been mentioned, differs greatly, so that no one cell of an ascogonium can be compared directly with any one cell of another, except in those cases where the number of cells is equal. Again, the ascogonia are usually so curled that in sections it is generally very difficult to determine the exact relative position of individual cells. The observations given below point to the probability of fusions taking place in every ascogonial cell or, rather, compartment.

In several ascogonia fusion stages were observed in two cells and, in many others, in three. In one ascogonium as many as five cells were observed containing nuclei in different stages of fusion. Also, although, as mentioned above, it is often difficult to determine the exact relative positions of ascogonial cells, there is no doubt that fusions can take place in all portions of the ascogonium, for such processes have been observed in apical, median, and basal parts.

Miss Welsford (27) in her *Ascobolus* paper reports fusions only in the middle central cell. It seems to me possible that at least some of the nuclei may fuse before they collect in this large structure, but this possibility does not seem to have been considered.

The observations given above were made on ascogonia, the cells of which were in open continuity by means of the secondary pores, and the mere fact that these fusions are able to take place in each of the partly-partitioned-off cells is no guarantee that the fusing nuclei are nuclei belonging to the cell in which they were observed fusing.

Relationship of the Fusing Nuclei.

As has been already mentioned, there is no antheridium or male organ in this form, and so there is no possibility of nuclei coming into the ascogonium from such a source ; still it is possible that some of the vegetative cells surrounding the ascogonium may fuse with its cells, and pass over nuclei. No signs of such a process were seen. The ascogonial side-walls were intact in all stages up to the dissolution of the ascogonium. There is also a possibility that nuclei may migrate into the ascogonium from the basal or tip-portions, or both, of the archicarp through the pores in the transverse walls before they are closed by the pads. Such a process would be very difficult to observe, and might easily take place without the cells showing

any indication of a loss of contents.[1] The cells in these regions at the different stages before and after the formation of the pad were carefully studied and compared, and neither was there any sign of nuclear migration, nor were there any differences noticeable in the contents, either cytoplasmic or nuclear, between these stages, but those which could be accounted for by the gradual increase in size of the cells of these regions. There was no sign, therefore, of a nuclear migration from these sources. The fusing nuclei very probably belong to the cells of the ascogonium only.

The next point to determine was whether the nuclei in these ascogonial cells were capable of fusing only with nuclei from another ascogonial cell or whether they only fused with others of the same cell, or again, whether they fuse indiscriminately with any ascogonial nucleus. Young ascogonia were examined in which the pads were still present, and it was found that fusions took place even in these young ascogonia (Fig. 5). If we neglect the possibility of nuclei migrating previously to the formation of the pad and, indeed, there seems to be no reason whatever for supposing this process to have occurred; it must be admitted that the nuclei in any one ascogonial cell are able to fuse with one another. When the cells are in open continuity, however, there is nothing to prevent nuclei wandering freely about, and I am strongly of the opinion that then the nuclei fuse indiscriminately, though, in the absence of any differentiation between the nuclei of the different cells, there can be no positive proof.

The Ascogenous Hyphae.

Some time after the nuclei of the ascogonium have begun fusing, and, as far as could be ascertained, soon after the formation of the secondary perforations, the ascogenous hyphae begin to be developed. It has already been mentioned that no special central cell is differentiated, as in *Ascobolus furfuraceus*; similarly here no special cell gives rise to ascogenous hyphae.[2] In some ascogonia two, three, or four cells have been seen to take part in their formation. I have only seen one ascogonium in which there are traces of ascogenous hyphae being given off by all the ascogonial cells, here four in number (Fig. 14). Whether this is the usual process or not could not be satisfactorily ascertained, for as the ascogenous hyphae soon lose their connexion with the ascogonium, and as the cells probably do not all begin forming ascogenous hyphae at precisely the same time, this particular point is difficult to elucidate. Cells, however, from all portions

[1] Harper records (20) that in *Pyronema* the nuclei move over from the antheridium to the ascogonium leaving behind most of the cytoplasm.

[2] Dangeard (10) mentions that in one of his sections of *Ascobolus glaber* he thought that two ascogonial cells were giving rise to ascogenous hyphae, but he is not quite sure of this. From his figures it would appear that the ascogonial cells in this species are very similar in size, and it is possible that his observation was correct.

of the ascogonium seem capable of giving rise to ascogenous hyphae. The two or three cells nearest the tip-portion of the archicarp were more usually found in this condition than those in any other portion of the ascogonium, and the balance of evidence seems in favour of the view that all the ascogonial cells are potentially capable of giving off these hyphae, but that usually those ascogenous hyphae which are formed from the tip-cells are found sufficient for the purpose of emptying the whole ascogonium of its contents, and that therefore the two or three lowest cells do not usually form ascogenous hyphae of their own, but pass on their protoplasm and nuclei to those cells that do. It is possible, however, that all of these ascogonial cells usually give off ascogenous hyphae.

Dangeard (10) is of the opinion that in *Ascobolus* the nuclei of all the archicarp cells, except the central cell, degenerate. The very open communication between the ascogonial cells, and the fact that ascogenous hyphae are given off by more than one cell in *Ascophanus carneus*, makes it seem very unlikely that any such phenomenon should occur in this species. This point, nevertheless, received careful consideration, and in no ascogonium was any evidence of such a wholesale degeneration of nuclei obtained. It does, indeed, often happen that some nuclei are left behind in the ascogonium (Figs. 21 *a* and *b*, 22) after the development of the ascogenous hyphae, and that these undergo a process of degeneration ; but these nuclei are about equally distributed amongst the cells of the ascogonium and not situated in any special cell or cells. Miss Welsford (27) has already denied the degeneration of the nuclei of the cells on either side of the central cell of *Ascobolus furfuraceus*, and my observations are against the occurrence of any such phenomenon in *Ascophanus carneus*.

The ascogenous hyphae when first formed are full of dense protoplasm which contains numerous nuclei. Harper (21), in his paper on *Phyllactinia*, attempted to account for the series of binucleate cells in the ascogenous hyphae of certain ascomycetous fungi by comparing the nuclear fusion in the ascus with that in the basidium. Because of this theory special attention was paid to these hyphae to try and find whether any such series of binucleate cells could be found in this form, and if so, in what manner it was brought about. No such series, however, was found, and the nuclei in the newly-formed hyphae seemed to be quite irregularly arranged.

Since the appearance of Claussen's preliminary note on *Pyronema* (8)—in which the author claims that the male and female nuclei do not fuse in the ascogonium, but enter the ascogenous hyphae in pairs, and that their descendants fuse to form the primary ascus nucleus—the search has been renewed, and other series of sections have been examined, but with the same result. The nuclei are not arranged in pairs, except at the formation of the asci. The ascogenous hyphae, when they have grown out to some little distance, form septa, and the major part of the protoplasm is aggregated

in the end cells which grow out. The cells near the ascogonium get more and more vacuolated, and their nuclei seem to degenerate. Sometimes a solitary nucleus may be seen in a cell of an ascogenous hypha ; at other times as many as three or even five in one section. This latter case is somewhat against the view of a binucleate series of nuclei in the ascogenous hyphae. Four nuclei in one cell may be regarded as an example of a nuclear division not yet followed by a cell-division but the presence of five nuclei is incapable of any such explanation.

The ascogenous hyphae make their way between the cells of the fruit and amongst the bases of the paraphyses, branching much during the process, and also twisting in all directions. The portions embedded in the hypothecium are distinguished from the surrounding cells by paucity of content, and the parts in the region of the paraphyses by the density of the protoplasm. As the paraphyses themselves are much richer in protoplasmic contents than the hypothecial cells, it will be seen that the difference between these two portions of the ascogenous hyphae is very marked indeed. A similar state of affairs is reported by Harper for *Ascobolus* (20). As in this latter genus it seems very probable that the young ascogenous hypha obtains most of its nourishment from the surrounding tissue and not from the ascogonium, from which indeed it soon loses its connexion, the young ascus probably receives most of its food from the paraphyses.

After the emptying of the ascogonium by the ascogenous hyphae, the remaining nuclei degenerate (Figs. 14, 21 *a* and *b*, 22), passing through stages very much like those described by Davis (12) for the degenerating nuclei in the oogonium of *Saprolegnia*, becoming more and more vacuolate, swelling up, and finally disappearing. The ascogonium-walls are forced inwards by the pressure of the surrounding cells, and soon the whole structure is unrecognizable. In the case of ascogonia which are vertically placed in the fruit, the ascogonial cells are first drawn out (Fig. 21 *a* and *b*) before their total disorganization.

The Ascus.

The ascogenous hyphae bend over at the tips (Fig. 15 *a*) and form the characteristic bent end, from the penultimate cell of which the ascus is formed with the usual fusion of two nuclei (Figs. 15 *a*, *b*, *c*, *d*, 16). This latter process is sometimes somewhat delayed.

As the ascus gets larger and larger a series of vacuoles make their appearance above and below the nucleus, and the protoplasm in these regions becomes more and more vacuolate until only the protoplasm immediately around the nucleus and at the extreme tip of the ascus is free from vacuoles (Fig. 19). At the tip the protoplasm is extremely dense, especially at that portion where the lid is formed, by which the ascus opens.

The ascus by this time has taken on the club-shape so commonly found in the Ascobolaceae, and resembles very much the figures which Overton gives of *Thecotheus Pelletieri* (24).

Although material has been fixed at all times of the day, no stages have been seen either in the divisions of the ascus-nuclei or in the formation of the wall of the spore. Most of the fruits that were examined were, however, much too young to show these stages, and those that were old enough were not examined in as much detail as the younger ones, so that it is still possible that such stages may be amongst the sections, and that such processes do take place during the day. The characteristic number of spores—eight—is formed in the ascus.

General Considerations.

In recent years evidence in favour of the sexuality of the Ascomycetes has been rapidly accumulating, and several cases of fertilization, both of the normal and of the so-called reduced type have been described.

Professor Harper has reported normal fertilization by means of male and female organs in *Sphaerotheca* (18, 19), *Erysiphe* (19), *Pyronema* (20), and *Phyllactinia* (21), and Blackman and Fraser (6) have confirmed his observations on *Sphaerotheca.*

The first recorded case of reduced fertilization was described by Blackman and Fraser (7) in *Humaria granulata.* Since then Miss Fraser has recorded other similar cases in *Lachnea stercorea* (14) and *Humaria rutilans* (15), and Miss Welsford a similar process in the development of *Ascobolus furfuraceus* (27).

Claussen (8) has recently described the state of affairs in *Pyronema confluens*, in which, as he holds, the male and female nuclei, arranged in pairs, pass out of the ascogonium into the ascogenous hyphae, and the sexual fusion is delayed until the formation of the ascus. From this one observation he concludes that in no ascomycete is there more than one fusion, and that the rule obtains throughout the group that the sexual fusion is delayed until the ascus formation.

Miss Fraser and Miss Welsford (17) have already pointed out that although such a shifting of the sexual fusion is possible, yet there is no reason whatever to regard it as the rule, rather than the exception, amongst the Ascomycetes.

In the paper that I have just quoted, the well-authenticated case of *Sphaerotheca*, in which there has been shown to be two fusions, is urged against this view of Claussen's, and also the fact of double reduction, which Miss Fraser has observed, in the ascus of *Humaria rutilans* and other forms (15, 17).

As this theory of Claussen's is fully discussed by Miss Fraser and

Miss Welsford (17) in their paper 'Further contributions to the Cytology of the Ascomycetes', the subject need not be further entered into here.

In *Ascophanus carneus* we have another example of a reduced fertilization, and in this case also two fusions have been seen, the one in the ascogonium representing the sexual fusion, and the other, the fusion which is the usual preliminary to the formation of the ascus.

The ascogonium of this form presents many points of similarity to that of *Ascobolus furfuraceus*, the two most striking differences being that, in the latter, only one ascogonial cell gives off ascogenous hyphae, and that, as has been described by Harper (19) and confirmed by Miss Welsford (27), the cells of the archicarp are uninucleate when first formed. This latter statement has been denied by Dangeard (10) who says that the ascogonium is multinucleate at its inception. The mere fact that the cells of the ordinary vegetative mycelium of the fungus are multinucleate at once presents a difficulty, when one tries to imagine the way in which an ascogonium with uninucleate cells could originate on it. Neither Harper nor Miss Welsford used pure cultures, nor did they succeed in obtaining anything like a complete series from the uninucleate to the multinucleate condition of the archicarp. In the absence of such a series it is not at all impossible that the fruit with the uninucleate ascogonium may belong to another fungus, and not to *Ascobolus furfuraceus*. It will also be remembered that the descriptions of this young fruit given by Harper and Miss Welsford do not agree, the former recording well-marked pores between the ascogonial cells and the latter finding no pores at all at this stage. Dangeard describes pores even in the earliest stages of the ascogonium of *Ascobolus*, and he regards these pores as comparable to the ones found in the vegetative hyphae. This description of their structure and of their origin as multinucleate cells would agree more closely with what I have found in *Ascophanus carneus*, but whether the ascogonium of *Ascobolus* is multinucleate at its origin or uninucleate, I think that it is comparable to that of *Ascophanus*.[1] Miss Fraser and Miss Chambers (16) mention that the archicarp of *Ascobolus furfuraceus* seems to have a sterile stalk, but whether it has a sterile tip-portion I have been unable to make out from the published accounts: however, Taf. XII, Fig. 44 of Harper's paper (19), and Pl. IV, Fig. 5 of Miss Welsford's paper (27) would seem to suggest that this is so.

In *Ascophanus carneus* the ascogonial cells are very nearly of the same size, fusions take place in all of them, and the majority, if not all, of these cells give off ascogenous hyphae; for these reasons I regard all of the ascogonial cells of *Ascophanus* as female. In *Ascobolus*, as Miss Welsford has pointed out, the central cell must be regarded as female, but the other

[1] The ascogonium of *Melanospora parasitica*, from the description given by Kihlmann (22), would also seem to be very similar to that of *Ascophanus*, but the details of its structure and development have yet to be worked out.

ascogonial cells may be regarded either as female or vegetative. If we allow that the ascogonia of these two forms are comparable structures, in the light of what has been said above for the ascogonium of *Ascophanus*, it would seem more than probable that all the cells of the ascogonium of *Ascobolus furfuraceus* are female. The isolated case which Dangeard (10) mentions, in which there was a suggestion of ascogenous hyphae coming off from two ascogonial cells in *Ascobolus glaber*, seems to strengthen this view.

Any attempts which have been made to trace out a monophyletic origin for the Ascomycetes have been met with two great difficulties: the various types of the male and also of the female organ met with in this group. Miss Fraser and Miss Chambers (16) in their paper on *Aspergillus herbariorum* have successfully disposed of the first problem, but the second still presents considerable difficulties.

In *Ascophanus carneus* we find an archicarp that is comparable to that of *Ascobolus furfuraceus*, and that also presents several points of similarity with the archicarps of other forms.

Like the Lichen archicarp it is divisible into three portions, a stalk, an ascogonial, and a tip-portion, which very probably represents a trichogyne, although in the absence of any trace of a male organ it is impossible to make sure of this. The number of cells in each of these portions of the archicarp, both in *A. carneus* and in Lichens, varies considerably; primary pores are also found connecting the cells, and these are broken down to form larger secondary pores. It is true that the pores in the 'trichogyne' portion of *Ascophanus* are permanently closed, but this is probably connected with the loss of function of this organ, while in the Lichen the secondary pores in this region are probably formed to allow of the passage of the male nucleus to the ascogonial region. In both plants a considerable number of cells give rise to ascogenous hyphae. The only marked point of difference lies in the uninucleate nature of the cells of the Lichen archicarp. Miss Fraser has suggested that the ascogonium of *Ascobolus* bridges over the gap between the uninucleate ascogonium and the coenogamete, but, as I have already shown, it cannot be regarded as proved that the ascogonium of *Ascobolus* is composed of uninucleate cells at its origin. Miss Dale (9) mentions that in *Gymnoascus* the ascogonium is uninucleate at the start and becomes multinucleate later, but in the case of neither of the species that she examined does Miss Dale give an account of the number of nuclei in the vegetative cells. It seems probable from the short, preliminary communications that Barker (1, 2) has made on *Ryparobius* that this genus may throw some light on the subject, and the appearance of his full account will be awaited with interest.

Fraser and Chambers, in discussing the different types of female organ in the Ascomycetes, point out that although several types are known in which the trichogyne is multicellular, yet the only case in which it

is reported that the ascogonium is multicellular is in the archicarp of the Lichen type. A complete investigation of such a form has yet to be made, and these authors suggest that the ascogonium may really be unicellular before fertilization and develop into a multicellular structure afterwards as in *Boudiera*,[1] *Gymnoascus*, and the Erysiphaceae. Whether this is really so further research alone can tell, but the facts so far recorded seem to be against this view. Baur (3) found that in *Collema* there were, from the beginning, a number of ascogonial cells, and he records the fact that after fertilization these cells divide, so that the number of ascogenous cells is thus increased. Darbishire (11) when working at *Physcia pulverulenta* paid especial attention to this point, and says that he does not find in this species the state of affairs which Baur stated for *Collema*; the ascogonial cells do not divide after fertilization. If, as Fraser and Chambers (16) suggest, the unfertilized ascogonium of the Lichen type is unicellular, it does not seem likely that the above-mentioned observers would have missed this point, especially as they were looking for divisions of the ascogonial cells.

It has been pointed out that the ascogonium of such forms as *Boudiera*, *Gymnoascus*, and the Erysiphaceae develops into a multicellular structure after fertilization. This structure, although analogous to the ascogonium, which is multicellular before fertilization, can scarcely be regarded as its homologue. Such a multicellular structure would seem to have some advantages over the unicellular form, as in *Humaria granulata* and *Lachnea stercorea*, in that it allows of the origin of ascogenous hyphae over a larger surface. The ascogenous hyphae are therefore, at their origin, placed under conditions better suited for their nourishment than when they arise crowded together on a more limited area. The septate 'ascogonium' of the Erysiphaceae might possibly be regarded as a structure adapted to the conditions best suited to the nourishing of the ascogenous hyphae. It will be remembered that in *Gymnoascus* (9) each of the cells of the septate 'ascogonium' gives rise to ascogenous hyphae, while in what would seem to be the undoubtedly homologous structure in *Erysiphe* only one cell produces ascogenous hyphae; in *Phyllactinia*, however, it would seem to be probable that more than one cell of the septate 'ascogonium' produces branches, and it is possible that in the ancestral form of the Erysiphaceae all the cells branched. Further research on allied forms might throw some light on this point, which seems to me to present another argument in favour of the reduction of the Erysiphaceae. It is possible that a comparable series of reductions is present in the Ascobolaceae, but is here shown in a series of ascogonia septate before fertilization.

Dangeard (10) records that in *Ascophanus ochraceus* he finds ascogonia like those of *Pyronema*, but without antheridia. On the strength of this he proposes to remove the genus *Ascophanus* from the Ascobolaceae. As the

[1] This plant may really be an *Ascodesmis*.

genus *Ascophanus* agrees very well with the characters of the Ascobolaceae (except in the ascogonium of *A. ochraceus* mentioned above), and as the ascogonium of *Ascophanus carneus* agrees very well with those of *Ascobolus* and *Lasiobolus*, it would seem desirable to retain the genus in the Ascobolaceae.

In concluding I wish to express my thanks to Prof. V. H. Blackman for the valuable help and advice he has given me throughout the progress of this investigation.

Summary.

I. The vegetative mycelium of *Ascophanus carneus* is composed of multinucleate cells. There is a pore in the middle of the transverse wall of each cell, and on either side of this pore a number of granules are situated.

II. The fruit is formed by one ascogonium, but the ascogonia often arise very close together, and in such cases the fruit-coverings of two adjacent ascogonia become common (cp. *Aspergillus*). In such cases each ascogonium still forms a separate hymenium.

III. The archicarp of *Ascophanus carneus* is of the type which Woronin described as a 'scolecite'. It is composed of a varying number of cells. It may be very simply curved but usually is curled round and round in a very complicated manner. There is no male organ.

IV. The cells of the archicarp are at first very similar, but they soon differentiate into three regions more or less marked off from each other. The middle portion behaves as an ascogonium, and the two side portions remain vegetative. The number of cells which go to make up these different portions varies greatly. The apical portion of the archicarp may possibly represent a trichogyne.

V. Each of the ascogonial cells is provided with a small pore in the middle of the transverse wall. This pore is guarded by small granules which early fuse together to form a pad closing the pore. After a time this pad disappears, and then a large secondary pore, as wide as the diameter of the pad, is found in the transverse wall. The ascogonial cells seem to be multinucleate at their formation.

VI. Nuclear fusions seem to take place in all of the ascogonial cells, and have been noted even in cells still cut off from their neighbours by the pads mentioned above. No nuclear migration into the ascogonium was noticed, either from the surrounding vegetative cells or from the other portions of the archicarp.

VII. All the ascogonial cells seem to have the power of giving off ascogenous hyphae, but it is not certain whether they always do so. Each ascogonial cell is regarded as female, and the nuclear fusions noticed within these are regarded as a reduced type of fertilization in which the female nuclei fuse in pairs.

VIII. The tips of the ascogenous hyphae bend over in the usual manner, and the ascus is formed from the penultimate cell after the fusion of the two nuclei which it contains.

IX. The ascus contains eight spores with walls of medium thickness. These spores germinate only in alkaline media (in one case a spore was observed to germinate in an acid medium) and under the influence of a medium temperature (about 35° C.).

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EXPLANATION OF PLATE XXVIII.

Illustrating Mr. Cutting's paper on the Ascocarp of *Ascophanus carneus*.

All the figures have been drawn with the aid of the camera lucida and the apochromatic objective 2 mm. apert. 1·30 of Zeiss, Figs. 1–4, 9–13, 15–17 and 20, with the ocular 12, and Figs. 5–8, 14, 18, 19, 21 *a* and *b*, and 22, with the ocular 6. Fig. 1 has been reduced about two-thirds in reproduction. The sections from which Figs. 16 and 17 were drawn were stained with Heidenhain's haematoxylin and erythrosin, the others with Fleming's triple stain.

Fig. 1. Restoration of typical archicarp made from several sections of a young fruit. × 1180 (about).

Figs. 2 and 3. Sections through cells of a young uncovered archicarp showing numerous nuclei. × 1770.

Fig. 4. Section showing transverse wall between contiguous ascogonial cells with the small primary pore and the granules on either side of it. × 1770.

Fig. 5. Ascogonium showing nuclear fusions in one of the cells entirely cut off from the adjacent cells by pads over the pores. In the transverse wall of the lowest cell a large, secondary pore is shown. The drawing is taken from the ascogonium which is reconstructed in Fig. 1, and is built up from two successive sections. × 830.

Fig. 6. Ordinary vegetative cell of fruit showing numerous nuclei. × 830.

Fig. 7. Cells from surface of fruit showing one of the secondary hyphae with pits in the transverse walls of the cells. × 830.

Fig. 8. Section through a young fruit showing three ascogonial cells in open contact. Only in the two lowest cells were any signs of fusion seen. × 830.

Fig. 9. Sections from the same ascogonium as Fig. 5, showing fusion-stages. × 1770.

Figs. 10, 11, and 12. Sections from the same ascogonium as Fig. 8, showing different fusion-stages in two contiguous cells. The secondary pore is well shown in Fig. 11. × 1770.

Fig. 13. Group of nuclei from an ascogonium showing nuclei before fusion, two in the act of fusing and a fusion nucleus. × 1770.

Fig. 14. Old ascogonium showing three portions; 'trichogyne,' ascogonium, and stalk-cells (most of the last have become disorganized). Ascogenous hyphae are shown coming off from all the ascogonial cells, but the majority are given off by the two cells adjacent to the 'trichogyne'. (Drawn from several sections.) × 830.

Fig. 15 *a*, *b*, *c*, *d*. Different stages in the formation of the ascus. × 1770.

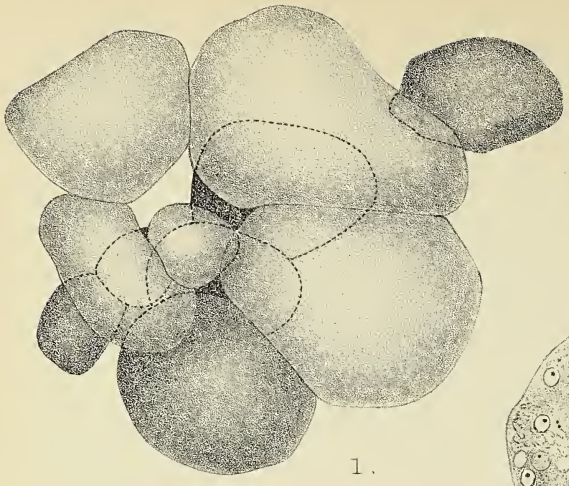
Fig. 16. Fusion of nuclei to form ascus-nucleus. × 1770.

Figs. 17 (× 1770), 18, and 19 (× 830). Young asci of slightly different ages.

Fig. 20. Section of ascospore as seen when still enclosed in the ascus. × 1770.

Fig. 21 *a*, *b*. Two sections through an old ascogonium showing degenerating nuclei and traces of ascogenous hyphae. The 'trichogyne' is also indicated. × 830.

Fig. 22. Section through another degenerating ascogonium. The secondary pores are well shown here. The nuclei are filled in from several sections. × 830.



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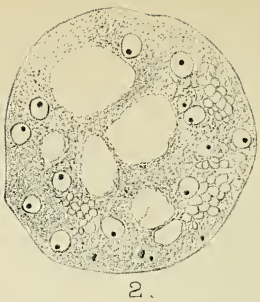

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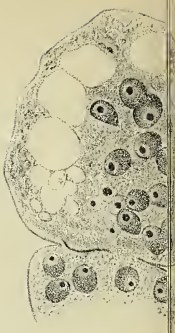

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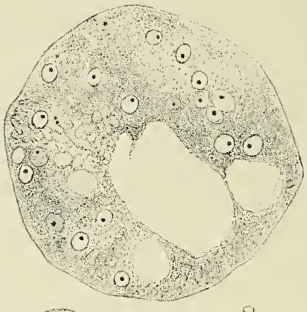

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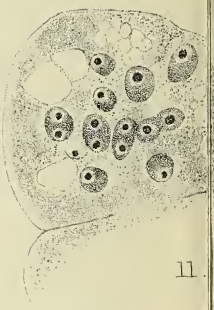

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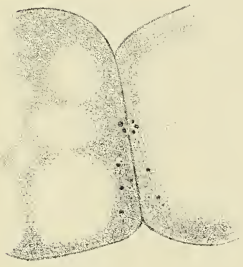

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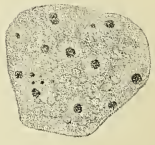

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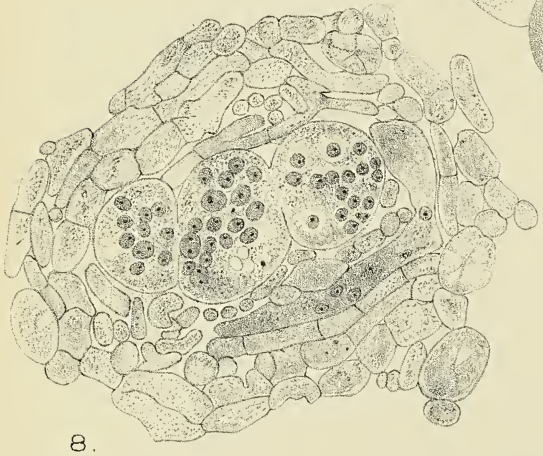

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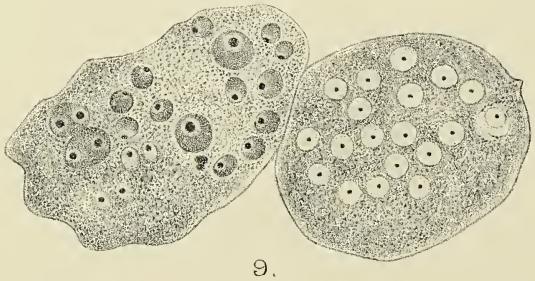

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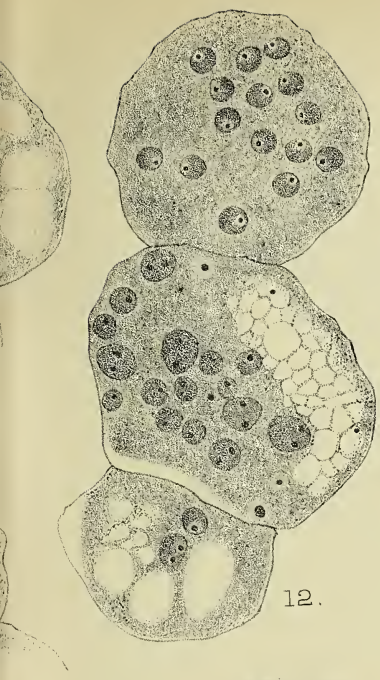
CUTTING — ASCOPHANUS CARNEUS



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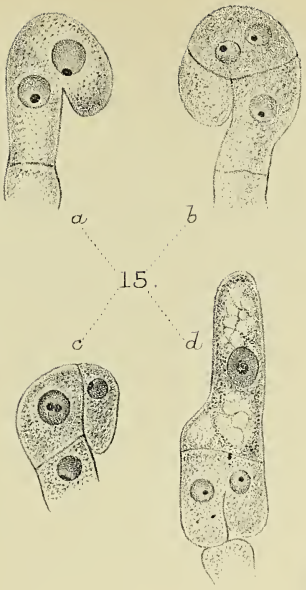

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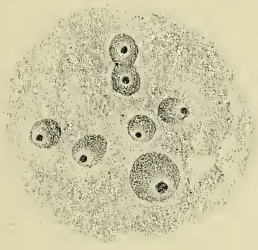

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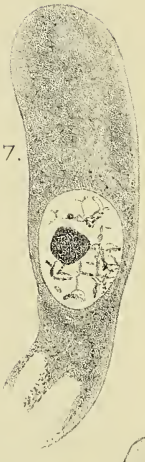

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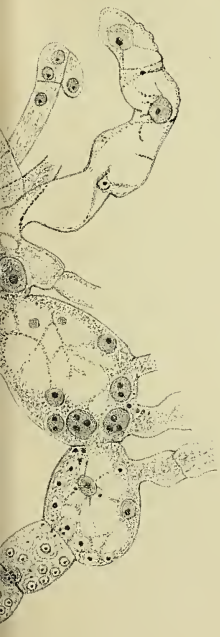

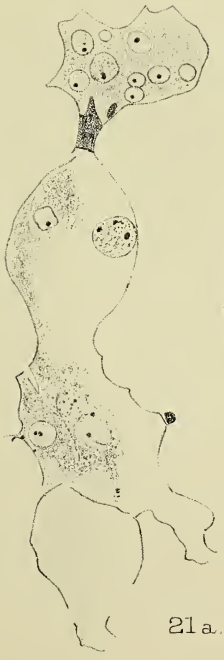

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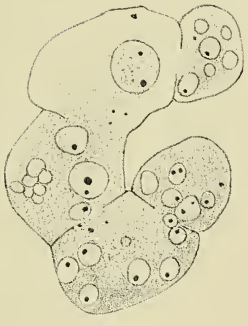

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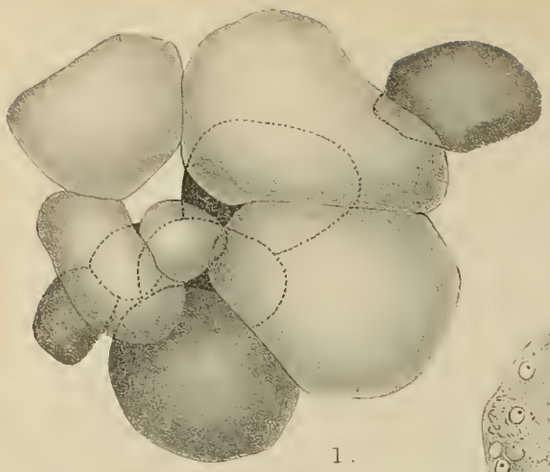
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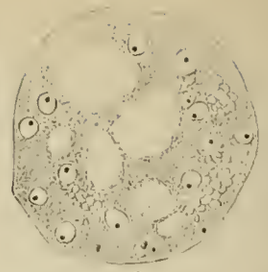
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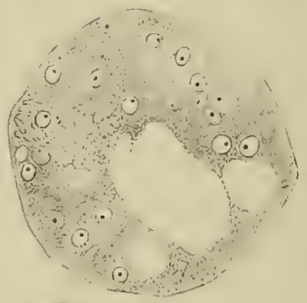
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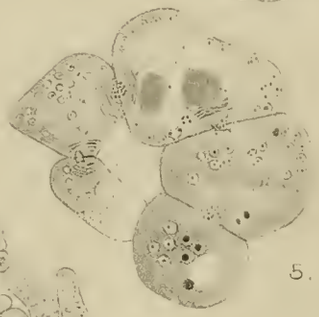
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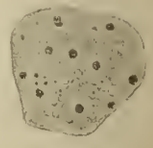
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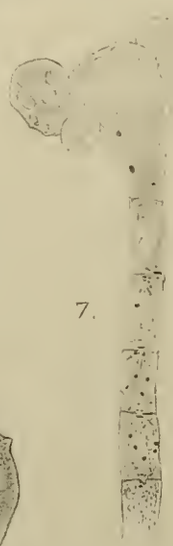
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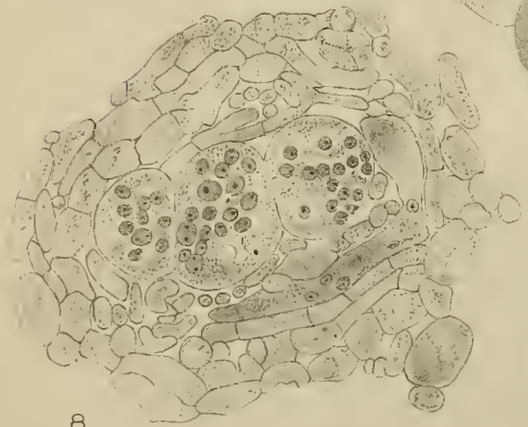
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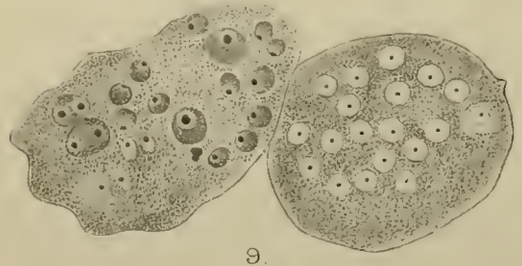
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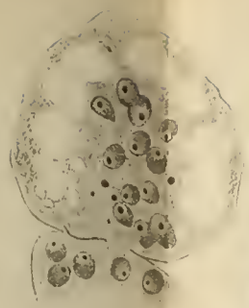
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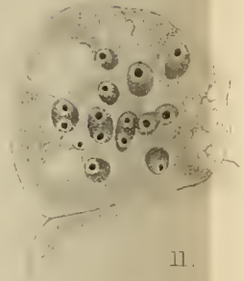
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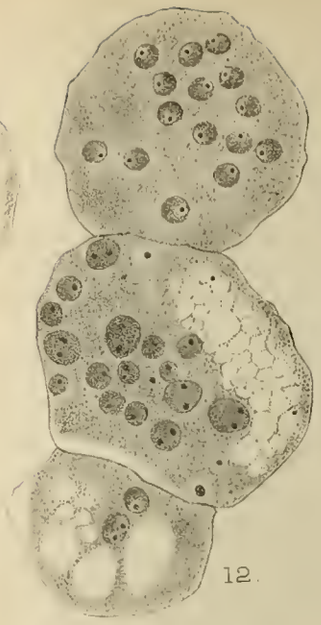
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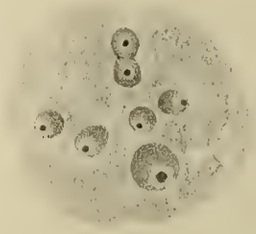
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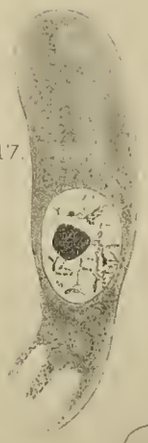
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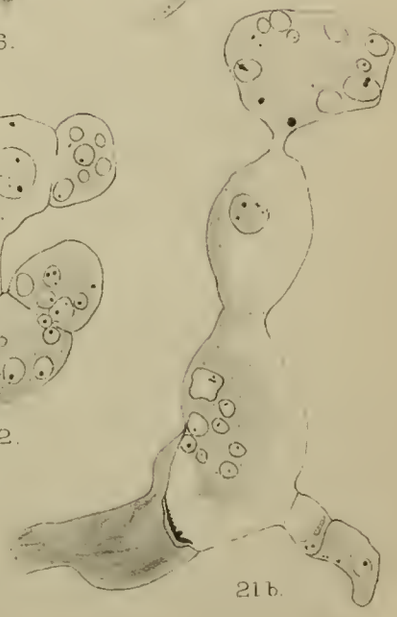
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E.M.C. del.

On the Vascular Structure of some Species of Gleichenia.

BY

L. A. BOODLE, F.L.S.,

AND

W. E. HILEY, B.A.,[1]

Queen's College, Oxford.

With Plate XXIX, and three Figures in the Text.

THE first full account of the anatomy of species of *Gleichenia* was given by Poirault in 1893. All the forms known to him were protostelic, and in these he carefully described the curious nodes, and the manner of departure of the leaf-trace. More recent observations on the vascular structure in this genus have been published by Boodle ('01), Jeffrey ('02), and Tansley ('07). In the first of these three papers the most important feature was the description of the anatomy of *G. pectinata* (Willd.), Pr., this species being specially interesting, as it differs from all the other members of the genus, as far as they are known, in having a solenostelic rhizome. The account of *G. pectinata*, given by Boodle ('01), was naturally incomplete, being founded on the examination of a small amount of dried material. Through the kindness of the late Mr. G. S. Jenman, who sent a supply of rhizomes preserved in spirit from British Guiana, we have been able to make a more extended study of the anatomy of this species. The results are given in the present paper, together with some observations on other species of *Gleichenia*,[2] which were re-examined for purposes of comparison.

G. PECTINATA.

G. pectinata is indigenous in tropical America, and appears to be confined to that region. It has a long trailing rhizome, which is approximately cylindrical, and bears the leaves at intervals of from four to six inches on its

[1] From the Jodrell Laboratory, Royal Botanic Gardens, Kew.

[2] *G. flabellata*, R. Br., *G. circinnata*, Sw., with var. *semivestita* and *G. dicarpa*, R. Br. The material was obtained from living plants grown at Kew.

[Annals of Botany, Vol. XXIII. No. XCI. July, 1909.]

upper side. The leaves are of the so-called dichotomous form,[1] and have long pinnules; the last feature being one of the characters of the subgenus *Mertensia*, in which this species is placed.

Internode.

A transverse section of a fully developed internode is represented diagrammatically in Plate XXIX, Fig. 1. The cortex (c.) consists of brown sclerenchyma, and is bounded externally by an epidermis, and internally by a well-marked endodermis. A zone of cortex, two or three cells in thickness, and just external to the endodermis, is distinguished from the rest of the cortex by the greater thickening of the cell-walls.[2] Three layers of thin-walled parenchymatous cells constitute the pericycle, within which there is a continuous ring of protophloem with metaphloem on its inner side. There is, as a rule, only one layer of parenchyma (mesocycle) between the phloem and xylem. The xylem, which has an annular form, is mesarch, and is composed of tracheae[3] and parenchyma, intermingled in the same way as in other species of *Gleichenia*.[4] The tissues within the ring of xylem form a series arranged in reversed order as compared with the similar tissues outside, and are as follows: inner mesocycle, metaphloem, protophloem, pericycle, endodermis, and lastly sclerenchyma. The sclerenchyma, which occupies the central space, is of the same nature as that forming the cortex, and its outer elements usually have smaller lumina than those nearer the centre, but there is nowell-marked zone of thick-walled cells like that of the inner portion of the cortex.

The rhizome is distinctly dorsiventral in structure, even where unaffected by the proximity of a leaf-trace, since the protoxylems of the lower half of a transverse section are differently situated (as regards immersion in the metaxylem) from those of the upper half. In the stem, a node of which is about to be described, there were seven of the lower kind of protoxylems and four of the upper, but these numbers are not constant, though their proportion is approximately maintained. The lower protoxylems (which are connected with the roots) are distinguished by having no large tracheae outside them; they are situated in xylem-promontories, in which they are covered by only a few small scalariform tracheae. On the other hand, the protoxylems of the upper part of the xylem appear deeply embedded, as the tracheae on the outside are large, like those on the inner

[1] See Goebel ('05), vol. ii, p. 319; Tansley ('07), p. 135; Bower ('08), p. 553.

[2] There is sometimes one layer of thin-walled cells between the endodermis and this zone of specially thickened cells.

[3] These are to be classed as vessels. Some of the mature tracheae were carefully examined, and the pit-membrane was found to be missing. See Gwynne-Vaughan ('08).

[4] The parenchymatous elements of the xylem may become lignified; one case was noted in which nearly all the xylem-parenchyma had undergone this change.

side of them. The outline of the xylem is not disturbed by the upper protoxylem-groups, hence there are no promontories on the upper surface of the xylem-ring.

Node, &c.

In the present instance the lowest cross-section affected by the node was at a distance of 2·9 cm. behind the axil of the petiole, or 2·6 cm. behind the point of complete separation of the leaf-trace from the stele. The first stage of preparation for nodal structure had been reached in the section shown in Plate XXIX, Fig. 3; here the protoxylem-elements at *y* 1 have become slightly scattered, and the parenchyma in their neighbourhood has increased in bulk, forming the beginning of a nodal island. In sections cut serially towards the growing apex it was next noticed that, towards the inner side of this mass of parenchyma, a few elements of protophloem, and subsequently metaphloem, arose; meantime the protoxylem branched, and formed two clusters, one at each end of the parenchymatous island. In the middle of the latter a few fibres were soon discernible; the lowest section containing them showed one fibre only, surrounded by an endodermis; but even this did not appear until the phloem had increased to about twenty elements, all of which were situated on the inner side of the group of sclerenchyma, and were separated from the endodermis by a single layer of parenchyma. By this time there was a radial band (Plate XXIX, Fig. 3, *r. b.*), composed of about three layers of xylem-parenchyma, connecting the parenchyma of the island with the inner mesocycle. This band of parenchyma next became broader, and the phloem spread into it from either end, until the phloem of the nodal island and the inner phloem of the solenostele were eventually united. At this stage a few elements of protophloem (Plate XXIX, Fig. 4, *ab. ph.*) were noticed in the nodal island on the abaxial side of the sclerenchyma. These elements were nowhere connected with the rest of the phloem, but they persisted for about 4 mm. along the rhizome, and then disappeared. The effect of the failure of this phloem on the petiolar bundle will be discussed later.

Groups of fibres now arise near the protoxylems *y* 2 and *y* 3, and eventually near *y* 4; they increase in size, and, one by one, become joined to the original nodal mass of sclerenchyma belonging to *y* 1, the parenchyma becoming united first in each case. Protophloem-elements also arise independently near each of the lesser sclerenchyma-groups, and afterwards unite to form a continuous band of phloem in the composite nodal island, on the inner side of the sclerenchyma. As the radial band (Pl. XXIX, Fig. 5) broadens, the sclerenchyma at the centre of the stele, and that of the nodal island spread into it, so that the two masses become united by a narrow strip having phloem on each side of it. The cross-section of the sclerenchyma is now T-shaped (cf. Boodle, '01, Pl. XXXIX, Fig. 26). Each of

the protoxylems $y3$ and $y4$ branches into two, thus giving $y3'$, $y3''$, $y4'$, and $y4''$ (cf. Plate XXIX, Fig. 6). The protoxylems $y3''$ and $y4''$ take no part in the formation of the foliar bundle.

The detachment of the leaf-trace from the stele of the rhizome takes place in the following manner. The radial band lengthens, and a gap is formed in the xylem between the protoxylems $y3'$ and $y3''$, and through this gap the internal sclerenchyma, phloem, and endodermis become connected with the corresponding external tissues, the process being similar to the differentiation of the radial band, as described above. One end of the arched leaf-trace is thus separated (Pl. XXIX, Fig. 6). Soon afterwards a similar interruption is formed on the other side between the protoxylem-groups $y4'$ and $y4''$, and the foliar bundle is then completely severed from the stele of the rhizome, leaving a leaf-gap, which, however, soon disappears. The protoxylems $y3''$ and $y4''$ by division give rise to the usual four upper protoxylems of the stele, and the normal internodal structure is restored. There is no distinct thickening of the 'margins of the leaf-gap', such as Gwynne-Vaughan ('03, p. 700), inferred from the earlier description of the node (Boodle, '01, p. 730).

The xylem of the leaf-trace is of the horseshoe type with incurved ends, and is endarch, several groups of protoxylem being scattered on the inner face of the arch. The phloem is continuous on the outer side of the xylem-arch, and on the inner surface of the incurved ends, but is absent in the inner middle region of the arch.

The description given above applies to nearly all the nodes examined, but in one node among the material sent by Mr. Jenman the structure was of a somewhat different type. In this case immediately the leaf-trace became detached from the stele of the rhizome one protoxylem-group of the leaf-trace ($y3'$ in Pl. XXIX, Fig. 6) became markedly mesarch, and a small group of fibres (accompanied by endodermis and phloem), branching off from the internal sclerenchyma, became immersed in the metaxylem in the neighbourhood of the protoxylem-group in question. In this way a group of tissue, resembling a small nodal island, came to be contained in the xylem of the leaf-trace near one of its extremities. Unfortunately the petiole had been cut off close to the base, so that it was not possible to trace the fate of the severed island. Another type of nodal structure was described by Boodle ('01, p. 730); in this case similar groups of tissue, resembling small nodal islands, became embedded in the xylem of the stele in the neighbourhood of the protoxylem-groups $y3''$ and $y4''$, and this took place just before the separation of the leaf-trace. These secondary islands, which differ from the typical nodal islands in being extended acropetally, were apparently not continued for any great distance.

Comparison with other Species.

In the species of *Gleichenia* examined two types of nodal island may be distinguished. In *G. pectinata*, *G. flabellata*, and *G. linearis*, Clarke,[1] which belong to the subgenus *Mertensia*, the first stage in the formation of a nodal island is an increase in the number of cells of xylem-parenchyma near a protoxylem-group, as described above for *G. pectinata*. As this nodal island increases in size (i. e. when traced acropetally), it becomes associated with the neighbouring protoxylem-groups. Since the extended nodal island thus constituted forms the inner limit of the leaf-trace, the latter has endarch structure when it separates from the stele. The other type of nodal structure is shown by *G. dicarpa*, *G. rupestris*, R.Br., and *G. circinnata*, with its variety *semivestita*, belonging to the subgenus *Eugleichenia*. Here the nodal island does not originate in connexion with a protoxylem-group, and the xylem of the leaf-trace separateswith one or more of the protoxylems mesarch (cf. Boodle, '01, Plate XXXIX, Fig. 22).

A very young node of *G. circinnata* var. *semivestita* will now be described, and will furnish an example of the second type of nodal island. The node was at a distance of about 1 cm. from the growing apex. The structure of the stem showed signs of dorsiventrality at an early stage; near the growing point the three lower protoxylems became lignified before the two upper groups, and soon gave off root-traces. Text-fig. 11 shows the structure of the stem below the node; 1, 2, and 3, are the lower protoxylems; 4 *a* and 4 *b* (derived from the branching of 4) and 5 are the upper groups. 4 *b* again branches to form 4 *b'* and 4 *b''*, and at the same time a nodal mass of parenchyma is differentiated (Text-fig. 12, *n.i.*), and is easily distinguishable from the surrounding immature tissue, which is destined to form normal xylem. The nodal mass of parenchyma is not immediately associated with a protoxylem-group, but is equidistant from the protoxylems 4 *b'*, 4 *b''*, and 5. It increases in size (when followed acropetally), and soon contains some phloem-elements. A new protoxylem-group (6 in Text-fig. 13) then arises, and has no connexion with any of the other protoxylems. Text-fig. 21 shows the lowest section of this protoxylem, the end-wall of the single trachea being seen in surface-view. In the transverse section this trachea was separated by a long distance from any other lignified element. It was itself very feebly lignified, but showed distinct sub-scalariform markings. Text-figs. 22 and 23 are from higher sections, two protoxylem-elements being present in Fig. 23.

To return to the general description of this node, in the next stage after that shown in Text-fig. 13, the xylem becomes cut across between the protoxylems 5 and 6, and between 4 *a* and 4 *b'*, the endodermis insinuating

[1] This species was referred to under the name of *G. dichotoma* in 'Anatomy of Gleicheniaceae' (Boodle, '01).

itself between the stele and the leaf-trace (the process beginning on the left) in such a way that the nodal mass of parenchyma (i. e. undifferentiated sclerenchyma) never comes into connexion with the cortex. The leaf-trace

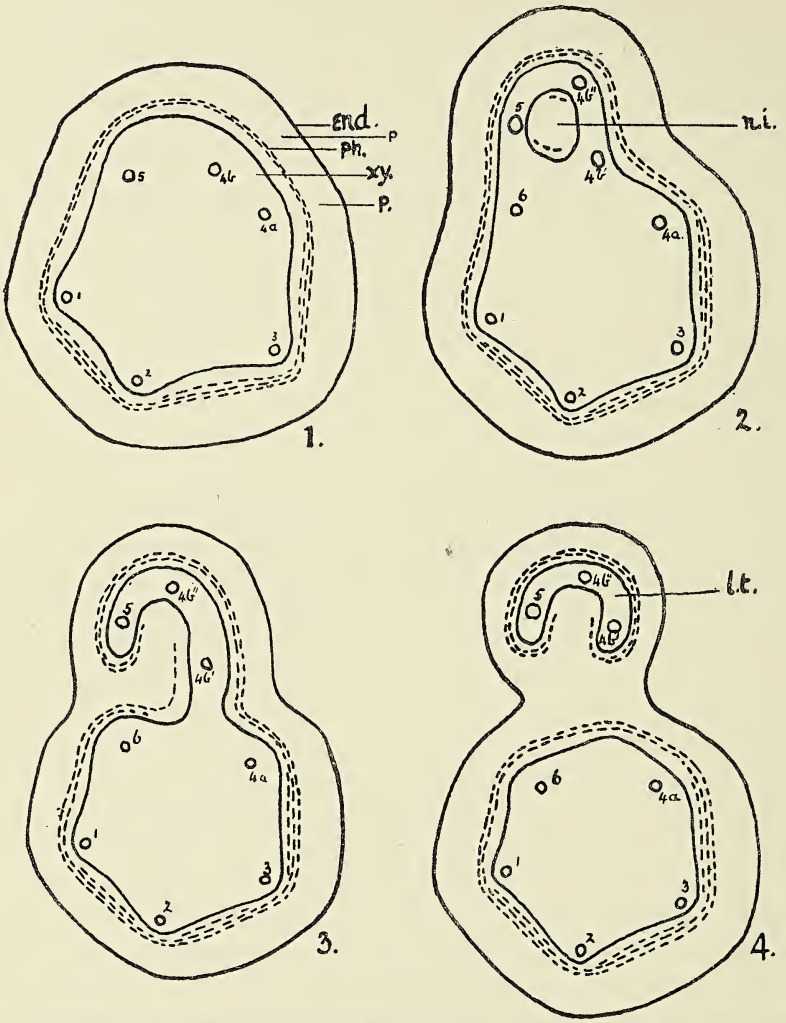

FIG. 1. *Gleichenia circinnata.* Transverse sections through a very young node. *end.* endodermis; *n.i.* nodal island; *p.* pericycle; *ph.* phloem. 1, 2, 3, 4*a*, 4*b*, 4*b'*, 4*b''*, 5, and 6, protoxylems. × 50.

takes with it the protoxylems 4 *b'*, 4 *b''*, and 5, leaving 4 *a* and 6 to supply the upper protoxylems of the stele (Text-fig. 1 4).

The mature node differs from the one just described in having a group of sclerenchyma surrounded by an endodermis in the nodal island. In some of the cases examined this sclerenchyma became connected for a short distance with the cortex, but this is apparently not a constant feature. The

number of fibres in the nodal island, or in its continuation in the petiole, varies considerably in different varieties; thus Boodle ('01, p. 725) found only three in *G. circinnata*, while for the var. *Speluncae*, Poirault ('93, p. 181) figures a petiole with thirteen fibres, and in var. *semivestita* we found sixteen. In *G. rupestris* only a single fibre was present in one case.

The internal pericycle of the leaf-trace and petiolar bundle may become sclerenchymatous,[1] this tissue being then composed of septate fibres with thick walls and numerous pits.[2] Examples of this were found in *G. circinnata*, and may be compared with the case of *G. alpina*, R.Br. (= *G. dicarpa*

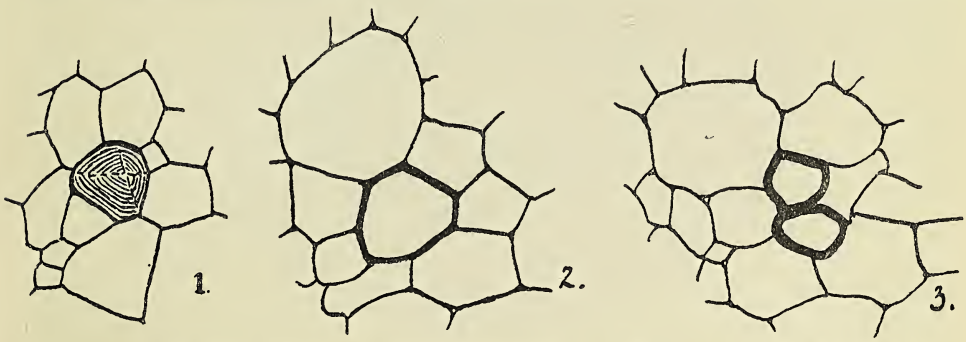

FIG. 2. *Gleichenia circinnata.* Protoxylem-element (6 in Fig. 1). 1. Lowest section of the element showing sub-scalariform markings. 2. Another section of the same, nearer the stem-apex. 3. Still higher section, showing two elements. × 540.

var. *alpina*, H.B.), in which both inner and outer pericycle in the petiolar bundle become sclerotic (Boodle, '01, p. 716). The xylem-parenchyma in the stele may also become lignified and fibrous; but this was only observed in the variety *semivestita*, while specimens of typical *G. circinnata* did not show this character. Poirault, however ('93, p. 173), describes the presence of 'quelques rares cellules scléreuses' among the scalariform tracheae in *G. alpina*. R.Br. (*G. hecistophylla*, Cunn.). In *G. circinnata* and many other species of *Gleichenia*, including *G. pectinata*, phloem-fibres are occasionally to be found in the petiolar bundle.

[1] The pericycle of the nodal island may also become sclerenchymatous, e.g. in *G. circinnata* var. *semivestita*.

[2] These septate fibres may be well shown by staining with Hofmann's blue, the preparations being made in the following way: Sections are soaked in eau de Javelle for about five minutes, then washed in spirit, and treated with a solution of Hofmann's blue (Grübler's) in weak glycerine (50 per cent.). The tracheae and all other lignified elements stain deep blue. Permanent preparations may be made by mounting the sections in glycerine-jelly coloured with Hofmann's blue. The brown fibres of the cortex and nodal island remain brown (unless bleached by long soaking in eau de Javelle); they are thus easily distinguished from fibres belonging to the pericycle of the leaf-trace or nodal island, since all these elements readily take up the blue stain. In the same preparations the dots on the radial walls of the endodermal cells were also well stained. Comparison with sections stained in phloroglucin showed that the action of Hofmann's blue, when used as described above, was limited to the walls which could also be stained by phloroglucin. Mäule's reaction (with permanganate of potash, hydrochloric acid, ammonia) showed staining of the same walls, excluding perhaps the Caspary's dots.

Branching of *G. Pectinata*.

In the rhizome of *G. pectinata* there are two kinds of branching, one monopodial, the other apparently dichotomous. The latter type appears not to be known to occur in the rhizome of any other species of the genus.

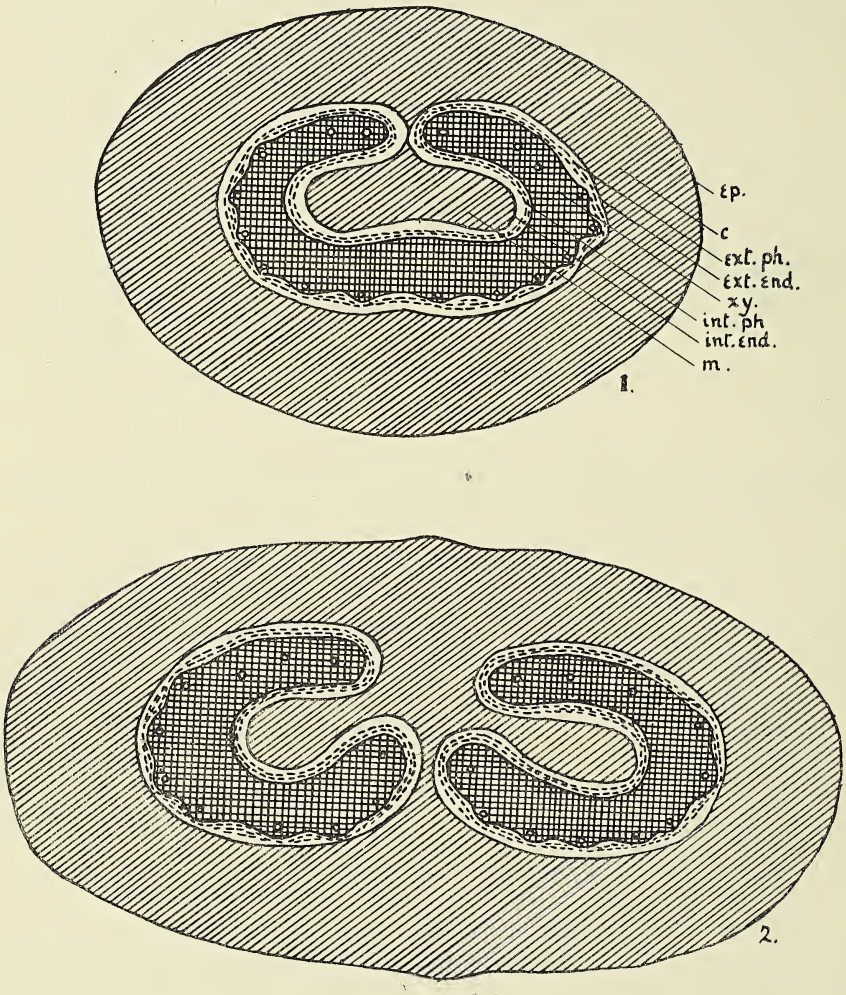

Fig. 3. *Gleichenia pectinata.* Transverse sections showing dichotomous branching. *c.* cortex; *ep.* epidermis; *ext. end.* external endodermis; *ext. ph.* external phloem; *int. end.* internal endodermis; *int. ph.* internal phloem; *m.* medulla; *xy.* xylem. × about 13.

In *G. pectinata* monopodial branching always takes place in the vertical plane, the branch being uppermost, and all the protoxylems of the branch owe their origin to the upper protoxylems of the original stele (Plate XXIX, Fig. 8). Two protoxylem-groups of the upper kind are left in the lower

stele, and these by division give rise to the full normal number. The two steles separate with open xylems, and in each of them the inner endodermis and phloem become continuous with the corresponding outer tissues. Thus a ramular gap is present (Plate XXIX, Fig. 9) but soon after their separation the two steles becomes closed again. The stele of the branch is smaller than that of the main rhizome. Plate XXIX, Fig. 9, shows that the separation of the two steles in monopodial branching bears a slight resemblance to the departure of a leaf-trace from the stele of the rhizome, but differs in the behaviour of the protoxylems and phloem; this may be seen by comparing Plate XXIX, Fig. 9 with Plate XXIX, Fig. 6. There is also the difference that in monopodial branching no nodal island is formed; the central mass of sclerenchyma simply enlarges so as to follow the changes in size and shape undergone by the stele before the separation of the branch.

Dichotomous branching is always in the horizontal plane, and the upper and lower protoxylems are equally concerned in supplying the two exactly similar branches. This necessitates an increase in number in both kinds of protoxylem. The stele becomes horizontally elongated, and divides first on the upper side, as shown in Text-fig. 3 1. In Text-fig. 3 2 the separation is complete, and the two steles are still open. Within a short distance they close again and resume the normal form. This is the usual mode of dichotomy, but in one case the two steles were closed when they separated, owing to the elongated stele having become constricted, and then nipped off in the vertical plane. Thus in the case of dichotomy a ramular gap may or may not be present.

One case was observed in which a dichotomy was closely associated with a node. A nodal island was formed in the xylem of a stele, which had become slightly elongated in the horizontal plane as a preliminary to dichotomy. The two processes of preparation for dichotomy and for separation of the leaf-trace then went on concurrently. The stele split on the upper side first, freeing one end of the leaf-trace; then the dichotomy of the stele was completed, the two steles being open, and the leaf-trace hanging on to the end of one of them, but very soon afterwards becoming free.

Theoretical Considerations.

Having described the nodal structure and the branching of the rhizome in *G. pectinata*, we may now consider the origin of the solenostelic structure of this species. It is to be expected that important data tending to the solution of this problem may be obtained from the anatomical study of some fossil Gleicheniaceae in the possession of Prof. C. Bommer, of Brussels, and possibly also from an examination of 'seedling' plants of *G. pectinata*, when these can be procured.

In the absence of evidence of this kind, an attempt may be made to

determine which is the most primitive type of stele among living species of *Gleichenia*, and provisionally to derive the other types from it.[1]

The genus *Gleichenia*, if *Platyzoma* be excluded from it, falls into two subgenera or sections,[2] *Eugleichenia* and *Mertensia*. Of these, *Eugleichenia*, which includes *G. circinnata*, *G. dicarpa*, &c., presents a series of apparently reduced forms, while most of the species of *Mertensia*, to which *G. flabellata* and *G. pectinata* belong, are not manifestly reduced.

The conclusion that reduction has taken place in *Eugleichenia* is based primarily on the comparative anatomy. In this subgenus the group of sclerenchymatous elements, which, as in most species of *Gleichenia*, is present in the nodal island, and is continued into the petiole,[3] has but slight connexion with the similar elements of the cortex, and in *G. circinnata* (Boodle, '01, p. 726, Tansley, '07, p. 138) it has no connexion whatever with the cortex. That this group of fibres was originally connected with the cortex of the petiole may be deduced from its resemblance to the cortical sclerenchyma, and from comparison with *Mertensia*, in which the sclerenchyma of the nodal island is continuous with the sclerenchymatous cortex filling the concavity of the arched petiolar bundle. The isolation of the group of fibres in *G. circinnata* is strong evidence that reduction has taken place, and the nearly circular or subcordiform petiolar bundle of *Eugleichenia* may be held to have been derived from the horseshoe-shaped bundle of the *Mertensia*-type by contraction, and by fusion of the free ends of the horseshoe, the sclerenchymatous cortex in the concavity of the latter thus becoming enclosed (see Boodle, '01, Fig. 23), or at a higher level suppressed. One species of *Mertensia*, viz. *G. linearis*, has undergone a similar reduction as regards its petiolar bundle, and is thus exceptional.

In two species of *Mertensia*, viz. *G. pectinata* and (one rhizome of) *G. flabellata*, a small amount of phloem was found on the abaxial side of the nodal island (Plate XXIX, Fig. 4, *ab. ph.*). As described above, this was a short strip, which had no connexion with any other phloem. Now from Plate XXIX, Figs. 2–7, it will be seen that, if this group of phloem-elements were slightly extended and continued up into the petiole, it would fill the gap in the phloem at present existing on the adaxial side of the petiolar bundle. This suggests, firstly, that in the ancestral type of *Gleichenia* the phloem probably formed a continuous band round the xylem-arch in the

[1] Cf. Boodle ('01), pp. 737, et seq., Tansley ('07), p. 142, &c., Bower ('08), p. 562, where this subject is dealt with. A point of view suggested by the branching of the rhizome makes a re-statement of the theoretical position advisable.

[2] 'Subgenera' according to Diels (Engler and Prantl, Natürl. Pflanzenfam.), who includes *Platyzoma* as a third subgenus; 'sections' according to Hooker and Baker (Synopsis Filicum), who exclude *Platyzoma*.

[3] In some species it is only continued for a short distance, i.e. into the basal part of the petiole, and in *G. Boryi* this group is entirely absent (Poirault, '93, p. 171). This would appear to be a case of extreme reduction.

lower part of the petiole, and, secondly, that *Eugleichenia*, in which this remnant seems to have entirely disappeared, is less primitive than *Mertensia*, in which it still remains.

Turning now to the external morphology, we find that in *Mertensia* the pinnules are usually long (10–40 mm.), while in *Eugleichenia* they are rounded and short (1–2 mm.). This is consistent with xerophytic reduction having taken place in *Eugleichenia*, and thus agrees well with the anatomical results.

For the reasons given above, *Eugleichenia* is to be regarded as consisting of a series of reduced forms, and as they appear to have been derived from forms of the *Mertensia*-type, the most primitive living species of *Gleichenia* should be sought for in the subgenus *Mertensia*, and *G. flabellata* may be taken as one of the most primitive species (Tansley, '07, p. 142). It has a simple protostele with a horseshoe-shaped petiolar bundle.[1] It is interesting that, in soral characters also, this species appears to be primitive. On account of its possessing a pre-eminently large number of spores in the sporangium, and a radiate-uniseriate type of sorus in its most regular form, Bower ('08, p. 559) regards this species as probably the most primitive of those which he examined.

The existence, however, of solenostelic structure in *G. pectinata* raises a doubt as to whether the protostelic forms may not have been derived from solenostelic ancestors by reduction; the nodal islands of the simpler forms might then be regarded as remnants of the original central core of the solenostele. But a careful examination of the structure of *G. pectinata* shows that there is no sound basis for this view, for in this species the same type of nodal island is present, in addition to the central solenostelic complications (Tansley, '07, p. 142). Thus, until further evidence can be obtained, it seems justifiable to consider protostelic structure as primitive for the genus *Gleichenia*.[2]

We may now consider the origin of the solenostelic structure of *G. pectinata*, deriving it from the protostelic type of structure found in *Mertensia*. Three possible courses of evolution for the solenostele may be suggested :—

1. By decurrence [3] from a leaf-trace, or

1*a*. From a branch of the rhizome.

2. By decurrence [4] from a branch of the rhizome, the protostele having previously become medullated.

3. Independently of decurrence, by a series of symmetrical changes in the rhizome.

1. Decurrence from the leaf-trace of *G. pectinata* gives rise to a nodal

[1] For a description of the nodal structure see Boodle ('01), p. 723.

[2] It is perhaps possible, but under the circumstances not likely, that the protostelic forms might have been derived from the *G. pectinata* type by suppression of the tissues within the xylem-ring and retention of the nodal pocket.

[3] This term is used metaphorically to imply basipetal extension by transformation of tissue in a phylogenetic series. See Boodle ('03).

[4] Or procurrence; see below.

island, which, at its base, is separate from the inner sclerenchyma, endodermis, and phloem of the solenostele, and also corresponds in position and structure to the nodal islands of other species of *Gleichenia*. This seems to show that in *G. pectinata* solenostely has not arisen by decurrence from the leaf-trace, but independently of the nodal islands.

1*a*. Decurrence of phloem and cortical tissues from the axil of a branch or dichotomy of the stele is not known in any species of protostelic ferns (without pith), and there would not be any probability that decurrence should occur at the junction of two solid cylindrical steles, transverse elongation, lateral constriction, and nipping off being the natural mode of subdivision of a solid stele.

2. In some rhizomes of *G. flabellata* the tracheae were found to be less frequent in the central part of the xylem, the parenchymatous elements being complementarily more numerous. In this way, by the exclusion of tracheae from the central region, the protostele might become medullated. It would then be possible for ramular gaps to be formed by the departure of the branches. Through these gaps the cortical tissue, phloem, &c., might become decurrent in the pith, thus forming an internal rod of these tissues (island in transverse sections), which would be central, if derived from a dichotomy. The decurrent tissues might be extended until they met the similar tissues at the next lower ramular gap, and a uniform stelar structure would be established, which, supposing leaf-gaps to be subsequently formed, would then become solenostelic. It may be objected that this theory requires an excessive and unlikely amount of decurrence, and for this reason it seems more probable that the converse of decurrence, i. e. an acropetal extension of tissues (which may be named 'procurrence'), may have been more largely concerned in the origin of the solenostely. At a ramular gap a slight upward as well as downward 'intrusion' of the tissues lying outside the xylem might occur (cf. the case of *Osmunda cinnamonea*, which may be an example of this kind; Faull, '01, Tansley, '07, p. 263, Kidston and Gwynne-Vaughan, '07, p. 775), and, when the meristem has begun to produce a certain type of structure, the acropetal extension of the latter would not perhaps be very surprising.

3. It may also be suggested that the solenostely has been developed by the rhizome, independently of the leaves and branches. The protostele having become medullated in the way described above, some elements of the medulla may have been differentiated as sieve-tubes forming a peripheral zone of phloem. Then it may be supposed that the inner medulla became sclerenchymatous, a change which necessitated the formation of an enveloping endodermal layer, when the formation of leaf-gaps would complete the solenostelic structure.

On general grounds the rarity (except in cases apparently to be explained by reduction) of a primarily disconnected tissue carries some weight against the likelihood of the last supposition (No. 3).

The problem of the origin of solenostely in *G. pectinata* cannot be satisfactorily solved at present, but it appears probable that the derivation from protostelic structure may have been somewhat as follows :—

1. *Mertensia*-type of protostele with nodal islands.
2. Enlargement of stele in connexion with increase in size of arched leaf-trace.
3. Replacement of central part of xylem by a medulla.
4. Formation of ramular gaps.
5. 'Intrusion' of phloem, &c., at ramular gaps.
6. Extension of these internal tissues throughout the length of the rhizome.
7. Formation of foliar gaps completing the solenostelic structure.

It is not easy to find any physiological advantage which the plant gains by its solenostely, apart from convenience of attachment for the phloem situated on the inner side of the hooks of the leaf-trace (see Pl. XXIX, Figs. 6, 5). Working on the suggestion that the presence of internal phloem might mean an additional amount of this tissue, the area of the phloem and xylem was measured in *camera lucida* drawings of *G. pectinata* and *G. flabellata*; it was found, however, that though the area of the xylem in *G. pectinata* was double what it was in *G. flabellata*, yet the area of the phloem in the two cases was approximately the same. Thus, as regards proportional amounts, a distinctly negative result was obtained, but possibly the possession of internal phloem may have carried some advantage at an earlier stage in evolution, e. g. when ramular gaps but no leaf-gaps were present, if such a state of things existed.

Summary.

Gleichenia pectinata shows regular solenostelic characters. The upper and lower protoxylem-groups differ in their degree of immersion in the xylem.

The structure of the node is similar to that of *G. flabellata*, but with the further complications due to the presence of leaf-gaps and solenostely. A nodal pocket is present; it is free in its lower portion, where it shows the typical structure of a nodal island, and in its upper course its different tissues become confluent with the corresponding internal tissues of the solenostele in relation to the formation of the foliar gap.

One curious feature at the node is the occurrence of a short disconnected strip of phloem-elements on the abaxial side of the nodal island; this may be considered as the remnant of a once continuous band of phloem, which formerly filled the gap at present existing in the phloem on the adaxial side of the xylem in the petiolar bundle.

In a node of *G. circinnata* var. *semivestita* a protoxylem-group was found to have a blind ending basally.

The rhizome of *G. pectinata* branches in two ways, monopodially in the

vertical plane, and dichotomously in the horizontal plane. There is nothing resembling a nodal island in connexion with the branching. A ramular gap is generally present in both types of branching, but in one case of dichotomy the two steles were closed when they separated.

It is considered that *Eugleichenia* represents a series of forms showing reduction from the typical *Mertensia*-type, represented, e. g. by *G. flabellata*, and that *Mertensia* includes the most primitive species of *Gleichenia*, as well as the most advanced, viz. *G. pectinata*. The solenostelic structure of *G. pectinata* is to be regarded as derived from a protostelic *Mertensia*-type like that of *G. flabellata*. Among the possible modes of derivation of the solenostelic structure of *G. pectinata* from the type referred to, there is some probability that the course of evolution may have consisted in the formation of a pith and ramular gaps, followed by 'intrusion' of phloem, &c., through a ramular gap into the pith, and subsequent extension of the structure thus attained throughout the rhizome.

List of Works referred to.

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Boodle ('03): On Descriptions of Vascular Structures. New Phytologist, ii, p. 107.

Bower ('08): The Origin of a Land Flora.

Faull ('01): Anatomy of the Osmundaceae. Botan. Gazette, xxxii, p. 381.

Goebel ('05): Organography of Plants, Eng. ed.

Gwynne-Vaughan ('03): Observations on the Anatomy of Solenostelic Ferns. Part II. Ann. Bot., xvii, p. 689.

Gwynne-Vaughan ('08): On the Real Nature of the Tracheae in the Ferns. Ann. Bot., xxii, p. 517.

Jeffrey ('02): Structure and Development of the stem in Pteridophyta and Gymnosperms. Phil. Trans. Roy. Soc., B., cxcv, p. 119.

Kidston and Gwynne-Vaughan ('07): On the Fossil Osmundaceae. Part I. Trans. Roy. Soc., Edinburgh, xlv, p. 759.

Poirault ('93): Recherches sur les Cryptogames Vasculaires. Ann. Sci. Nat., Bot., 7^{e} sér., t. 18, p. 113.

Tansley ('07): Lectures on the Evolution of the Filicinean Vascular System. New Phytologist, vi, p. 135.

EXPLANATION OF FIGURES IN PLATE XXIX.

Illustrating Messrs. Boodle and Hiley's Paper on the structure of *Gleichenia*.

Lettering used in the illustrations: *ab. ph.* abaxial phloem; *c.* cortex; *ep.* epidermis; *ext. end.* external endodermis; *ext. ph.* external phloem; *int. end.* internal endodermis; *int. ph.* internal phloem; *m.* medulla; *n.i.* nodal island; *ph.* phloem; *r.b.* radial band; *r.t.* root-trace; *sc.* sclerenchyma; *xy.* xylem. Protoxylem-groups are marked *y* 1, *y* 2, &c., *y* 3′, *y* 3″, &c.

All the figures are of *Gleichenia pectinata*.

Fig. 1. Transverse section through internode. × 15.

Fig. 2. Radial section through a node. × 7½.

Figs. 3–7. A series of transverse sections through the nodal region of the rhizome, arranged opposite their correct positions in the longitudinal section (Fig. 2). × 15.

Figs. 8 and 9. Transverse sections through a rhizome undergoing monopodial branching. × 11.

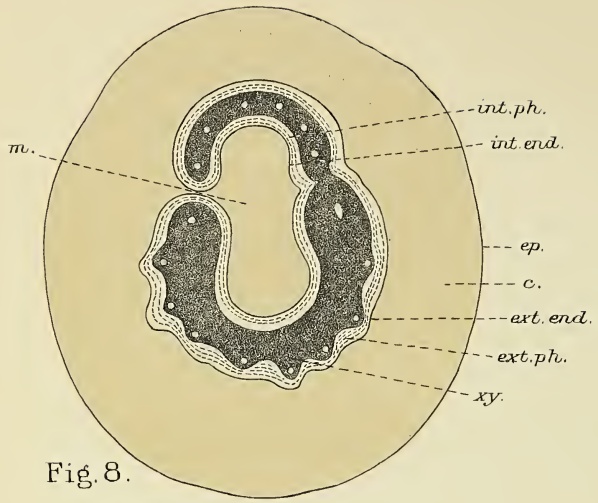
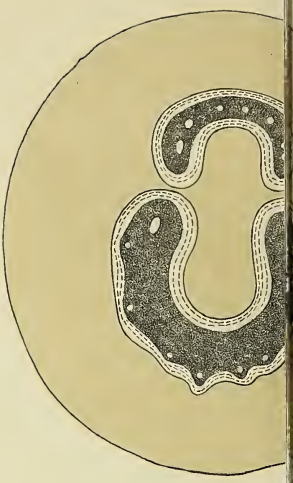


Fig. 8.



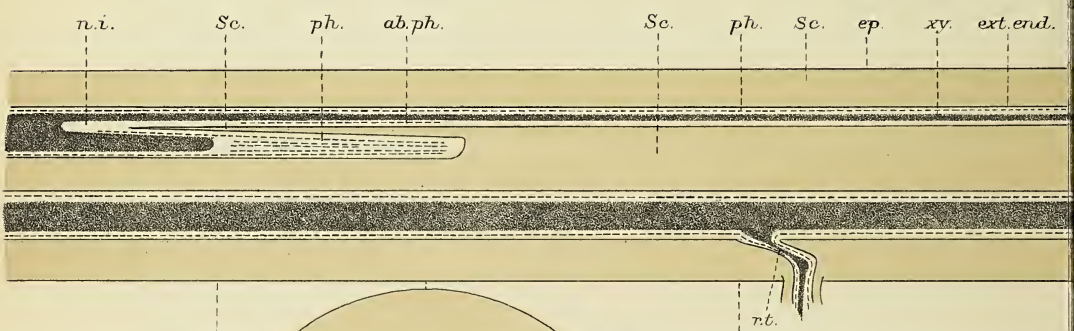


Fig. 4.

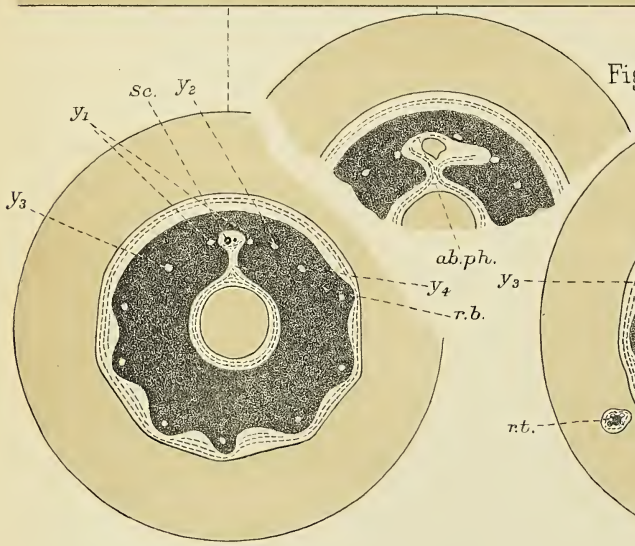


Fig. 3.

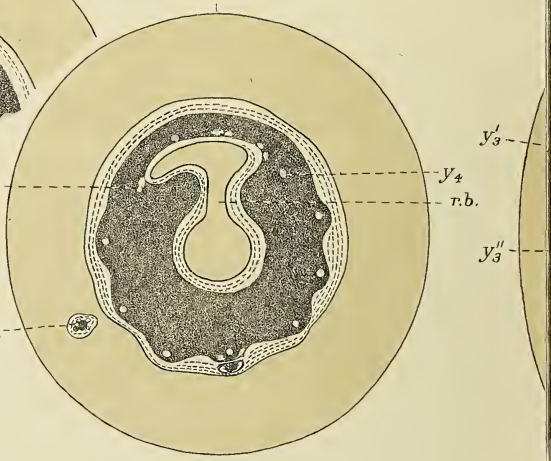


Fig. 5.

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BOODLE AND HILEY — GLEICHENIA.

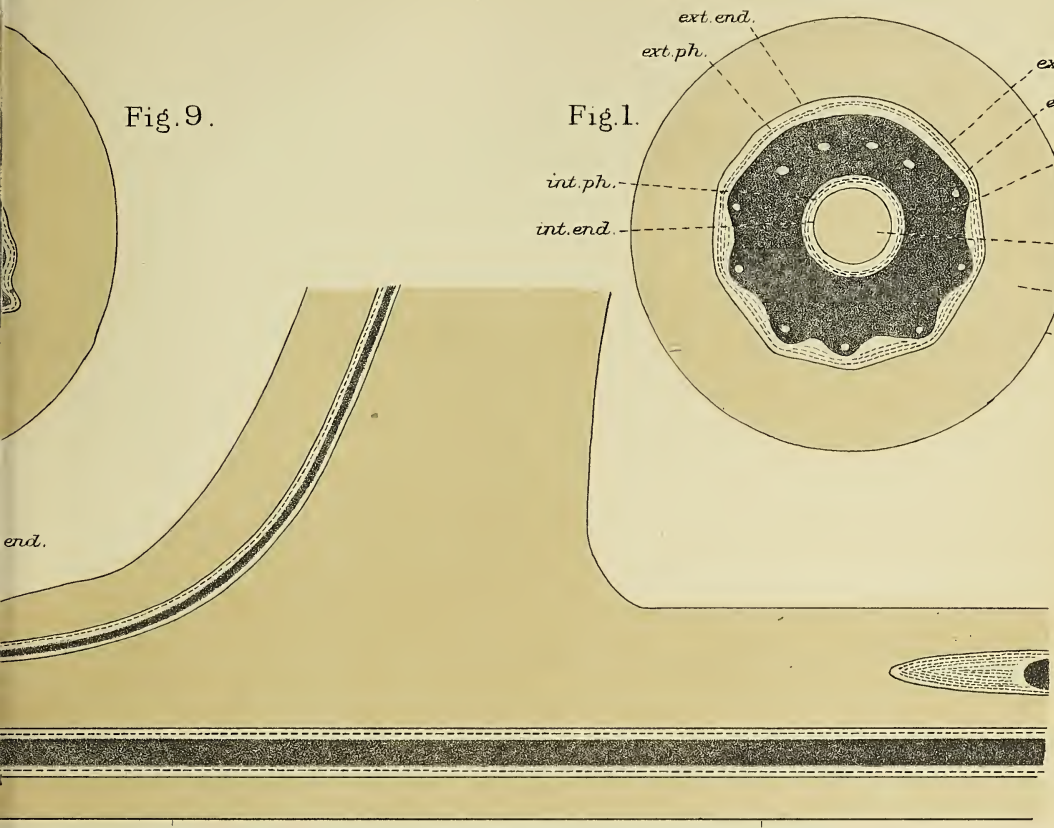

Fig. 9.

Fig. 1.

Fig. 2.

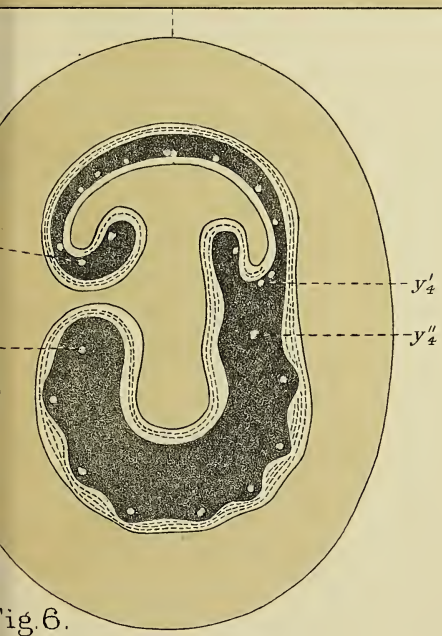

Fig. 6.

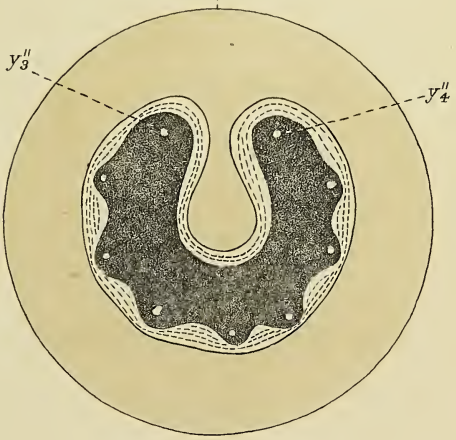

Fig. 7.

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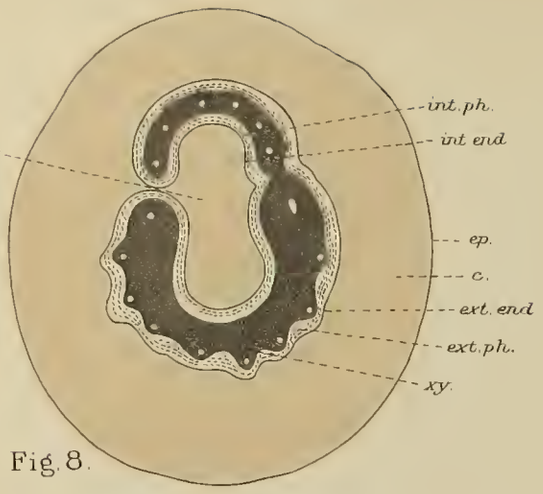

Fig. 8.

Fig. 9.

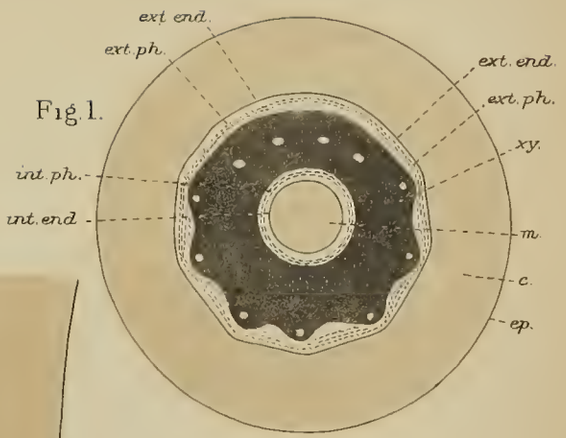

Fig. 1.

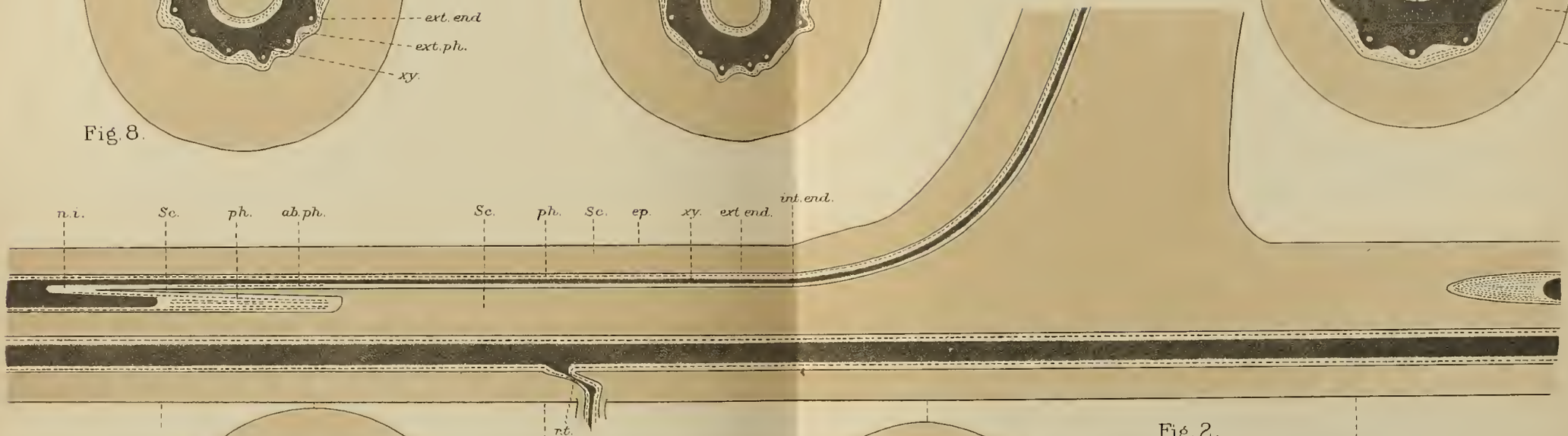

Fig. 2.

Fig. 3.

Fig. 4.

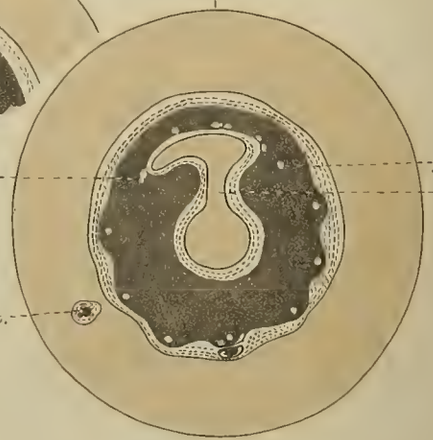

Fig. 5.

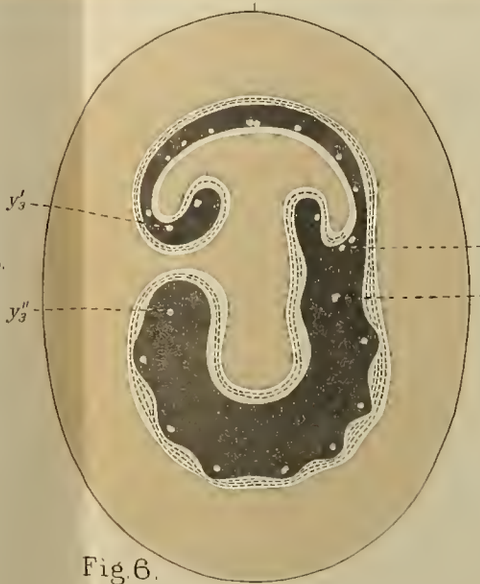

Fig. 6.

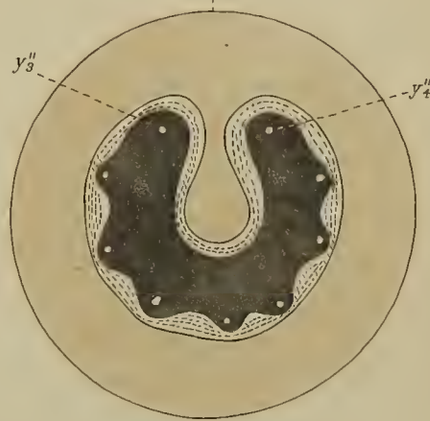

Fig. 7.

On the Seedling Structure of Gymnosperms. III.

BY

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AND

E. DE FRAINE, F.L.S.

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With Plate XXX, and four Figures in the Text.

GINKGOACEAE.

Ginkgo biloba.

THE appearance of the seedling of this plant, and the germination of the seed, have already been described and figured by Sprecher,[2] and are sufficiently well known to render an extended description unnecessary. Figs. 1–3, Pl. XXX will suffice to recall its more obvious features: the cotyledons usually are two in number but three sometimes occur (Fig. 3, Pl. XXX), a fact already recorded by Lyon [1] and Sprecher.[2]

The seed-leaves are frequently unequal in length; they exhibit a more or less well-marked lobing, and throughout their existence, remain embedded within the seed where they are closely adpressed by their ventral surfaces. These facts have already been remarked upon by Strasburger,[3] Worsdell,[4] and Seward and Gowan.[5] According to Lyon [6] the seed-leaves are normally equal and entire. Towards the basal region the cotyledons separate in the manner indicated in Fig. 2, Pl. XXX. Although the details of the histology of the seed-leaves have been investigated by other authors, more especially by Sprecher,[7] it will not be out of place to draw

[1] Lyon: Embryogeny of *Ginkgo* (Minnesota Bot. Studies, 1904).

[2] Sprecher: Le *Ginkgo biloba* (Genève, 1907).

[3] Strasburger: Die Coniferen und die Gnetaceen (Jena, 1872).

[4] Worsdell: On Transfusion Tissue: its Origin and Function in the Leaves of Gymnospermous Plants. (Trans. Linn. Soc. Lond.; Bot., 2nd Ser., v.)

[5] Seward and Gowan: The Maidenhair Tree (*Ginkgo biloba*). (Ann. Bot. xiv, 1900.)

[6] loc. cit.

[7] loc. cit.

[Annals of Botany, Vol. XXIII. No. XCI. July, 1909.]

attention to some of the more salient facts. Their structure is simple; the mesophyll is homogeneous with its parenchymatous elements densely crowded with starch grains; also stomata occur, a feature which has been fully considered by Wigglesworth [1] and Sprecher.[2]

Resin-ducts are present, they appear first at the corners of the cotyledons, but at lower levels several may be present (Diagram 1, Figs. 2 and 6); also secretory cells are abundant.

The chief feature of interest in the structure of the vascular bundles is their mesarch structure, a fact which has been recorded by Worsdell [3] and other observers. On the outer side of the soft bast is a band of fibrous elements precisely similar to the same tissue alluded to in the case of many of the plants already considered [4] (Diagram 1, Figs. 3 and 5).

Transition.

Each seed-leaf has four vascular strands at the apex arranged in two pairs (Diagram 1, Fig. 1). In one plant examined a cotyledon had but three strands at its distal end; but, at a lower level, an extra bundle developed next to the odd one, so that equality was constituted. Tracing these strands downwards, the individuals of each pair approach each other, and a union is brought about by the fusion of the fibrous elements on the outer side of the soft bast (Diagram 1, Fig. 2). This condition is maintained for some distance: the bundles, as viewed in transverse section, appear semi-circular in outline with the two xylem masses facing each other in the manner indicated in Diagram 1, Fig. 3. At the same time the wood has become more compact, and new xylem elements may appear between the centripetal tracheides; this before the actual fusion of the two groups of vascular tissue has taken place. At a lower level the union is consummated so that each cotyledon now has two mesarch bundles with the vascular arrangement indicated in Diagram 1, Figs. 4 and 5.

This condition persists for some distance downwards: then, as the strands are traced towards the cotyledonary node, they are seen gradually to approach one another and ultimately to fuse, first by the fibrous elements (Diagram 1, Fig. 6), and then by the soft bast. The metaxylems are connected together by the protoxylem elements which are tangentially extended; they—the metaxylems—may or may not fuse (Diagram 1, Figs. 7 and 8) and in this state the bundles enter the hypocotyledonary axis.

It may be remarked here that some earlier authors (Worsdell,[5] Seward and Gowan,[6] and Sprecher [7]) are all agreed upon the presence of two bundles throughout the main length of each cotyledon and the occurrence of one trace at the base of the seed-leaf, but they have not remarked upon

[1] Wigglesworth: The Cotyledons of *Ginkgo biloba* and *Cycas revoluta* (Ann. Bot., xvii, 1903).
[2] loc. cit. [3] loc. cit. [4] Parts I and II, Ann. Bot., 1908, 1909.
[5] loc. cit. [6] loc. cit. [7] loc. cit.

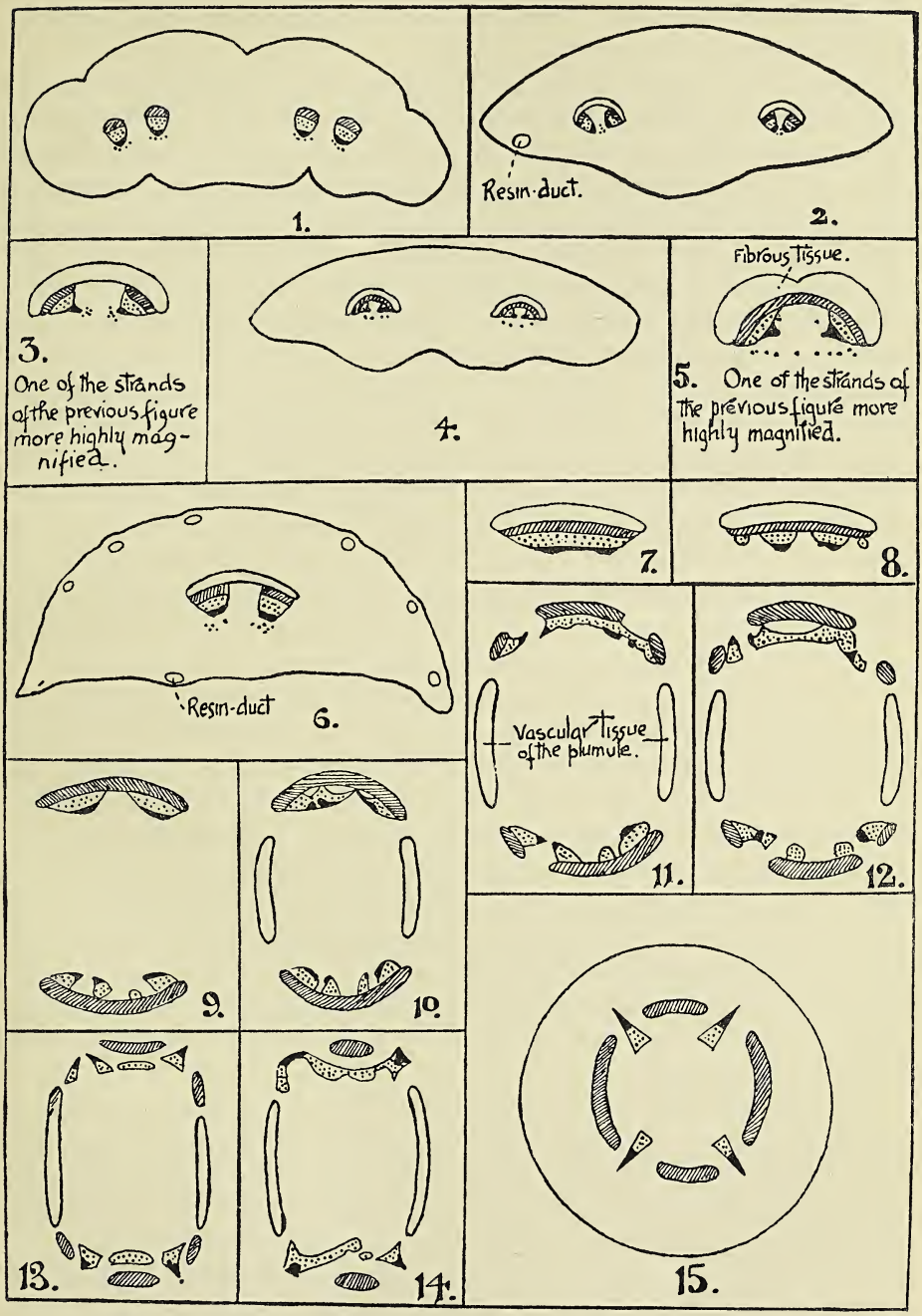


DIAGRAM 1. *Ginkgo biloba.*—In this, and in the following text-figures, the protoxylem is indicated by black areas, the metaxylem by dots, and the phloem by shading.

the presence of four strands at the apex. This feature has, however, been commented upon by Miss Thomas,[1] who draws attention to the similarity, in this respect, to some of the Araucarias.

The actual transition takes place along fairly simple lines and with some degree of rapidity. The bundles from each seed-leaf travel gradually towards the centre of the axis, and the metaxylem, if it had previously joined to form a continuous tissue, divides first into two parts (Diagram 1, Fig. 9); then each portion again divides into two, so that at each end of the central cylinder there occurs an uninterrupted arc of phloem bounded on its inner side by two more or less well-marked pairs of groups of xylem elements. The individual masses of each pair then commence a rearrangement, which is generally a distinct rotation towards one another and outwards, which tends to bring the protoxylems into an exarch position (Diagram 1, Fig. 10). While these latter movements are taking place the arc of phloem divides into three parts, the division occurring opposite the place where the protoxylems are becoming exarch (Diagram 1, Figs. 11 and 12). Of these three portions of bast, the central one remains *in situ*, while the two lateral parts travel towards the intercotyledonary plane and effect a junction with the corresponding tissue of the plumular strands (Diagram 1, Figs. 13 and 14). The completion of these movements results in the formation of a tetrarch root-structure.

In no case have we observed a rotation of the protoxylem within the cotyledons themselves as indicated by Tansley and Miss Thomas[2]: at the top of the hypocotyl each seed-leaf-trace is a large tangentially elongated structure from which most of the centripetal elements have disappeared, so that it is practically endarch. As before mentioned the number of cotyledons is usually two, but among the plants at our disposal there was one which had three seed-leaves. Hitherto our experience has been that in similar cases in other plants, the transition is the same as in an example with the normal number of cotyledons, and the root instead of being diarch is triarch. Applying this experience to *Ginkgo* we should expect a tricotyledonous plant to have a hexarch root since a dicotyledonous one has a tetrarch root-structure.

But this was not found to be the case in this tricotyledonous specimen of *Ginkgo biloba*. The facts are as follows: as in the normal seedlings, a single tangentially elongated bundle enters the axis from each cotyledon and, omitting unessential features, the xylem of each divides into two principal parts which rotate towards each other and outwards so as to form the three protoxylem poles of the triarch root-structure. The phloem of each strand divides opposite each protoxylem, and each half passes

[1] Thomas: a Theory of the Double Leaf-Trace founded on Seedling-Structure (New Phytologist, vi, 1907).

[2] Tansley and Thomas. Discussion on Seedling-Structure. Brit. Assoc. York, Sect. K. 1906.

to one side and fuses with the adjacent bast of the epicotyledonary structures, so there results a triarch root-structure.

Thus there is exhibited a striking variation. The observations of Tansley and Miss Thomas,[1] and also our own, show that the normal occurrence is for the vascular tissue derived from each cotyledon to form two poles of the root-structure, so that the number of poles in the root is double the number of cotyledons ; but, in the case under consideration, the seed-leaf-traces organize a root-structure having the same number of poles as there are cotyledons. This variation does not appear to be uncommon, for Sprecher[2] describes the root-structure as being hexarch in the higher regions which at lower levels becomes diarch, and Miss Thomas,[3] in a later publication, states that the tetrarch arrangement is fugitive, giving place to a diarch structure at a lower level.

This tricotyledonous specimen also showed a few other, but minor, differences; thus the mesarch structure of the cotyledonary bundles persisted relatively for a longer time than in the case of dicotyledonous examples ; also, one seed-leaf joined the axis at a higher level than the other two.

It remains to draw attention to a few outstanding features of general occurrence.

The protoxylem of the cotyledonary bundles is well marked, it becomes less in amount as the seed-leaf-traces pass towards the centre of the hypocotyl, and it becomes increasingly difficult to distinguish it from the metaxylem. Further, there is often seen an addition of xylem elements on the outer side of the protoxylem after the root-structure has been attained, a feature which has already been remarked upon as occurring in other plants, e. g. *Juniperus virginianus* and *Libocedrus decurrens*.[4] An endodermis is differentiated as soon as the root-structure is arrived at, and it occurs some distance out from the outermost vascular elements; in other words the 'pericycle' is several cells in thickness. The upper region of the root is protected by a well-marked exodermis situated just beneath the superficial layer of cells.

CYCADACEAE.

The seedlings of these plants have received much attention from botanists during the last few years, and reference will be made to these researches as the occasion demands. We regret that we have been enabled to examine so few seedlings of these plants; seeds of other genera and species were obtained but failed to germinate, and in other cases, even when germination was successful, many damped off before a stage suitable for our investigation was reached.

[1] loc. cit.
[2] loc. cit.
[3] loc. cit.
[4] Part I, Ann. Bot. 1908.

Finally, it may be remarked that as this particular series of papers is already somewhat detailed and lengthy, it has been considered desirable to make little or no mention of histological features of the Cycadaceae, more especially of the vascular bundles, here. They may, if necessary, form the subject of a separate communication.

Macrozamia.

Macrozamia spiralis.

The form of the seedling closely resembles that of *Ginkgo* and is illustrated in Fig. 4, Pl. XXX. There are two hypogeal cotyledons which, throughout their existence, remain embedded in the endosperm of the seed ; they are of unequal size, and the larger one is also often slightly longer, its tip sometimes being folded over the free end of the smaller.

The seed-leaves are very closely adpressed by their ventral surfaces ; at the apex, and a little below, the epidermis of each member forms a line of demarcation, but at a slightly lower level the fusion appears to be quite complete, the ventral epidermis can no longer be distinguished, and a transverse section has all the appearances of a section of a stem.

At a still lower level the boundary line again appears and, towards their bases, the seed-leaves partly separate to form a cotyledonary tube which encloses the plumule.

As regards structure little need be said. Stomata, sunken below the general level of the epidermis, have been observed, and they are restricted, as far as has been seen, to the dorsal surface where the cuticle is fairly well pronounced and thicker than on the ventral surface. The internal structure resembles very closely that of *Ginkgo*. The compact mesophyll is homogeneous, and its parenchymatous cells are densely packed with starch grains. Secretory cells are abundant, and also mucilage canals of a large size ; the number of these latter varies, about five in each seed-leaf are present.

The vascular bundles are not particularly large, and they vary in number in each seed-leaf; the smaller cotyledon generally has one less than the larger, which usually has five ; six, however, were observed in one case. The vascular strands are very markedly mesarch in structure ; most, in some cases practically all, of the metaxylem is centripetal.

At the extreme apex of each cotyledon there is often but one vascular strand consisting of a broad tangentially elongated mass of tracheides but no phloem ; this strand, as it is traced downwards towards the base, quickly divides up into four, five, or six bundles, according to the size of the cotyledon, and phloem is differentiated. The number of bundles thus produced generally remains constant, so that five strands enter the hypocotyledonary axis from one seed-leaf and four from the other. But in one case it was observed that the larger cotyledon had six strands and the

smaller five, some of which fused together so that four entered the axis from each seed-leaf.

These observations are in agreement with those of Worsdell,[1] who has briefly investigated the seedling of this plant and draws attention to the similarity of *M. spiralis* and *Cycas* in the structure of the cotyledons.

Transition.

Taking the commoner occurrence, as regards the number of cotyledonary traces, nine vascular bundles enter the hypocotyledonary axis and travel towards the centre with some degree of rapidity, so that, in transverse sections, the traces are always very oblique, which renders it almost impossible to distinguish the protoxylem elements from the metaxylem, for which reason the protoxylem has not been indicated in all the figures of the second and succeeding Diagrams. During the passage various fusions take place between adjacent bundles; thus, to take a concrete case which is illustrated in Diagram 2, *i* joins up with *a* (Diagram 2, Fig. 1); *c* and *d*, as they pass inwards, approach each other and fuse (Diagram 2, Figs. 2 and 3); and likewise the bundles *f* and *g*. At a slightly lower level *b* joins with the compound bundle *cd* and, similarly, *h* fuses with *fg*. So far *e* has remained isolated, but it very soon effects a junction with *fgh*, by which time the xylem-masses of the cotyledonary strands have fused with what wood is present belonging to the plumular traces. There is thus formed a very irregular core of xylem, which, sometimes, is roughly three-rayed, in the centre of the axis. The phloem-masses also fuse so that the vascular tissue derived from the cotyledons and from the plumule forms a single concentric cylinder (Diagram 2, Fig. 6). This condition obtains only through a very short length of the axis; the phloem separates into two masses situated in the intercotyledonary plane and, concurrently, the xylem elements, which by now are rather less numerous than before, become organized to form a well-marked diarch plate situated in the cotyledonary plane.

In no case has a transient tetrarch root-structure been observed, and, so far as has been seen, it is generally diarch, an observation which is in agreement with Worsdell's[2] experience. During these later changes, as has already been remarked, it is impossible to trace the protoxylem or to distinguish it from the metaxylem owing to the numerous and complicated anastomoses. It is therefore impossible to say whether or not all the cotyledon-traces are equally important in the formation of the resulting root-structure. It is obvious, from the strongly mesarch nature of the cotyledonary bundles, that the rotation of the protoxylem is of very little importance for the amount of centrifugal wood is so small that only a slight

[1] Worsdell: The Comparative Anatomy of certain Genera of the Cycadaceae (Journ. Linn. Soc. Lond., Bot., xxxiii).

[2] loc. cit.

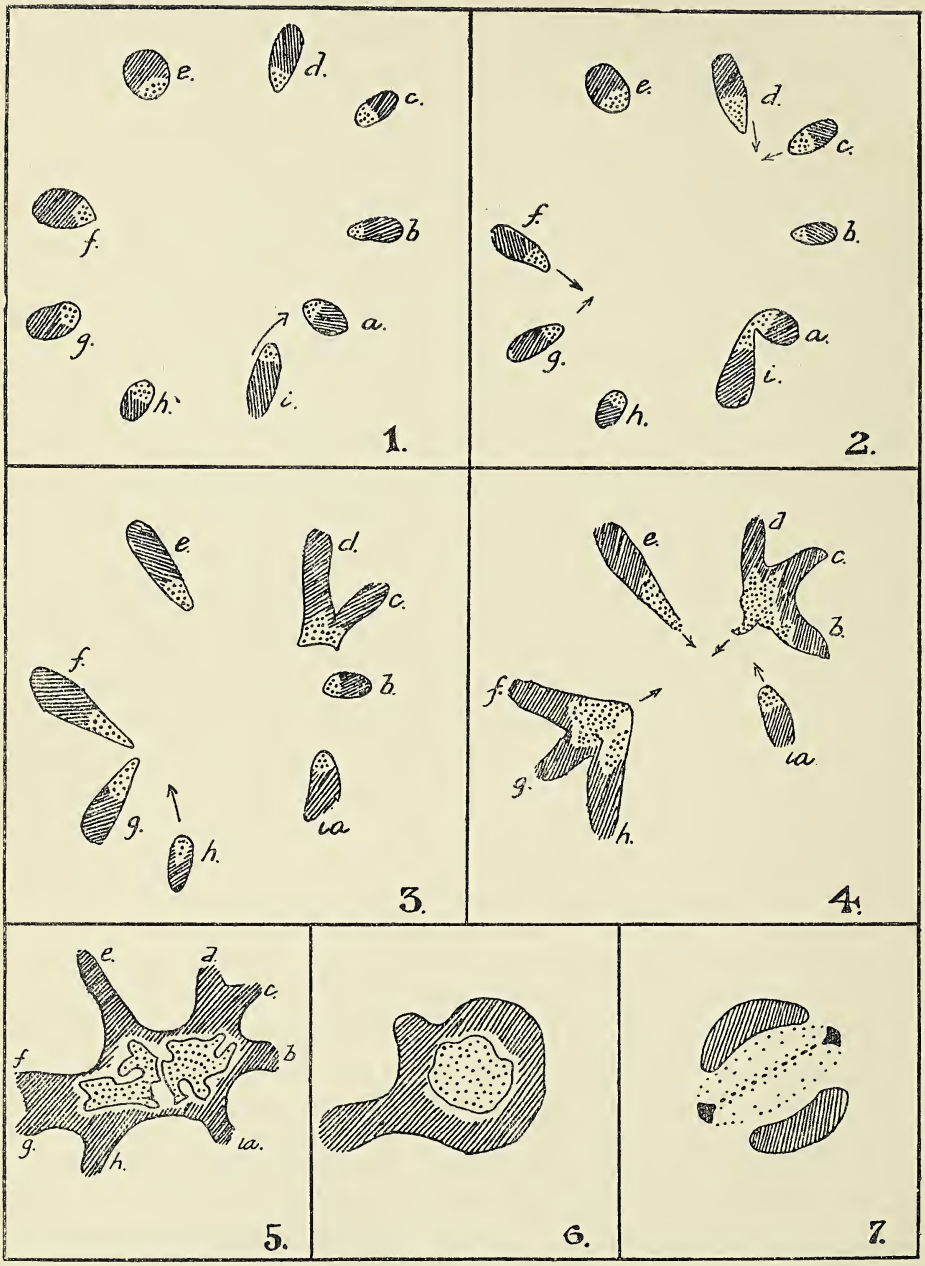


DIAGRAM 2. *Macrozamia spiralis.*

amount of rearrangement is necessary in order to bring the protoxylem into its exarch position.

As regards minor features, an endodermis does not appear until a much lower level has been reached, but a well-marked exodermis, two or three layers of cells in thickness, is conspicuous.

The metaxylem situated between the phloem-masses of the root soon disappears and, at the same time, the protoxylems become tangentially expanded (Diagram 2, Fig. 7). It is almost unnecessary to remark that the smaller details of the transition region are not precisely the same in the different individuals examined, but the above account is typical of the species as regards essential features, so far as can be judged from the material at our disposal.

Attention may be drawn to another seedling of this species which was the only one that germinated out of a number of seeds sent to us as *M. corallipes*; its external morphology is precisely similar to that of the other seedlings of *M. spiralis*, and so also is the structure of the cotyledons, with the exception that the secretory cells are less numerous and the mucilage-ducts fewer and not so well marked.

With regard to the transition, the number of bundles towards the apex of the seed-leaves is more numerous than in the other examples, there being ten in the larger cotyledon and eight in the smaller. These strands, as they are followed downwards towards the node, fuse together to form four, which are arranged in two pairs, in each seed-leaf. The units of each pair of strands fuse together, either at the base of the cotyledon or in the outer regions of the uppermost part of the hypocotyl, so that four traces are ultimately produced. These rapidly pass inwards and fuse with what plumular bundles may be present and form a broad U-shaped and compact mass of vascular tissue. This undergoes a rearrangement resulting in a triangular-shaped vascular cylinder, as viewed in transverse section, with the protoxylems situated at the corners and surrounded by phloem which speedily separates into three masses so that a triarch root-structure results.

As regards the part played by the cotyledonary traces in the formation of the root-structure, the bundles of one seed-leaf formed one pole of the root, while the traces of the second cotyledon produced the remaining two poles.

The chief features of difference between the transition-phenomena of this plant and the other examples of *M. spiralis* are that in the former the number of cotyledonary bundles are more numerous, and the resulting root-structure is triarch instead of diarch.

In all cases the actual transition within the axis takes place with extreme rapidity, hence the hypocotyl for the major part of its length shows a root-like structure.

Brief mention may also be made of an abnormality occurring in this aberrant example of *M. spiralis*. In the cortex of the hypocotyl, at about the level of the transition region, there obtained a short spindle-shaped vascular strand which, throughout its course, was entirely isolated and unconnected with the rest of the vascular tissue. It consisted of a central core of short tracheides, about sixteen in number at the broadest part, and surrounded by a zone of cambium and cambiform cells. Sieve-tubes were absent, and what few immature xylem elements were present were developed centrifugally from the centre of the bundle. This probably represents an early commencement of the anomalous cortical strands which are a characteristic feature of the root of certain other Cycads, *Cycas revoluta* for example. Worsdell[1] comments on the absence of cortical strands on vascular tissue in the stem of *M. spiralis*, although they are present in *M. Fraseri*.

Stangeria.

Stangeria sp.

The seedlings of *S. paradoxa* have been investigated by Worsdell[2] and Matte,[3] both of whom illustrate and describe the external appearance and enter into a detailed description of their anatomy: more especially the latter author, who gives some information regarding the transition-region which Worsdell did not deal with.

The seedlings are illustrated in Figs. 5–7, Pl. XXX. Fig. 5 represents quite a young seedling in which the plumule is breaking through the cotyledons; Fig. 6 illustrates an older seedling in which the plumule is more conspicuous and the primary root dichotomously branched; and Fig. 7 is that of a still older example in which the seed had dropped off and the cotyledons decayed. Of these the seedling represented in Fig. 6 is of some interest; Prof. Pearson informs us that this bifurcation of the tap-root is not of unusual occurrence, and both Worsdell and Matte have recorded that dichotomous branching of the lateral roots obtain in *Macrozamia spiralis* and *Ceratozamia mexicana*, respectively.

The morphology and structure of the cotyledons does not differ in any important feature from that of *Macrozamia*; the cotyledons, however, do not appear to fuse so completely by their ventral faces, judging from the material at our disposal; secretory cells are much less abundant and mucilage ducts apparently are absent; finally, although the bundles have more centripetal wood than centrifugal, the relative amount of the former is perhaps not so much, in all cases, as in *Macrozamia*.

As regards the number of vascular bundles in each seed-leaf there is much variation. Worsdell[4] states that 'each cotyledon has, in its upper

[1] loc. cit. [2] loc. cit.

[3] Matte: Sur le développement morphologique et anatomique des germinations des Cycadacées.

[4] loc. cit.

part, four bundles arranged in a row parallel to the greatest width. . . . In the lower part of the lamina of the cotyledon and in its stalk, besides the row of four or five bundles, there are two or three others towards the ventral side, which may be orientated like those of the normal row or may lie sideways.' These inner bundles, on tracing them upwards, lose their phloems, and their xylems become united each with one of the normal strands. Towards the basal region the majority of the strands become concentric, a fact which Worsdell regards as of considerable phylogenetic importance.

Matte [1] found four or five normal bundles in each seed-leaf in addition to a few strands towards the ventral surface. As regards these last, Matte does not find any phloem, 'ils se montrent simplement constitués par des vaisseaux isodiamétriques très larges, en groupes plus ou moins compacts qui réunis vers le haut aux faisceaux voisins de l'arc normal . . .' Further, he disagrees with Worsdell regarding their significance. The strands here referred to are wanting in our material; they, therefore, are probably nothing more than individualistic variations of no theoretical import in the present connexion.

Passing on to our own observations, it was found that in one case the larger seed-leaf had five strands which, by the time the cotyledonary node had been reached, were reduced to three by the fusion of adjacent traces, while the smaller cotyledon had two throughout; in another example the smaller seed-leaf had two vascular bundles and the larger three towards the apex, but another was differentiated at a lower level which, at the cotyledonary node, fussd on to its nearest neighbour. The following description of the transition-phenomena is based upon the latter seedling, owing to the fact that the former one was so young that the vascular bundles of the hypocotyl were insufficiently differentiated.

Transition.

Matte [1] has described some phases of the vascular rearrangements in the transition region. Our results are in accordance with his so far as the treatment coincides. At the cotyledonary node five traces enter the axis (Diagram 3, Fig. 1), two from one seed-leaf and three from the other. These gradually pass to the central region of the axis, much more slowly than in *Macrozamia*, where the plumular bundles form conspicuous objects (Diagram 3, Fig. 2). Terming the seed-leaf-traces *a*, *b*, *c*, *d*, and *e*, and the plumular traces *p.* 1, *p.* 2 . . . ; the strand *d* passes between *p.* 1 and *p.* 2 and fuses with them, and, at a slightly lower level, the trace *a* effects a junction with *p.* 4 (Diagram 3, Fig. 3). The trace *e* entirely fuses with *d* (Diagram 3, Fig. 4), there is then a general union of the remaining bundles, which leads to the formation of a horseshoe-shaped vascular cylinder as viewed in transverse section (Diagram 3, Fig. 5). During these changes the

[1] loc. cit.

wood has been becoming gradually less, especially the metaxylem, so that, in transverse section, it is impossible to distinguish with certainty the protoxylem from the metaxylem elements.

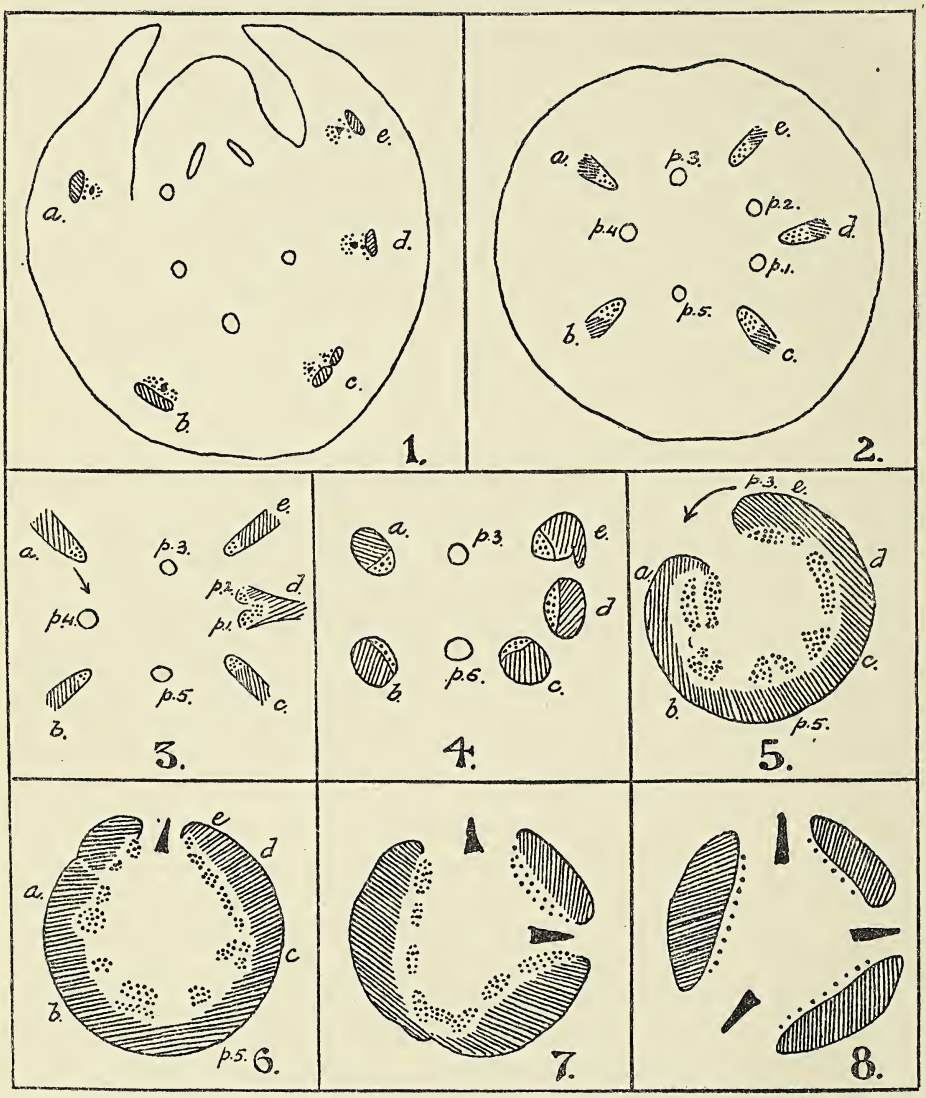


Diagram 3. *Stangeria sp.*

That part of the cylinder marked *p. 3* now becomes detached and joins on to the opposite horn; in the gap thus produced a group of xylem elements is left *in situ*, and forms one pole of the root-structure (Diagram 3, Fig. 6). At a slightly lower level the phloem in the region between *c* and *d* divides, exposing another small mass of wood which forms another pole of the

root-structure (Diagram 3, Fig. 7). A precisely similar vascular rearrangement takes place in the region of *b* leading to the formation of the third pole of the triarch root-structure (Diagram 3, Fig. 8). In no case has a definite rotation of the protoxylem been made out; this tissue occupies its final position chiefly by the dying out of the metaxylem elements which would otherwise be in the way.

From the above facts it is seen that the vascular tissue from the smaller cotyledon forms one pole of the triarch root, while the other two poles are formed from the bundles of the larger seed-leaf. In this process the relative contributions of the different strands varied; and although in the later stages of the transition, owing to the compactness of the cylinder, it is almost, and sometimes quite, impossible to accurately delimit one strand from another, the positions of the xylem poles of the root, when compared with the relative positions of the seed-leaf traces, point to the fact that, in the seedling described, the bundles *b*, *c*, and *e* are more important than *a* and *d*.

As in *Macrozamia* an endodermis does not appear until a much lower level has been reached.

Worsdell found that the upper part of the primary root was triarch which appeared to be reduced to a diarch structure towards the apex; this was not so in the example described above, the dissimilarity is probably due to the difference in age between the seedlings investigated. Matte found that in one example the root was diarch at its base which, by the organization of another pole, became triarch nearer the apex; while in an older seedling he found that 'la structure-racine, avec apparition de deux pôles trachéens, s'établit un peu plus bas, en même temps que, toujours sous l'influence des recloisonnements tubérisants, les lames radiales libéro-ligneuses secondaires s'espacent notablement les unes des autres avec contournements fréquents de leurs éléments constituants.

'Vers le bas cet espacement s'accentue régulièrement et aboutit finalement, à des niveaux très espacés, à la formation de trois, puis de quatre masses à peu près égales, avec différentiation progressive d'un troisième, puis d'un quatrième pointement trachéen suivant le mode indiqué précédemment.

'A un centimètre de la pointe, la structure primaire subsiste seule et, contrairement à ce qui se passe dans la germination *a* [the younger seedling referred to above], les quatre pôles sont réunis entre eux par un bois primaire compact occupant tout l'axe de la racine.'[1]

In a footnote he makes the following observation: 'On remarquera que le nombre des pôles radicaux, de plus en plus grand à mesure qu'on se dirige vers l'extrémité de la racine chez le *St. paradoxa*, devient au contraire de plus en plus petit, dans ce même sens, chez *Ceratozamia mexicana*'.

These are significant statements, more especially in view of the like

[1] loc. cit. p. 44.

observations and theoretical conclusions of Shaw[1] on *Araucaria Bidwillii*, and the similar facts recorded by others besides ourselves both for Gymnosperms and Angiosperms. It is, however, not desired to discuss them here; their theoretical importance will be considered in our general conclusions.

Dioon.

Dioon edule, Lindl.

The seedling has been described and figured by Matte[2] and Thiessen,[3] with whose observations we are in agreement. It resembles the seedlings of other Cycads, e.g. *Macrozamia*, very closely both as regards its general appearance, and also in its internal structure, with the minor exception that in *Dioon* the mucilage canals and secretory cells are more numerous.

The seed-leaves are closely apposed one to the other, but the line of demarcation is always obvious. At their apices the cotyledons are very irregular in outline, and there is no definite arrangement in the disposition of the vascular bundles (Diagram 4, Fig. 1); at a lower level the strands take up a normal position, when it is seen that the larger seed-leaf has more bundles than the smaller. Following the traces downwards they are seen to become reduced in number which is brought about by the fusion of the extreme laterals. Thus, in one case, at the apex of the seed-leaves there were seven and six bundles respectively, but, at the base, the numbers were five and four (Diagram 4, Fig. 3); in another instance each cotyledon had four strands at the cotyledonary node.

These observations are of the same nature as those of the two authors cited above. Thus Thiessen remarks that the tip of each cotyledon has one concentric strand which abuts immediately against the epidermis. At lower levels this bundle divides into eight, which fuse in pairs so that four strands result. In two seedlings it was found that one of the cotyledons had five bundles at its base.

In the two seedlings described by Matte, one had four traces in each cotyledon, while the other example had four in one seed-leaf and two in the other together with two much smaller ones which, in the hypocotyl, fused on to the nearest plumular traces and played no part in the transition; the resulting root-structure was triarch, while in the former case it was tetrarch.

Both authors enter fully into histological details, which it is not proposed to consider here.

Transition.

Matte and Thiessen also describe and illustrate, more or less briefly, the transition-phenomena. The latter writer remarks that 'the four strands

[1] Shaw: The Seedling Structure of *Araucaria Bidwillii* (Ann. Bot., xxiii, 1909).
[2] Matte: Recherches sur l'appareil libéro-ligneux des Cycadacées (Caen, 1904).
[3] Thiessen: The Vascular Anatomy of the Seedling of *Dioon edule* (Bot. Gaz., xlvi, 1908).

of each of the petioles of the cotyledons may be said to join two by two. Just before reaching the central cylinder the inner strands of each fuse, and the outer strands of the one fuse with the outer strands of the other, the four strands thus formed giving rise to the four protoxylem groups'.

Series A. The top of the hypocotyledonary axis is occupied by nine cotyledonary traces, five derived from one seed-leaf and four from the other (Diagram 4, Fig. 3, *a* . . . *i*). These pass with some degree of rapidity towards the centre, and the three traces *b*, *c*, and *d*, derived from the larger cotyledon, fuse to form one structure; at the same level *e* and *f*, of the smaller seed-leaf, have approached one another pretty closely (Diagram 4, Fig. 5). At a lower level *e* and *f* effect a junction as they enter to form part of the central cylinder, and so also do the bundles *g* and *h* (Diagram 4, Fig. 6). The strand *a* enters the cylinder by itself and so also, at a lower level, does *i*. The vascular cylinder is now concentric, and the bundles have lost their identity, that is to say it is impossible to delimit their boundaries with absolute certainty.

The organization of the root-structure proceeds rapidly, two xylem poles, in the plane of the cotyledons, are differentiated quickly; then two other poles in the intercotyledonary plane are formed. The phloem is still intact; but, as soon as these last xylem rays are produced, the bast divides first opposite the xylem rays first differentiated, and then, at a lower level, opposite the intercotyledonary poles.

With regard to the relative value of the cotyledonary strands in the formation of the root-structure, *g* and *h* together form one pole; *e* and *f* another pole; *i*, by itself, the third; and *b* and *c*, the fourth pole. The strands *c* and *d* appear to be of no particular value. A comparison of Diagrams 2 and 4 shows that the transition of this example of *Dioon edule* and *Macrozamia* is in many respects identical.

Series B. This seedling differed from the first in the fact that the hypocotyl, at its upper end, had eight vascular strands, an equal number being derived from each seed-leaf. The behaviour of each of these was very regular: the bundles of each cotyledon consist of a central pair which is bounded on either side by one strand. Each central pair of traces organize that pole of the root-structure situated in the plane of the cotyledons; and each of the lateral bundles gives origin to one pole so that a hexarch root-structure results. There is thus a considerable difference between these two seedlings of *Dioon edule*. On the other hand there are important points of similarity between them. In each case those two poles of the root-structure situated in the plane of the seed-leaves was organized first and were the strongest; those poles in the intercotyledonary plane were differentiated at a lower level; further, the division of the phloem-ring first took place opposite the two protoxylem rays first organized.

In no case has a rotation of the protoxylem been seen, the remarks on

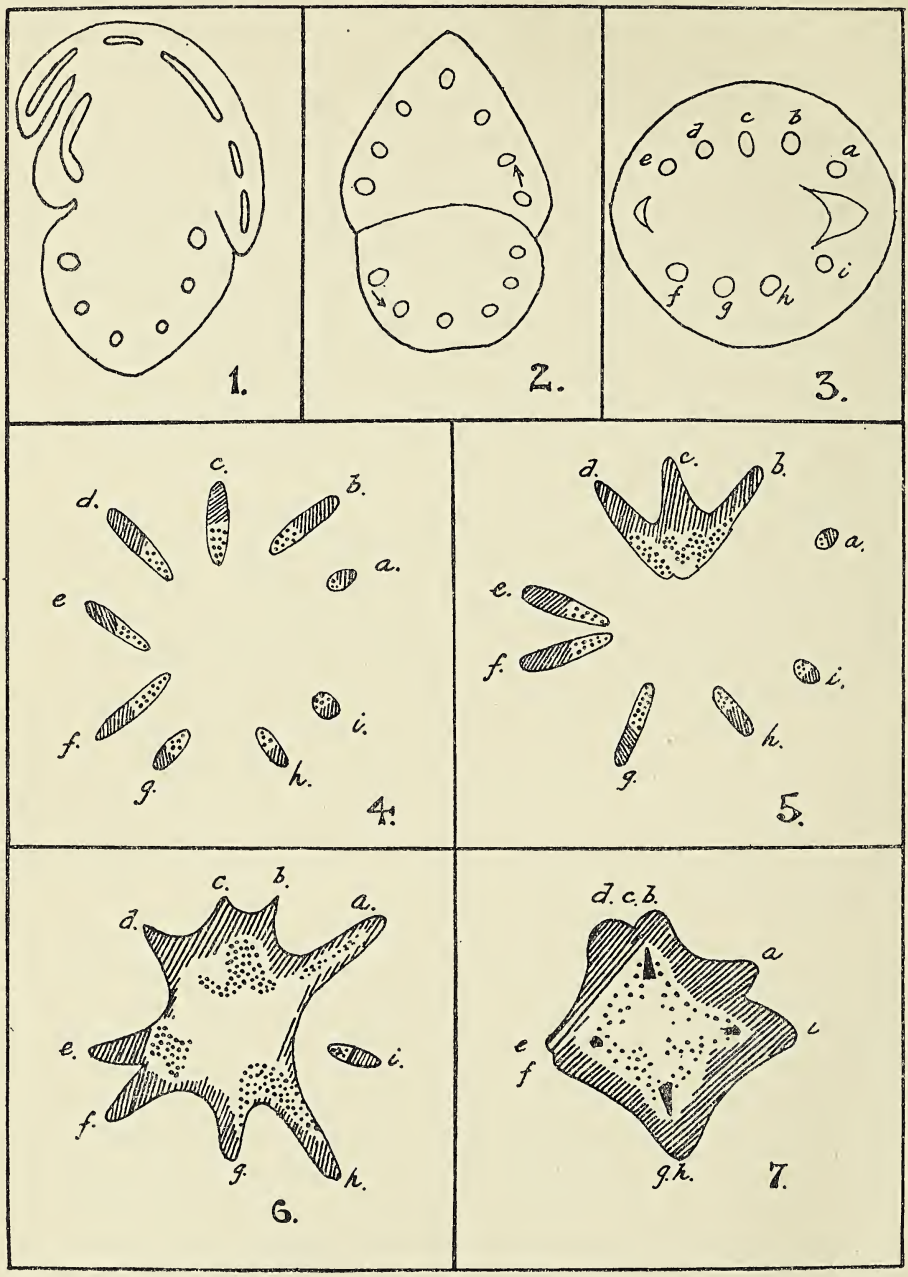


DIAGRAM 4. *Dioon edule.*

this feature made at the end of the description of *Macrozamia* apply equally well here.

A comparison of the above account with that given by Matte and Thiessen shows that there is much variation ; our second seedling (Series B) most closely conforms with what Thiessen found, but instead of the four lateral cotyledonary bundles forming two poles of the root they produced four, so that the resulting structure was hexarch instead of tetrarch. In the first seedling described above the two poles in the cotyledonary plane were formed from the three central bundles of one seed-leaf, and from the two corresponding strands of the other ; and the intercotyledonary poles were formed, one from the extreme lateral seed-leaf-traces on the one side, and from a single lateral bundle on the other. Further, the intercotyledonary protoxylem rays of the root-structure were in each example organized at a level lower than those in the cotyledonary plane.

Encephalartos.

Encephalartos Altensteinii, Lehm.

The seeds of this plant are larger than those of *Dioon*, *Stangeria*, and *Macrozamia*, and the seedling is much stouter, especially older ones which have a thick hypocotyl resembling, in many features, those of some hypogeal species of *Araucaria*. Different stages are represented in Pl. XXX ; Fig. 8 shows the cotyledons embedded in the prothallus to be of unequal size, and it will be noticed that the tip of the longer one is wrapped around the apex of the shorter ; Fig. 9 indicates the external appearance of a seedling a little older than the previous one ; and, lastly, Fig. 10 represents a still older seedling with a thickened hypocotyl. Periderm formation begins early, and is indicated in these drawings by the reticulate markings.

The cotyledons, both as regards their morphology and anatomy, do not differ in any feature of importance from those of the plants previously considered ; a cotyledonary tube is formed.

Transition.

Each cotyledon has five vascular bundles, a central strand bounded on each side by a pair of laterals, all of which are much the same in size, although, in some examples, the extreme lateral traces may be a little smaller than the rest. These bundles travel towards the centre of the hypocotyl very gradually, much more slowly than in *Dioon* and *Stangeria*, and, during this passage, the individual strands of each pair of laterals join together so that, when the central region of the axis is reached, there are six cotyledonary traces, three derived from each seed-leaf, alternating with the bundles from the plumule. Their arrangement is quite regular, the central cotyledonary bundles being in the plane of the cotyledons and bounded on each side by a lateral.

A complicated series of anastomoses takes place between the cotyledonary and plumular traces, which results in the formation of a closed vascular ring, enclosing a mass of parenchyma, of the same nature as has been described above for *Dioon*. The protoxylem rays are now organized, but not all at the same level. First, a ray is formed from the central bundle of one of the seed-leaves, and situated in the plane of the cotyledons; then, at successively lower levels, of course not far distant one from the other, two other protoxylem rays are formed, each from the two lateral strands of the other seed-leaf, and inclined at an angle to the cotyledonary plane. The phloem-ring divides opposite each protoxylem group, and thus there is formed a triarch root-structure.

This triarch arrangement persists for some little distance downwards towards the root-apex, but, at a lower level, a new protoxylem group comes into being and the band of phloem opposite it divides, thus a tetrarch structure results; at a still lower level a similar occurrence takes place in a corresponding position on the other side of the central cylinder, so that a pentarch arrangement obtains. This increase in the number of poles in the root-structure appears to be a general feature of this plant, for in two other cases the initial triarch arrangement became tetrarch at lower levels, a character shared by *Stangeria*, *Ceratozamia mexicana*, and other plants.[1] We have found no instance of the pentarch or tetrarch structure giving place to a triarch arrangement such as Matte described for *Stangeria*, *Ceratozamia*, and *Encephalartos Barteri*; this is probably due either to the fact that the seedlings examined by us were either too young or, when sufficiently old, to the decay of the greater length of the root. It may be remarked here that the above account does not agree in certain respects with that given by Tansley and Miss Thomas.[2] They describe each cotyledon as having six vascular bundles which organize a tetrarch root-structure. In the examples examined by us the seed-leaves each had five bundles at the node, with one exception, when four were present and a triarch root-structure invariably was formed first.

Matte[3] gives a relatively brief description of *Encephalartos Barteri*. The specimen examined by him had three cotyledons, of which one was larger than the others, this larger one had two vascular bundles in its tip, whereas the smaller seed-leaves had but one each. At a lower level more strands arose, so that at the base each cotyledon had four. These traces remained separate one from the other until they had entered the hypocotyledonary axis, when those of each seed-leaf fused together to form a single concentric strand; the three bundles thus produced fused with the plumular vascular tissue at different levels, and ultimately produced a pentarch root-structure, which, at a lower level, became reduced to triarch.

[1] Matte, Shaw, loc. cit.

[2] British Association, Section K, York, 1906.

[3] loc. cit., 1904.

The behaviour of the cotyledonary traces of this species is obviously different to that of the same structures of *E. Altensteinii*, and we have seen nothing exactly like it in other genera. It is interesting to find that Karsten [1] has described each cotyledon of *Zamia muricata* as receiving one vascular trace from the axis which branches in the higher regions of the seed-leaves. This apparently does not obtain in all species of *Zamia*, for Miss Dorety, in her paper on *Ceratozamia*,[2] states that some species of *Zamia* are of the same type, as regards their structure, as *Dioon edule*. To return to *E. Barteri*, the later vascular rearrangements leading to the formation of the root-structures apparently are similar to those of *Macrozamia spiralis*.

The following descriptions deal with plants which we have not had the opportunity of examining. In many cases the authors cited have followed in their descriptions of the transition-phenomena a method different to that pursued by us; in such instances we have described the facts in our own way for the sake of uniformity.

Cycas.

Cycas siamensis, Miq. According to Matte [3] the transition-phenomena are similar to those described by him for *Dioon edule* with this difference, that the lateral cotyledonary bundles do not form any intercotyledonary poles, so that the resulting root-structure is diarch. As regards *Cycas* in general the following paragraph is quoted from Miss Thomas [4] as it embodies a summary of some of the facts observed by her and Tansley.[5]

'It appears that Cycad cotyledons are almost without exception destitute of a midrib, and have four or six bundles at their base; that the transition features take place so near the cotyledonary node that the hypocotyl is root-like in structure; and that the primary root is in most cases tetrarch, with a strong tendency to reduce near its apex to diarch. The genus *Cycas* would appear to be most aberrant, for while *C. revoluta* forms a tetrarch root, *C. siamensis* . . . forms a link between *C. revoluta* and *C. Rumphii*, which is diarch through the absence of intercotyledonary poles. Further, *C. revoluta* may have its two central bundles replaced by a single one, which, however, plays the part of two.'

There is apparently some variation, for Bower [6] states that in *Cycas Seemanni* there may be in the cotyledon a median bundle, or two equal ones disposed symmetrically near the centre of the cross section, and between these extremes intermediate modes of arrangement may occur. He

[1] Karsten: Organographische Betrachtungen der *Zamia muricata* (Abh. d. Berlin. Akad., 193. 1856).
[2] Bot. Gaz., xlvi, 1908.
[3] loc. cit.
[4] loc. cit.
[5] British Association, Section K, York, 1906.
[6] Bower: On the Comparative Morphology of the Leaf in the Vascular Cryptogams and Gymnosperms (Phil. Trans. Roy. Soc., Lond., 175. 1884).

goes on to remark that 'it might be assumed that the median bundle, when present, is merely the result of the coalescence of two equal bundles, which might be found to be distinct in the upper part of the cotyledon ; but this is not the case, since the median bundle has been found to maintain its individuality in an upward direction'.

Further, Miss Thomas and Tansley found that *Cycas revoluta* had a tetrarch root, whereas Van Tieghem [1] describes it as being diarch, and Worsdell [2] as triarch or tetrarch.

Finally, as regards the number of bundles in the cotyledons, Worsdell describes those of *Cycas revoluta* as having five strands in the apical parts, which at lower levels fuse together so that three enter the axis.

Ceratozamia.

Ceratozamia mexicana, Brongn. is of particular interest, owing to the presence of one seed-leaf only, the other being frequently aborted. Our knowledge of the seedling structure of this plant is practically complete, and is due to the researches of Miss Dorety [3] and Matte.[4] Considering the work of the former author first, the cotyledon, which in many cases is lobed at the tip, has many bundles in its distal region which increase in number towards the base and then undergo a reduction in number so that, finally, three strands only enter the axis. Reading the serial sections in the opposite direction three traces enter the seed-leaf from the hypocotyl, and each dichotomizes several times, hence, in the upper parts, the number of bundles may be fifteen, which undergo reduction as the apex is reached. The three traces each behave in the same manner, 'the median one being no more a "double" bundle than any of the others.' The transition takes place rapidly, each cotyledonary strand giving rise to one pole of the root-structure, which is either triarch or tetrarch ; whether the vascular arrangement of the root be triarch or tetrarch seemingly depends upon the degree of development attained by the median bundle of the aborted cotyledon.

When the second seed-leaf is made to develop, the transition-phenomena closely resemble those described for *Dioon edule* by Matte [5] and Thiessen [6] ; three strands enter the axis from each seed-leaf, one central and two lateral, the two central ones organize those two poles of the tetrarch root-structure situated in the cotyledonary plane, and the corresponding, i. e. opposite, lateral strands of the seed-leaves fuse together and form the two poles in the intercotyledonary plane.

Turning to the results obtained by Matte, who enters very fully into the anatomy and morphology of seedlings of different ages, it is clear that

[1] loc. cit. [2] loc. cit.

[3] Dorety : The Embryo of *Ceratozamia*, a physiological study (Bot. Gaz., xlv, 1908). The Seedling of *Ceratozamia* (Bot. Gaz., xlvi, 1908).

[4] loc. cit., 1908. [5] loc. cit., 1904. [6] loc. cit.

the same kind of variations obtain in this plant as we have shown to occur in others. As regards morphology, the material at his disposal contained one seedling with two cotyledons naturally developed, of these one was slightly larger than the other, and each had in the upper regions ten or twelve vascular bundles, which by fusion became reduced to three in the basal parts. There is no cotyledonary tube such as obtains in *Encephalartos Altensteinii.* The cotyledonary strands enter the axis and fuse on to the plumular traces, thus forming an elliptical concentric vascular cylinder from which a diarch root-structure is organized, the two poles being in the plane of the cotyledons. At a lower level a third pole is differentiated. In another example having but one cotyledon eight vascular strands entered the axis, fused in pairs, and gave rise to a triarch root-structure.

Ceratozamia longifolia ♀ × *C. mexicana* ♂. The seedlings of this hybrid have been described by Van Tieghem,[1] who does not enter at all fully into the transition-phenomena. Monocotyledony appears to be the rule, but one out of four examples examined seemingly had two cotyledons of very unequal size ; Van Tieghem concludes that this specimen was really monocotyledonous, and the apparent smaller second seed-leaf was nothing more than a lobe of the larger one. The cotyledons generally have eight vascular strands which fuse in pairs as they are traced downwards, so that four traces enter the axis and give rise either to a triarch or a tetrarch root. The apparent dicotyledonous specimen had six seed-leaf bundles and the root-structure was triarch.

Microcycas.

Microcycas calocoma. The germination and structure of this plant has been described by Miss Dorety.[2] The cotyledons are two in number, and for the greater part of their length are fused very closely together by their ventral surfaces ; basally they form a tube. Each seed-leaf has in its upper region eight to ten vascular bundles, all of which are derived from the branching of three. The transition is of the same type as that occurring in *Dioon edule*, as described by Matte and Thiessen. The corresponding lateral bundles of each cotyledon fuse together, thus four strands obtain. 'The metaxylem and phloem divide, as usual, and the resulting portions swing to right and left, the right half of the phloem of each joining with the left half of that of the next, with sometimes the lowermost extremities of leaf-trace phloem intervening. There is thus produced the characteristic root-structure, four groups of phloem alternating with four double-fan-shaped xylem groups.' In some cases the tetrarch arrangement became reduced to triarch towards the apex.

[1] Van Tieghem : Symétrie de la structure des plantes (Ann. Sci. Nat., Bot., xiii, 1873).

[2] Dorety : Vascular Anatomy of the Seedling of *Microcycas calocoma* (Bot. Gaz., xlvii, 1909).

Bowenia.

Bowenia spectabilis. Our knowledge of the seedling structure of this plant is due to the work of Pearson,[1] who gives an extended description of the morphology and structure of the plant in question. Each cotyledon contains from four to seven vascular bundles, the neighbouring ones of which may fuse together so that four strands enter the hypocotyledonary axis from each seed-leaf. These, in the axis, join together, and with the traces derived from the plumule form a concentric pithless vascular cylinder, the centre of which is occupied by protoxylem elements. From this central strand there is quickly organized a diarch root-structure which becomes triarch at a slightly lower level, which arrangement in turn gives place to a pentarch root-structure which persists for some distance downwards. Whether, in older seedlings, this pentarch organization becomes reduced to a diarch structure, such as Matte found to obtain in *Stangeria* and *Ceratozamia*, Pearson does not state.

From Pearson's account it appears that the transition-phenomena in *Bowenia spectabilis* are of the same type as generally obtain in *Macrozamia spiralis*.

Zamia.

As far as has been seen there exists no account of the seedling-structure of species of this genus of any importance in the present connexion. Van Tieghem [2] states that in *Zamia furfuracea* the number of cotyledons are usually two, but sometimes one and sometimes three obtain. In dicotyledonous examples each seed-leaf has four vascular bundles; in the monocotyledonous specimen there were eight cotyledonary strands; and, finally, in the tricotyledonous plant, each seed-leaf had two vascular bundles. A cotyledonary tube commonly is formed, and the root-structure is tetrarch.

Zamia spiralis sometimes has the tips of the cotyledons lobed to such a degree as to suggest a pinnate lamina [3]; a fact which has also been observed by Miss Dorety,[4] who states that species of *Zamia* have seed-leaves with four to ten lobes, and that the transition is of the same type as in *Dioon edule*.

Summary.

Cotyledons.

1. In all the plants examined the seed-leaves are hypogeal, and are embedded in the prothallus throughout their existence.

2. They are very generally two in number; three have been observed in *Ginkgo biloba*.

[1] Pearson: Anatomy of the Seedling of *Bowenia spectabilis* (Ann. Bot., xii, 1898).
[2] loc. cit. [3] Sachs: Text Book, 2nd Ed., p. 501 (Oxford, 1882). [4] loc. cit., 1909.

3. The seed-leaves are frequently unequal in size, the larger one overlapping the tip of the shorter ; there is a marked tendency to the formation of lobes, especially at the apex ; and a short basal cotyledonary tube is formed in some cases, e. g. *Encephalartos Altensteinii.*

4. In the Cycadaceae the cotyledons are more or less intimately fused by their ventral surfaces, especially in the upper parts which are embedded in the prothallus.

5. As regards structure, stomata generally are present ; the mesophyll is homogeneous ; secretory cells and canals are common ; and the vascular bundles are mesarch or exarch in varying degrees.

6. The number of bundles in each cotyledon varies (see table below) ; if the seed-leaves of a seedling are unequal in size, the smaller one generally has fewer bundles than the larger.

7. In all cases the bundles are more numerous in the central region than in the basal parts and the tips ; or, in other words, the strands passing into the seed-leaves from the axis branch or dichotomize sometimes very regularly as in *Ginkgo*; the branches may subsequently fuse together in the apical regions.

Transition-region.

8. In all cases the transition-phenomena take place rapidly, so that the hypocotyl throughout the greater part of its length shows root-structure. These changes are most rapid in *Macrozamia spiralis* and slowest in *Encephalartos Altensteinii. Dioon edule* and *Stangeria sp.* occupy an intermediate position as regards the features in question.

9. In all the plants examined the vascular rearrangements take place within the hypocotyl.

10. In no Cycad has a rotation of the protoxylem of the seed-leaf-traces been observed ; the amount of centrifugal wood is so small that only a slight rearrangement is required in order to bring the protoxylem into the exarch position. In *Ginkgo* such a rotation does take place within the hypocotyledonary axis.

11. In *Ginkgo* each cotyledonary bundle, in dicotyledonous examples, gives rise to two poles of the root-structure ; in the tricotyledonous specimen examined each seed-leaf-strand gave origin to one pole, so that the resulting root-structure was triarch.

12. In the Cycads examined—*Macrozamia spiralis, Stangeria sp., Dioon edule*, and *Encephalartos Altensteinii*—the cotyledonary bundles fuse with the plumular traces, and ultimately form a central cylinder which consists in *Macrozamia spiralis* either of a central rod of xylem surrounded by phloem, or of an open U-shaped strand which soon closes to form a triangular-shaped vascular cylinder, as viewed in transverse section, enclosing a central mass of parenchyma ; in *Stangeria*,

of a horseshoe-shaped central cylinder enclosing a central mass of parenchyma; in *Dioon edule* and *Encephalartos Altensteinii* of a concentric cylinder with a central pith.

13. The fusion of the cotyledonary traces with one another, or with the plumular bundles, or with the central cylinder, does not always take place at the same level.

14. The seed-leaf bundles are not of equal value in the production of the root-structure; some traces play a wholly subordinate part, and the behaviour of similarly situated bundles in different individuals of the same species varies. Thus in *Dioon edule*, in one example, the two poles of the root-structure situated in the plane of the seed-leaves were organized from the three central bundles of one cotyledon, and the two central strands of the other seed-leaf; and the intercotyledonary poles were formed, one from the extreme lateral traces on the one side, and from a single lateral bundle on the other. In another example the four lateral traces, two from each cotyledon, each formed one pole of the root-structure, so that there were four intercotyledonary poles in addition to the two situated in the plane of the cotyledons.

In *Stangeria* it is more difficult to allocate values; one pole was formed chiefly from a lateral strand of one of the seed-leaves, the second pole from the other lateral bundle and part of the central trace of the same cotyledon, while the third pole was organized chiefly from the vascular tissue of one of the two bundles of the other cotyledon.

In *Encephalartos Altensteinii*, the central bundle of one cotyledon organizes one pole of the root-structure; the other two poles are formed from the two lateral traces of the other seed-leaf.

15. The poles of the root-structure are formed successively in *Stangeria* and in *Encephalartos Altensteinii*. In the case of *Dioon edule* the two poles situated in the plane of the cotyledons are the first to appear, those in the intercotyledonary plane being organized at a slightly lower level.

16. In one seedling of *Macrozamia spiralis* an isolated concentric strand of vascular tissue occurs in upper regions of the hypocotyl.

Root.

17. In *Ginkgo* an addition of protoxylem elements may occur after the root-structure has been attained.

18. In *Stangeria sp.* the primary root may dichotomize.

19. After the initial root-structure has been attained the number of poles may be increased at lower levels; thus, in *Encephalartos Altensteinii*, the initial structure may be triarch but it becomes tetrarch or pentarch at lower levels.

20. The following table shows the variation in the number of bundles in the base of the cotyledons of the plants named, and also the relation between the number of poles in the root-structure and the cotyledonary strands.

This table is based on observations recorded in the literature on the subject, together with our own.

Plant.	*No. of bundles at base of each cot.*			*Root.*
	C. 1.	*C.* 2.	*C.* 3.	
Ginkgo biloba	1	1		4-arch
	1	1		4→2-arch
	1	1		6→2-arch
	1	1	1	3-arch
Macrozamia spiralis	5	4		2-arch
	4	4		2-arch
	4	4		3-arch
	3	2		3-arch
Stangeria paradoxa	4 or 5 + 2 or 3			3→2-arch
				2→3-arch
				2→3→4→2-arch
Dioon edule	2	4		3-arch
	4	4		4-arch
	4	4		6-arch
	5	4		4-arch
Encephalartos Altensteinii	5	4		3→4-arch
	5	5		3→4→5-arch
	6	6		4-arch
Encephalartos Barteri	4	4	4	5→3-arch
Cycas revoluta	3	3		3 or 4-arch
	5 or 6			4-arch
				2-arch
Cycas siamensis	4	4		2-arch
Cycas Rumphii				2-arch
Ceratozamia mexicana	3			3 or 4-arch
	8			3-arch
	3	3		4-arch
	3	3		2→3-arch
Ceratozamia longifolia × *C. mexicana*	4			3 or 4-arch
	6			3-arch
Microcycas calocoma	3	3		4-arch or 4→3-arch
Bowenia spectabilis	4	4		2→3→5-arch
Zamia furfuracea	8			4-arch
	4	4		4-arch
	2	2	2	4-arch

EXPLANATION OF PLATE XXX.

Illustrating Mr. T. G. Hill's and Miss de Fraine's paper on the Seedling Structure of Gymnosperms. III.

All the figures are natural size.

Fig. 1. A seedling of *Ginkgo biloba.*

Fig. 2. An older example showing a greater development of the plumule.

Fig. 3. A tricotyledonous specimen of *Ginkgo.*

Fig. 4. A young seedling of *Macrozamia spiralis.*

Fig. 5. A young seedling of *Stangeria sp.*; the plumule is just showing.

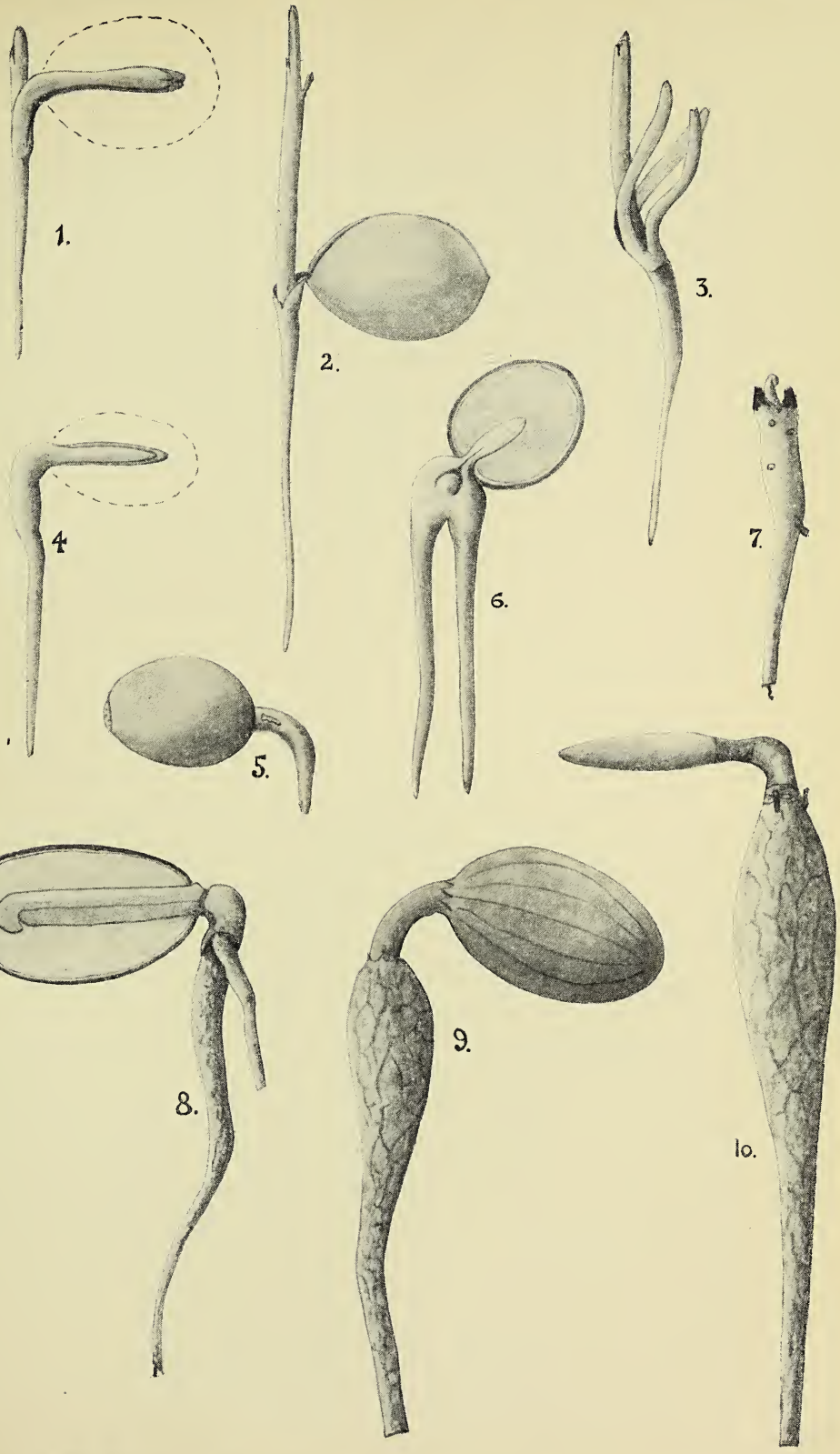
Fig. 6. An older example showing the cotyledons embedded in the endosperm, a greater development of the plumule, and a dichotomously-branched primary root.

Fig. 7. A still older seedling of *Stangeria sp.* in which the cotyledons have decayed and the hypocotyl enlarged.

Fig. 8. A seedling of *Encephalartos Altensteinii* showing the unequal cotyledons embedded in the prothallus.

Fig. 9. An older specimen.

Fig. 10. A still older example with a swollen hypocotyl.



T. G. H. del.

HILL & DE FRAINE—SEEDLING STRUCTURE OF GYMNOSPERMS III.

The Perception of Light in Plants.

BY

HAROLD WAGER, F.R.S.

With Plates XXXI and XXXII, and three Figures in the Text.

THE extremely interesting observations by Haberlandt upon the sense organs for light in foliage leaves [1] have stimulated anew the interest in this question which has been taken ever since the discovery of Charles and Francis Darwin, that in grass seedlings 'sensitiveness to light is sometimes confined to a small part of the plant; and that this part, when stimulated by light, transmits an influence to distant parts, exciting them to bend.' (The Power of Movement in Plants, 420, 1880.)

It is now well known that the stimulus which causes the foliage leaves of many plants to place themselves in such a position as to receive the fullest advantage from the incident rays of light, is perceived mainly by the leaf-blade, and that this stimulus is transmitted to the petiole, by which the turning of the leaf is brought about.

The question therefore arises: by what means does the leaf-blade, or the plant for that matter, perceive that it is or is not in the most advantageous position for the incident rays? Haberlandt has attempted to explain how this is brought about. He has shown that in many leaves the upper epidermal cells are shaped like convex or planoconvex lenses, and being filled with a clear sap are able to bring about a convergence of the light rays; in others, special cells or local thickenings of the cuticle act in the same way. The epidermal cells have a thin layer of protoplasm on their basal walls. When the leaf is at right angles to the light, the central portion of this layer in each cell is illuminated, the peripheral zone remaining dark. Under the microscope this appears as a bright central disk of light on a dark ground (Fig. 7). In oblique illumination, the bright spot of light moves to one side; and this alteration in the position of the light spot, according to Haberlandt, sets up the stimulus which results in the orientation of the leaf into a more favourable position. The evidence for this conclusion is based upon the optical behaviour of the epidermal cells and upon experiments by which the lens function is eliminated.

[1] Lichtsinnesorgane der Laubblätter, 1905.

[Annals of Botany, Vol. XXIII. No. XCI. July, 1909.]

Various observations which have been made by recent observers to test the truth of this explanation would seem to show that, in many respects, both the morphological and the physiological evidence upon which it is based is unsound; and in any case it appears to me Haberlandt has not sufficiently considered (1) that the orientation of the leaf may be bound up with the absorption of light by the chlorophyll grains, and not with the action of light upon the protoplasmic layer of the epidermal cells, and (2) that the light rays which are brought to a focus in the tissues of the leaf may be functional, in part at any rate, in the more efficient illumination of the chlorophyll grains.

The Optical Behaviour of the Epidermal Cells.

The various modifications by which, according to Haberlandt, a differential illumination of the basal wall is brought about in the epidermal cells of diaheliotropic leaves may be grouped as follows:—

Type I. Outer wall flat; inner or basal wall curved or angular.

Type II. Outer wall curved; inner wall flat.

Type III. Combination of I and II. Both outer and inner walls curved. As Seefried [1] points out, Haberlandt does not give this as a separate type, but it is convenient so to regard it.

Type IV. Papillate projections of a portion of the upper cell-wall, which act as lenses. These are included by Haberlandt under papillate epidermal cells.

Type V. Lens-shaped thickenings of portions of the upper cell-wall, which act as lenses.

Type VI. Local light-perceiving organs of various kinds, reduced or rudimentary hairs, special lens cells, oil drops, and tannin-containing receptacles.

To these may perhaps be added the special cases described by Gaulhofer [2] as:—

Type VII. Flat epidermal cells with very thick walls which bring about a differential illumination of the basal protoplasmic lining layer through the passage of the rays of light from the highly refractive cell-wall into the less refractive cell-sap.

[1] Ueber die Lichtsinnesorgane der Laubblätter einheimischer Schattenpflanzen. Sitzungsber. d. k. Akad. d. Wiss. in Wien, cxvi, 1907.

[2] Die Perzeption der Lichtrichtung in den Laubblättern mit Hilfe der Randtüpfel, Randspalten und der windschiefen Radialwände. Sitzungsber. d. k. Akad. d. Wiss. Wien, cxvii, pp. 153–90, 1908.

The curved upper surface of the epidermal cells can be as a rule very easily seen with a pocket lens, as Haberlandt has pointed out is also the case with well-marked papillate cells; in bright sunlight or near a window a bright spot can usually be seen reflected in the curved surface of each cell. If examined under a low power by reflected light, images of various objects, reflected in the convex surface as in a convex mirror, are visible. *Tradescantia fluminensis*, *Orchis bifolia*, *Adoxa moschatellina*, and many other plants show this clearly.

With the exception of Type I, the differential illumination of the basal wall is always due to convergence. In Type I, where the outer cell-wall is flat, and there are no special thickenings or other contrivances for the convergence of light, the curvature of the inner wall is sufficient, according to Haberlandt, to bring it about. The lateral portions of such a wall are placed obliquely to the incident rays, and are therefore less illuminated than those portions near the middle which are more or less horizontal. How far this arrangement is capable of bringing about a sufficient amount of differentiation to set up an orientation stimulus will be discussed later.

An excellent example of the way in which these convergent rays act in the illumination of the basal wall is seen in *Saxifraga Geum*. The curved outer walls of the upper epidermal cells have a well-marked papillate projection about the middle of the cell-wall. This, even without a diaphragm, produces a clear spot of light on the basal wall (Pl. XXXI, Fig. 7). If the mirror is moved a little to one side, this spot of light moves, and is now not exactly in the centre (Fig. 8). If the mirror is moved still further to one side, the spot of light moves correspondingly further to one side of the cell (Fig. 9). In this plant the light is brought to a focus actually on or very near the basal wall, and, under all circumstances, the differential illumination of the basal wall is very marked; in the majority of plants it is usually not so clear.

As Haberlandt points out (loc. cit., p. 54), the usual case is for the rays of light to be brought to a focus behind (below) the basal wall. The basal wall cuts the converging cone of light so as to give a bright middle region surrounded by a darker field. The further this is from the apex of the cone of light, the larger the middle field, and vice versa. If the diaphragm is taken away, the dark zone becomes narrower, but is always visible. If the focus is in front of (above) the inner wall, then the clear middle field is formed by cutting through the divergent cone of light. In either case, if the mirror is moved laterally, so that the light falls at an angle on the epidermis, the field of light makes a corresponding movement to one side (Figs. 8, 9).

Haberlandt points out (loc. cit., p. 56) that if the diaphragm opening is 1 mm. and this is at a distance of 5 mm. from the epidermis, a cone of light is admitted which is equal to a circular opening in the woods of a diameter of 1 m. at a distance of 5 m. high from the surface of the leaf illuminated.

Smaller openings in the woodland green would produce a correspondingly smaller cone of light and a smaller middle field.

Cells with a very regular outline, such as occur in *Tradescantia fluminensis* and *Zebrina pendula* on both sides of the leaf, the upper epidermal cells of *Ligustrum ovalifolium*, various Orchidaceae, &c., may be compared to the corneal facets of the compound eyes of insects. They act as very efficient lenses, and not only bring the light to a focus but are capable of forming clear and distinct images of objects near them. In one case Haberlandt (loc. cit., Pl. IV, Fig. 2) was able to obtain a somewhat indistinct photograph of a microscope stand which was focused upon the basal wall of the cell. Guttenberg [1] placed a preparation of the epidermis of *Adoxa moschatellina* firmly under the microscope, and, after removing the mirror and diaphragm, directed the instrument towards various objects. He then saw the images of the objects quite clearly focused through the cells. Similar observations were made with other plants. It is probable that the upper epidermal cells of the majority of ordinary foliage leaves will be found to possess to a greater or less extent this power. I have examined some hundreds of plants, and only in very few has it been absent. Moreover, in the great majority the cells of the lower epidermis, although not in all cases equal to the upper epidermal cells, function in the same way.

By appropriate manipulation with the microscope and the Gordon photo-micrographic apparatus made by Messrs. Beck, it has been found possible to obtain photographs of a variety of objects through cells both of the upper and lower epidermis of leaves of many species of plants. Among these are portraits from life, reproductions of photographs of various kinds, flowers and other objects direct, and it has even been found possible to photograph trees, houses, and landscapes, and to reproduce simple diagrams in colour on the autochrome plates of Messrs. Lumière.

The methods employed are perfectly simple, and do not call for any special skill in manipulation. A strip of epidermis is sliced off with a razor and floated gently upon a layer of dilute glycerine placed on a thin cover-glass. As much of the glycerine as possible is then soaked up with blotting-paper, leaving the layer of epidermis adhering closely to the glass. The upper surface of the epidermis should be kept dry. The cover-glass is then inverted over a damp chamber and placed on the stage of the microscope, so that the upper surface of the epidermis will be nearest the mirror. Remove the sub-stage condenser but retain the diaphragm ; place the object to be photographed at some distance from the microscope ; whether this should be two or three feet or several hundred yards depends upon the size of the object. Then by means of the plane mirror reflect the image on to the epidermal cells, and examine with a 1-inch objective. A clear view

[1] Die Lichtsinnesorgane der Laubblätter von *Adoxa Moschatellina*, L. und *Cynocrambe prostrata*, Gärtn. Ber. d. d. bot. Gesell., xxiii, p. 265, 1905.

of the epidermal cells should be first obtained ; then slowly raise the tube of the microscope and the images of the object focused through each of the epidermal cells will gradually come into view. If the images overlap reduce the size of the diaphragm opening until they are clear ; in most cases it will be found that an opening of from two to three millimetres is suitable. Now focus the image clearly by means of the special eye-piece supplied with the photo-micrographic apparatus ; replace it by the camera, in which a suitable plate has been placed, and expose. The correct exposure must be found by experience ; but using a fairly rapid plate with 1-inch objective and ocular A (a moderately deep eye-piece) an exposure of from 2 to 30 seconds is sufficient. With higher powers correspondingly longer exposures must be given. Thus with a bright image, using a $\frac{2}{3}$-inch objective and the apochromatic ocular No. 8 of Zeiss and Cadett extra-rapid plates, an exposure of one minute was given.

The following method of mounting the epidermis of *Tradescantia fluminensis*, or other epidermis which possesses fairly regular lens cells, is useful. Float the strip of epidermis on a 5 per cent. solution of chromic acid for a short time ; then transfer it to water. Cover a glass slide with glycerine jelly and allow to cool. Lay the epidermis on it and soak up the superfluous water. The cells will remain in good condition for observation for several hours. As the epidermis dries the outer walls of the epidermal cells sink in and form concave lenses through which images can still be focused, but below instead of above the layer of epidermal cells.

By means of the autochrome plates of Messrs. Lumière, simple colour photographs have been obtained through the epidermal cells of *Tradescantia fluminensis* and the special lens cells of *Mesembryanthemum cordifolium*. The objects used were bright-coloured flowers photographed by reflected light, and simple patterns constructed of coloured sheets of gelatine photographed by transmitted light. In bright sunlight in July, using a 1-inch objective and a No. 4 eye-piece, an exposure of from 20 to 30 minutes was given, but in a good light, out of the sun, an exposure of $1\frac{1}{2}$ to 2 hours was necessary. The $3\frac{1}{4} \times 3\frac{1}{4}$ plates were cut into four $1\frac{5}{8} \times 1\frac{5}{8}$ plates for this purpose. No difficulty was experienced in the development of these cut plates beyond a slight tendency to frill at the edges of the film where it had been broken across.

A few illustrations produced from some of the photographs obtained are given in the plates which accompany this paper. Fig. 1 shows a reproduction of a photograph taken through the epidermal cells of *Tradescantia fluminensis*, and Fig. 6, a photograph of a geometrical figure taken through the cells of *Zebrina pendula*. In both cases, the image was brought to a focus below the basal walls and the outlines of the cells cannot be seen. Fig. 12 shows images brought to a focus almost exactly upon the basal wall in *Zebrina pendula* through papillate projections in the middle of the

curved outer cell-walls. In this case two images are formed at different levels, one by the cell as a whole (Fig. 6), the other by the more or less central papilla in the upper wall of the cell (Fig. 12). If the epidermal cells are focused so that the image formed by this papilla is first of all brought into view, then, as the tube is moved upwards, this disappears, and the image formed by the whole cell appears.

The production of such clear images shows that the epidermal cells are capable of functioning as very efficient lenses. But it is not probable that the plant is capable of perceiving images, nor is such a supposition necessary to Haberlandt's hypothesis. The epidermal cells have very little resemblance to the highly organized eyes of animals, although, as in the case of *Fittonia Verschaffeltii*, instanced by Haberlandt, in which there is a lens-shaped cell at the apex of a larger cell, the two have some analogy to an animal eye in their general arrangement. Haberlandt points out, if we reserve the term 'eye' only for those organs which bring about an image perception, then the foliage leaves and many animals are eyeless. But if the term is extended to include organs capable of perceiving a difference in the intensity of light, then plants must be said to possess eyes.

Cases in which the Lens Cells do not function in Light Perception.

That the lens function is of very common occurrence is shown not only by the number of instances adduced by Haberlandt, but by many more recent observations, notably those of Seefried (loc. cit.) and Sperlich,[1] all of which lend support to Haberlandt's hypothesis. On the other hand, there are numerous instances where lens cells and even special cells and local thickenings of the cuticle occur, which can have no special function in the perception of light for the orientation of the leaf.

In the majority of leaves which I have examined, the cells of the under epidermis show a distinct lens action; they produce a less regular convergence than those of the upper epidermis as a rule, but in many cases equally good and clear. Albrecht[2] has also shown that papillate cells are of common occurrence on the under sides of leaves. This had, however, not escaped Haberlandt's observation. On p. 125 of his chief paper[3] he refers to it as of frequent occurrence. He states, however, that it cannot be considered as an objection to his hypothesis regarding the function of the upper epidermal cells. All papillate epidermal cells do not necessarily function in this way; they may serve for very different purposes such as the prevention of wetting of the leaf. But whenever the curved outer wall of the upper epidermal cells is specially clearly marked,

[1] Die optischen Verhältnisse in der oberseitigen Blattepidermis tropischer Gelenkpflanzen. Sitzungsber. d. k. Akad. d. Wiss. Wien, cxvi, 1907.

[2] Ueber die Perzeption der Lichtrichtung in den Laubblättern. Ber. d. d. bot. Gesell., xxvi, p. 182, 1908.

[3] Lichtsinnesorgane der Laubblätter, 1905.

as in velvet leaves, or when a specially differentiated small lens is produced, as in *Vinca major*, &c., then these contrivances fail completely on the under side. In such cases he considers it is clear, therefore, that these structures are connected with a definite function which is peculiar to the upper epidermis, and this can only be the function of light perception. Moreover, the local sense organs for light (ocelli) are found exclusively on the upper side of the leaf.

Seefried, however, points out (loc. cit.) that in *Aquilegia vulgaris* optically efficient papillae are found on the outer walls of the epidermal cells of the lower epidermis as well as on the upper. The papillae of the lower epidermis are, however, not so regularly formed, and are placed more excentrically. From this he would seem to imply that they cannot be functional in Haberlandt's sense. So far as my own observations go, this is not the case. The papillae are much the same in structure on both sides, as indeed Seefried's own figure shows; the lower epidermal cells are more irregular in outline than the upper, but in both cases the papillae may be excentrically placed, although not so much so as in *Adoxa moschatellina*, as figured by Guttenberg (loc. cit.), to which no exception is taken.

In *Viscum album*, according to Albrecht (loc. cit.), epidermal swellings occur on both sides of the leaf; and he adduces numerous instances in which papillae are found on leaves which do not assume the fixed light position. He also shows that special cells or groups of cells (ocelli) may occur on the under sides of leaves.

Haberlandt's contention, that special lens cells or lens thickenings fail completely on the under sides of the leaves, therefore falls to the ground, as also does his conclusion that they are specially adapted for the perception of light. Two other examples which show this very clearly may here be given. In *Mesembryanthemum cordifolium* we have an excellent example of local lens cells. The leaves and stem of this plant are covered with glistening spots which can be quite easily seen by the naked eye. On the leaves these consist of enlarged epidermal cells, more or less regular in outline (Fig. 2) and of a biconvex shape in section; on the stem they are in the form of spherical cells, as in the ice-plant, which stand out from the surface. The cells appear to contain a mucilaginous substance, which may occur in the form of bright refractive granules (Fig. 4), and probably serve to store up water. These cells are very efficient lenses (Fig. 5); they occur equally on both sides of the leaf and in both cases equally good images can be photographed through them. In Fig. 3 is shown—somewhat indistinctly, owing to the interference of the mucilage granules—a photograph of some houses taken through the cells on the under side of the leaf. Between these large cells there are the much smaller normal epidermal cells. These also bring about a very clear convergence of the light rays (Fig. 4). The leaves always place them-

selves with the upper surface exposed to and more or less at right angles to the rays of light. In the leaves of *Garrya elliptica* both the upper and the under epidermal cells are covered by a thick cuticle, in which lens-shaped thickenings occur more or less regularly spaced (Fig. 23). These function as very efficient lenses and produce clear spots of light about the level of the basal walls of the epidermal cells. Fig. 24 shows the image of a cross photographed through them. The images formed are equally clear on both sides of the leaf. So far, however, from producing a centric differential illumination of the basal walls of the epidermal cells, they appear to have no definite relation to them. They are sometimes placed in the middle of the cell-wall, but are just as often near one side, and frequently occur just over the partition wall between two cells (Fig. 23). In these circumstances it is difficult to conceive that their function is that of light perception.

If we consider both upper and lower epidermal cells and also ortho-heliotropic as well as dia-heliotropic organs, we find that we can distinguish three different forms of cells according to their optical behaviour.

Form I. Cells with more or less regular outline, which bring the incident rays of light to a focal point—spherical or stigmatic lenses.

Form II. Cylindrical cells which bring the rays to a focal line instead of a focal point, and may therefore be designated astigmatic lenses. These occur mainly on orthotropic organs.

Form III. Cells with very wavy or irregular outlines, intermediate between I and II, each one capable of bringing rays of light to a focal point in one or more regions of the cell, and to a focal line in other parts of the same cell. These occur commonly on the under sides of leaves, but are also found occasionally on the upper surface (e.g. *Eranthis hyemalis*).

In cells with an irregular or wavy outline, such as occur on the leaves of *Eranthis hyemalis, Ranunculus Ficaria, Veronica Beccabunga,* various species of Anemone, many Ferns, and numerous other plants, a distinct differential illumination can be seen when a small diaphragm opening, 2 to 3 mm., is used. The light area is, however, very irregular (Fig. 17), and, although not incapable of explanation by Haberlandt's hypothesis, introduces complications in the light perception which the plant must find some difficulty in disentangling. In some cases the variation in curvature of the outer cell-wall results in the formation of two or more bright spots of light in each cell, each of which is capable of forming an image of an object focused through it.

Anything which brings about a state of turgor in transparent cells tends to the development of a more or less spherical or cylindrical form, and the cells may thus become optically active. This occurs in many cases where it can be no question of the lens function being of special

importance to the plant for light perception. Such are, for example, the cells of the pith of the Rush, cortical cells in the stems of certain plants, cells in the pileus of species of *Russula,* and various hair cells and gland cells. Turgidity plays a very important part in the growth of plants and especially in the unfolding of leaves. The cells of the epidermis on the concave side of many expanding leaves are more turgid than those on the outer convex side. This is well shown in *Hyacinthus romanus.* The young leaves just as they appear above ground are very much curved; the outer epidermis exhibits no turgidity whatever; the inner epidermis, on the contrary, is composed of elongated cells which are very strongly turgid and are capable of bringing the rays of light to a focus in the shape of a clearly defined bright line. The same phenomena can be seen in the strongly incurved young leaves of *Cyclamen.* Both exhibit a pronounced heliotropism in the young state.

In the case of all leaves which remain flat and rigid, the maintenance of a certain amount of turgidity in the epidermal cells is doubtless necessary unless they possess a thick cuticle. It may be suggested, therefore, that possibly the amount of turgidity and the arching of the cell-walls bear some relation to the thickness of the cell-wall and cuticle. In some cases, for example, the leaves show little or no turgidity in their epidermal cells, but their flatness and rigidity are maintained apparently (in part, at any rate) by much-thickened cell-walls. Such leaves may exhibit a very pronounced dia-heliotropism, and yet show no lens action at all (*Prunus lauro-cerasus*). Gaulhofer (loc. cit.) has apparently appreciated the importance of this in respect to Haberlandt's theory, and has brought forward a number of observations to show that in such cases a differential illumination can be brought about, which gives similar results to those described by Haberlandt, by refraction of light through the more highly refractive thick cell-walls into the less highly refractive cell-sap.

The greater turgidity of the epidermal cells of many shade plants may be due largely to their thinner cell-walls and to the fact that they have probably more water to store up owing to their diminished transpiration. Haberlandt refers to turgidity (loc. cit., p. 126) in speaking of aphotometric leaves, but considers apparently that it is of little importance. If, he says, such leaves possess either an arched outer or inner wall in their epidermal cells, we should only perceive in them those physiologically unimportant early stages due to turgor which formed the starting-point in euphotometric leaves of their adaptation to light perception. The problem, however, is one which deserves a much fuller investigation from this point of view than has yet been given to it.

The papillate epidermal cells of many petals show a very distinct light convergence, and are capable of bringing about a very clear centric illumination of the basal walls (Fig. 18). This is well seen in a large number of

plants, but there is no evidence that it plays any part in orienting the flower with respect to the light.

Dia-heliotropic Leaves which do not possess Contrivances for Centric Differential Illumination.

Albrecht (loc. cit.), on the ground of numerous observations on the microscopic structure of the epidermis, comes to the conclusion that in many cases, in the absence of contrivances for light perception, the leaves are still found to assume the fixed light position. Haberlandt,[1] however, considers that all the cases cited by Albrecht which he examined have contrivances for differential illumination of the basal wall, and are thus capable of light perception.

The problem to be determined, however, is not merely the presence of curved outer or inner walls to the epidermal cells, but to what extent these are capable under normal conditions of producing a definite and visible differential central illumination. In the case of markedly papillate cells where the rays of light are actually brought to a focus on or very near the basal walls, the central brighter area of illumination is quite clear and distinct, but in a very large number of plants the focus of the rays is so far below the basal wall that although theoretically a differentiation is possible, in practice, even with the most careful illumination, no such differentiation is observed. Thus in *Saxifraga hirsuta* or *S. Geum*, the papilla on the upper surface of the cell produces a very distinct spot of light, whether the diaphragm is open or no, and a corresponding irregular illumination of the field when the light is oblique. Figs. 7, 8, 9, show this. In *Tradescantia fluminensis*, on the other hand, no differentiation of the lower wall can be observed when the light is perpendicular (Figs. 13, 14). When oblique, one side of the wall is illuminated, the other dark (Fig. 16). This would of course be sufficient to explain the orientation, but in a slightly different manner from that given by Haberlandt. When the leaf is at right angles to the incident light, the whole of the basal protoplasm is equally bright; there is no differentiation into a light central spot surrounded by a darker zone, and, consequently, there is no necessity to regard different portions of the cytoplasmic layer as sensitive to light of varying intensities. The whole of the basal plasma layer may be regarded as attuned to light of higher intensity and no part of it to darkness. When one portion of it is illuminated whilst the other remains dark, as in the case cited (see Fig. 16), this variation may act as the stimulus by which the orientation is brought about. It occurs much more commonly than the centric illumination required in Haberlandt's hypothesis, and will probably be found very widely distributed among plants

[1] Ueber die Verbreitung der Lichtsinnesorgane der Laubblätter. Sitzungsber. d. k. Akad. d. Wiss. Wien, mathem.-naturw. Klasse, cxvii, 1908.

which possess only a slight or moderate curvature of the outer walls. It is, however, only a variation on Haberlandt's hypothesis.

But there are numerous other leaves mentioned by Haberlandt, and of which some instances are given by Albrecht, where the outer wall is flat or only very slightly curved, but the inner wall distinctly angular or arched, which assume a distinctly dia-heliotropic position under the influence of light, and are more difficult to explain. In these cases, Haberlandt assumes that a sufficient amount of light differentiation would be brought about by the difference in the area covered by the rays of light passing through the cell, and impinging on the lower wall. If the lateral walls were very steep, there might be a perceptible difference; but in all the cases which I have examined they show absolutely no light differentiation at all under the microscope. Even when the light falls obliquely there is no differential illumination. The illumination becomes less bright equally all over the basal wall so far as can be observed under the microscope, and as the light becomes more and more oblique, and more of it is reflected from the surface, the basal wall becomes less and less bright, until finally the light is so oblique that none of its rays is able to penetrate the cell.

The number of species in which epidermal cells of the kind just described are found is probably small. The following are a few examples of such leaves, which possess flat-walled or only very slightly curved epidermal cells: *Hedera helix*, *Prunus lauro-cerasus*, *Buxus sempervirens*, *Rhododendron*, *Berberis vulgaris*, and *Ilex aquifolium*. In the majority of other plants which I have examined, the outer walls of the epidermal cells are curved to some extent and therefore capable of causing, in an oblique light, an unequal illumination of the basal wall.

In *Hedera helix*, according to Haberlandt,[1] the upper epidermal cells of the leaf are even, but in consequence of the presence of a somewhat uneven cuticle are not quite flat. He states that the inner walls of the cells are more or less angular or arched, and that about 24 per cent. possess the structure suitable to a differential illumination, and only these cells are able to perceive precisely changes in the direction of light. The leaf is clearly dia-heliotropic, but although I have examined a large number of specimens I have not been able to observe any differential illumination capable of demonstration under a microscope. If it exists, therefore, it must be extremely small, and can only be inferred from the cell structure as Haberlandt has done.

In *Prunus lauro-cerasus*, the most careful microscopic examination, both with and without a diaphragm, fails to reveal anything in the nature of a differential illumination. Fig. 10 shows this clearly. The basal walls were clearly focused; the lateral walls are thick and the illumination is seen to be equal all over the cell. The same results were obtained in the other species mentioned.

[1] Lichtsinnesorgane, &c., 1905.

In all these cases, therefore, both Haberlandt's hypothesis, and the variation of it which I have brought forward above, fail to account for the orientation of the leaf.

Ortho-heliotropic Organs.

The epidermal cells of many ortho-heliotropically sensitive organs show a pronounced optical activity. They are usually elongate, more or less cylindrical cells, and in consequence bring a beam of light to a focal line instead of a focal point. It will be convenient to speak of them as astigmatic lens cells. They are, however, probably not functional in light perception. Some of the most sensitive heliotropic organs do not possess them. They frequently occur on leaves which do not show any heliotropic sensitiveness; in some cases such leaves even possess clearly marked and optically active papillae or lens-shaped thickenings of the cuticle.

C. and F. Darwin point out (loc. cit.) that the exciting cause of the light response in positively heliotropic organs, such as seedlings, is the difference in illumination of the two sides. Haberlandt also considers that this is sufficient to account for the stimulus, and that in consequence no special light-perceiving cells or cell groups are necessary. The observations which I have made, although they show that optically active epidermal cells frequently occur, tend to confirm this view.

In grasses the leaf-blade shows very little if any sensitiveness to light, but, as is well known, the young seedlings are strikingly heliotropic. According to Figdor [1] the first sheathing leaf or cotyledon and the basal sheathing portions of the leaves are entirely responsible for the response observed in grass seedlings. The epidermal cells on both sides of the leaf-blade of *Avena sativa* and the inner epidermal cells of the cotyledon exhibit a very pronounced and beautiful astigmatic convergence (Fig. 22); the outer walls at the ends of the cells are slightly more curved, and are capable of forming clear images, such as those of a pipe, shown in the figure. The epidermal cells on the outside of the cotyledon, which are the only ones exposed to the light, are, on the other hand, optically inactive.

The aphotometric leaves of *Glyceria fluitans* possess well-marked papillate upper epidermal cells. These papillae occur near the end of each cell and produce very clear spots of light on the basal walls. Their function is, however, to prevent the wetting of the leaf and apparently has nothing to do with heliotropic activity.

The cells of both the upper and lower epidermis of *Deschampsia caespitosa* show a well-marked convergence due to long astigmatic lens cells. The leaves are aphotometric: they spread out and bend downwards

[1] Ueber Heliotropismus und Geotropismus der Gramineenblätter. Ber. d. d. bot. Gesell., xxiii, 1905.

so as to expose their upper surfaces to the light, but this appears to be due to epinastic growth.

The young leaves of *Hyacinthus romanus* grow towards the light if they are subjected to a lateral illumination. They have a dorsi-ventral structure and soon begin to bend outwards and downwards so as to present their upper surfaces to the light. This appears to be due to epinastic growth, and by its means the leaf is brought into such a position that its upper surface is more or less at right angles to the rays of incident light. The upper epidermal cells are optically active and converge the light as in Iris (Fig. 20). The lower surface, which is the only one exposed to the light when the leaves are heliotropically responsive in the early stages, possesses elongate cells which are flattened and show no trace of convergence.

The hypocotyls of the seedlings of many dicotyledons and the leaf-stalks of many leaves, such as *Ranunculus Ficaria*, *Eranthis hyemalis*, *Cyclamen*, &c., all of which are heliotropically responsive, possess well-developed astigmatic lens cells similar to those already described (Fig. 20).

Young buds of various bulbous plants, such as *Chionodoxa*, Tulip, *Freesia*, &c., are heliotropically sensitive, but the adult leaves of these plants, although they possess well-marked optically active cylindrical cells, are not, or only very slightly, sensitive to light.

In *Freesia refracta, var. alba*, the young sheathing leaves bend strongly towards the light. The leaf-blade is not heliotropically sensitive and grows in the direction in which it issues from the bud. As in Grasses, the young sheathing leaves and the basal sheathing position of the ordinary leaves are resposible for the heliotropic response. The epidermal cells of all parts of the adult leaf and the sheathing leaves show a very distinct differential illumination of the basal walls due to thickenings of the cuticle. On the basal sheathing part of the leaf and on the outer sheath leaves the epidermis, both on the inner and the outer surface, consists of elongate astigmatic cells, which have an elongate cuticular thickening in the middle of the outer wall of each cell. In the upper non-sensitive parts of the leaf, there are, instead of these elongate cuticular thickenings, one to three lens-shaped thickenings of the cuticle in each cell which produce clear circular spots of light on the basal walls. These are quite as well developed and as clearly marked as in many of the special cases described by Haberlandt, but are not functional as light-perceiving organs.

Aphotometric Leaves.

If it could be shown that aphotometric leaves were destitute of light-converging epidermal cells, it would be distinctly favourable to Haberlandt's hypothesis. Only one case is, however, adduced by Haberlandt (loc. cit., '05,

p. 126), and this does not appear to hold good for all specimens. He states that the leaves of *Convallaria majalis* are aphotometric, and that both the inner and outer walls of the epidermal cells nearly perfectly even. This apparently implies that they have little power of light convergence. I find, however, that this is not so; the cells of the upper epidermis, in all the specimens which I have examined, act as cylindrical lenses, and show an unmistakable convergence (Fig. 21) which, in an oblique light, is capable of producing a very definite variation in the illumination of the basal walls.

The leaves of *Iris*, *Gladiolus*, *Montbretia*, *Tradescantia virginica*, and numerous other plants possess elongate cylindrical lens cells capable of bringing the light rays to a focal line, but appear not to be heliotropically sensitive.

In support of Haberlandt, Seefried (loc. cit., p. 17) has some interesting observations on *Pirola secunda* and *P. chlorantha*. Both species often occur near one another in the shade of woods. The leaf-blade of *P. secunda* is pan-photometric and turns irregularly towards the light on both sides. *P. chlorantha*, on the other hand, is euphotometric, and takes up the fixed light position. The latter is clearly dorsi-ventral with well-developed palisade tissue; the upper epidermis has biconvex lens-shaped cells; the cells of the lower are flattened. In *P. secunda* the leaf is iso-lateral with no, or only slightly developed, palisade tissue and flattened epidermal cells, a few only of which show any light convergence.

Many Fern leaves, which appear to possess no power of response to light, show in their epidermal cells a distinct and often very beautiful convergence, capable of producing in oblique light a distinct difference in the illumination of the basal walls of the cells.

The only leaf known to me which is aphotometric, and does not possess epidermal lens cells, is *Aspidistra lurida*. The cell-walls, both inner and outer, are quite flat, and the outer wall is very thick. They appear not to have any power of causing a differential illumination. In many of the cells, however, there is another means by which this can be brought about. Such cells occur generally in groups, and are not visible over all parts of the leaf. Each cell contains a well-marked finely punctate nucleus more or less spherical in shape, generally lying on the lower wall. Its diameter is about $\frac{1}{2}$ to $\frac{2}{3}$ that of the cell itself. Sometimes it is at one end of the cell sometimes near the middle. It is more highly refractive than the substances around it, and is capable of causing the convergence of light rays and of producing bright spots of light. Images can be focused through the nuclei, and with care photographs can be obtained (Fig. 11). We have here an efficient apparatus for convergence of light, but it is obvious that it is not functional in any way in causing the orientation of the leaf.

In the leaves of the Carnation (*Dianthus Caryophyllus*) both the upper

epidermal cells and the lower show a convergence, due partly to the slightly arched outer and inner walls, partly to an irregular sculpturing in the middle region of the outer wall. This produces a number of little spots and lines of light which unite together to form a more or less homogeneous band of light which falls on the lower wall. The position which the leaves take up is, however, as C. and F. Darwin have shown (loc. cit., p. 269), probably mainly due to epinastic growth, and seems 'to be very little affected by geotropism or heliotropism'.

Experimental Observations.

The experimental observations upon which Haberlandt's hypothesis are based consist in the elimination of the lens function either by submerging the leaf in water, or by covering the surface of the leaf with a layer of water under a thin strip of mica. In all cases where the leaf was properly wetted and the lens function eliminated, Haberlandt found that the heliotropic movements were inhibited (loc. cit., 1905). In *Tropaeolum majus* the leaves have a waxy covering which prevents wetting, and thus allows the lens function to be maintained even when submerged; the leaves are, under these conditions, still able to respond to the light stimulus. If the surface of the leaf is washed in dilute alcohol, however, it is easily wetted; its lens function is eliminated, and it no longer responds to the light. These experiments appear to be conclusive, but more recent observations show that leaves do not always behave in this way, and that some other factors than the elimination of the lens function must be taken into account in explaining the loss of heliotropic response.

Fitting[1] suggested that possibly some deep-seated disturbance in the life of the plant had been brought about by the water. Haberlandt,[2] however, showed that a leaf wetted on the under side exhibited no loss of power to reach the fixed light position. This does not prove that some modification may not be brought about by the contact of water with the upper surface. That it is extremely important the leaf should not remain wet for any length of time is seen in the very perfect arrangements, such as waxy bloom, smooth epidermis, &c., for preventing it. Haberlandt[3] himself shows that when a leaf has been covered with water for some time, it is either not able to gain its power of response to light, or only imperfectly, although the lens function remains as good as ever.

In a later experiment with leaves of *Tropaeolum majus*. Haberlandt[4] found, however, that when the upper surface only of the leaf was wetted he

[1] Bot. Zeit., 1906.

[2] Die Bedeutung der papillösen Laubblattepidermis für die Lichtperzeption. Biol. Centralbl., xxvii, 297, 1907.

[3] Ein experimentaler Beweis für die Bedeutung der papillösen Laubblattepidermis als Lichtsinnesorgan. Ber. d. d. bot. Gesell., xxiv, 361–6, 1906; and Bedeutung, &c., p. 297.

[4] Bedeutung, &c., p. 300.

got a distinct turning towards the fixed light position. This, he explains, is due to the fact that the inner walls of the cells are curved, and that there was still, therefore, a sufficient light differentiation to produce the given result.

Kniep [1] eliminated the lens function by covering the leaf with a thin layer of paraffin oil under a thin sheet of mica or a thin piece of paper. The refractive index of the oil (1·476) is greater than that of water, and tends to produce a divergence of the light rays instead of a convergence, and in some cases he obtained, instead of a bright central spot of light, a dark central area and a lighter peripheral zone. Notwithstanding this, the leaves responded to the light stimulus just as ordinary leaves.

I find that this reversion of the differential illumination only takes place when the lens function is very pronounced. It is very easily seen in *Saxifraga Geum,* which produces under normal conditions a very marked central spot of light on the basal wall. But it is not visible in *Tradescantia fluminensis*, where the epidermal cells, althoughvery efficient as lenses, show no differential illumination on the basal wall.

In discussing the results obtained by Kneip, Haberlandt [2] points our that, theoretically, the divergence produced by the concave oil layer over the curved wall of the epidermal cell ought still to result in a slightly brighter central region on the basal wall, but that actually what is seen under the microscope is the reversion described by Kniep. The brighter peripheral zone appears to be due to reflection of the outer rays of light by the lateral walls and to the passage of rays through the walls from neighbouring cells. By experiments on leaves of *Begonia semperflorens* he confirms Kniep's observations. When the leaves are covered with a layer of paraffin oil, they respond to the light just as ordinary leaves; when they are covered with water, however, they cease to respond. It is evident, therefore, according to Haberlandt, that the power to respond, which is maintained in the oil-covered leaf, must still be connected with the differential illumination of the basal wall, although under these conditions it is completely reversed. Instead of, as he formerly suggested,[3] the central region of the basal protoplasmic layer being attuned or sensitive to light of higher intensity, he now concludes that this sensitiveness cannot be considered as an unchangeable inherent property of the different parts of the plasma layer, but only as an acquired adaptation phenomenon (analogous to the local adaptation of the human retina) capable of modification, when placed under conditions which bring about an inverse illumination.

It is, however, not clear how the plasma layer is able to adapt itself to such extremes, which are unlikely to occur in nature. Moreover, the difference in illumination is nothing like so pronounced with the paraffin oil,

[1] Ueber die Lichtperzeption der Laubblätter. Biol. Centralbl., xxvii, 97, 1907.
[2] Bedeutung, &c., p. 289. [3] Lichtsinnesorgane, &c.

as it is under the normal conditions, and if so marked a difference is required in the one case, it is reasonable to suppose that it would be equally required in the other. From experiments which he has recently made, however, upon the amount of light differentiation required for heliotropic response in plants, Haberlandt [1] comes to the conclusion that it is sufficient to bring about a response.

Gius [2] concludes from experiments which he has made upon various seedlings, and upon leaves of dia-heliotropic plants submerged in water, that it is not the perception but the power of movement which is inhibited. The response to light is markedly later and slower than in air. If, however, the submergence in water is limited to the period required for the perception to take place, there is no delay in the motor response. The explanation offered is that the difference in turgor of the cells required to bring about the movement is prevented by the surrounding water. This explanation cannot apply when the surface only of the leaf-blade is covered with water. But it is possible that even here the water may produce some change in the leaf, even if only a very slight one, detrimental to the perception of light or to the motor reaction. It is quite possible that leaves may vary in their power to resist the detrimental action of water, as indeed is seen in water plants and those grown in marshy places, as compared with plants normally accustomed to drier conditions, and which, when submerged in water, soon begin to decay.

Haberlandt [3] shows that even when covered with water the highly curved or pyramidal epidermal cells still maintain the power to some extent of light differentiation. In cells in which the outer wall is less curved, but still moderately strongly curved, as in *Tropaeolum Lobbianum*, the whole basal wall is equally illuminated when covered with water, but in oblique light a distinct differentiation can be observed. Under such conditions the orientation of the leaf would still take place when submerged in water, but not so perfectly or so quickly as in air.

Haberlandt describes (loc. cit., 1909) some interesting experiments in which separate parts of the upper surface of a single leaf were illuminated from opposite sides by equal light, one half of the leaf being covered with a layer of water, the other half dry. With almost equal illumination from opposite sides of corresponding parts of the leaf, and with the wetted part of the leaf 2·2 to 4·8 times as large as the dry part, the inclination of the lamina towards the light was always in the direction of the dry part of the leaf. If the two halves of the leaf were of equal size and the wetted half twice as strongly illuminated as the dry half, the leaf still turned in the direction of the dry half of the leaf, that is, towards the weaker light. The experi-

[1] Zur Physiologie der Lichtsinnesorgane der Laubblätter. Jahrb. f. wiss. Bot., xlvi, 377, 1909.

[2] Ueber den Einfluss submerser Kultur auf Heliotropismus und die Lichtlage. Sitzungsber. d. k. Akad. Wien, cxvi, 1907.

[3] Zur Physiologie, &c.

ments were made with *Tropaeolum majus*. They show clearly enough that in the case of the leaves of this plant the dry leaf is more sensitive to heliotropic stimulation than the wetted leaf; in other words, the leaf in which the lens function is not interfered with is more sensitive than the one in which it is eliminated. This, however, does not prove that the lens function stands in direct causal relation to the heliotropic response. The power to respond when covered with water seems to vary with different leaves, and it is quite probable that the leaves of *Tropaeolum majus* are sensitive to water in some way which interferes with the heliotropic response other than elimination of the lens function.

Nordhausen[1] brought about the elimination of the lens action by means of a thin layer of 5 to 10 per cent. of gelatine spread over the surface of the leaf. As the refractive index (1·341 in 5 per cent., 1·347 in 10 per cent. solution) is very near that of the cell contents, there can be no question of reversed illumination. Experimenting on similar plants to those used by Haberlandt, Nordhausen shows that the response to light is not inhibited, and therefore he concludes that the lens function is not directly connected with the perception of light by the leaf-blade.

Haberlandt (loc. cit., 1909) criticizes Nordhausen's experiments and shows that, as regards the lens function, leaves covered with a 5 to 10 per cent. solution of gelatine behave very much like those covered with water, and therefore in certain cases a light response would be obtained.

From a few experiments which I have made to test the heliotropic response when leaves are submerged in water, results were obtained which seemed to show, when taken in conjunction with those already described by Haberlandt and others, that the evidence on the experimental side is at present so contradictory and unsatisfactory that a very much more complete investigation of the various functions involved will be necessary before any satisfactory conclusions can be arrived at.

Ranunculus Ficaria. A complete plant was submerged in water and exposed to one-sided illumination. The leaf-stalks curved very markedly towards the light, and the leaf-blade was placed more or less at right angles to the falling light rays. The response was just as active and as rapid as in plants not submerged. Leaves removed from the plant did not respond so quickly, but in all young leaves there was a definite response in a few hours. The cells both of the upper and lower epidermis of the leaves are irregular in outline, with both inner and outer walls arched. With a small stop there is a slight differential illumination of the basal walls, but not with an open stop. Below the basal wall is an irregular light area surrounded by a dark one (Fig. 17), with brighter spots here and there where the cell-wall was slightly more curved. When the outer wall was covered with water

[1] Ueber die Bedeutung der papillösen Epidermis als Organ für die Lichtperzeption der Laubblätter. Ber. d. d. bot. Gesell., xxv, 398, 1907.

no differential illumination of the basal walls was visible. When the leaf-blade was cut off, the leaf-stalks also curved towards the light, but not so quickly or so definitely as when the leaf-blade was present, and the movement was soon inhibited. These experiments show clearly that, although the lens function is eliminated, the heliotropic response of the leaves of *Ranunculus Ficaria* is almost as pronounced in water as in air.

Adoxa moschatellina. Both in water and in air, a one-sided illumination causes the leaf-stalk to curve towards the light so as to bring the leaf-blade into a position more or less at right angles to it. The position taken up by the submerged leaves was exactly like that given by Guttenberg in his figure illustrating the heliotropic response of the leaves in air (loc. cit., Fig. 5, Pl. II). In one experiment which I made, a submerged leaf, which had curved sharply to the light, was turned completely round so that its upper surface was turned away from it. Almost immediately a second curvature was induced a little higher up the leaf-stalk, and the leaf again curved towards the light (Text-Fig. 1). The upper epidermal cells exhibit a beautiful convergence, due to a small projecting papilla in the middle of each outer wall, as Guttenberg (loc. cit.) has shown, which disappears entirely in water.

Text-Fig. 1. Leaf of *Adoxa moschatellina* submerged in water and exposed to a one-sided illumination. The double curve is due to the leaf having been completely turned round from its first position and exposed a second time to the light.

Saxifraga Geum. The old leaves turn towards the light very slowly in air, but in water apparently not at all. The young shoots, in air, turn very markedly towards the light, so as to bring the young leaves into a position more or less at right angles to it, but are not responsive in water. The minute papillate projection in the middle of the outer wall of each of the upper epidermal cells brings about a very distinct bright spot of light on the basal wall, which disappears entirely in water.

In both these plants the lens function disappears entirely in water: there is no differentiation visible even in oblique light, and yet when submerged in water there is a definite heliotropic response in the one, but not in the other.

The Function of the Lens Cells in the more Efficient Illumination of the Chlorophyll Grains.

It is clear that the orientation of the leaf with respect to the light has for its main purpose the more efficient illumination of the chlorophyll grains; and this must be taken into account in considering the lens function of the epidermal cells. Haberlandt[1] had already in 1882 suggested that they might bring about a more intense illumination of the chlorophyll grains by concentrating the light upon the chlorophyll-containing cells. Later, Stahl[2] showed that if sunlight is allowed to pass through the epidermis of a somewhat thin leaf, such as *Begonia falcifolia*, and observed through the under side by means of the microscope, the chlorophyll grains would be seen glistening in the bright concentrated light. Any one who will take the trouble to examine carefully the incidence of light upon the chlorophyll grains after passing through the lens-shaped epidermal cells of the leaves of species of *Selaginella*, *Tradescantia fluminensis*, *Adoxa moschatellina*, Narcissus, Hyacinth, and many other plants will be struck by the very efficient illumination which is observable. As Stahl points out, however (loc. cit.), the concentration of the light would only be useful to a certain number of the assimilating cells, as there may be several under each epidermal cell. This is well seen in *Oxalis acetosella*. In species of *Peperomia*, Stahl points out, the light would be brought to a focus in the water-containing tissue, and not on the chlorophyll grains; this indicates that the concentration must have some other purpose. He suggests that the papillate or dome-shaped form of the epidermal cells, which are found in many shade plants, may serve not so much for the concentration of the light as for the purpose of collecting those rays which, coming in a nearly parallel direction to the surface of the leaf, would be reflected from it if the cells were flat. The conical shape of many of the epidermal cells of shade plants would seem to favour this view, and Stahl shows by means of models that the collection of light in this way, and its utilization by the chlorophyll grains, is quite possible. This explanation would hardly hold good, however, in the case of cells which possess only slightly curved outer walls.

The numerous observations which have been made upon *Schistostega osmundacea*, some Selaginellas, and *Hepatica* all clearly indicate that in some cases the lens cells are for the purpose of bringing about a concentration of light upon the chlorophyll grains.

The researches of Vuillemin[3] and Noll[3] showed this very clearly in

[1] Die physiologischen Leistungen der Pflanzengewebe. Schenk's Handbuch der Botanik, ii, 579, 1882.

[2] Ueber bunte Laubblätter. Ann. du Jardin Bot. de Buitenzorg, xiii, 137, 1896.

[3] L'appareil reluisant du *Schistostega osmundacea*. Journ. de l'anatomie et de physiologie, vol. xxiii, p. 18, 1887; and Noll, Ueber das Leuchten von *Schistostega osmundacea*, Schimp. Arbeiten

Schistostega osmundacea. The protonema of this moss, which is found in caves, is luminous. The cells are shaped like a convex lens above, and the chlorophyll grains are found in the basal part of the cells, which is conical. The rays of light which fall upon the cell are refracted and concentrated upon the chlorophyll grains. Owing to the shape of the cell, some of the rays are totally internally reflected from the basal walls of the cell and are again emitted, which gives the luminous appearance. Noll showed also that a variation in the direction of the light rays was accompanied by a corresponding movement of the chlorophyll grains, in order that they might always be in a position to take the fullest advantage of the concentration of the light.

The arrangement of the chlorophyll grains in such plants as *Oxalis acetosella*, various species of *Begonia*, and other shade plants favours this view. Schürhoff[1] has also shown that in some species of *Peperomia* the upper convex walls of the palisade cells, together with the contained calcium oxalate crystal, act as lenses for the concentration of light on the chlorophyll grains, as well as for light perception.

In *Botrydium granulatum* the chlorophyll grains are distributed more or less evenly over the whole interior surface of the nearly spherical cell, the rest of the cell being filled with a clear cell-sap. As the plant grows in shady places, such as roadside ditches and damp clayey soil under trees, and possesses no means of orientation, a portion of the chlorophyll grains would be always in obscurity, if it were not that the spherical shape of the cell brings about a total internal reflection of some of the rays of light which pass into it. In this plant, however, it is not so much the condensation of the light as the more efficient distribution of it that is important.

In leaves with more or less elongate palisade cells, it is also probable that the direct condensation of the rays of light upon the chlorophyll grains is not so important as the more efficient dispersal of them among the chlorophyll grains, due to their being brought to a focus in the palisade cells, instead of passing straight through them, as would be the case if no convergence were possible, as in leaves with flat outer walls.

In those shade plants, and in the species of *Peperomia* cited by Stahl (loc. cit.), where the rays of light are brought to a focus above (in front of) the cup-shaped or short palisade cells, we should also have, not a condensation of the light rays, but a more efficient distribution of them over the chlorophyll grains, due to their divergence after passing the focal point. This would be the greater owing to the number of different focal points produced, a consequence of the spherical aberration due to the conical shape of the cell.

aus dem botanischen Institut zu Würzburg, iii, 1887. See also W. West, Luminosity of *Schistostega osmundacea*. Naturalist, 256, 1907.

[1] Ozellen und Lichtkondensoren bei einigen Peperomien. Beil. z. bot. Centralbl., xxiii, p. 14, 1908.

The evidence before us, therefore, seems to justify the conclusion that in special cases the lens-shaped epidermal cells may be functional in the more efficient illumination of the chlorophyll grains, either by concentrating the light upon them, or by a more efficient dispersal of it among them. It is possible that this may be also bound up with light perception in the manner suggested in the following section.

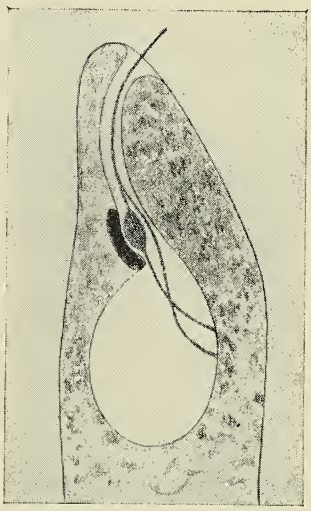
Chlorophyll Grains as Organs for Light Perception.

In formulating his hypothesis that it is in the cytoplasm lining the walls of the epidermal cells that the light perception takes place, Haberlandt based it fundamentally upon the statement of Noll (1878), that the outer layer of the cell protoplasm is the seat of sensitiveness for light and gravity. In order to explain this sensitiveness, Haberlandt suggests that it may be due to the mechanical pressure exerted by light. The stimulus set up by the differential illumination of the plasma layer on the basal wall of the epidermal cells would thus be brought about by the difference in pressure between the illuminated and the dark areas. But this must be extremely small. Clark Maxwell showed that, on a perfectly reflecting surface on which the pressure would be the greatest, sunlight should exert a pressure of about 0·8 milligram per square metre, and on a black surface about 0·4 milligram per square metre. As a writer in the Botanical Gazette remarks[1]: 'The pressure on a cell (from figures calculated by Maxwell and determined experimentally by Nicholls and Hull) 0·01 mm. square in full sunlight would scarcely amount to 7×10^{-11} milligram! To believe that a plant could discriminate between 0 and 0·000,000,000,07 mg. pressure makes a severe test of one's credulity.' It is well known that heliotropism depends upon the quality of the light rays, and not merely upon variation in light intensity. The less refrangible rays are important in photosynthesis, the more refrangible rays are more important in growth and formative processes, irritable movements, and curvatures. Haberlandt himself has shown, by the use of chlorophyll screens, that the only rays which are functional in heliotropism are those which are absorbed by the chlorophyll; and, as we know from the researches of Sachs, it is only the more refractive half of the visible spectrum, the blue and violet rays, which produce the stimulus. If it were merely a difference in the illumination, we ought to obtain a result in red or yellow light.[2] This points to the conclusion that the light perception is bound up with the absorption by the chlorophyll. Haberlandt, indeed, considers this possibility, but dismisses it for the reason that certain plant organs which do not contain chlorophyll are

[1] C.R.B. Bot. Gaz., xxxviii, 157, 1904.
[2] Cf. E. Pringsheim, junr., Ber. d. d. bot. Gesell., 556, 1908.

still found to be heliotropically sensitive. He points out, for example, that in *Acer Negundo* and *Cornus sanguinea* there are sometimes whole shoots in which the leaves are almost bleached and destitute of chlorophyll. These show, notwithstanding the absence of chlorophyll, in their young stage, a dia-heliotropic response, which is, however, in most cases lost as the leaves become older. The weak point in this argument seems to me to be that, although the leaves are destitute of chlorophyll, they may contain, at any rate in the earlier stages, a certain amount of yellow colouring matter by which the more refractive rays of the visible spectrum would be absorbed, and thus satisfy the requirements for a heliotropic response.

Light is able to bring about an orientation of the chloroplasts in many foliage leaves and in some Algae. Under the influence of too strong or too weak a light, they are brought into more suitable positions, either by turning completely round, as do the chlorophyll bands of *Mesocarpus*, or by moving into different positions in the cell, as in *Elodea*, *Oxalis acetosella*, *Schistostega osmundacea*, &c. Just as in the orientation of the leaf itself, the stimulus which brings about the orientation of the chloroplasts is produced by the more refrangible rays of the spectrum absorbed by the chlorophyll.



Text-Fig. 2. Side view of the anterior end of a cell of *Euglena viridis*, showing the flagellum and its enlargement in front of the eye-spot. (From Journ. Linn. Soc. Zool., xxvii, 1899.)

Light sensitive motile organisms, such as *Euglena* and the swarm spores of Algae, behave in red light just as they do in the dark, but exhibit pronounced heliotaxis in light blue. This is the more significant as the red spot, which is supposed to function in this heliotactic stimulation, absorbs just those rays which are active.[1] In these cases, therefore, it is fair to assume that the movement is brought about by absorbed rays and not by any kind of mechanical action on the cytoplasm. The association of the spherical or oval swelling on the basal portion of the flagellum in *Euglena*, with the red pigment-spot (Text-Fig. 2), is analogous to the association of the rods and cones of the animal eye with their pigment layer. The light absorbed by the pigment-spot probably sets up chemical changes which affect the swollen part of the flagellum, and this in turn acts upon the

[1] Wager: On the Eye-spot and Flagellum in *Euglena viridis*. Journ. Linn. Soc. Zool., xxvii, 463, 1900. Mast: Light Reactions in Lower Organisms, II. *Volvox*. Journ. Comp. Neurology and Psychology, xvii. 112, 1907.

flagellum itself, and thus brings about those modifications in its vibrations by which the direction of movement of the organism is regulated.

At the red end of the spectrum, Engelmann has shown that the curve of assimilative activity corresponds very nearly with the curve of absorption ; but at the blue and violet end, a comparison of the two curves shows that the amount absorbed is much greater than can be accounted for by the assimilative activity, and the suggestion may be made that the excess of light absorbed at the blue end of the spectrum is partly functional in heliotropism. This would perhaps partly explain the heliotropic activity of young seedlings, which are of a yellower colour than fully developed leaves, for the yellow colouring matter readily absorbs the blue end of the spectrum.

One important difference between the light-perceiving organs of animals and those of plants is the presence of pigment in the former. The exact function of pigment in the animal eye is still uncertain. Some physiologists think that it serves merely for the absorption of superfluous light rays, and, taking this view, Haberlandt suggests that there is no need to explain its absence in the plant cell, for light perception could very well take place without it. He points out, however, that the chlorophyll grains might act as a screen to keep extraneous light away from the sensitive percipient layer of cytoplasm, and that, in *Selaginella Martensii* and similar forms, there is a chlorophyll grain at the base of the epidermal cell which could be compared to a pigment layer, the cytoplasm between it and the cell-sap being the percipient organ. But it is doubtful whether the absorption of superfluous light is the sole function of the pigment in the animal eye. The alternative view is that the pigment is not merely concerned with superfluous rays, but that the rays which are active in vision are absorbed by it and produce a chemical change in it, and that it is this change which affects the rods and cones and causes the stimulus of light perception. There is in the eyes of many vertebrates a substance called visual purple, associated with the percipient organs, which is possibly derived from the pigment layer and is very sensitive to light. How far the experiments made by Kühne[1] and others upon the changes which take place in this pigment may be found to have a bearing upon the problem, we cannot say, but the destruction or bleaching of chlorophyll by light is perhaps somewhat analogous to the changes in colour and bleaching of the visual purple. The most active rays in the bleaching of visual purple are those of the yellow-green part of the spectrum, and these are the rays which are most readily absorbed by the colour itself.

Cannot this be taken as the basis of a more satisfactory hypothesis for light perception in plants? It is difficult to believe that the sensitive

[1] On the Photochemistry of the Retina and on Visual Purple. Eng. trans., edited by Sir M. Foster, 1878. See also Text Book of Physiology by Foster and Rivers, Part IV, p. 1369, 1900.

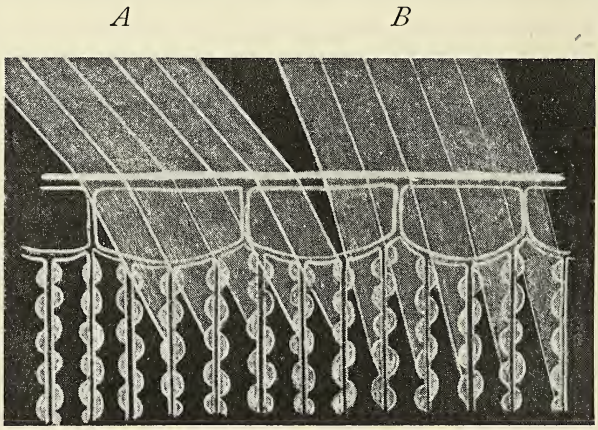
chlorophyll grains, which in some plants show definite movements of orientation in response to light, have no other function in light perception than that of a screen. If the light had a merely mechanical action such as is suggested by Haberlandt, it is difficult to see why the most energetic rays, which are at their minimum in the blue, but rise rapidly to a maximum in the yellow-green, and extend through the whole of the red and far beyond into the infra red, should not be functional. The rays which are active are, in fact, the chemical rays, and it appears to me to be much easier to explain the heliotropic reaction upon the assumption that it is due to chemical activity than that it should be merely due to mechanical action.

Perhaps the most important and attractive feature of Haberlandt's hypothesis is that it affords, for the first time, a definite explanation of how a plant is able to perceive the right direction in which its leaf must be turned to bring it into the most advantageous position for the illumination of the chlorophyll grains. It is true that he has not brought forward any evidence to show whether the movement takes place at once in the right direction, or whether there is anything in the nature of a trial and error movement on the part of the leaf before the right direction is perceived. From the general trend of his observations we are, I think, led to conclude that the leaf at once perceives and turns in the right direction.

It is an important question, therefore, whether the leaf would be able to orient itself as readily and as surely if the chlorophyll grains were the percipient organs. There seems to be no reason from the evidence available why it should not do so. The chlorophyll grains are probably much more sensitive to light than the protoplasm, and any differential illumination of them would be likely to set up a much more definite stimulus than a differential illumination of the cytoplasm lining the basal walls.

To bring about such a stimulus, an unequal illumination of the chlorophyll grains would be necessary in oblique light. There is no difficulty in seeing that this would be the case (Text-Fig. 3). The passage of rays of light obliquely through the epidermal cells into the palisade tissue would bring about a partial illumination of the chlorophyll grains. The rays of light would be bent on passing into the epidermal cells, and would then pass on in straight lines to and through the palisade cells. The chlorophyll grains on one side of each palisade cell would be more strongly illuminated to an extent depending upon the angle of the incident rays, than those on the other side, which would remain in obscurity, or at least would only be exposed to rays which had already passed through one, and possibly more than one, layer of chlorophyll grains, being thereby much weakened in the active heliotropic rays. This would set up the stimulus required to bring about the orientation of the leaf in a direction at right angles to the incident light, in which position the chlorophyll grains in each palisade cell would be equally illuminated. That this unequal illumination of the chlorophyll

grains is sufficient to account for a direct movement towards a right position, without the intervention of trial and error movements, is perhaps not so certain as in the cases of clear centric illumination of the basal walls of the epidermal cells described by Haberlandt: but it would take place quite as surely and as readily as in those cases of unequal non-centric differential illumination which, as I have shown (p. 468), occur in a large number of leaves with epidermal lens cells of long focus; and it certainly affords a much more satisfactory explanation for those cases in which the epidermal cells are flat or possess only curved basal walls. It would also enable us to account for those cases in which a heliotropic response takes place when the lens fuction is eliminated. Even if we have to assume a certain amount of trial and error movement before the right direction is perceived, and I am



Text-Fig. 3. Diagram showing how the chlorophyll grains in the palisade cells of a foliage leaf with flat outer walls would be partially illuminated in oblique light. *A* and *B* show the difference in the illumination at different angles of incidence.

not sure that this is not also necessary in Haberlandt's hypothesis, we have a basis for this in the autonomous movements of circumnutation which have been so fully described by Charles and Francis Darwin[1]. They point out (p. 3) that:—'The movements of various organs to the light, which are so general throughout the vegetable kingdom, and occasionally from the light, or transversely with respect to it, are all modified forms of circumnutation.' And again (p. 419)—'Heliotropism seems always to consist of modified circumnutation. Any kind of movement in relation to light will obviously be much facilitated by each part circumnutating or bending successively in all directions, so that an already existing movement has only to be increased in some one direction, or to be lessened or stopped in the other directions, in order that it should become heliotropic, apheliotropic, &c., as the case may be.'

[1] The Power of Movement in Plants. London, 1880.

So also, in discussing the connexion between circumnutation and positive heliotropism it is pointed out (p. 436) that:—'A plant, when exposed to a lateral light, though this may be bright, commonly moves at first in a zigzag line, or even directly from the light; and this no doubt is due to its circumnutating at the time in a direction either opposite to the source of the light, or more or less transversely to it. As soon, however, as the direction of the circumnutating movement nearly coincides with that of the entering light, the plant bends in a straight course towards the light, if this is bright.'

In the case of dia-heliotropism, a similar explanation probably holds good; 'cotyledons and leaves place themselves so that their upper surfaces may be exposed to the light, and this movement is regulated, though not directly caused, by the direction whence the light proceeds' (p. 442).

In both orthotropic and diatropic organs, the final result aimed at—the equal illumination of the chlorophyll grains—is the same. Ortho-heliotropic organs are symmetrical; dia-heliotropic organs are bi-symmetrical. In the latter case the position of the chlorophyll-containing tissue, not the epidermis merely, must determine finally the light position of the leaf, just as, in the former case, the symmetrical distribution of the chlorophyll tissue must finally determine whether the organ shall place itself parallel to the rays of light, or laterally with respect to them.

So far as the chlorophyll grains are concerned, the one-sided illumination of ortho-heliotropic organs corresponds almost exactly with the oblique illumination of dia-heliotropic organs. The only difference is that, in the one case, the whole of the chlorophyll grains on one side of the organ are exposed to the light, while the other side remains in obscurity; in the other case, the differential illumination takes place in each palisade cell in such a way that the chlorophyll grains on one side of the cell are more brightly illuminated than on the other. If this one-sided illumination is sufficient in the one case to bring about a regulation of the fundamental movements of circumnutation, it is fair to conclude that it is equally sufficient in the other.

In dia-heliotropic leaves which possess papillae, or other contrivances in their epidermal cells, capable of causing a convergence of the light rays, we should still have a one-sided illumination of the chlorophyll grains, probably more efficient than in leaves with flat-walled epidermal cells. It is quite possible, therefore, that in such cases the lens cells, although not absolutely necessary, may be of advantage, especially to shade plants, in bringing about the light perception.

Summary.

1. Haberlandt has shown that the cells of the upper epidermis of dia-heliotropic leaves are so constructed that they cause a convergence of the rays of light, and has suggested that they are functional as ocelli or primitive eyes, capable of setting up a stimulus which results in the heliotropic orientation of the leaf.

2. The central and peripheral areas of the layer of cytoplasm lining the basal walls of the epidermal cells are, according to Haberlandt's hypothesis, attuned to light of different intensities. When the leaf is in equilibrium, the central area is bright, the peripheral zone dark. When the light is oblique, these relations are altered, and it is this variation which sets up the stimulus by which the orientation of the leaf is brought about.

3. The efficiency of these lens cells is shown by the fact that in many leaves they are able to form clear images of objects focused through them, which may be easily seen under the microscope and can be photographed. It is not probable that the plant can perceive these images.

4. Haberlandt's hypothesis is open to criticism both on morphological and on physiological grounds. The phenomenon of the convergence of light by the cells of plants is, of very widespread occurrence. Not only the epidermal cells of leaves, but all cells which through turgidity assume a spherical or cylindrical form are capable of bringing it about.

5. Lens cells are present in many cases on leaves and other organs where there can be no question of their functioning in light perception.

6. The cells of the lower as well as the upper epidermis of leaves are in most cases capable of light convergence.

7. Special lens cells and lens-shaped thickenings of the cuticle often occur on the lower as well as the upper epidermis. The position of the lens-shaped thickenings of the cuticle in *Garrya elliptica*, which occur on both sides of the leaf, has no relation to the position of the epidermal cells.

8. According to the form and outline of the cell, the rays of light may be converged to a local point, a focal line, or to an irregular figure intermediate between these. Cells with very irregular outlines, as in *Eranthis hyemalis*, commonly have more than one series of converging rays. As many as three or more may be seen, each producing a bright spot of light.

9. Cylindrical cells which bring rays to a focal line are present on some orthotropic organs, such as stalks of leaves, pedicels of flowers, and hypocotyledons of seedlings, also on the long, narrow, non-sensitive leaves of such plants as Grasses, Hyacinth, *Freesia,* &c.

10. Papillate cells and lens-shaped thickenings of the cuticle are found on leaves which are not heliotropically sensitive.

11. The extent to which the phenomenon of light convergence is simply a result of cell-turgor and not an adaptation to light perception

cannot be definitely determined, but it is suggested that the curvature of the lens cells of the epidermis may be found to bear some relation to the thickness of the cell-wall and cuticle. It is possible that this turgidity may be the starting-point for an adaptation to (1) either light perception or, as Haberlandt suggests, to (2) the more efficient illumination of the chlorophyll grains, or (3) both, but the evidence is not very conclusive.

12. The papillate epidermal cells of petals exhibit a very pronounced convergence of light, with a clear differentiation on the basal wall of a central bright area, and a dark peripheral zone.

13. It is only in a very few leaves, where the cells are highly papillate, or where there is a well-marked local thickening of the cuticle, that we get the differential illumination of the basal wall required by Haberlandt's hypothesis. In some, it is not visible at all under any conditions, in others only when a small stop is used, and in a large number of leaves, probably the majority, there is no differential illumination as defined by Haberlandt, but only an unequal illumination of the basal wall when the light falls obliquely.

14. The experiments which have been made upon the elimination of the lens function by submerging the leaves in water, or by covering them with a layer of paraffin oil, have given results which are so contradictory and unsatisfactory that a much more complete investigation is necessary before any definite conclusion can be based on them.

15. In a few special cases, the lens cells appear to bring about a concentration of the light on the chlorophyll grains. In some leaves the general arrangement of the lens cells with respect to the chlorophyll grains seems to indicate that they are effective in promoting a more efficient illumination of the chlorophyll grains.

16. Haberlandt suggests that the stimulus may be brought about by the difference in pressure exerted by the light upon the cytoplasm; but this is so very slight that it is hardly probable it can be effective.

17. There seems to be no good reason why the epidermal cells should be the percipient cells more than the chlorophyll-containing cells, except that the presence of chlorophyll would interfere with the incidence of the light upon the percipient protoplasm.

18. There is, however, some evidence that the perception of light is bound up with its absorption by the chlorophyll grains, in which case the palisade cells would be the percipient cells, and the chlorophyll grains with the cytoplasm in connexion with them the actual percipient organs. The evidence for this is as follows:—The heliotropic response depends mainly upon the quality of the light and not upon its intensity; the rays which are active are those which are absorbed by the chlorophyll; of these the more refrangible rays are the most important; if it were merely the intensity and

not the quality of the light, there seems to be no reason why the red and yellow rays should not be just as active as the blue and violet rays; in the more refractive half of the spectrum the amount of light absorption is greatly in excess of that required for assimilation; when a chlorophyll screen is interposed between the leaves and the light, the heliotropic response either ceases altogether or is much reduced; in motile organisms, such as *Euglena*, the heliotactic response is bound up with the absorption of light by the pigment-spot; in the large majority of animal eyes the presence of a layer of pigment in connexion with the actual percipient organs seems to be necessary; light exerts a very definite stimulus upon the chlorophyll bodies of some Algae and foliage leaves, resulting in their movement into positions in which they can be more effectively illuminated; why should not a similar stimulus bring about the orientation of the leaf itself, if the chlorophyll grains are incapable of movement? the rays absorbed by the chlorophyll, which are functional in heliotropism, are the chemically active rays; chemical changes taking place in the chlorophyll would afford a more satisfactory explanation of the origin of the stimulus than the pressure of light upon the cytoplasm.

19. With the exception possibly of the few special cases in which the light is concentrated upon the chlorophyll grains, there is no satisfactory evidence to show that the lens-shaped cells or local cuticular thickenings can be regarded as special adaptations, either for light perception or for the more efficient illumination of the chlorophyll grains, although it is possible that they may be of use for both purposes.

EXPLANATION OF PLATES XXXI AND XXXII

Illustrating Mr. Wager's paper on the Perception of Light in Plants.

PLATE XXXI.

The figures are reproduced from photographs taken by means of the Gordon photo-micrographic apparatus with the objectives of Leitz Nos. 3 and 4, and oculars 4 and 8.

Fig. 1. Reproduction of a photograph taken through the upper epidermal cells of *Tradescantia fluminensis*. Some of the cells are in much better focus than others.

Fig. 2. Special cells from the upper epidermis of *Mesembryanthemum cordifolium*. They contain a mucilaginous substance, and are equally well developed on the under side of the leaf.

Fig. 3. The special cells on the under side of the leaf of *M. cordifolium*, showing distant houses photographed through them. These special cells vary in size, and, being irregular in outline, do not usually give a circular disk of light.

Fig. 4. Lower epidermis of *M. cordifolium*, showing the special cells with highly refractive granular mucilaginous substance through which the light is shining.

Fig. 5. Rays of light brought to a focus through the granules of mucilage shown in Fig. 4. These granules interfere with the passage of light through the cells as a whole, and render the image more or less indistinct, as shown in Fig. 3.

Fig. 6. A geometrical figure with coloured segments photographed through the cells of *Tradescantia fluminensis* on an ortho-chromatic plate.

Fig. 7. *Saxifraga Geum.* Cells of upper epidermis, showing more or less central disks of light thrown on to the basal walls by the minute papillae which occur in the middle of the outer cell-walls.

Fig. 8. Shows the same disks of light moved laterally when the light is oblique.

Fig. 9. Shows the extreme lateral movement of which they are capable when the rays which fall upon the leaf are still more oblique.

Fig. 10. Upper epidermal cells of *Prunus lauro-cerasus.* The photograph shows the illumination of the basal walls by light which has passed through a small diaphragm $\frac{3}{16}''$ in diameter. There is no differential illumination of the basal wall. The outer walls of the cells are flat, the inner walls slightly curved. In spite of this absence of differential illumination, the leaves undergo a definite orientation into the position of equilibrium as regards the light.

Fig. 11. *Aspidistra lurida.* Upper epidermal cells showing images of a six-sided figure photographed through the transparent nuclei.

Fig. 12. *Zebrina pendula.* Image of a cabinet photograph, taken through the papillate projection on the outer cell-wall, and focused very nearly on the basal wall in each cell.

PLATE XXXII.

Fig. 13. *Tradescantia fluminensis.* Basal walls of epidermal cells showing that convergence of light produces no differential illumination.

Fig. 14. The same preparation. Light focused slightly below the basal walls after passing through a small diaphragm opening.

Fig. 15. The same preparation, under same conditions, but focused at some distance below the basal walls. At this level there is a distinct differential illumination as shown by the separate irregular disks of light.

Fig. 16. Basal walls of the cells in the same preparation when light falls obliquely upon the outer walls. Half of each basal wall is in the light and half in darkness.

Fig. 17. Upper epidermis of *Eranthis hyemalis.* Shows the irregular light areas below the basal walls of the epidermal cells when allowed to fall upon the outer curved walls through a small diaphragm opening. Here and there in these light areas in each cell are to be observed clearer circular disks of light, due to more pronounced curvature of the cells. Each one of these is capable of forming an image of an object near it.

Fig. 18. Basal walls of papillate epidermal cells of the petals of Phlox showing a clear disk of light in the centre of each cell.

Fig. 19. Elongate cells of epidermis of *Tradescantia virginica* showing lines of light brought to a focus on the basal walls. The guard cells of the stomata also bring about a convergence.

Fig. 20. Elongate cells of epidermis of *Iris alata* showing very bright illumination of basal walls when light is allowed to pass through a small diaphragm opening.

Fig. 21. Cells of the upper epidermis of *Convallaria majalis* showing bands of light which are brought to a focus near the basal walls when a small diaphragm opening is used.

Fig. 22. Inner epidermal cells of the sheathing leaf of *Avena sativa* showing the image of a pipe focused on or near the basal walls. At the ends of the cells, owing to a more prominent curvature of the outer walls, the outline of the pipe is distinctly seen, but between these there is a more or less continuous dark line which represents the pipe.

Fig. 23. Epidermal cells of the leaf of *Garrya elliptica* showing the special lens-shaped thickenings of the cuticle. These are distributed more or less regularly over the whole surface of the leaf, independently of the position of the cells. Some of the thickenings occur exactly over the walls which separate neighbouring cells.

Fig. 24. The same preparation showing the image of a cross brought to a focus near the basal walls of the epidermal cells.

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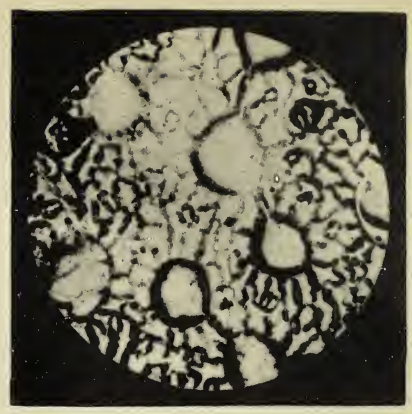

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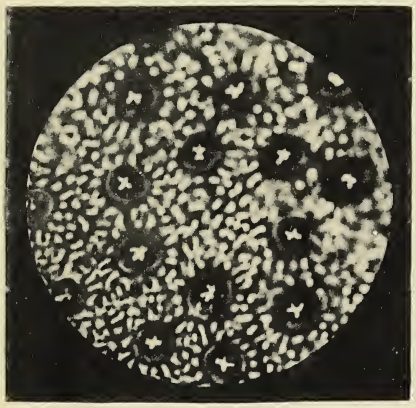

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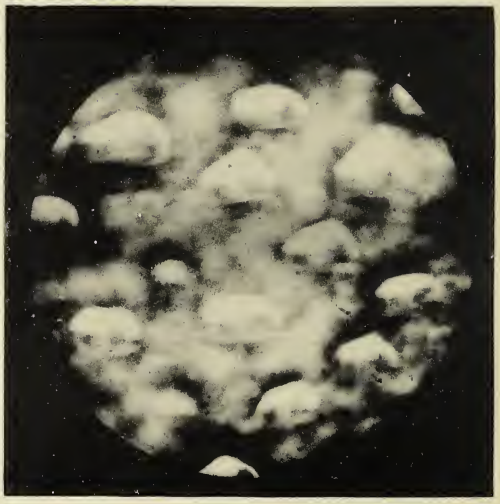

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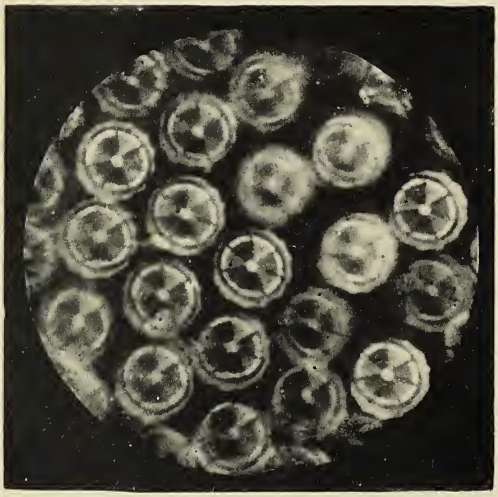

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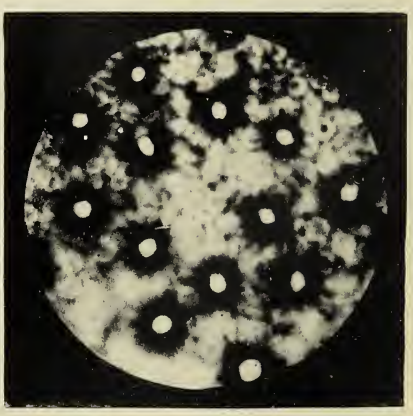

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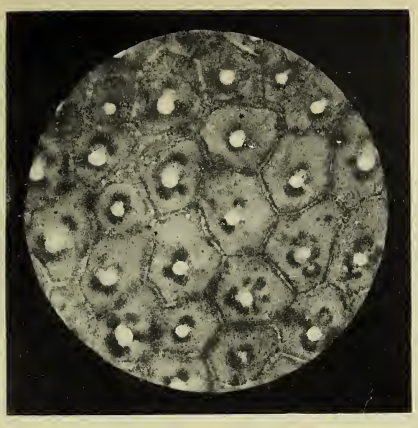
WAGER—PERCEPTION OF LIGHT IN PLANTS.



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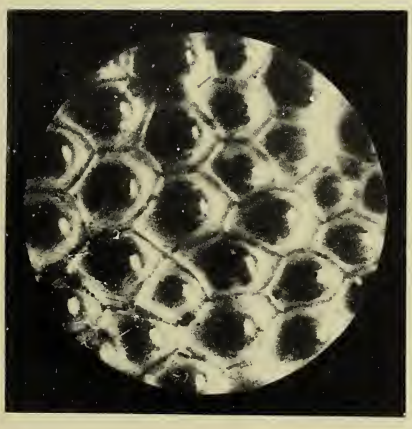

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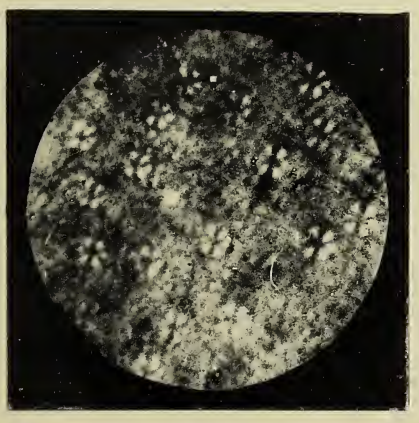

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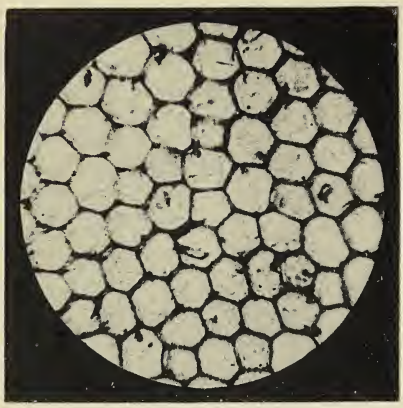

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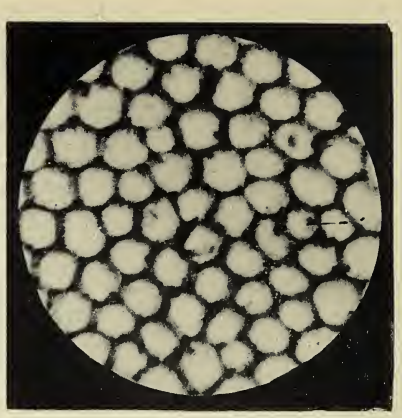

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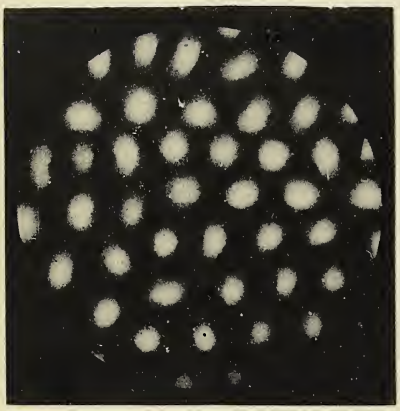

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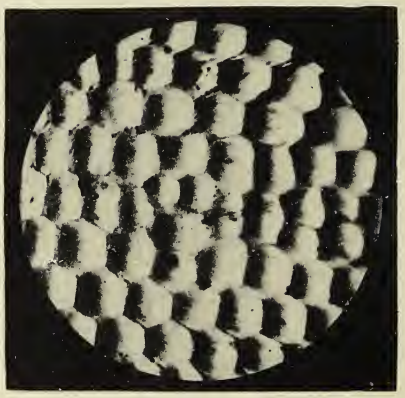

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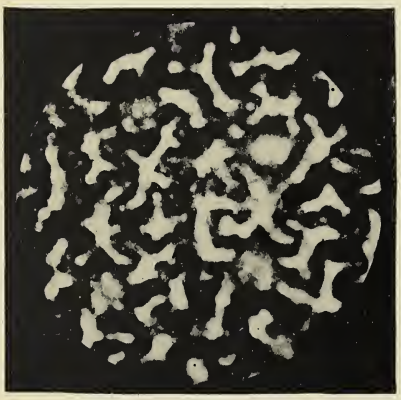

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WAGER—PERCEPTION OF LIGHT IN PLANTS.

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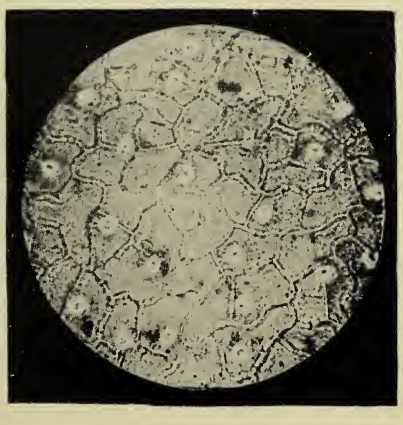

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Observations on 'Chromatin Bodies' and their Relation to the Nucleolus in Galtonia candicans, Decsne.

BY

L. DIGBY.

With Plates XXXIII and XXXIV.

Introduction.

In the course of an investigation of the first meiotic division of the pollen mother-cells of *Galtonia candicans*, it was noticed that during the pre-synaptic and synaptic nuclear stages a most constant extrusion of 'chromatin bodies' takes place. This process is so striking in these cells that it seemed advisable to follow it out in detail, especially as somewhat similar phenomena have lately been described by several writers both in animal and plant cells.

Methods.

Care has been taken to check the work by using various methods both in 'fixing' and in staining. Buds have been fixed on bright warm days between 11 a.m. and 1 p.m. in (1) Strong Flemming, (2) Hermann, (3) Alcohol and Acetic. The material, for the most part, has been run up through the glycerine method, and has been left in paraffin, and that always at a low temperature, for three to three and a half hours. The stains used have been many, including Heidenhain with a counter stain; Flemming's Triple; Breinl (17); Iodine Gentian-Violet and Orange G.; Thionin Blue and Ruthenium Red; Methylene Blue and Eosin. The sections have been cut of various thicknesses, the greater number at 3 μ, but some at 6 μ and at 9 μ.

Presynapsis and Synapsis.

When the nucleus is preparing to go into synapsis there is a gradual concentration of the nuclear contents (Pl. XXXIII, Fig. 1) which eventually mass together at one side of the nucleus. At synapsis the nucleus lies excentrically in the cell (Pl. XXXIII, Figs. 7, 8, 9, &c.), the chromatin mass being always on the side of the narrow strip of cytoplasm. This mass, as

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a rule at full synapsis, stains uniformly and resembles a heap of tightly compressed wood chips in which no structure can be seen. Sometimes in a superficial section it is possible to recognize the linin matrix with more deeply staining patches of chromatin embedded in its substance. In such a section favourably stained with Breinl's method the chromatin is coloured purple, the linin blue, and the nucleolus yellow. The spherical nucleolus, more or less hidden by the synaptic knot (Pl. XXXIII, Fig. 4), projects into the clear nuclear cavity. In exceptional cases two nucleoli are present (Pl. XXXIII, Fig. 6). There is no definite nuclear wall, the nucleus being bounded by cytoplasmic fibrils.

The 'chromatic bodies' may originate either from the nuclear framework (Pl. XXXIII, Figs. 1, 4, 5, 6), or from the nucleolus (Pl. XXXIII, Figs. 7, 8). It is not uncommon to find both modes of origin proceeding simultaneously in the same nucleus (Pl. XXXIII, Fig. 9).

Origin of 'Bodies' from the Synaptic Knot.

At synapsis buds are seen to be given off from the knot, often from a part in close proximity to the nucleolus (Pl. XXXIII, Figs. 4, 5, 6). These buds become round, or pear-shaped, and are composed of linin in which chromatin of varying amount and density is carried (Pl. XXXIII, Figs. 5 and 6). They arise from the knot in many different ways. Sometimes large pieces round themselves off, and separate in part from the general mass (Pl. XXXIII, Fig. 4); or finger-like portions protrude (Pl. XXXIII, Fig. 2); or a piece of the linin matrix may escape from the general tangle (Fig. 6), and this gradually takes up a chromatin stain and becomes modified to form a 'body'; or rounded globules of eu-nucleolar substance (Fig. 3) may escape from the knot, apparently independently of the nucleolus, these by degrees show a 'chromatic' staining reaction, and become 'chromatin bodies'. In whatever way the buds originate, they always remain in connexion with the 'chromatic' portion of the parent nucleus (Pl. XXXIII, Fig. 4), and this connexion is retained until the final disintegration of the 'body'. As each 'body' becomes differentiated, it draws out with it as a long thick process, that portion of the knot to which it is attached (Pl. XXXIII, Figs. 6, 10). Gradually the 'body' moves away, and the process, in response to the continuous strain exerted on its viscous substance, becomes pulled out into a fine thread (Pl. XXXIII, Fig. 4). Having separated themselves from the synaptic knot, the 'bodies' pass through the clear nuclear space (Pl. XXXIII, Fig. 4), and the surrounding cytoplasm (Pl. XXXIII, Fig. 4). They may, and often do, penetrate the cell-wall (Pl. XXXIII, Figs. 5, 6), and enter an adjacent cell (Pl. XXXIII, Figs. 5, 6). There is no evidence to show that the 'bodies' secrete a ferment which dissolves the wall. Their passage leaves no trace, the hole

appears to close in and to become obliterated immediately. Arrived in the neighbouring cell, the 'bodies' remain stationary close to the wall (Pl. XXXIII, Fig. 9), so that the cell-wall is invariably seen to lie across the fine thread close to its junction with the body (Pl. XXXIII, Figs. 9, 10).

Great caution needs to be exercised before one can admit the conviction that the 'bodies' do come to lie in the cell adjacent to that from which they originated. There are two main difficulties which have to be considered. Firstly, the refractive index of the wall may be slightly different at the places where it has been perforated ; secondly, the walls run obliquely. Although these two possible sources of error have been fully appreciated, there seems, nevertheless, to be no other explanation than that the 'bodies' do migrate from their parent cell into one of the neighbouring cells. Colouring the walls with a contrast stain to that of the nucleus, such combinations as Ruthenium Red and Heidenhain, Congo Red and Heidenhain, Ruthenium Red and Thionin Blue, Methylene Blue and Eosin, have given striking and unmistakable results all confirming the foregoing conclusion.

There is always a clear space in the cytoplasm round each 'body' (Pl. XXXIII, Figs. 1, 4, 5, 6, 9, 10, and Pl. XXXIV, Figs. 11, 15, 16). This is possibly due to an electrical condition connected with the metabolism proceeding in its substance. Possibly it may be compared to the space, which, during certain stages of nuclear division, surrounds the nucleolus. This possibility is strengthened by the fact that the 'bodies' may be of direct nucleolar origin. 'Bodies' belonging to the same nucleus may pass to any of the adjacent cells (Pl. XXXIII, Fig. 6), and they may lie singly or in groups (Pl. XXXIII, Fig. 9). Thus both in transverse and in longitudinal sections of an anther, nuclei are seen, sending out processes and attachments in opposite directions. The fine connexions vary in length according to the distance which the 'bodies' have had to travel before they enter the neighbour cell. Those that come off the knot on the side abutting on to the cell-wall have a short connexion (Pl. XXXIII, Fig. 9), whilst those that have travelled in the opposite direction, and have passed through the broad area of cytoplasm, have a relatively long connexion (Pl. XXXIII, Fig. 10). At complete synapsis nearly every pollen mother-cell of an anther is seen to possess these bodies, and the threads by which they are joined to the parent nucleus are most conspicuous.

Origin of 'Bodies' from the Pre-synaptic Framework.

The close synaptic stage is the most active time for the formation of 'chromatic bodies', but they may often be seen to be budded off when the nuclear contents are being massed together prior to synapsis (Pl. XXXIII, Fig. 1). Then they can be recognized in the nuclear framework as rounded or oval aggregations of 'chromatin' (Pl. XXXIII, Fig. 1). These bodies behave like those formed during synapsis.

When the nuclei have completed their synaptic rearrangement, and the 'bodies' have been extruded and are lying in the neighbour cells, more or less abutting against the wall dividing the parent cell from the invaded cell (Pl. XXXIII, Fig. 9), the knot begins to loosen and the cells to separate. It is not uncommon in *Galtonia* to find at this time that the whole synaptic knot breaks up into 'chromatic bodies'. This invariably indicates that the nucleus, instead of passing into the subsequent spireme stage, disintegrates and dies. Often two loculi, or even all four, may have their pollen mother-cells in this aborted condition.

Walls of the Pollen Mother-cells.

It is necessary here to sketch the history of the walls of the pollen mother-cells, which come into such close relationship with the 'chromatic bodies'. During the stages leading to synapsis, the cells are polyhedral in shape, and form a closely fitting and compact mass (Pl. XXXIII, Fig. 9). The cell-walls are thin and evidently are composed both of pectose and of cellulose, as they stain brightly with Methylene Blue and with Ruthenium Red (pectic stains), and even more brilliantly with Congo Red (cellulose stain). They consist of a clear middle lamella, which stains homogeneously, and in which occasionally granules, apparently cytoplasmic, may be found ; this layer is bounded on either side by fine membranes. The cytoplasm of the cells extends to the walls. When the cells are preparing to separate the middle lamella swells considerably but unevenly (Pl. XXXIV, Fig. 11): in some places it may be considerably thickened, whilst in others it remains thin ; at the same time it loses its staining capacity and appears as a colourless space.

Some chemical change is apparently proceeding in the interior of the wall, that is to say in the colourless layer, which causes the swelling, and forces the delicate membranes severally to round off and limit the cytoplasm of the cell with which they are in contact. In *Funkia Sieboldiana* a thin membrane, a specialized part of the middle lamella, has been seen in the clear space of the wall, but in *Galtonia* this is not visible. Protoplasmic connexions across the walls are most obvious (Pl. XXXIV, Fig. 14). Thus each pollen mother-cell is enclosed in its own wall (Pl. XXXIV, Fig. 17). The cytoplasm gradually contracts, so that by the time that the nucleus contains definite chromosomes there is a wide space between the circular enveloping wall and the enclosed, somewhat irregularly outlined cytoplasm (Pl. XXXIV, Fig. 18). The wall has by this time resumed its staining reaction, and appears as a thin membrane, often with many adhesions and granules on its surface. The wall persists, with slight modifications in its structure, throughout the first and second meiotic divisions, and is only finally broken down when the pollen grains are set free in the cavity of the pollen-sac.

Further History of 'Chromatic Bodies'.

As the pollen mother-cells begin to separate from one another, the fine threads, connecting the 'bodies' with the parent nucleus, may be seen traversing the clear space in the wall. As events proceed and the walls swell, forcing the cells apart, the connexions are still to be seen (Pl. XXXIV, Fig. 11). It is usual at this stage, when the nuclei are coming out of synapsis, to find the 'chromatic bodies' lying at the extreme edge of the cytoplasm, united by long connecting threads to the mother-nucleus (Pl. XXXIV, Figs. 15, 16).

Whereas at synapsis nearly every nucleus has attached 'chromatic bodies', at the later nuclear stages only a small percentage of nuclei possess them. This fact indicates that the 'bodies' are gradually disintegrating.

As the synaptic knot loosens and grows out into beaded or thready loops, so likewise the 'bodies' become granular (Pl. XXXIV, Fig. 16). Often the connexions are in direct continuity with the loops of the spireme, and it is just possible that they are transferring some of their substance back into the parent-nucleus. The connexions are broken when the spireme has become more or less unravelled, and the loops are distributed throughout the nuclear cavity. The 'bodies' generally remain in the cytoplasm of the invaded cell, but sometimes they appear to be dragged across the space to the nucleus from which they originally came, as very occasionally a 'chromatic body' or a piece of one is seen adhering to the exterior of the nucleus. In any case the 'bodies' now disintegrate. Often portions revert to the eu-nucleolate condition, fragments of eu-nucleolar staining substance being found attached to the disorganizing 'chromatin' fragments. When the nucleus has returned to the centre of its surrounding cytoplasm, there is a sudden disappearance of the 'body' remains. The destruction must be extremely rapid, as in most cases at the 'open spireme' the cytoplasm appears homogeneous, but under favourable fixing and staining conditions the 'chromatic bodies' can still be recognized as brightly staining granules scattered in the cytoplasm (Pl. XXXIV, Fig. 17). This rapid fragmentation has its parallel in the sudden disappearance of the nucleolus when ejected from the equatorial plate in the somatic division.

Origin of 'Bodies' from the Nucleolus.

It is also common to find 'bodies' originating as buds from the nucleolus, but they are not given off in such numbers as from the nuclear framework.

When newly formed at the close of the telophase of the last archesporial division, the nucleolus takes up a chromatin stain. As the nucleus passes on into the early meiotic prophase, the nucleolus gradually takes a cytoplasmic stain and becomes a typical true or 'eu'-nucleolus (20). It is especially colourless in *Galtonia* as compared to other plants. Chromidia

are always associated with a plasmosome of this nature. The nucleolus is approximately spherical, but its outline, though definite, may be undulating.

From the early prophases onwards the nucleolus may show what appear to be vacuoles (Pl. XXXIII, Figs. 3, 5). These may be scattered in the nucleolus, or they may form a small group towards the centre. Although these nucleolar buds may be found at the early prophases, it is at complete synapsis that the nucleolus becomes the most active. Then it may be studded with buds, some in the act of breaking off (Pl. XXXIII, Fig. 9), whilst others may be free in the nuclear cavity (Pl. XXXIII, Fig. 7), and others again may have already travelled into the surrounding cytoplasm (Pl. XXXIII, Fig. 7). Wherever they are, they instantly begin to assume the character of 'chromatic bodies'. Sometimes the buds, while still attached to the nucleolus, initiate this activity, and may be seen to have a tiny cap of 'chromatin' on their outer surfaces (Pl. XXXIII, Fig. 8), just as Page May and Walker (11) have described in the nucleolar buds of nerve cells of certain mammals.

With appropriate staining a marked differentiation can be obtained between the small globule of nucleolar material and the angular block of 'chromatin' to which it is attached. Stained with combinations of basic and acid dyes, the nucleolus and the newly differentiated buds take up the acid stain; but as the buds get into the cytoplasm they absorb the basic dye in an ever increasing degree. In time the nucleolar fragments are absorbed, and there remains no trace of the portions of eu-nucleolus adhering to the 'chromatic bodies'. Whether the 'bodies' so formed secondarily connect themselves with the parent-nucleus, and whether they pass through the cell-walls, it is impossible to say. Isolated, unconnected 'bodies' may be often seen in the cytoplasm, but whether the connexions are so cut as to render them invisible, or whether they never existed, could not be determined.

The nucleolus persists throughout the later nuclear stages, and is always in close connexion with the spireme, and later with the chromosomes, especially with one of the small pairs. As the chromosomes are forming, it begins once more to show vacuoles, and the nucleolus finally falls to pieces in a shower of globules (Pl. XXXIV, Fig. 19), much like the buds that were given off in the prophases and at synapsis; but they are less definite at this stage of the mitosis. Some pass into the cytoplasm where they disappear, while others apparently become absorbed in the nucleus. This final destruction of the nucleolus has been recorded and figured by Miyake in *Galtonia* (9). Sometimes when the chromosomes are being differentiated, the nucleolus breaks up into several rounded nucleoli, each small nucleolus adhering to one of the paired chromosome segments (Pl. XXXIV, Fig. 18).

Megaspore Mother-cell.

No definite formation of 'bodies' has been seen in the synaptic stages of the megaspore mother-cell. The knot resembles that of the pollen mother-nuclei; it is a dense mass thinning out towards the periphery where shreds of linin escape (Pl. XXXIV, Figs. 12, 13). Fragments of linin may become detached; these round themselves off and lie in the nuclear cavity (Pl. XXXIV, Fig. 13). Occasionally similar bodies may be found in the adjacent cell (Pl. XXXIV, Fig. 13), but no connexion with the parent-nucleus is retained. As a rule these portions remain colourless, but they may also become chromatic. They are not always present, and even when found they are indefinite and insignificant as compared with the 'chromatic bodies' of the pollen mother-cells.

The nucleolus may be of the nature of a plasmosome (Pl. XXXIV, Fig. 13), and take an acid stain, but more often it is densely chromatic in staining reaction (Pl. XXXIV, Fig. 12). Wilson (20) states that 'plasmosomes sometimes seem to have no envelope, but in many cases (e.g. in leucocytes) are surrounded by a thin layer that stains like chromatin'. This would explain the differently staining nucleoli found in the megaspore mother-cells; moreover, a pale nucleolus has been seen emerging from one that stains more deeply. Nevertheless, in most cases the chromatin in the 'chromatic nucleolus' is more than a 'thin layer', as in a section through such a nucleolus both portions stain with equal density. The deeply staining nucleolus is generally vacuolated (Pl. XXXIV, Fig. 12). It may be close to, and partly enveloped by, the synaptic knot, or it may lie apart. In the latter case it is united to the 'chromatin' mass by a narrow strip of linin (Pl. XXXIV, Fig. 12), recalling the 'chromatic body' with its thread-like connexion.

When the nucleolus is of a plasmosome nature, it may protrude from the synaptic mass as in the nuclei of the pollen mother-cells (Pl. XXXIV, Fig. 13). It is vacuolated and often throws off tiny buds. These buds remain colourless, and are inconspicuous. Occasionally the plasmosome is hour-glass shaped, and becomes nipped into two at the constriction. This mode of duplication of the nucleolus is often seen in the early prophases of the somatic divisions of the root, only in these cases the nucleoli take a basic stain. Rosen (12) has figured these two lobed nucleoli in the root of *Vicia Faba* var. *megalosperma*, but he concludes that they are in the act of fusing, and not of separating.

General Comparisons.

There exists a considerable literature dealing with the elimination and casting out of substance from the nucleus. This elimination has been observed both in animals and in plants, and both in vegetative and reproductive cells.

Balbiani (1) in 1864 noticed the vacuolated nucleoli in the ova of *Geophilus longicornis* which he believed functioned as excretory organs. Since then many writers have confirmed Balbiani's views.

Tangl (16) has described vacuolated nucleoli in the pollen mother-cells of *Hemerocallis fulva*. These bud off small colourless nucleoli, which pass out into the cytoplasm where they are reabsorbed.

Scharff (13) found 'a small nucleolus being constricted off from a large one' in the intra-ovarian ovum of *Conger vulgaris*. In the ovum of *Gadus virens* he figured 'spots' in the cytoplasm, which he thought might possibly be 'escaping nucleoli which have travelled through the nuclear membrane into the surrounding protoplasm'. In *Trigla gurnardus* protuberances appear on the nucleus, and into these fragments of nucleolus are drawn. The protuberances separate from the nucleus and appear as vesicles. They travel towards the surface of the egg. The vesicles with their nucleolar contents are the yolk-spherules.

Miss Nicholls (10) in *Sarracenia* has figured granules in connexion with the nucleolus, and mentions that the nucleolus, after synapsis, is vacuolated.

Cardiff (3), in *Acer platanoides*, found that a small papilla often projects at the point of contact of the linin thread with the nucleolus. 'In some preparations this nucleolar papilla looked like one of the vacuoles escaping.'

Wilson (21), in the spore mother-cells of *Mnium hornum*, has described the presence of a small deeply staining rounded body in the nucleus, in addition to the nucleolus. 'A careful examination leads to the conclusion that this body arises from the nucleolus by a process of budding.'

In 1891 Brauer (2), in the course of his work on *Hydra*, found that the large nucleolus budded off small portions which tended to group themselves round the large one.

Recently Walker and Embleton (18) have investigated *Hydra fusca*, and have shown that nucleolar budding takes place in the resting cells both of the ectoderm and endoderm. The buds pass out of the nucleus, and eventually disintegrate in the cytoplasm. They may remain joined to the nucleus by a membranous process for a considerable period.

Page May and Walker (11) have found in the nerve cells of some mammals that an excrescence is formed on the nucleolus. This separates and grows until nucleolus and excrescence are indistinguishable. Then one of these two nucleoli passes out of the nucleus into the cytoplasm and wanders to the periphery of the cell. It may pass bodily through the surface membrane, and be set free among the surrounding cells. In other cases the nucleolus lies on the inside of the surface membrane of the nerve cell, and 'here the substance of the nucleolus seems to pass piecemeal through several small openings, and to be absorbed into the cytoplasm of the neighbouring cell'.

Walker and Tozer (19) have made further investigations into the nucleolar budding in the vegetative cells of animals and plants. The buds pass out of the nucleus into the cytoplasm. While in the nucleus they take a basic stain, but arrived in the cytoplasm their staining reaction gradually alters, until they take the acid stain more deeply than the surrounding cytoplasm. In the formation of the chromatic bodies from the nucleolar buds in *Galtonia* the staining reaction is reversed.

Chamberlain (4), in the body cells of *Dioon edule*, has described 'black granules' in the cytoplasm. He believes that they have come from the nucleus and have passed through the nuclear wall without becoming soluble. The black granules increase in size by imbibing liquid, until the granule becomes a thin pellicle enclosing a liquid. 'As the pellicle stretches, granules pass through it into the watery interior, and the colour with iron alum hematoxylin gradually changes from black to gray.' [1]

There is a suggestive similarity between the above-mentioned cases of the extrusion of nuclear material in the higher animals and plants and the 'chromidia' of the lowly forms. A vast literature has accumulated round the subject of chromidia. Richard Hertwig (7 and 8) was one of the first to insist on the importance of the chromidia and introduced the terms 'chromidia' and 'chromidial net'. The chromidia are believed to be always extruded from the nucleus, and are composed partly of nucleolar, and partly of chromatic substances. They may be scattered in the cytoplasm or arranged in a net-like system. Often, as in *Arcella vulgaris*, each chromidial aggregation forms a new nucleus. Comes (5) has shown in *Gregarines* that the chromidial apparatus varies according to the amount of nutrition absorbed by the animal. In the summer, when well nourished, the chromidia are most abundant, whilst in the winter, when food is scarce, there are very few to be found.

Hartmann and Prowazek (6) have recently confirmed Schaudinn's (14) work on the Trypanosome, *Haemoproteus noctuae*. Before the animal encysts the large nucleus sheds out a portion of its karyosome and this becomes the second nucleus. Both nuclei send off two small nuclear buds which degenerate. The two large nuclei then retreat to the centre of the cell and fuse.

In 1899 Siedlecki (15) observed an elimination of nuclear substance in the *Sporozoa*, and this has since been corroborated by several writers. At the formation of the macrogametes the karyosome divides into small portions. These go to the periphery of the cell and become surrounded by chromatin. The edge of the Coccidium cell is seen to be studded with

[1] 'Nuclear gemmation' and 'fragmentation' have been found to take place in the fungus *Synchytrium*. The karyosome of the parent-nucleus buds or fragments, and each portion becomes a new nucleus. Griggs, R. F.: Some Aspects of Amitosis in *Synchytrium*. Bot. Gaz., xlvii, Feb., 1909, pp. 127–38.

these aggregates, and round each there is a clear space in the cytoplasm, as is always to be seen round the 'chromatic bodies' of *Galtonia*. Each aggregate forms a nucleus. Almost identically the same events take place in the formation of the microgametes. During the maturation of the female element the macrogamete extrudes part of its substance which lies as a mass at the side of the nucleus. Siedlecki regards this action as 'une épuration nucléaire'.

It is difficult to resist the conclusion that, in phenomena such as those so strikingly exemplified in *Galtonia*, we are confronted with processes leading to a definite elimination of nuclear substance, and further that the analogy which exists between these higher organisms and the *Protozoa*, in this respect, may turn out to possess a far-reaching significance. Beyond this it would at present be perhaps unsafe to go; but it is plain that the comparison is worth further investigation.

Summary.

1. 'Chromatic bodies' are found to be present in abundance in the pollen mother-cells of *Galtonia candicans* at the pre-synaptic and synaptic nuclear stages.

In the megaspore mother-cell only a few inconspicuous chromatic bodies have been seen.

2. 'Chromatic bodies' may originate as portions of the nuclear framework, or of the synaptic knot, or as nucleolar buds.

3. They are composed of linin, in which chromatin is embedded, or of nucleolar material.

4. Those 'bodies' that come from the 'chromatic' portion of the nucleus retain their connexion with the nucleus, by means of a fine thread, until their final disintegration.

5. The 'bodies' pass from the nucleolar space into the surrounding cytoplasm, penetrate the cell-wall, and enter the neighbour cell.

6. On the separation of the pollen mother-cells, the 'bodies' remain at the extreme edge of the invaded cell. Their connecting threads pass through the space between the cells.

7. As the nucleus comes out of synapsis, the threads break up and the 'bodies' begin to disintegrate.

8. At the 'open spireme' nuclear stage they can be recognized only as refractive granules.

9. The 'chromatic bodies' which originate as buds from the nucleolus at first take an acid stain, but as they pass into the cytoplasm they become increasingly 'chromatic'. It is not known whether 'bodies' so formed become secondarily attached to the nucleus.

10. The nucleolus in the synaptic nucleus of the megaspore mother-

cell may take an acid or a basic stain. Insignificant 'bodies' may be found in the nuclear cavity. These are derived either from nucleolar buds or from shreds of the synaptic mass. When deeply staining the nucleolus may lie independently of the knot to which it is attached by a linin strand.

In conclusion I wish to express my gratitude to Professor J. Bretland Farmer for the constant help, advice, and criticism that he has given to me throughout the course of this work.

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London.

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EXPLANATION OF PLATES XXXIII AND XXXIV.

Illustrating Miss L. Digby's Observations on 'Chromatin Bodies' and their Relation to the Nucleolus in *Galtonia candicans*.

All the figures were drawn with the camera lucida under a 2 mm. Apochr. Hom. Imm. Zeiss, N.A. 1.40, with Comp. Oc. 18. ×2250.

Figs. 1–11. Nuclei of pollen mother-cells.

Figs. 12 and 13. Nuclei of megaspore mother-cells.

Figs. 14–19. Nuclei of pollen mother-cells.

PLATE XXXIII.

Fig. 1. Nucleus in presynaptic stage showing 'chromatic bodies' being given off from the nuclear framework. Three 'bodies' have passed through the wall.

Fig. 2. Nucleus in synapsis. Finger-like portions protruding from the synaptic knot.

Fig. 3. Synaptic knot showing globules of nucleolar material escaping apparently independently of the nucleolus.

Fig. 4. 'Bodies' still in the parent cell, and united to the nucleus by fine threads.

Fig. 5. Two 'bodies' have passed through the wall and have entered the neighbouring cell. Note the 'chromatin' concentration in the substance of the 'bodies'.

Fig. 6. 'Bodies' from the same nucleus have passed into two neighbouring cells. Two nucleoli present in nucleus.

Fig. 7. Shows the origin of the 'bodies' as nucleolar buds. One bud is still attached to the nucleolus, one is in the clear nuclear space, while one has entered the cytoplasm.

Fig. 8. Nucleolar buds beginning to show 'chromatin' aggregations.

Fig. 9. Three cells showing nucleolar budding and 'bodies' arranged against the wall separating the invaded cell from the parent cell.

Fig. 10. Two adjacent cells with their 'bodies'. One nucleus shows long, the other short, connexions.

PLATE XXXIV.

Fig. 11. Shows the uneven swelling of the cell-walls. The connexions joining the 'bodies' to the nucleus traverse the clear space in the wall.

Fig. 12. Nucleus of megaspore mother-cell, in synapsis, with 'chromatin' staining nucleolus which is joined to the knot by a strand of linin. Nucleolus is vacuolated.

Fig. 13. Synaptic knot of megaspore mother-cell with plasmosome showing buds. Insignificant 'bodies' in nuclear cavity, and one in the neighbouring cell.

Fig. 14. Protoplasmic connexions across the walls.

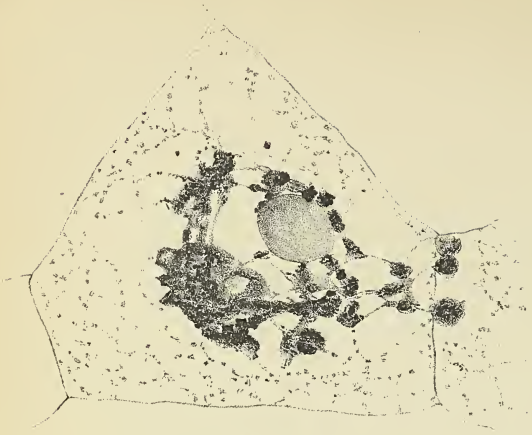
Fig. 15. The nuclear spireme is unravelling. The cells have separated. The 'bodies' lie at the extreme edge of the cytoplasm of the invaded cell, and are still in connexion with the parent nucleus.

Fig. 16. The spireme shows a beaded arrangement. There is a corresponding appearance in the attached 'bodies'.

Fig. 17. Nucleus in the 'open spireme' stage. Nucleus returned to the centre of the cell. The remains of the 'bodies' appear as bright refractive granules. Note the definite cell-wall, and the contraction of the cytoplasm.

Fig. 18. The nucleolus has divided into several small nucleoli. These are attached to the paired chromosome segments.

Fig. 19. The final disintegration of the nucleolus at the formation of the chromosomes.



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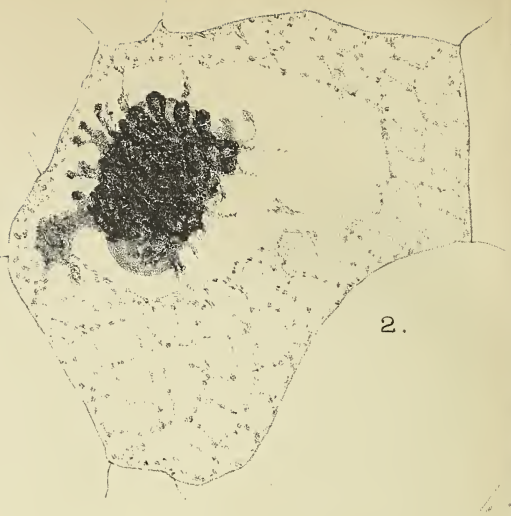

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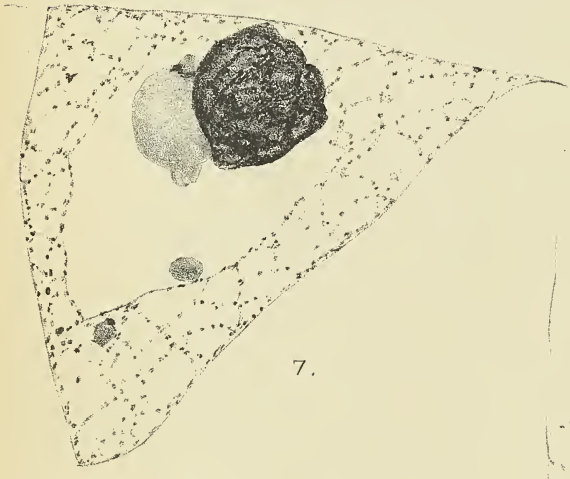

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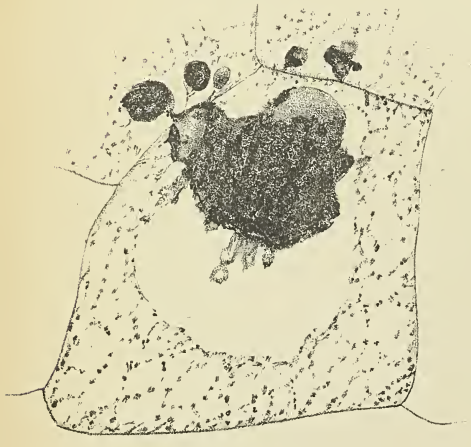

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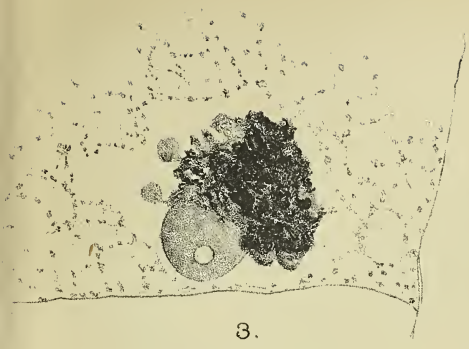

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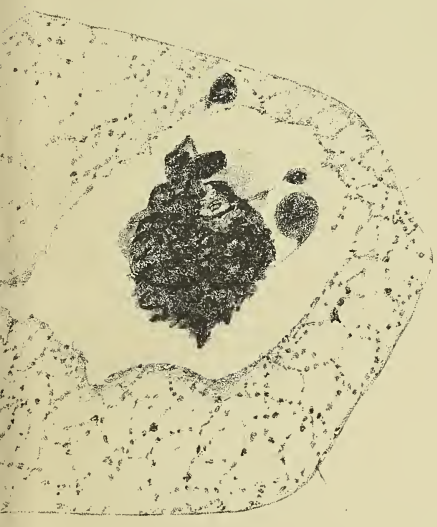

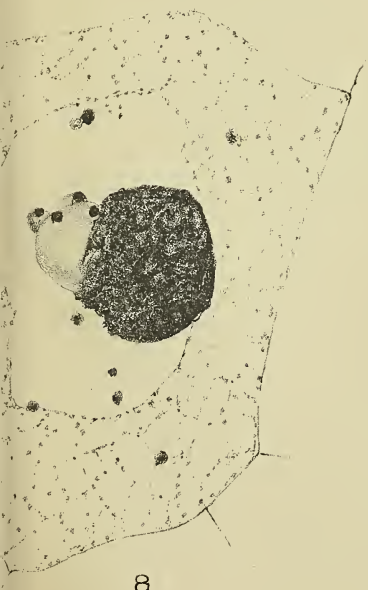

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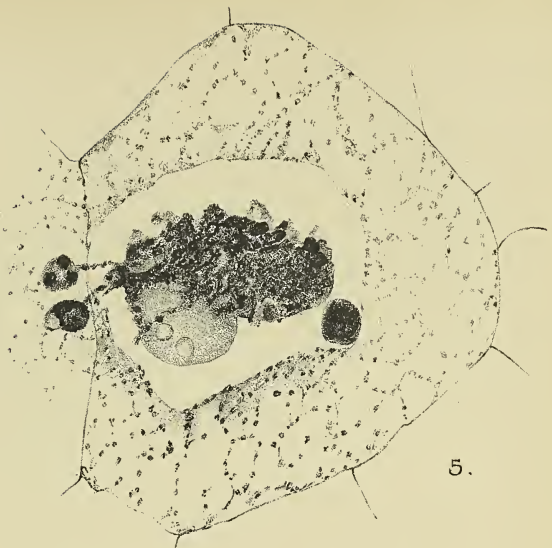

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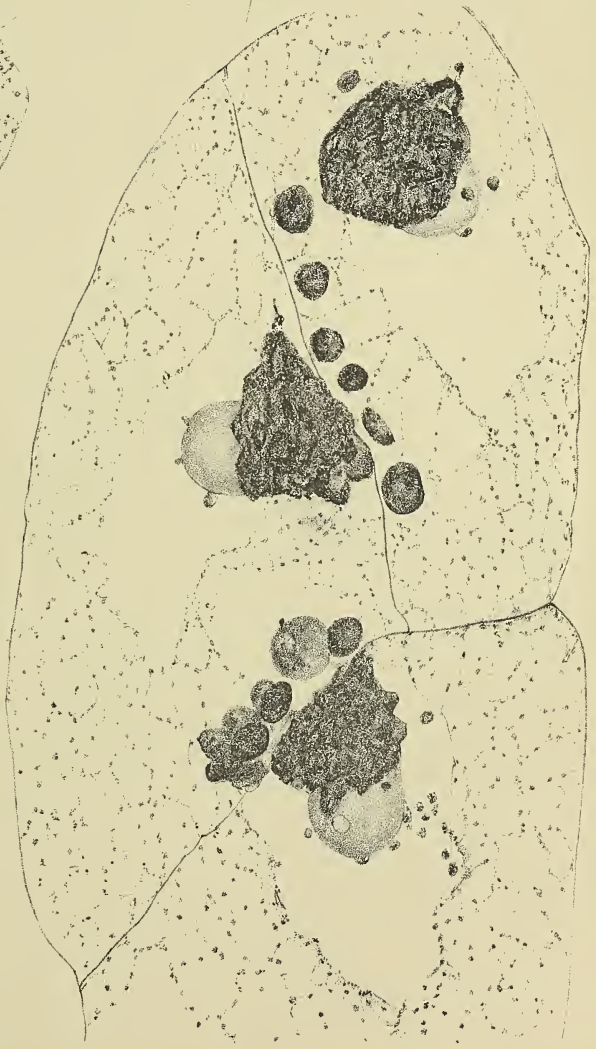

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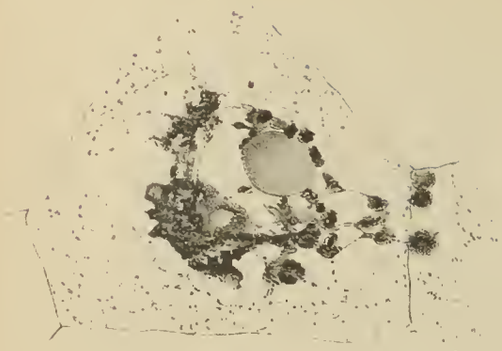

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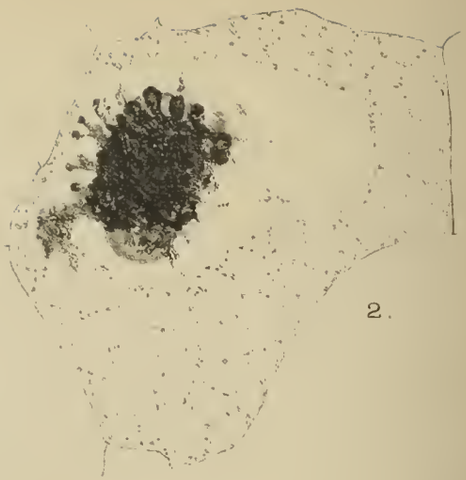

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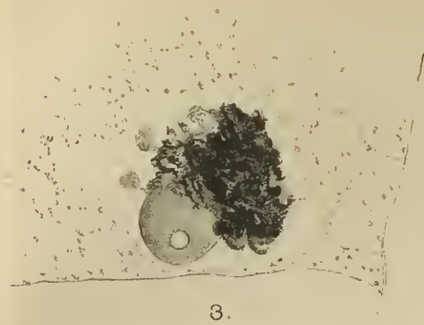

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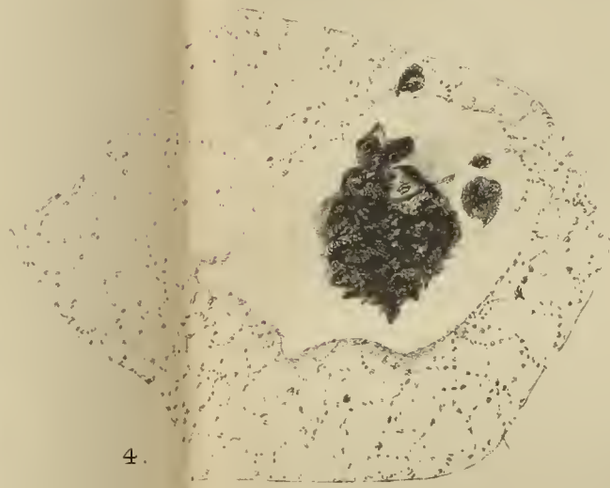

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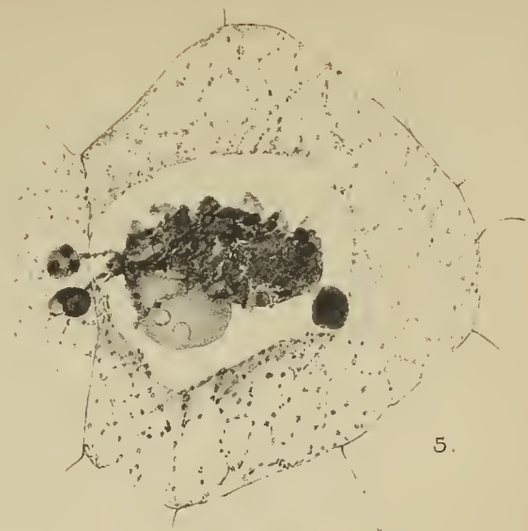

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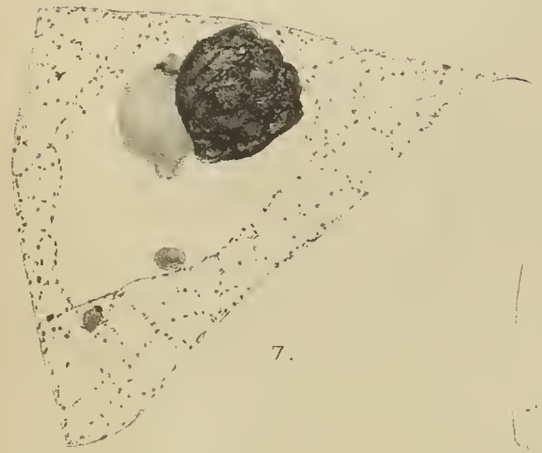

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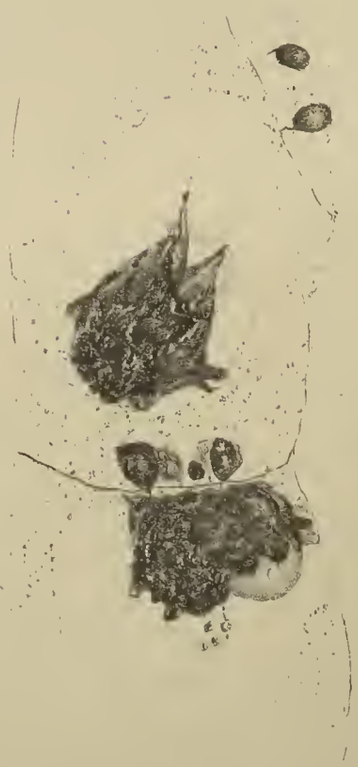

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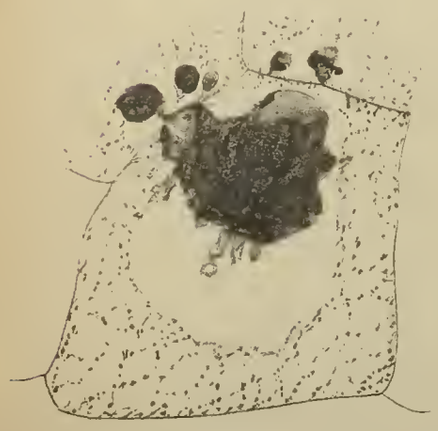

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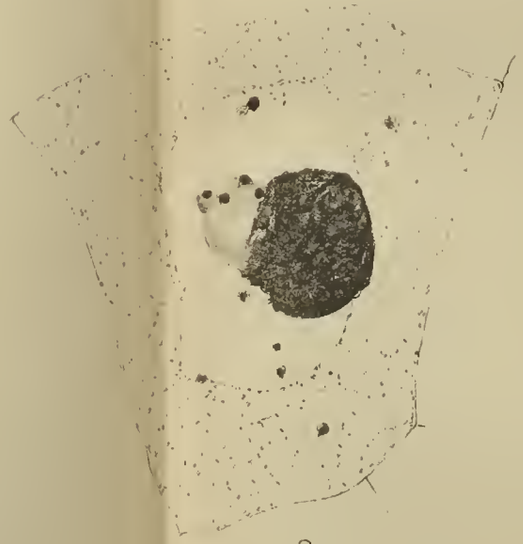

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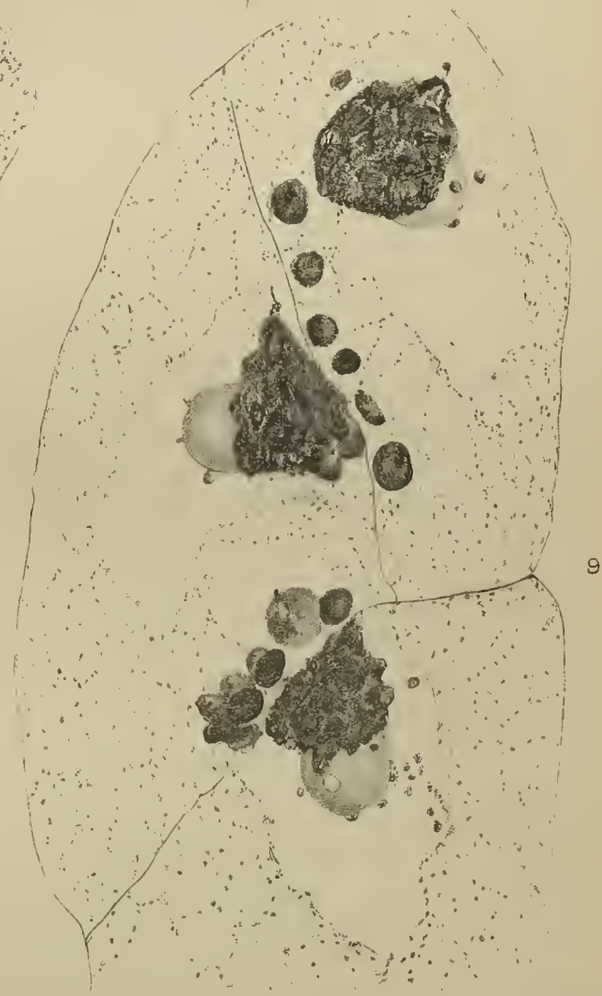

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DIGBY — CHROMATIC BODIES.

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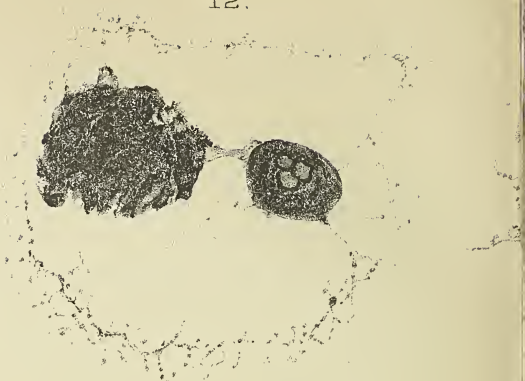

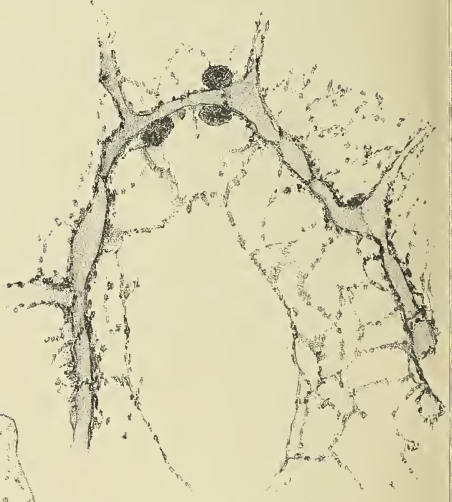

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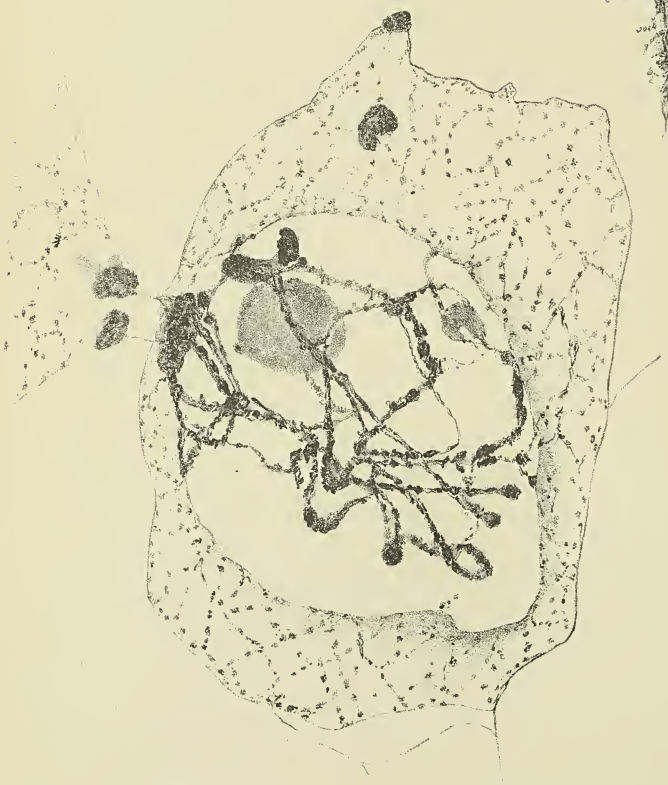

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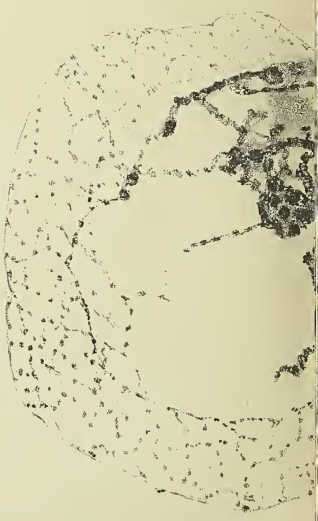

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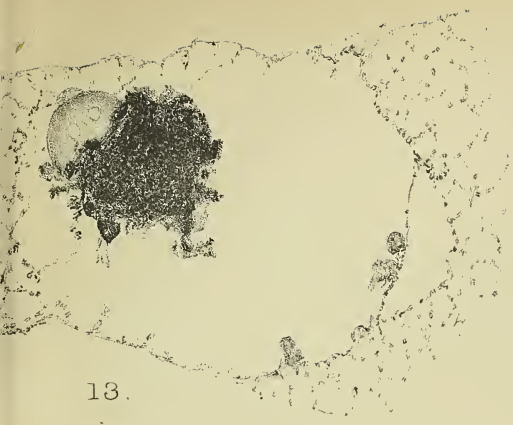

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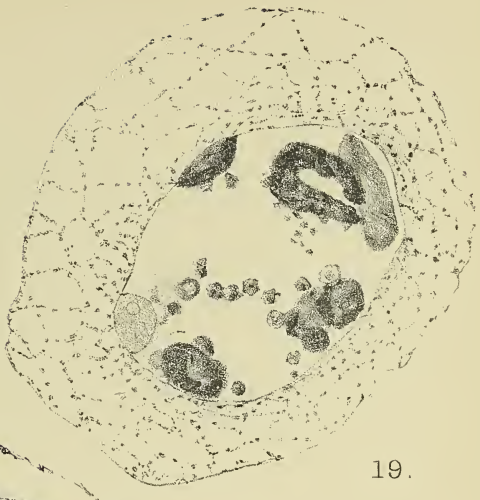

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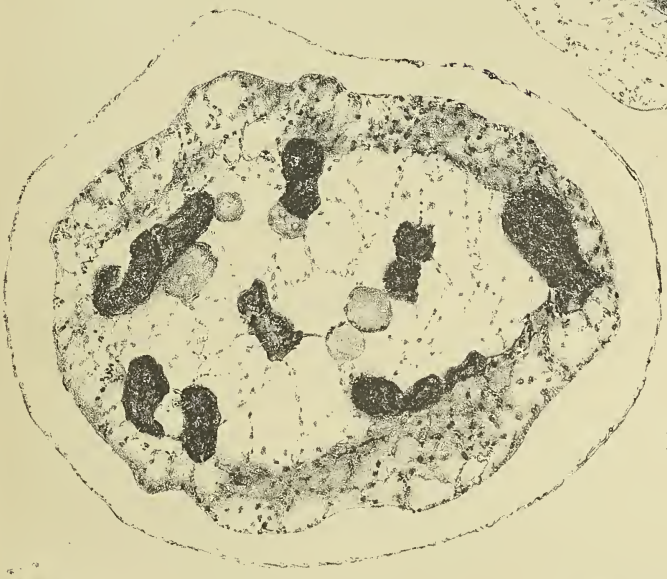

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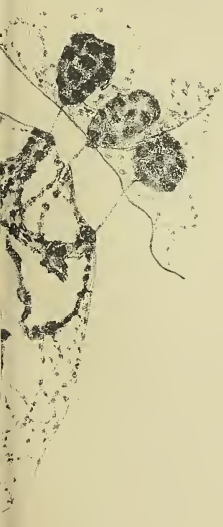

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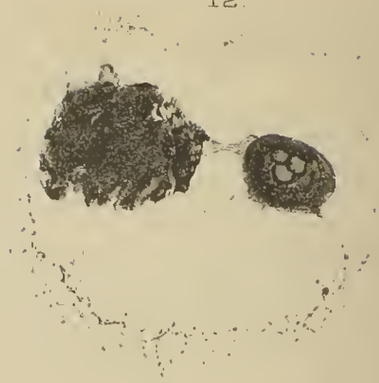

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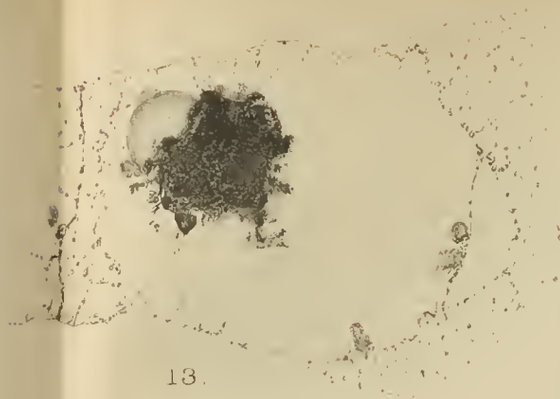

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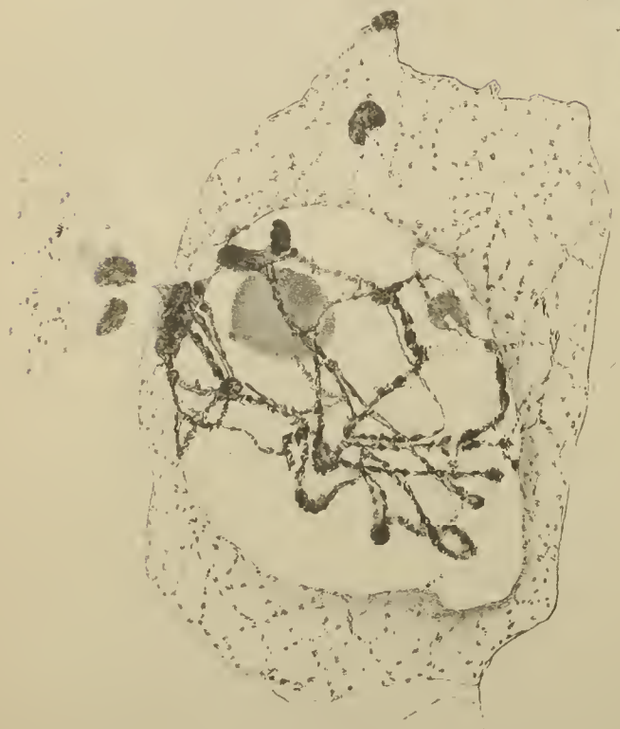

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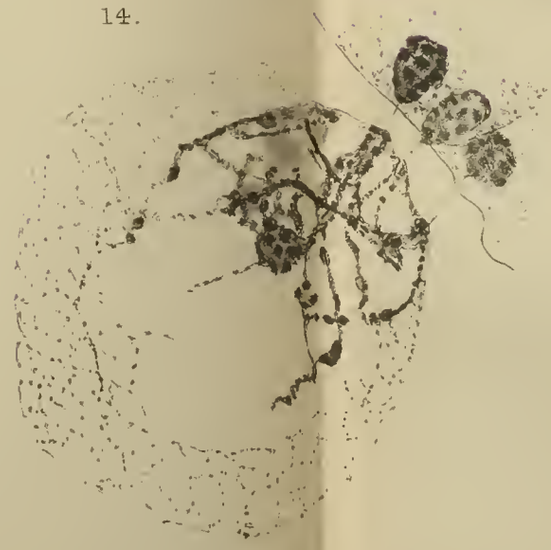

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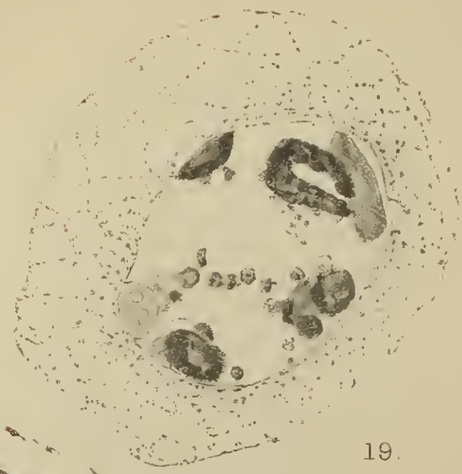

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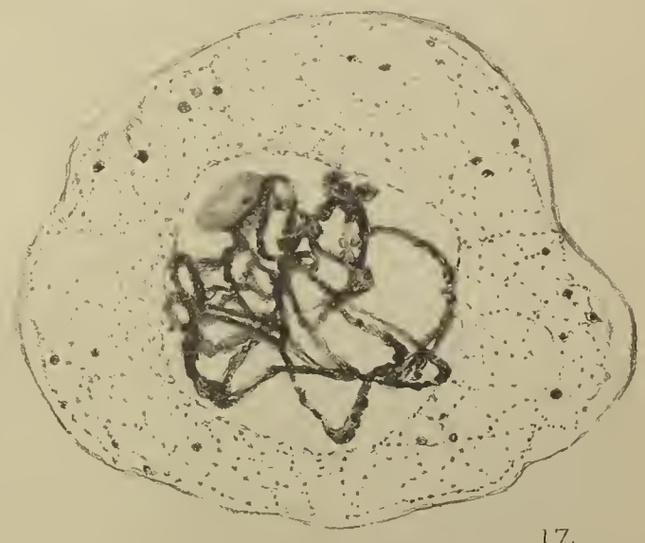

17.

Huth, lith. et imp.

On the Development of the Fructification of Armillaria mucida, Schrad.

BY

CECIL C. E. FISCHER,

Indian Forest Service.

With Plate XXXV.

ARMILLARIA MUCIDA is a hymenomycetous fungus commonly met with in England wherever the beech grows. Its fruit-bodies appear in autumn, growing in tufts on the diseased portions of beech trees, and are readily identified by the (usually) intense white of the pileus, which, further, is covered with a mucilaginous coating.

The fungus is fairly abundant on the older beech trees in Windsor Park, where it seems to be doing considerable harm.

There appears to be some diversity of opinion as to its true parasitic nature, and partly for this reason I undertook a study of its life-history, the results of which will be embodied in a separate paper. The opportunity for tracing the course of the development of the fructification naturally presented itself, and this forms the subject of the present note.

Comparatively little attention has been given to the development of the fructification in Hymenomycetes. One of the most recent papers on the subject is that of G. F. Atkinson on *Agaricus campestris* (1), in 1906. Prior to this paper no detailed study of the development appears to have been made since Fayod's work (3) in 1889.

Atkinson gives an historical sketch of previous work in this direction in the paper quoted above; consequently no lengthy *résumé* need be given here.

Atkinson suggests, apparently with justification, that the earlier investigators did not examine sufficiently young specimens, and they seem to have been led to general conclusions which do not hold good at any rate in all cases.

De Bary in the case of *Agaricus campestris* (2) and R. Hartig in that of *Armillaria* (*Agaricus*) *mellea* (5) describe the veil (*velum partiale* of Fries) as developing by new growth. They find that at an early stage a circular

[Annals of Botany, Vol. XXIII. No. XCI. July, 1909.]

channel, in the form of a narrow ring open towards the outside, arises in the upper part of the body by means of separation of the tissues. The portion above becomes the pileus and that below the stipe. By later growth, downwards from the pileus and upwards from the stipe, two interweaving series of hyphae are formed, thus giving rise to the veil, which covers the air-space. Atkinson observed a very different course of development in *Agaricus campestris*. He supposes that De Bary may not have studied the very young stages, and states that it would seem that 'he adopted Hartig's suggestion that *Agaricus campestris* followed the same course as that described by Hartig for *Agaricus melleus*', and in this way the statements current in textbooks (4) may have originated.

Having obtained a number of young fructifications of *Armillaria mucida* in pure cultures, I undertook a study of the development of the veil in that species, and, as will be seen, my observations agree essentially with those of Atkinson.

Atkinson seems to accept Hartig's account of the development in *Armillaria mellea* as substantially correct, but believes that De Bary was mistaken in admitting a similar process for the case of *Agaricus campestris*. As a result of my own investigation I am led to the conclusion that either Hartig's observations must be faulty, for the reason put forward by Atkinson, or else the development in two such closely allied species as *Armillaria mellea* and *A. mucida* is entirely divergent. I venture to suggest that such a divergence is not probable, and that the course of development is similar to that described by Atkinson for *Agaricus campestris* and as set forth below for *Armillaria mucida*. It must also be remembered that at the date of Hartig's work the improved methods we can now command were unknown.

The fungus was grown on bread and on sterilized beech wood, and sporocarps in all stages were taken for the investigation. Three naturally-grown very young carpophores were also obtained for comparison; no differences were found between those from the two sources.

At first I failed to realize at what a very early period differentiation of the tissues took place, and had to take specimens of a much younger age. Before there is any external indication of a distinct separation of the pileus, there is an internal differentiation of the tissues, as described later.

The sporocarps were fixed in chromo-acetic acid and microtomed. The sections were stained with various stains. Eosin gave very good results, though, unfortunately, the stain is not lasting. Gentian violet followed by orange G or Bismarck brown was also used, as well as Heidenhain's alum-haematoxylin followed by orange G. The latter method gave the most satisfactory results. All the drawings were done with the aid of the *camera lucida*.

The youngest stages examined, about half a millimetre in length,

presented a homogeneous tissue ; the hyphae ran longitudinally, interweaving freely. Soon, the tissue at the apex becomes looser in texture, leaving, however, an unaltered layer round the periphery (Pl. XXXV, Fig. 1). The hyphae near the apex, which hitherto all ran in the same direction, now begin to display a tendency to radiate towards the periphery, and rapidly form a subcuticular layer of hyphae at right angles to the outer surface (Fig. 2). These hyphae, which are somewhat closely packed to form a sort of palisade tissue, are not differentiated, but are rich in protoplasm, and take a deeper stain. The universal veil can be clearly distinguished now as a layer of hyphae, two or three deep, traversing the ends of the hyphae composing the palisade tissue (Figs. 3 and 4). The veil generally takes a brown stain with haematoxylin and orange G, contrasting well with the violet tint of the adjacent cells.

Hartig considered that *Armillaria mellea* was devoid of a universal veil, but Fayod (3) has shown that such a tissue always exists, though it is sometimes so tenuous as to escape attention.

We have seen that the differentiation of tissues begins at a very early stage and marks off the stipe from the pileus, the latter being defined by the palisade layer. This layer rapidly becomes more confirmed and spreads inwards from its outer and lowermost edge, at the same time assuming a marked palisade character. By this time, too, the pileus is externally distinguishable as a minute cap. The radial palisade layer spreading inwards forms the *primordium of the hymenium.*

Strong surface-growth now causes the pileus to assume greater convexity, at the same time, owing to cessation of growth of the tissues immediately below, the primordium of the hymenium becomes separated from them and a small air-space is formed, which is the origin of the 'gill cavity'. Meanwhile, the outline of the future stipe has become more clearly defined by the outer layers growing looser in texture, so that a mass of lacunae filled with air arises, whereas the future stipe remains densely felted. This, no doubt, assists in supplying the necessary air for the formation of the gill cavity, as pointed out by Atkinson (Fig. 5).

Up to this stage the universal veil has kept pace with the growth of the inner tissues, now, however, its hyphae begin to disintegrate and break up, not only over the surface of the pileus, but also down the sides of the stipe (Figs. 6 and 7).

It may be noted here that at this time the decomposing universal veil is very delicate and apt to be torn off even by the microtome blade, so that in some of the sections no veil can be seen. Further, the pileus has taken a more rounded form and is growing in diameter more rapidly than the stipe, so that it bulges somewhat beyond the latter. The disappearance of the veil accentuates this overlapping (Fig. 7). It would seem possible that these phenomena combined to mislead Hartig.

The pileus assumes a globular shape by continued growth, still further separating the hymenium from the 'neutral tissue', which increases the depth of the gill cavity (Fig. 8).

The size of the sporophores does not necessarily correspond with the degree of development, so that it is not possible to indicate the stages of growth by external description. This will be seen by comparing Figs. 5 and 6, both drawn to the same scale, 5 being the younger stage.

Soon after the last described stage the lower portion of the pileus begins to grow out and upwards, stretching the neutral tissue to which it is still attached by its margin (Fig. 8).

By this time the universal veil has entirely disappeared, as also the loose lacunar tissue which surrounded the stipe and was previously covered by the veil. It is at this stage that the mucilaginous coating begins to appear on the surface of the pileus.

The neutral tissue is much drawn out by the outward growth of the pileus, and becomes very loose and almost diaphanous by the separation of its elements. This is the *velum partiale*. Sooner or later it is ruptured at the edge of the pileus, sometimes not till the latter is nearly horizontal, remaining attached to the stipe as the *annulus superus*.

During this last period of development the hymenial layer differentiates its elements of basidia, paraphyses, and cystidia. The spores are formed before the rupture of the veil, and I found them capable of germination even when the veil had to be pierced to obtain them.

The mucilage of the pileus is derived from the disintegration of the extremity of the palisade layer laid bare by the disappearance of the universal veil, the remainder of the palisade layer persisting. That the mucilage is due to decomposition and is not merely a secretion is evidenced by the particles and rounded cells found embedded in it (Fig. 9). The mucilage gave an acid reaction with litmus paper.

Summarized, the results are as follows:—

I. A universal veil exists which disintegrates after the differentiation into primordial stipe, pileus, and hymenium.

II. The origin of the hymenium is *endogenous*.

III. There is no separation of pileus and stipe by the formation of an annular air-space.

IV. The marginal veil (*velum partiale*) *is not an aftergrowth*, but is formed by the neutral tissue, which is present from the beginning.

V. The mucilaginous coating of the pileus is derived from the degeneration of the apical portion of the palisade tissue.

An early examination of the very young stages of the sporophores of *Armillaria mellea* seems called for. This species is readily cultivat in the laboratory (see Brefeld's 'Untersuchungen aus dem Gesamtgebi

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Line 10 from top. *For* 8 *read* 7.
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P. 507. In description of Fig. 6. *For* 35 *read* 470.
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For Fig. 8 *read* Fig. 7.
For Fig. 9 *read* Fig. 8.

der Mykologie'), so that a sufficient supply of carpophores in all stages of development could be secured without difficulty.

I embrace this opportunity to express my best thanks to Professor J. B. Farmer, F.R.S., at whose suggestion this study was undertaken, for his kind encouragement, and to acknowledge my indebtedness to his valuable advice.

Literature quoted.

1. Atkinson, G. F.: Development of *Agaricus campestris*. Botanical Gazette, vol. xlii, 1906.
2. De Bary: Morphologie und Physiologie der Pilze. 1886.
3. Fayod: Prodrome d'une histoire naturelle des Agaricinées. Ann. Sci. Nat., Bot., vii, 9, 1889.
4. Goebel: Outlines of Classification and Special Morphology of Plants. English edition. 1887.
5. Hartig, R.: Wichtige Krankheiten der Waldbäume, &c. 1874.

EXPLANATION OF FIGURES IN PLATE XXXV.

Illustrating Mr. Fischer's paper on the Development of the Fructification of *Armillaria mucida*, Schrad.

Fig. 1. Young stage showing loose texture in centre of the undifferentiated sporophore. × about 35.
Fig. 2. Subcuticular palisade layer forming the primordium of the pileus. × about 35.
Fig. 3. The same enlarged to show details of structure and the *velum universale*. × about 470.
Fig. 4. Somewhat further accentuated palisade tissue. × about 470.
Fig. 5. Sporophore showing primordium of the hymenium, the gill cavity and the lacunar texture of the subcortical tissue covering the stipe. × about 35.
Fig. 6. Further stage—the universal veil beginning to disintegrate. × about 35.
Fig. 7. The same enlarged to show details. × about 470.
Fig. 8. The universal veil and loose tissue around the stipe have entirely disappeared. The neutral tissue is still attached to the margin of the pileus. × about 25.
Fig. 9. Section through pileus of ripe carpophore to show decomposition of the outer part of the palisade tissue to form mucilage. × about 315.

Royal College of Science, South Kensington.
May, 1909.

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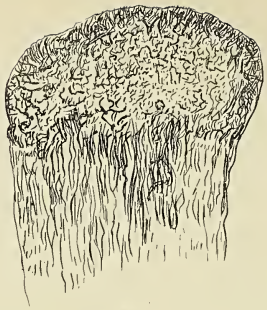

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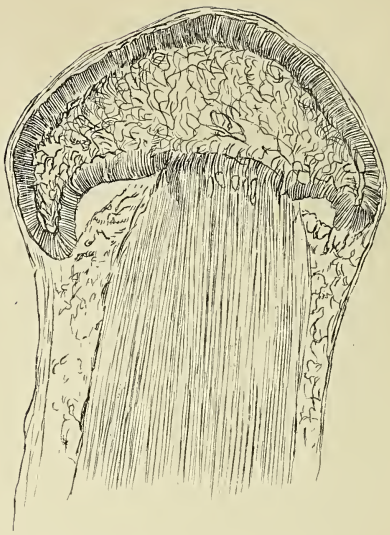

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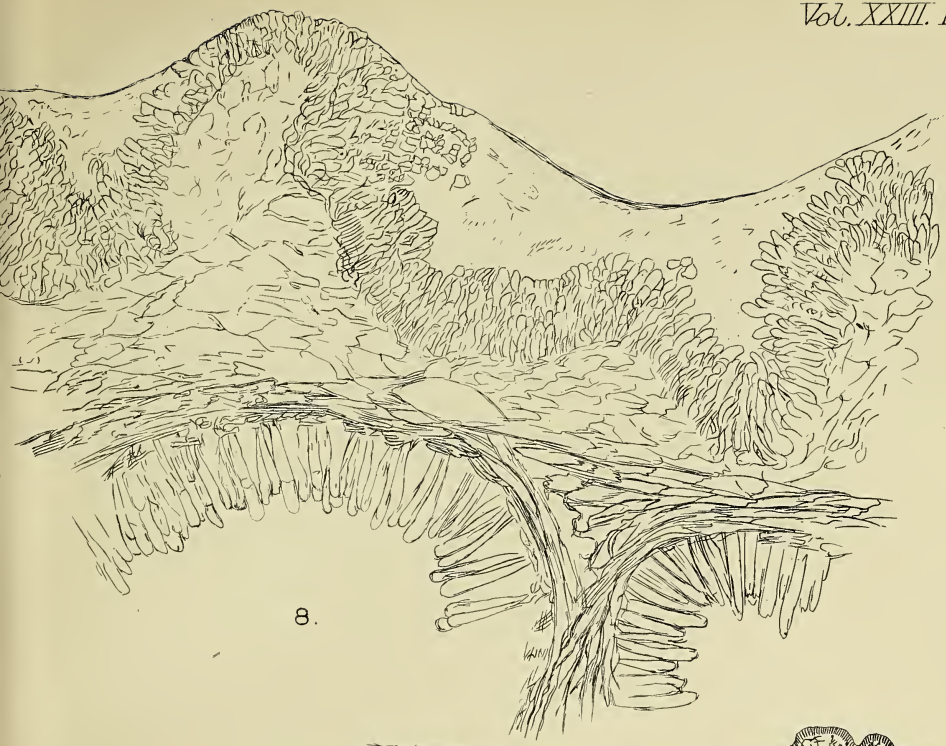
FISCHER — ARMILLARIA MUCIDA.



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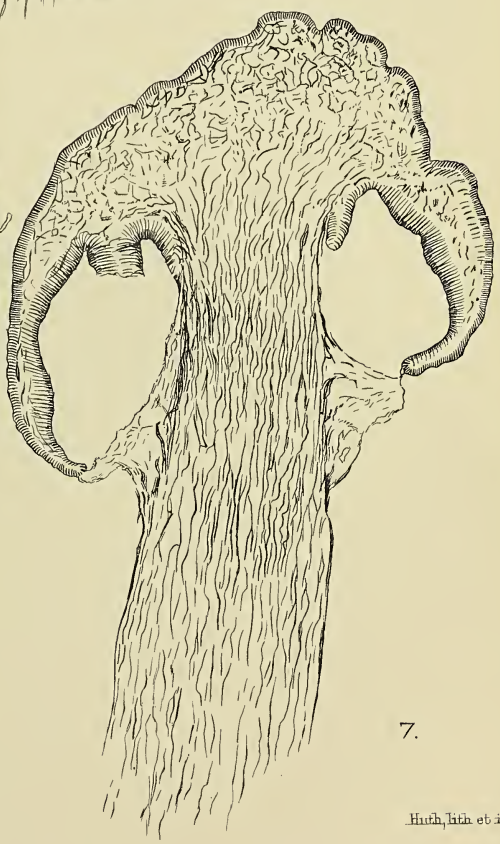

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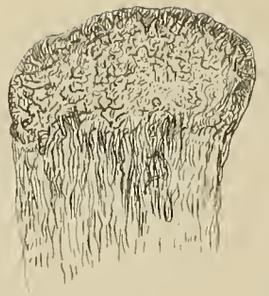

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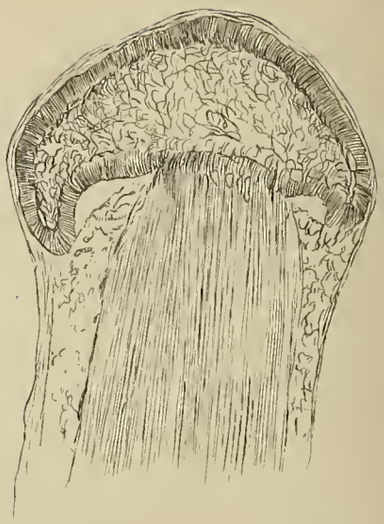

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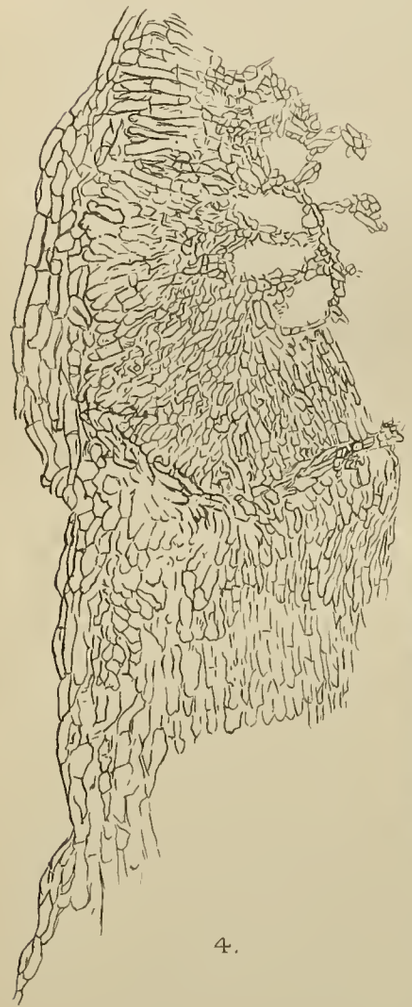

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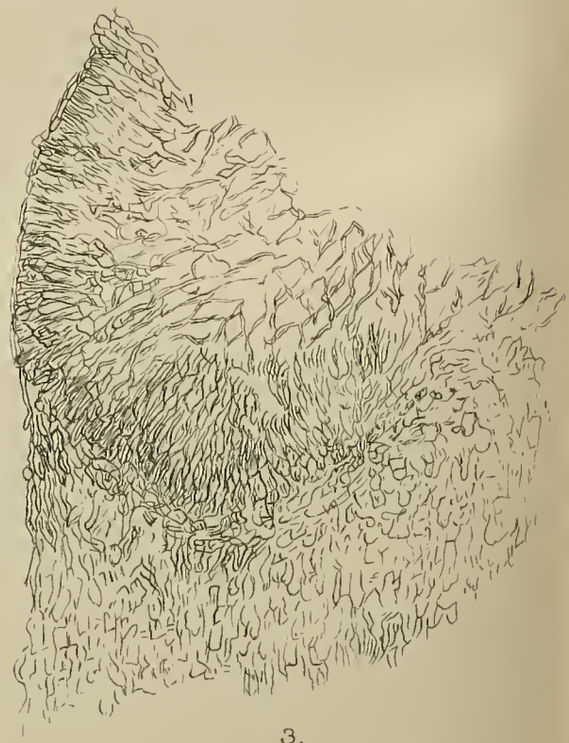

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FISCHER — ARMILLARIA MUCIDA.

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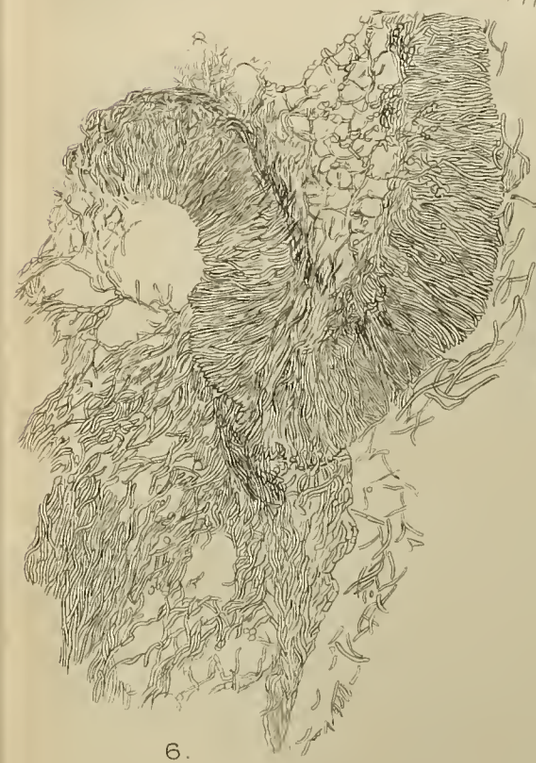

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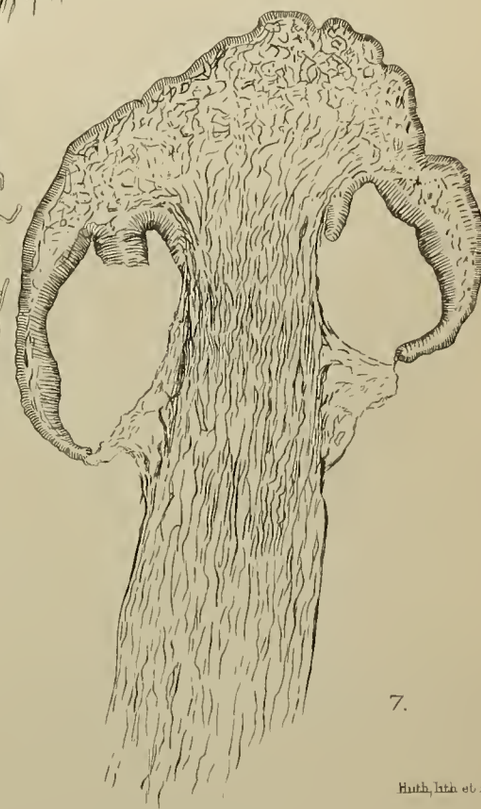


7.

Huth, lith et imp.

Fertilization in Ephedra altissima.

BY

EMILY M. BERRIDGE, B.Sc., F.L.S.

With Plate XXXVI.

IT is somewhat remarkable that, although the development of the prothallium and archegonium in three species of *Ephedra*, *E. helvetica*, *E. trifurca* and *E. distachya*, has been very fully described, and the embryogeny in the first two carefully investigated, our knowledge of the important intermediate stage, that of fertilization, is still very imperfect. In the two cases of fertilization observed by M. Jaccard [1] and Dr. Land [2] the fusion of gametes is so advanced, that the individual nuclei are almost indistinguishable; moreover, in the figure given by the latter, the smallest nucleus, interpreted as the ventral canal nucleus, is evidently the second male, while that which more nearly approaches the egg in size is certainly the ventral canal nucleus.

It therefore seemed desirable to give a short account of some preparations obtained from ovules of *E. altissima*, illustrating the period of development from the germination of the pollen-grain and the separation of the ventral canal nucleus to the formation of the fusion nucleus.

The ovules were gathered, by kind permission of Lady Hanbury, in the garden of La Mortola, near Ventimiglia; most of them were fixed in acetic alcohol and stained with Heidenhain's iron haematoxylin. The material was collected on March 9, 11, and 12, but as it was obtained from five different plants in varying stages of development, a close series was secured. By March 13 all the fertilized ovules contained embryos.

The organization of the ovule in *E. altissima* before division of the central nucleus is almost identical with that of *E. trifurca*, as described by Dr. Land. The nucleus, which is closely applied to the apex of the archegonium, is large and contains a considerable amount of chromatin material collected in the middle, with a clear space surrounding it; the nucleolus also is large and prominent.

[1] P. Jaccard: Recherches embryologiques sur l'*Ephedra helvetica*. Bulletin de la Société Vaudoise, xxx, 1894.

[2] W. J. G. Land: Fertilization and Embryogeny in *Ephedra trifurca*. Bot. Gaz., xliv, 272–290, 1907.

[Annals of Botany, Vol. XXIII. No. XCI. July, 1909.]

While in process of division, however, to form the egg and ventral canal nuclei, it is very inconspicuous owing to the diffuseness of the spindle and slenderness of the chromosomes, which number about twelve. The spindle is parallel to the long axis of the archegonium, and is limited at the sides by a series of vacuoles. The egg nucleus is usually somewhat larger than the ventral canal nucleus, but otherwise, when first formed, they resemble one another very closely. The former retreats into the egg-cell, but the latter remains close to the neck, and, like the synergidæ of the Angiosperms, is either pushed aside or broken up by the entry of the pollen-tube; in cases where no tube reaches the egg-cell it sometimes outlives the egg nucleus itself.

The earlier stages in the germination of the pollen-tubes could be followed within the pollen chamber, for the grains in the majority of cases germinate on the surface of a drop of liquid, the fixed remains of which form a structureless film across the mouth of the chamber. The pollen-grain shown in Fig. 2 occurred in some material of *E. distachya* collected by Miss Sanday in Brittany; it shows the intine escaping from the exine, and indicates that the body cell has already divided, while still within the grain, into two equal gametes, as described by Dr. Land for *E. trifurca*. Fig. 3 *a* shows a slightly later stage in the development of the pollen-tube in *E. altissima*, while in Fig. 3 *b* the tube has elongated considerably; in this case the stalk-cell could not be traced. All these three figures show the two gametes enclosed in a well-marked, but not very dense sheath of cytoplasm, and in this they appear to travel through the neck down to the egg-cell, for in Fig. 4, where the tip of the tube is in actual contact with the wall of the egg-cell, the nuclei still occupy the same relative positions, and some trace of the sheath is still to be seen. In this last case this pollen-tube has been forestalled by another, which has evidently caused great disturbance of the cytoplasm of the egg; the ventral-canal nucleus has been broken up and swept aside by the inrush of the contents of the pollen-tube.

Figures 2, 3 *a*, and 4 are all drawn to the same scale and illustrate the increase in size of the two gametes as they pass down the tube. Their average diameter in Fig. 3 *a* is 10 μ, in Fig. 4 it is 14 μ, while of those represented in Fig. 5 within the archegonium and drawn on a smaller scale, the first sperm (m_1) measures about 19 μ, and the second male gamete (m_2), which is probably beginning to enlarge and degenerate, about 21 μ in diameter. The statement was made in a previous paper[1] that the male gametes are unequal in *E. distachya*; in several pollen-tubes they appear distinctly unequal; the second one, however, is usually very irregular in shape, and shows signs of breaking up into two or more. That fragmentation

[1] E. M. Berridge and E. Sanday. Oogenesis and Embryogeny in *Ephedra distachya*. New Phytologist, vi, 5, 6, 7, 1907.

does occur seems probable from the fact that, more than once, five or six small nuclei are found in the egg-cell near the mouth of the pollen-tube; also it is likely that the small nucleus associated with a male gamete, as represented in Fig. 13 of that paper, is really one of these secondary nuclei.

In several archegonia fusion of the gametes was taking place at the time of the fixing of the ovule. In the preparation represented in Fig. 5 the two nuclei although in contact are quite distinct. The male nucleus is closely granular in structure, and therefore appears darkly stained, the egg contains much larger fragments of chromatin material and shows a clear area just within the nuclear wall. No layer of dense cytoplasm is to be observed round the fusing nuclei, but they are surrounded by delicate radiations of the general cytoplasm of the egg-cell, which extend up to, and enclose the second sperm, which is more vacuolate and slightly larger than the first, probably because it has already begun to degenerate. These cytoplasmic radiations become more marked as fusion progresses, they also surround the daughter nuclei of this nucleus when they are first formed. The ventral canal nucleus still persists, though it also shows signs of degeneration. In another archegonium fusion of the gametes is almost complete, and here again no sheath of denser cytoplasm can be discerned, only a wide band of delicate radiating strands of cytoplasm. In this case the second male gamete has remained close to the neck and resembles the first gamete of Fig. 5 in its size and closely granular structure. The ventral canal nucleus still persists as an irregular diffuse nucleus lying against the apical wall of the archegonium.

Nothing like the post-fertilization activity of the jacket cells in *E. distachya* is to be observed in *E. altissima*; the nuclei divide once by mitotic division as a rule, just after fertilization has occurred in the egg-cell, and then degenerate and die.

A comparison of *E. altissima* with *E. distachya* emphasizes the abnormality of the latter; it is hoped that a fresh gathering of ovules this season may determine whether this abnormal development in the latter is habitual or only due to adverse circumstances.

Summary.

The nucleus of the central cell of the archegonium divides and gives rise to a ventral canal nucleus very similar to the egg nucleus, but slightly smaller. This persists as a rule without degenerating till after fertilization has occurred.

The body-cell of the pollen-grain divides into two equal gametes before the male gametophyte leaves the exine; these travel down the tube enclosed in a cytoplasmic sheath.

Both gametes enter the egg-cell, but no cytoplasm was observed to pass with the first sperm to the egg nucleus. The first male gamete is very small, round, and densely granular; it applies itself to the pointed end of the egg nucleus, which is directed to the neck of the archegonium. A zone of radiating strands of cytoplasm appears round the fusion nucleus.

The nuclei of the jacket cells divide once, and then die after fertilization is accomplished.

EXPLANATION OF FIGURES IN PLATE XXXVI.

Illustrating Miss Berridge's paper on *Ephedra altissima*.

Abbreviations.—ar. egg-cell of archegonium; *n.* neck-cell; *p.t.* pollen-tube; m_1. first male gamete; m_2. second male gamete; *t.* tube nucleus; *s.* stalk-cell; *e.* egg nucleus; *v.c.* ventral-canal nucleus.

Fig. 1. The nucleus of the central cell undergoing division. × 375.

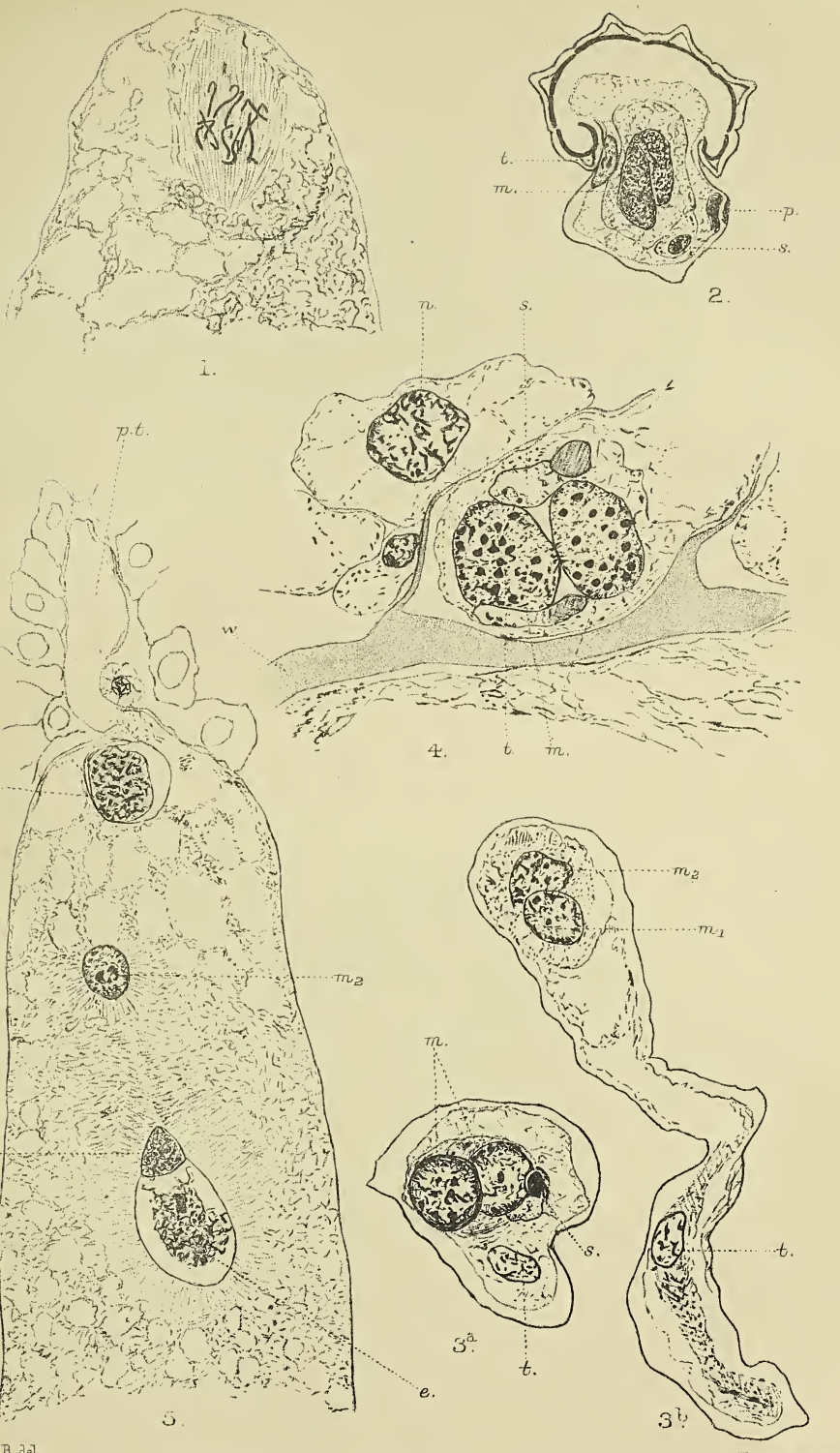
Fig. 2. Male gametophyte of *E. distachya* escaping from the exine of the pollen-grain. × 1000.

Fig. 3*a*. Commencement of pollen-tube in *E. altissima*. × 1000.

Fig. 3*b*. Older pollen-tube with male gametes and tube nucleus. The full length of the pollen-tube is not represented. × 580.

Fig. 4. Tip of the pollen-tube fused with the wall (*w*) of the egg-cell. × 1000.

Fig. 5. Fusion of first male gamete and egg nucleus. × 320.



E.M.B. del.

Huth lith. et imp.

BERRIDGE — EPHEDRA ALTISSIMA

NOTE.

A NOTE ON THE STRUCTURE OF THE CORTEX OF SIGILLARIA MAMILLARIS, Brongn. In a paper[1] on the structure of *Sigillaria scutellata*, Brongn., published last year, we drew attention to some new points of interest in regard to the ribs, leaf-bases, and also the leaf-traces of the stem of this species. We have since had an opportunity of examining the anatomy of an excellent specimen of *Sigillaria mamillaris*, Brongn., recently acquired by the British Museum (Nat. Hist.)[2]. The material was obtained originally by Mr. Lomax from Shore-Littleborough, Lancashire. Mr. Lomax was able to expose the external surface, which, in the case of one rib, proved to be extremely well preserved. Thus, as with our specimens of *Sigillaria scutellata*, there has been no difficulty in determining the species by its external characters.

The structure of the stele of another example, referable to the same species, has been already described by Dr. Kidston,[3] in a preliminary note published some two years ago. No reference, however, was there made to the anatomy of the cortical tissues, or of the leaf-bases.

We do not propose to attempt a full account of the anatomy of the British Museum specimen, but rather to compare its structure on certain points with that of *Sigillaria scutellata*, especially in regard to the ribs and leaf-bases.

Sigillaria mamillaris, like *S. scutellata*, is an Eusigillarian stem of the Rhytidolepis type; that is to say the stems are ribbed, and the ribs are separated by straight furrows. While, however, in *S. scutellata* the bracket-shaped leaf-bases are long and distant from one another, in *S. mamillaris* they are shorter (about 13 mm. in length) and closely approximated. In the former species, the successive leaf-bases of the same rib are separated by small areas of primary cortex. In the latter, the leaf-bases are merely marked off, above and below, by small transverse grooves, partly filled by a growth of cork. These grooves are more or less oval in form at the surface, and are not as broad as the rib. Their breadth decreases as we pass inwards from the surface, and more internally they assume a triangular shape.

Thus the ribs of *S. mamillaris* are interesting as affording a transition stage between a Sagillaria with distant leaf-bases, such as *S. scutellata*, and a Favularian type such as *S. tessellata*, where the leaf-bases are very short but much broader, though equally approximate. The zigzag course of the grooves between the ribs in Favularian species appears to be due to the fact that the leaf-bases are more hexagonal

[1] Arber and Thomas: Phil. Trans. Roy. Soc., Ser. B, vol. cc, p. 133, 1908; Proc. Roy. Soc., Lond., B, vol. lxxx, p. 148, 1908.

[2] Nos. V. 11,403–11,418 in the General Collection of Sections in the Geological Department.

[3] Kidston: Proc. Roy. Soc., Edinburgh, vol. xxvii, p. 203, 1907.

in form, and that their breadth is greater than that of the rib, and equal to, or greater than, their length.

No trace of the ligule has been found in the present specimen. This organ may or may not have occupied the groove between the successive leaf-bases. At present the evidence as to its exact position does not seem satisfactory.

As in *S. scutellata*, the ribs of the present specimen, which are about 9–10 mm. broad, consist largely of phelloderm, of 2–3 mm. in thickness, probably developed on the inner side of a meristematic zone, although no actual evidence of cambial activity can be seen. The prismatic fibres of the phelloderm appear to be rather shorter and smaller than in *S. scutellata*, and we have not noticed any evidence of their being chambered.

External to the phelloderm, a small zone of primary cortex is found in the longitudinal grooves, as in *S. scutellata*. The leaf-bases are likewise bracket-shaped structures, though, as has been already remarked, of a different shape. The external layers of the cortex appear to have been more highly suberized than in our previous specimens, three or four rows of cork-like cells being observed in some cases.

The leaf-trace bundle, when passing through the leaf-bases, also possesses a *double xylem strand*, as we found to be the case in *S. scutellata*. The leaf-traces are usually not preserved very clearly, as is frequently the case in petrifactions of this genus, and it is only in a few instances that the xylem elements can be distinguished. There is some evidence, however, to indicate that the two xylem strands united as the leaf-trace passed inwards through the rib.

The form of the parichnos appears to agree with that of *S. scutellata*. The various stages can be followed, leading up to the state where the two arms unite below the bundle; but we have seen no example in which they are also united above, and completely surround the trace. It may be that the absence of tangential sections, cut deep through the ribs, will account for the lack of evidence of this stage.

In conclusion we find that *S. mamillaris* confirms several of the main conclusions of our previous paper on *S. scutellata*, as regards the structure and relation of the ribs and the leaf-bases. Although the leaf-bases are entirely different in form, the relationships remain unaltered. The discovery of a second case among the Eusigillariae in which the leaf-trace, when passing through the leaf-base, possesses a double xylem strand, closely similar to that found in the leaf *Sigillariopsis*, confirms Dr. Kidston's conclusion as to the leaves of this species, and would seem to imply that this peculiarity may be of frequent occurrence, if indeed it be not the rule, among members of this group of the Sigillarias.

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The Biology of Armillaria mucida, Schrader.

BY

C. E. C. FISCHER,

Indian Forest Service.

With Plates XXXVII and XXXVIII.

ARMILLARIA MUCIDA is a widely distributed gill-fungus wherever the beech occurs. Saccardo (23) gives its habitat as '. . . totius Europae et Americanae federatae, frequens,' and according to McIlvaine (16) its distribution in America extends to the States of North Carolina, Pennsylvania, and West Virginia.

There appears to be no special literature on the subject of this plant, and a perusal of the references to it in textbooks and floras leaves some doubt in the mind as to whether it should be described as a parasite of the beech or as a saprophyte on dead beech wood. Generally speaking, we may take it that the older works treat it as a saprophyte and more recent ones as a parasite, as will be seen from a perusal of the following extracts.

Schrader, 1794 (24), says: 'Hab. ad Fagos emortuas'; Fries, 1821 (10): 'In truncis vetustis, apprime Fagi'; Patouillard in 1886 (19) gave: '. . . sur les troncs pourris'; Lambotte (13) has: '. . . parmi le gazon, sur les racines ou sur les troncs coupés du hêtre.'

On the other hand, we have Massee's account (15) of a successful infection: 'At High Beech, Epping Forest, . . . a healthy branch of a beech having been broken off, the wound was inoculated with the spores of *A. mucida*. At the end of the second season after the inoculation the branch was killed for a considerable distance, and the sporophores of the fungus appeared in abundance. The spores also germinate readily on very small wounds made in the bark.'

It is obvious that sufficient information has not been given, and in the absence of statement to the contrary we are justified in believing that no precautions were taken to exclude infection by other fungi. Some parasitic fungus, e.g. *Polyporus fomentarius*, may have gained entry and prepared the way for *A. mucida* by killing the tissues.

Cooke (6), in 1906, wrote of this same fungus: '. . . so commonly seen on beech trees . . . has been charged with being a wound parasite, capable

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of attacking a healthy branch when broken or wounded and causing death and decay.' This statement is probably inspired by Massee's report quoted above. Sorauer, in 1906 (25), also evidently accorded it parasitic rank, since he refers to it in his work: 'Von der Gattung *Armillaria* wäre in erster Linie *A. mucida* zu erwähnen,' but he adds: '. . . über die Art des Parasitismus wissen wir nichts.' Finally, we have the following authors who make no reference to it as a parasite: Tubeuf (26), Prillieux (21), and Frank (9) omit all mention of it; Saccardo (23) states: 'Hab. ad truncos Fagi'; Gillot and Lucaud, 1891 (12): '. . . exclusivement sur les vieux troncs de hêtres'; Gautier (11), in 1884: '. . . sur les troncs de hêtres languissants ou coupés depuis peu'; McIlvaine (16): '. . . on beech trees and roots . . . pushing up through the soil'; Rabenhorst (22): 'An Buchenstangen.'

It seems, therefore, highly desirable that further tests be applied to determine whether the fungus is a parasite or a saprophyte. The experiments described later clearly demonstrate that it can attack dead beech wood, but so far give no evidence of a true parasitic nature. However, let me at once admit that my experiments were neither sufficiently numerous nor extended over a sufficient space of time to traverse the theory of parasitism. Indeed, I am far from claiming to have proved that *Armillaria mucida* is *not* a parasite, but I am of opinion that more evidence, and of a positive character, is required before we accept that view. It is true that perfectly healthy trees in full vigour bear branches from which the fructifications of this fungus extrude, but in all cases the tissues in the neighbourhood of the tufts of carpophores are dead, and this is also the case when the tufts are seated on diseased parts of the main trunk itself. It should not be forgotten, moreover, that our knowledge of the causes predisposing to disease is almost negligible. Buller (4) failed to infect living maple and horse-chestnut trees with the spores of *Polyporus squamosus,* and in his paper remarks: 'Possibly in nature other organisms serve to prepare the wounds for infection by the fungus.' This 'preparation' might be the killing of the tissues in advance of the fungus under consideration, which then would be an *obligate saprophyte* and no longer even a *facultative parasite*. This should be borne in mind in connexion with Massee's account of his experiment commented on earlier. This point will be dealt with further when we come to discuss the results of the inoculation experiments.

Description of the Fungus.

A description of the fruit body of *A. mucida* will not be out of place, and the following is compiled from those of Cooke (5) and Massee (14):—

Pileus 1 to 4 inches across, flesh thin and almost diaphanous, hemispherical, then expanded, obtuse, often rugulose; glutinous, whitish or tinged with grey (by some authors described as often much darker); often

growing in clusters (Pl. XXXVII, Figs. 1 and 2); gills white, rounded behind, and broadly adnexed with a line-like decurrent tooth; *stipe* 2 to 5 inches long, rather slender, but thickened at the base; white, but base often with sooty squamules, rigid, stuffed; ring near apex of stipe, white tumid; *spores* elliptical, very shiny, 14 to 16 by 8 to 9 μ.

Massee adds: 'Very variable in size. Readily known by the very shiny pileus, which is usually whitish, but sometimes sooty or olive brown. Solitary or caespitose.'

It may be added that there are three vulgar names for this fungus in England: 'Beech Tuft,' 'Beech Agaric,' and 'Clammy Armillaria.'

The descriptions by all other authors that I have consulted agree with that given above, with the exception of the statement as to the shape of the spores. Brefeld (2) refers to them as '. . . fast runden Sporen,' and Patouillard (19) figures them spherical or nearly so. This discrepancy is to be explained in the same way as Münch settled the controversy between Möller and Malenconvié as to the shape of the spores of *Merulius lachrymans* (17). Münch showed that those spores when quite dry are collapsed and boat-shaped, but swell out when moistened, though only by the breath, to an oval outline. I find the same phenomenon in the case of *A. mucida*, and that when moist the spores are almost perfectly spherical with a small insertion papilla and granular contents (Figs. 3 and 4), with an average diameter of 14 μ. The utility of this adaptation for dispersal is obvious, for the convexity when dry, coupled with the lightness due to absence of water, gives a better prospect of transport by wind. Dr. Münch himself showed me a similar process with the spores of other fungi, among them one or two Ascomycetes. It seems probable that it occurs in most, if not all, fungi with smooth-walled spores that depend on wind for dispersal.

Subject of the Investigation.

The object of this inquiry was threefold:—

1. To trace out the life-history of the fungus, at the same time determining its true nature—parasitic or saprophytic.
2. To investigate its action on the wood.
3. To suggest appropriate preventive and remedial measures.

Each of these three will be dealt with separately and in the above order.

I. Life-History.

Methods.—The fruit bodies from which all the cultures were started were obtained from beech trees in Windsor Park on November 18 and 27, 1908. On the second occasion I took a sterilized Petri-dish with me, and one sporophore was placed directly in it. The spores shed on the

bottom of the dish were used for starting cultures, as also were spores obtained in a similar way in the laboratory from the carpophores brought in on the first occasion. These spores were taken off with a sterilized needle and inoculated into nutritive media.

Some of the cultures were started by means of a neat contrivance invented by Dr. Münch and described by him in his inaugural dissertation (18). A piece of cloth (velvet is very suitable) with a circular hole cut out of its centre, of a smaller diameter than the mouth of the vessel that is to contain the culture, is sewn on to a piece of wire mesh. It is best to cut the cloth so as to allow a sufficient overlap to fold back and cover any object that is placed on the wire.

The stipe is cut off a carpophore and after testing it to make sure that it is actively shedding its spores, the cap is deposited on the wire net in such a position that a portion shedding spores comes directly over the hole in the cloth. The whole is thoroughly wetted, and the superfluous moisture is vigorously shaken out in order to avoid any flow of water. With one hand the vessel containing the culture solution is opened, and the wire net with the cap is slipped on with the other with the least possible interval, and is so fitted that the hole in the cloth coincides with the mouth of the vessel. After the lapse of a few minutes, long enough to allow of the deposition of a sufficient number of spores, but not for the drying up of the cloth, the apparatus is removed and the stopper slipped back with the same caution as before. As long as the apparatus is thoroughly damp it is only the spores that are thrown off by the fungus and which pass through the meshes without touching the wire that can fall on the nutritive solution. All spores that touch the damp wire, including foreign ones that may have been previously adhering to any part of it or to the cap, will stick to it and will not fall through. It will be seen that in this way one can secure, practically with certainty, and without previous tedious separate *eliminating cultures*, a quite pure culture. Dr. Münch has employed this method with practically unfailing success in the course of many hundreds of pure cultures, and I have also obtained good results with it on a smaller scale. Dr. Münch, however, will be the first to agree that its results must not be accepted uncorroborated by other cultures started in other ways. Of course, this appliance can only be used with fungi which throw off their spores more or less violently.

The culture solutions employed were the following :—

For observation of the germination of the spores: hanging drops in moist chambers of prune juice, beer-wort jelly, sterilized water, and meat and malt extract jelly.

For observation of the development of the mycelium and as a source from which to obtain other cultures by inoculation: Petri-dishes containing beer-wort jelly and meat and malt extract jelly.

Finally, for the permanent cultures in which the life-history was to be traced, the substratum used was chiefly bread moistened with water only, or with a decoction of horse-dung. Beech twigs and small cubes of beech wood were also made use of.

The permanent cultures were in bulb or Erlenmeyer flasks.

All glass receptacles were sterilized in the dry oven up to a temperature of 165° C. Bread and beech twigs or cubes were similarly heated in the dry oven, the bread until it was toasted a rich brown, and subsequently moistened.

Liquids were placed in the steam sterilizer for at least one hour after strong steaming had begun on not less than three consecutive days. In addition, in order to exclude bacteria, a little acid was added to the liquids.

The manual methods employed do not essentially differ from those advocated by Brefeld (3), so they need not be described in detail. It will be sufficient to add that I took every care to avoid intrusion by foreign bodies and to keep the cultures absolutely pure. Cultures in which impurities—Bacteria, *Penicillium*, *Mucor*—appeared were promptly destroyed. The flasks were stoppered with cotton-wool, which was invariably burnt in a Bunsen flame before removing from or replacing in the mouth of a flask. The interior of the neck of the flask was similarly subjected to the Bunsen flame.

Altogether the following cultures were instituted: 8 hanging drops in moist chambers; 25 Petri-dish cultures in jelly; 12 on jelly or liquids in flasks; 6 on twigs or wood cubes; 2 on bread in large crystallizing dishes under bell-jars; 23 in flasks on bread; and 4 in liquids in test-tubes.

The meat and malt extract jelly was made up as recommended by Professor von Tubeuf as follows:—

Liebig's meat extract	10 gr.
Malt extract	20 gr.
Gelatine	100 gr.
Citric acid	4 to 5 gr.
Water	1 litre.

In some cases a decoction of horse-dung was added to the above, both with and without the gelatine.

Two other solutions were made up as follows:—

I.	Peptone	·25 gr.
	Dextrose	1·25 gr.
	Potassium phosphate	·075 gr.
	Magnesium sulphate	·005 gr.
	Citric acid	1 gr.
	Water	250 cc.

II.	Ammonium nitrate	2·5 gr.
	Potassium phosphate	1·25 gr.
	Magnesium sulphate	·25 gr.
	Citric acid	·5 gr.
	Water	250 cc.

These were used either as jelly, when 25 gr. of gelatine were added, or in the liquid form.

The permanent cultures were started either by sowing the spores directly on the substratum or by inoculation from other obviously pure cultures.

Germination and Subsequent Growth.

In hanging drops of pure water, prune extract, or beer-wort jelly, as well as in Petri-dishes in beer-wort jelly, the spores germinated abundantly and at once, i. e. in less than twenty-four hours. Placed in the solution one morning they were found with germ-tubes on the following morning, twenty-one hours later. No germ-pore was to be distinguished in the spore-wall, and the germ-tube did not emerge at any fixed point, but in varying positions on the several spores. Prior to germination the spores became vacuolated. On germination the vacuoles enlarge, and the protoplasmic contents of the spore pass into the germ-tube (Fig. 6). The protoplasm advances with the end of the hypha, which becomes septate and empty behind. The hyphae produced in pure water, however, formed few septa.

In addition to spores small pieces from the interior of the stipe and the cap of a sporophore were placed on jelly in a Petri-dish. New growth of hyphae appeared from both in two days (Fig. 7).

A small section of the gill of a fruit body was suspended in a hanging drop ; a few spores were scattered among the basidia. These spores germinated and put out normal hyphae, and the basidia and paraphyses also grew out into curiously branching and distorted and multi-septate hyphae (Figs. 8 and 9).

For the first day or two after germination the hyphae remain unbranched, but then fork and ramify rapidly. The hyphae often fuse at the points of contact. There is a marked contrast in the habit of growth when there is sufficiency or deficiency of moisture. In the first case the hyphae are thick, long, and but little branched ; when too dry they remain fine, shorter, and much branched (Figs. 10 and 11). The hyphae often assume somewhat fantastic shapes: abruptly and angularly branched (Fig. 12), or, on the contrary, gracefully curled (Fig. 13).

In the Petri-dishes the mycelium soon showed above the surface of the jelly as a perfectly white felt, which spread rapidly till it covered the whole surface. In course of time, about twenty-five days, the mycelium

assumed an orange hue underneath. Here, as well as in the cultures on jelly in flasks, the jelly was soon liquefied, showing that the fungus exudes some enzyme capable of liquefying gelatine. No crystals were formed as occurs with *Cordyceps* in jelly.

In most of the cultures clamp-connexions appeared on the hyphae (Figs. 14 and 15), in some cases in abundance. They were also seen in the stipe of a carpophore grown in pure culture, and in one case on a hypha in a wood vessel. This traverses Brefeld's assertion: '. . . ohne Schnallen . . .' (2).

No further changes were observable in any of the jelly cultures, whether in dishes or in flasks, or in hanging drops, or in any of the fluids experimented with. No fruit bodies or reproductive organs of any kind presented themselves. It would seem, therefore, that *A. mucida* is devoid of all form of conidia, a conclusion not altogether unexpected. In one rather doubtful case, in a hanging drop of water, something recalling *oidia* formation took place. The chamber had been allowed to dry and was nearly devoid of moisture, and the hyphae broke up into short lengths. This, however, was in one corner only, and I was unable to separate the short rods and test them for germinative power. Though I attach no importance to the occurrence, the fact is here stated for what it is worth. The rods are delineated in Fig. 16.

Attempts were made to stimulate the mycelium to produce conidia by altering the nutriment, but in vain. The amount of moisture, too, was varied; in one case the mycelium being allowed eventually to dry up altogether. The kind and quantity of acid was changed, lactic, gallic, and even oxalic acid being tried. With 0·4 per cent. of the last named the fungus grew just as well as in any of the other solutions.

Spores were also sown in the liquid solutions detailed previously. Though they gravitated to the bottom of the test-tubes, they nevertheless germinated, and the mycelium eventually made its way to the surface of the liquid, where it formed a dense felting.

An attempt was made to grow the fungus from spores, and also the mycelium from another culture, on sterilized elm twigs. No infection took place in spite of repetition, which apparently indicates considerable specialization in favour of beech.

As already stated no difficulty was met with in the germination of the spores when fresh. Some observations were made with regard to the endurance of germinative power. A cap obtained from a beech tree in Windsor Park was placed in a sterilized Petri-dish on November 20, and spores were shed on it, after which the cap itself was removed. The spores in the dish were kept quite dry, and were tested from time to time. By December 29 they had suffered already, for they no longer germinated at once, but took ten days to produce germ-tubes. Spores sown in fluid on

January 5 germinated in a similar period, but in this case it was obviously difficult to make certain of germination at once on occurrence. Sown on the same solution with gelatine added on January 6 they failed altogether to germinate. But on the next day spores were sown on a jelly of meat and malt extract mixed with a decoction of horse-dung and acidulated with oxalic acid, and these germinated in ten days. Attempts made with spores from the same source at later dates were entirely unsuccessful.

This indicates no great endurance of germinative power, and as the fruit bodies ripen from August to December, infection must take place in autumn and the first half of winter; in other words, at a time when there is least sap in the host tree.

The spores formed on the carpophores grown in pure culture (as described later on) were slightly smaller than those found in nature, having an average diameter of 12 to 13 μ as against 14 μ (Fig. 5). They germinated readily in water and in meat and malt jelly within twenty-four hours when fresh. These spores kept in a dry Petri-dish failed to germinate when four months old.

From the jelly cultures single germinated spores and pieces of mycelium were taken to start the permanent cultures on bread.

On the surface of the dense felted mycelium, both on bread and on jelly, beads of a clear brown fluid appeared.

On bread and on wood the fungus first showed itself in the shape of erect white flocculent hyphae about $\frac{1}{4}$ inch long. These soon became denser and formed a thick layer of perfectly white felted mycelium.

Pieces of mycelium grown on bread were found to be capable of further development on a jelly made with 12 per cent. gelatine and pure water, the jelly being liquefied in a few days. Also, in a solution of 20 per cent. glucose, both fluid and in a jelly with 12 per cent. gelatine, similarly inoculated mycelium grew on actively, liquefying the jelly as before.

Sporophores.

We will now follow the development of the fruit bodies in the cultures.

On November 28 a culture was started in an Erlenmeyer flask on beer-wort jelly by Münch's method of direct sowing described above. By the 30th of the same month it was evident to the naked eye that germination had taken place, and by December 24 the whole surface of the jelly was covered with a film of white mycelium. On that date a very small piece of mycelium was extracted and placed in another flask on bread moistened with a decoction of horse-dung acidulated with gallic acid. In two days it became obvious that the inoculation had been successful, and in a few days the white mycelium completely concealed the bread. On January 27 the first signs of fructification appeared, and eight days later the first sporophore attained maturity. Thus from germination of the

spore to the first visible signs of fruiting sixty days elapsed, and in sixty-eight days the life-cycle had been accomplished.

In another flask, on December 15, on bread moistened with sterilized water only, spores were deposited with a needle. The first incipient carpophores showed themselves on January 29. Six days later there was a fully developed fruit body. So that in this case the generation occupied only fifty-eight days.

In other cultures fruit bodies also appeared, but only one more will be specially mentioned here.

Cubes of beech wood were sterilized in the dry oven at a temperature of 165° C. They were inoculated on December 21 with a piece of mycelium taken from a culture in a Petri-dish on a jelly of meat and malt extract with agar. In two days a growth of flocculent white mycelium appeared around the inoculated section. This spread rapidly and covered the surfaces of all the blocks and bound them firmly together. After some time the mycelium in places assumed the orange hue already referred to in connexion with the Petri-dish cultures, and finally, after about five months, became blood-red in patches. Four carpophores arose in this flask, the first appearing on March 10. This gives 79 days from the inoculation, and 109 days from spore to spore. In this case the fruit bodies stood singly and not in clusters as is usual.

In order to ascertain the effect of desiccation on the mycelium, three pieces from the culture in which fructifications appeared on January 29 were taken out on the 27th (i. e. before any signs of fruiting had shown themselves) and were placed in an empty sterilized flask. Twelve days later several incipient fruit bodies were to be seen on each fragment. They were unable, however, to grow further and mature, apparently owing to insufficient nutriment. This seems to indicate that when the mycelium as a whole has reached the fruiting-stage it acquires a stimulus to form fruit bodies, which asserts itself under comparatively adverse conditions.

So as to give them more food the three pieces were eventually transferred on to a jelly of meat and malt extract. In a few days they all three started vigorous vegetative growth, and the jelly was soon completely liquefied. Though the whole surface became covered with mycelium, the incipient sporophores did not develop, but, on the contrary, gradually disappeared, and no signs of new ones presented themselves during the four months for which the culture was kept on. This, coupled with the fact that no fructification ever came up on any of the cultures on jelly, seems to show that such a substratum cannot afford the conditions necessary for the production of fruit bodies. No experiment, however, was carried out with more concentrated solutions than those described in the formulae.

The earliest traces of the carpophores are recognizable as diminutive conical protuberances above the surface of the felted mycelium (Fig. 17).

Usually these arise in clusters of a large number of individuals crowded together. In twenty-four hours, in vigorous growths, a small cap is already differentiated, the protuberances having elongated appreciably (Fig. 18). The increase in size and bulk goes on rapidly, and in three or four days the cap presents a very definite and separate structure; the veil is already stretched out and transparent, allowing the gills to be seen through it (Figs. 19 to 22). In another two or three days the veil has separated off altogether from the edge of the pileus, which then begins to expand, first to a horizontal position, and finally its edges curl up so that the lamellae are exposed laterally (Pl. XXXVIII, Figs. 23 and 24). In another paper I have described the anatomical development of the fruit body (8).

Owing to want of space in these cultures and the consequent approximation of several fruit bodies, compound carpophores are liable to be formed in which three or four distinct ones agglomerate into a single stipe and pileus, the lines of union remaining recognizable.

In this crowded condition some of the sporophores may grow downwards (Fig. 19), though they never do so when space allows.

In one case the fruit bodies appeared as rounded instead of conical protuberances, and eventually had bulbous bases to their stipes (Fig. 25).

The average size of the artificially produced fruit bodies was considerably smaller than those found in nature; in no case did the expanded pileus exceed two inches in diameter. The stipes, too, are rather more slender and elongated. As they were mostly grown in the dark, this was only to be expected. In this connexion a curious feature presented itself. All cultures grown in the dark produced pure white carpophores, which is the prevalent colouring in nature, whereas those grown in the light were invariably dark brown or, at least, a decided fuscous grey, turning somewhat lighter with maturity. Moreover, if when still young the culture was transferred from the light to the dark, or vice versa, the hue changed to that appropriate to the altered conditions. If the transfer was effected at a late stage, however, no appreciable change in tint was observable.

These facts may perhaps be taken to throw some light on the differences in colour noticed by the several authors in the naturally grown fructifications.

The fructifications reached maturity equally well when grown in the dark or in the light, but, on the whole, they were a little less robust when kept entirely in the dark and matured a little less rapidly.

There remains but one more culture to be given special notice. In a bulb flask a bread culture was made in the usual way, but was moistened with a 10 per cent. watery solution of glucose and a small quantity of 4 per cent. lactic acid. This was inoculated with a small piece of bread permeated with mycelium, taken from one of the cultures that eleven days later began to fructify. The inoculation was effected on January 18, and

the usual precautions to ensure purity were observed. It is important to note that in the parent culture no impurity presented itself at any time, and the subsequent development of normal carpophores of *A. mucida* in it demonstrated that the fungus growing actually was the one desired.

In two days growth from the inoculated section was perceptible, and was of the normal type of white mycelium. Before this had spread further than an inch, however, a new description of growth was seen. The flask had been kept in the light by a window, and a plate of smooth tissue of a light brown colour, rather of the hue of many of the fructifications developed in the light, projected itself above the surface of the mycelium. Other similar outgrowths appeared alongside the first, and it seemed as if the tissues producing the fruit bodies were unable to form the usual form of erect carpophores, but were giving rise to a series of folds standing out vertically from the surface (Fig. 26). The mass presented an appearance somewhat recalling that of a tremelloid fructification.

On microscopical examination it was found that the inside of the folds consisted of a palisade layer at right angles to its surface entirely resembling the basidial layer of the normal hymenium. Many spores of the normal shape were scattered about, but none connected with the basidia could be seen, nor even, at first, were any sterigmata to be found. After repeated cutting of sections, however, some sterigmata were seen, which in no particular differed from those of basidia on normal fructifications.

This abnormal growth progressed somewhat slowly, and very gradually spread over half of the available area. Meanwhile the mycelium grew out in the opposite direction, and from the latter, on April 8, the first indication of a normal carpophore came to light. Instead of being erect, however, it projected horizontally from the side of a cube of bread. By April 24 it had grown out to a length of $2\frac{1}{2}$ inches, with the distal $\frac{1}{2}$ inch swollen to a club. This swelling no doubt represented what should have been the cap, but actually no cap was differentiated. The stipe remained prostrate, and the swollen end rested on the bread, where it reverted to vegetative growth. At the base, however, and later on at the apex as well, a mass of incipient carpophores arose (Fig. 27). Some of these secondary fructifications attained a length of $1\frac{1}{2}$ inches, but none produced a cap visible to the naked eye. Sectioned with the microtome a differentiation into primordial pileus and hymenium could be distinguished. No further progress towards ripening a fully developed carpophore was made, and the fruit bodies drooped and degenerated. Some evidenced a tendency to produce tertiary fructifications a little below the apex (Fig. 28); eventually they also either degenerated or began to grow out into vegetative mycelium.

From all the above-mentioned facts it seems certain that the abnormal fructifications, extraordinary as it may seem, can only be very aberrant

resupinate forms of *Armillaria mucida*. Abnormalities of this nature are not altogether unknown in the case of other fungi.

It must be remembered that the inoculation was made from a culture that never showed any trace of impurity and produced normal carpophores, proving its identity. Also, the daughter-culture under consideration produced a mycelium of normal appearance, and on one part of it incipient fruit bodies which entirely resembled those of *A. mucida* appeared, though they failed to mature. Unfortunately, I was unable to separate out the spores from the abnormal fructifications so as to germinate them, nor could I obtain vegetative growth from pieces of the fructification in separate cultures.

In the normal carpophores it was observed that, as laid down by Fayod (7), the spore does not arise apically on the sterigma, but somewhat laterally, and only assumes the central position later on as it approaches maturity (Fig. 29 *a*, *b*, *c*).

Inoculations.

The experiments carried out to test for parasitic habit were of two descriptions: on living trees in the open and on living and dead twigs in the laboratory.

On November 27 a healthy branch about $2\frac{1}{2}$ inches in diameter was selected on a sound beech tree, and two holes some eight inches apart were bored to its centre with a centre-bit, which had been previously sterilized by dipping it first in corrosive sublimate and then in absolute alcohol. Spores taken from a carpophore obtained in the neighbourhood were placed in one of the bore-holes only, the other being left intact. Both holes were closed with grafting-wax. A little further along the same branch a small wound just exposing the wood was made and was also infected with spores, and similarly occluded with wax. The branch was cut off on April 17, on which date it was quite healthy and full of sap. No infection had taken place at either of the points at which the spores had been placed. Spores were still adhering in a mass to the surfaces of the wounds; most had not even germinated, and those germ-tubes that had appeared had failed to effect an entry into the wood. The section of wood containing the inoculated bore-hole was put in a damp vessel on April 19. By June 5 it was found that hyphae had penetrated about 50 μ into the tissues, which though now dead were still full of moisture. The block was then placed in a dry jar. Sixteen days later, the wood being then comparatively dry, it was ascertained that the hyphae had penetrated much deeper and were far more abundant.

On January 11 two further inoculations were made on living beech trees. One rather slender branch on each of two healthy trees was picked out. In each case a twig was broken off so as to leave a jagged surface,

which was then inoculated with a piece of mycelium from a pure culture on bread, on which a carpophore had grown. The wound was bound up with a length of bast that had been previously sterilized in absolute alcohol, which was allowed to evaporate off before binding. As a control, neighbour twigs were also broken off but not inoculated. The twigs were removed on April 17 for examination. In one case there were no signs of infection ; in the other the stub of the twig broken off, half an inch in length, was dead and discoloured throughout, and fungal hyphae could be seen to have penetrated to a depth of about one milimetre into the wood, but nowhere into still-living tissue.

Four beech plants five feet high and of a rather bushy habit were procured and planted out. One of them was inoculated in two places with sections of mycelium grown on bread—on the wound surface where a twig had been broken off, and on a cut on the stem. A control plant was similarly mutilated, but was not infected.

A third bush was grafted at two points with pieces of wood permeated with the hyphae of *A. mucida* taken out of a pure culture, and a control was cut in the same manner, but not grafted. All four inoculation spots were bound with bast steeped in alcohol, and were then smeared over with grafting-wax.

They were cut off on June 22, when it was found that no infection of living tissues had taken place. The stub left on breaking off a twig had died and was dry and discoloured, and in this alone had hyphae penetrated, but only as far as the point to which the tissues had died.

At the end of November some beech sticks, ¾ to 1 inch in diameter, were brought in from Windsor Park while still quite fresh and green. The cut ends were occluded with sealing-wax to hinder the evaporation of the moisture contained, and the sticks were inoculated with spores at places where the bark had been cut away sufficiently to discover the wood. They were then placed in a closed sterilized jar. On March 13 all were dead, no trace of green being revealed on incisions being made. One was cut up, but no signs of penetration by the hyphae could be detected. The remaining twigs were placed in a dish on moist earth, and on examination on May 22 were found to be grown through and through with hyphae.

Two other twigs, each with a side shoot and a bud attached, were inoculated with spores in incisions, and were placed in a jar of water. Early in February the buds had opened and fresh buds had formed, so that both twigs bore leaves, and consequently their tissues were still living. On March 16 one of the twigs had lost its leaves, and its tissues were found to be dead, but the leaves of the other were still fresh. In neither case had infection taken place. The inoculated spots of the dead one were then cut off, and the exposed surfaces were sterilized in the flame. The whole twig was then rubbed over with absolute alcohol, and after being allowed to dry

was reinoculated with pieces of mycelium from a pure culture, in incisions extending to the wood. It was then placed in a vessel of water standing in a stoppered jar. In a short time white mycelium in abundance appeared on the outside of the twig and soon spread around, attaching the twig to the side of the vessel. On microscopical examination it was ascertained that the hyphae had penetrated right to the centre of the wood, and no starch reaction was obtained with iodine (the fungus absorbs the starch, as will be seen later). In places the mycelium had found its way between the epidermis and the cortex, and had separated the two in waved lines, raising the epidermis in a blister-like manner.

The two last described experiments were repeated with the same results, which seems to show that the fungus is unable to effect an entry so long as a certain proportion of moisture in the wood is exceeded, with a corresponding lack of air. This is quite in accord with the results of Münch's researches with *Nectria ditissima*, *Cerastomella coerulea*, and other facultative wood parasites (18).

On sterilized thin unbarked beech twigs, mycelium inoculated from a pure culture grew very rapidly, entering the wood at the cut surfaces. An abundant dense, pure white mycelium overspread the twigs and extended all round and over the inside of the glass receptacle, and eventually it gave rise to two carpophores.

Deductions as to Parasitism.

We can now consider how all the experiments described above affect the question of the parasitic nature of *Armillaria mucida*. We have seen that attempts to grow the fungus as a parasite have failed, but on the other hand it was readily cultivable as a saprophyte on several nutritive media. It could also be grown on dead beech wood, on which it produced fructifications. In fact, the evidence so far available nowhere records the appearance of fruit bodies on living wood, though it is to be seen on dead parts of living trees. It is possible that it is a facultative parasite demanding specially favourable conditions to enable it to attack living tissues. It seems possible that it must first develop saprophytically and then, after a start, during which it has gained vigour, is able to enter upon a parasitic existence. These conditions would be secured at a point where a branch has broken off and the surface tissues have died from loss of moisture, but as yet we have no certain evidence in support.

It may be noted, also, that no one has recorded the occurrence of the fungus on young beech plants.

II. Action of the Fungus on the Tissues.

Methods.—In order to investigate the action of the hyphae on the wood of its host the following methods were employed.

Diseased wood brought in from Windsor Park was sawn into convenient sections and fixed in one of the following solutions :—

Merkel's solution.
Flemming's weak solution.
Absolute alcohol.
Picric acid.

Sections were cut freehand and tested with iodine, chlor-zinc-iodine, phloroglucin, alkalin, &c., and were stained by the methods detailed below.

At all stages sections of sound wood were treated in the same manner for comparison.

The stains employed were :—

Gentian violet and Congo red, as described by Biffen (1).

Ruthenium red and methyl green, first soaking in a solution of 75 per cent. alcohol and 25 per cent. hydrochloric acid and washing in 50 per cent. ammonia.

Safranin and Hoffmann's blue. With this stain after many trials the most satisfactory procedure was found to be the following: The sections were placed in safranin for fifteen minutes and then washed rapidly in 50 per cent. alcohol. They were then left in the Hoffmann's blue for from fifteen to twenty minutes and then washed in water. They were then passed rapidly up to absolute alcohol, and through xylol, and mounted in canada balsam.

Lastly, the diamant fuchsin and light green method was tried and gave the best results, especially for following the course of the hyphae in the tissues.

Disorganization of the Tissues.

The portions attacked can be recognized by the discolouration of the wood, which assumes a dark grey hue with the large medullary rays standing out unchanged (Figs. 30 and 31). Just as Biffen observed with *Bulgaria polymorpha* (1), the medullary rays seem to withstand the attack longer, but eventually they too succumb.

The hyphae apparently disintegrate after they have done their work, for where the wood had undergone considerable change (as described below), few or no hyphae were to be met with, whereas in places in which the hyphae were abundant the decomposition had hardly begun. This can be easily seen by comparing Figs. 32 and 33.

The tests applied showed that there was an early disappearance of the starch, followed by a gradual delignification of the secondary layers of the cell-walls of the wood. These layers become swollen and detached from the middle lamellae, and, owing to the want of space in the lumina in their expanded condition, they are curled up and contorted (Fig. 34). Gradually these disorganized tissues decompose and are absorbed by the hyphae, until only the middle lamellae are left (Fig. 33). In this state the wood is full of air and has become soft and lighter in colour.

The early stages tested with iodine or chlor-zinc-iodine gave no starch reaction. At a later stage with phloroglucin results similar to those obtained by Marshall Ward in the case of *Stereum hirsutum* (28) presented themselves. The middle lamellae stained a deep bright pink, the layers next to them were of a lighter pink, and the innermost swollen layers remained colourless, showing delignification from the lumen outwards. The cells of the medullary rays and of the wood parenchyma retained their lignin intact for a longer period, showing up in the advanced stages more or less bright pink in the midst of colourless fibres, vessels, and tracheids.

In the advanced stages the secondary layers of the cell-walls before their absorption gave a cellulose reaction with chlor-zinc-iodine, demonstrating the reduction to cellulose. This was too general and too complete to be referred to want of lignification as suggested by Potter (20) ; moreover, sound wood similarly tested gave no violet colour.

Evidence in the same direction was obtained with the gentian violet and Congo red stain. The lignified walls took on a blue stain, and those converted to cellulose coloured pink with the Congo red. The reactions, however, were not so very definite as the results obtained by Biffen.

With ruthenium red and methyl green the middle lamellae stained a cherry red, and the secondary layers either remained colourless, especially where much swollen and contorted, or took on a light green tint.

After the disappearance of the starch, products of decomposition of a brown colour are present in the cells of the medullary rays and of the parenchyma. Tested with ferrous sulphate they showed no trace of tannin, and the alkalin test betrayed no oil.

In the cubes of beech wood on which the mycelium had grown in pure culture, no structural change could be detected after two months, and no chemical alteration was revealed by the phloroglucin test. After the lapse of five months, however, the secondary walls were affected. With the diamant fuchsin and light green stains the structural changes described above for advanced stages of attack were verified; the detachment and partial disappearance of the secondary layers being evident.

The course of the hyphae in the wood can be followed in the sections stained with the diamant fuchsin and light green method (Fig. 35). This

stain colours the walls that are still lignified a light pink, with the middle lamellae standing out a darker shade, and the secondary layers remain uncoloured or stain green. The hyphae are stained olive green.

With safranin and Hoffmann's blue the host tissues are pink and the hyphae blue.

The hyphae may pass from cell to cell through the pits, but also bore their own way through the intact walls. The diameter of the hyphae varies considerably, ranging from 2 to 5 μ.

Pieces of a diseased branch that actually bore carpophores of *A. mucida*, and the wood of which was in an advanced stage of decay, were ground up into a powder, which was left to soak in 50 per cent. glycerine for about five weeks. In the filtrate obtained from this, small pieces of sound beech wood were immersed for $2\frac{1}{2}$ months. No structural change was visible, but treated with Schultz's solution, though the starch was found to be practically intact, there were distinct signs of delignification of the secondary layers of the cell-walls, which were stained a more or less deep violet to pale lilac, according to the degree of decomposition, with the middle lamellae yellow. In places, too, the secondary layers were detached from the middle lamellae and contorted, much as was the case in the diseased wood described above, though not to the same extent.

I feel justified, therefore, in concluding that the fungus secretes an enzyme, or enzymes, capable of reducing the starch and of reconverting the xylem to cellulose.

III. Remedial and Protective Measures.

Whatever the eventual conclusions as to the parasitic nature of *A. mucida* may be, it is certain that it causes serious detriment to beech timber, rendering it unfit for anything but fuel of a poor quality. It is possible that it is not a parasite at all, but it is none the less a foe of the beech forest and, like some other xylophile fungi, may require a preparation of the tissues by other organisms or by special conditions before it can avail itself of the nutriment they afford. Therefore, it is advisable to consider what precautions can be adopted to prevent its gaining an entrance into beech trees, and what can be done to eradicate it once it has established a footing.

The taking of active steps against fungoid diseases must ever be controlled by the question of cost. In maladies such as the one under consideration it too often happens that the necessary expenditure is out of proportion to the benefit to accrue. Nevertheless, it will be readily conceived that where beech is grown commercially this fungus, given suitable conditions, might inflict such losses that heavy expenditure on its eradication, and in subsequently protecting the forest against its inroads, would be

amply justified, and that failing drastic action some other species of timber tree would have to be grown in its place.

Prevention is better than cure, and in the case of small parks and avenues it is worth while to protect the trees against the entry of the disease. From its nature it is obvious that the fungus can enter a tree only by means of its spores, and these must alight on a wound from which the bark has been completely removed, so that they can reach the mature wood. Therefore, all wounds that give this opportunity should be promptly painted over with an antiseptic ; one of the compounds of tar is most suitable. Tubeuf (27) has shown that the normal formation of wound tissue is not a sufficient protection, hence the necessity of an antiseptic which, of course, will also keep the wound secure from the attacks of other timber-infecting fungi.

Where the disease has established itself all diseased branches must be cut off and destroyed, and the exposed surfaces must be painted over as before. Care must be taken to cut off the limb at a point which will assure the removal of all trace of diseased tissue. For this purpose, where there is any doubt, the aid of the microscope should be called in.

This eradication may in some cases demand the sacrifice of the whole tree. As an attacked tree may live for many years, owners of ornamental parks may not be prepared to fell. In that case the tree must be kept under watchful observation, and the carpophores must be destroyed as soon as they appear and before they can ripen and shed their spores. This precaution, if strictly enforced, will preclude infection from diseased trees within the property, but will not insure against infection from outside by wind-borne spores. In any case decaying beech stumps or timber should on no account be left lying about, since the fungus may live saprophytically in the wood and develop its fruit bodies, and become a centre of infection.

When one remembers that a single spore may infect a tree, and that according to Buller's calculation (4) a single fruit body of *Polyporus squamosus*, 250 sq. centimetres in area, produces 11,112,500 spores, the importance of a careful watch for the appearance of the fruit bodies will be realized, if one desires to keep the trees free of the disease.

In forests of considerable extent such measures cannot well be applied. Here, the manager must content himself with removing, as far as possible, all diseased trees and stems exhibiting wounds at the periodical fellings. Also, felled timber must not be left lying in the forest. But these are the platitudes of Forest Science, and no stress need be laid on them here.

Summary.

1. *Armillaria mucida* can be grown saprophytically on various substrata: bread, dead beech wood and twigs, jellies of beer-wort, meat and malt extract, &c.

2. The spores germinate at once and abundantly in water and prune juice as well as on the above media.

3. The attempts to infect *living* beech wood failed, and no proof of its alleged parasitism can be preferred.

4. The time elapsing between the sowing of the spores to the ripening of the carpophores in pure cultures varied from 51 to 109 days.

5. The sporophores produced in the pure cultures were identical with those found in nature on beech trees, but the average size was considerably less, and the spores too were somewhat smaller, but were nevertheless fully capable of germination.

6. No secondary spore forms were obtained.

7. Clamp connexions were observed.

8. The fungus secretes enzymes which liquefy gelatine, dissolve starch, and reduce lignin to cellulose.

9. The products of decomposition contain neither tannin nor oil.

10. The preventive measures consist in occluding wounds on the trees with an antiseptic.

11. The remedial steps involve the removal and destruction of diseased parts and of the sporophores before they reach maturity.

I desire here to acknowledge the very great assistance I have received from Professors J. B. Farmer, F.R.S., and P. Groom, D.Sc., who suggested this research, and to express my sincere thanks for much kindly advice, many valuable suggestions, and a great deal of instruction tendered by them. My thanks are also due to Mr. M. Wilson, B.Sc., and to Mr. F. J. F. Shaw, B.Sc., for unfailing kind assistance. The latter gentleman also very kindly took the photograph reproduced as Fig. 23. Finally, I have to acknowledge the kindness of Dr. Somerville Hastings, F.R.C.S., who gave permission for the reproduction of two admirable photographs (Figs. 1 and 2) of *A. mucida*, which have already been published in Messrs. Gowans and Gray's excellent booklet 'Toadstools at Home'.

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EXPLANATION OF PLATES XXXVII AND XXXVIII.

Illustrating Mr. Fischer's paper on *Armillaria mucida*.

Figs. 1, 2, and 24 are from photographs. Figs. 3 to 16, 29 *a*, *b*, *c*, and 32 to 35 from drawings by the author with the camera lucida. Figs. 17 to 23, 25 to 28, 30 and 31 from freehand drawings by the author.

PLATE XXXVII.

Figs. 1 and 2. *Armillaria mucida* on beech. Photographs by Dr. Somerville Hastings, F.R.C.S. 1 × $\frac{1}{10}$: 2 × $\frac{1}{2}$.

Fig. 3. Collapsed, boat-shaped dry spores. × about 475.

Fig. 4. Turgid spherical spores. × about 475.

Fig. 5. Spores from fruit body grown in pure culture. × about 475.

Fig. 6. Germinating spores. × about 260.

Fig. 7. Section from stipe putting out new hyphae in jelly. × about 100.

Fig. 8. Germ tube and vegetative growth from basidium. *a*. Germ tube from spore; *b*, much branched hypha from basidium. × about 475.

Fig. 9. Multi-septate hypha grown out of a basidium. × about 475.

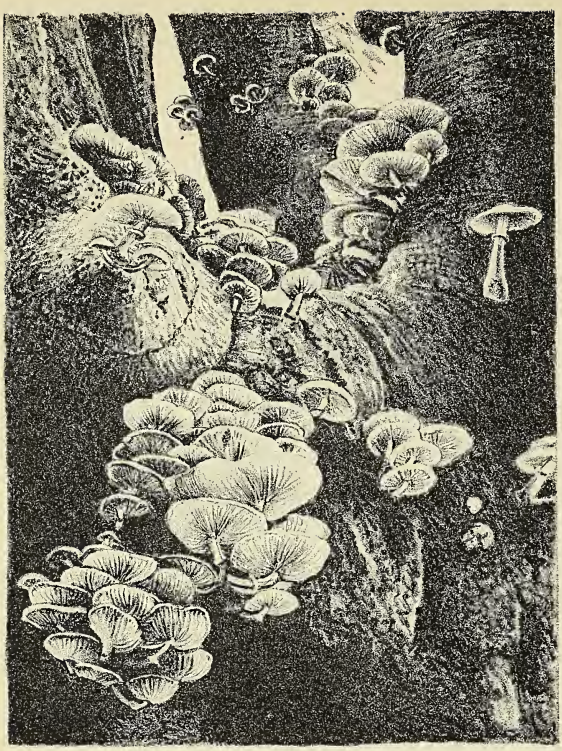
Fig. 10. Hypha in moist jelly. × about 100.

Fig. 11. Hypha in drying jelly. × about 100.
Fig. 12. Hypha in jelly. × about 300.
Fig. 13. Curled hypha in jelly hanging drop. × about 400.
Fig. 14. Clamp connexion. × about 700.
Fig. 15. Clamp connexion on a hypha from a basidium. × about 475.
Fig. 16 *a*, *b*, *c*. Hyphae breaking up into rods—*oidia*? × about 475.
Fig. 17. Group of incipient carpophores on bread. × about $\frac{1}{1}$.
Fig. 18. The same 24 hours later. × about $\frac{1}{1}$.
Fig. 19. The same 24 hours older than Fig. 18. × about $\frac{1}{1}$.
Fig. 20. The same 48 hours older than Fig. 19. × about $\frac{1}{1}$.
Fig. 21. The same 24 hours older than Fig. 20. × about $\frac{1}{1}$.

PLATE XXXVIII.

Fig. 22 *a* and *b*. The same carpophore as in Fig. 21, 24 hours later. Veil much stretched. × about $\frac{1}{1}$.
Fig. 23. Mature carpophore. × about $\frac{1}{1}$.
Fig. 24. Group of carpophores grown on a pure culture of bread. × about $\frac{1}{4}$. Photograph kindly taken by Mr. J. F. J. Shaw, A.R.C.S.
Fig. 25. Young carpophores grown in the light; dark brown with bulbous bases. × about $\frac{1}{1}$.
Fig. 26. Abnormal resupinate fructification. × about $\frac{1}{1}$.
Fig. 27. Aberrant undeveloped prostrate carpophore with secondary carpophores arising on basal portion of stipe. × about $\frac{1}{1}$.
Fig. 28. The same 30 days later. Secondary carpophores arising at apex, and indications of tertiary ones near apex of two of the secondary carpophores. × about $\frac{1}{1}$.
Fig. 29 *a*, *b*, *c*. Basidia showing sterigmata and development of spores. × about 475.
Figs. 30 and 31. Discoloration of wood. × about $\frac{1}{1}$.
Fig. 32. Hyphae in the vessels, cell-walls as yet but little affected. × about 475.
Fig. 33. Advanced stage of attack, hyphae have disappeared and practically middle lamellae only remain. × about 475.
Fig. 34. The secondary layers of cell-walls detached from middle lamellae and swollen and contorted. × about 360.
Fig. 35. Hyphae in cells of large medullary ray. × about 475.

Imperial College of Science and Technology,
South Kensington,
June, 1909.



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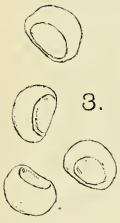

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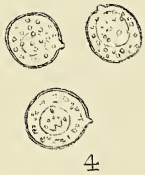

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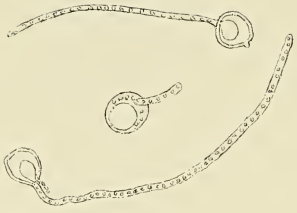

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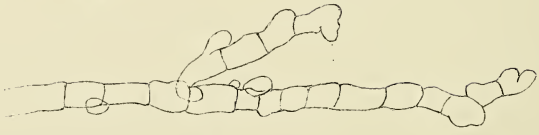

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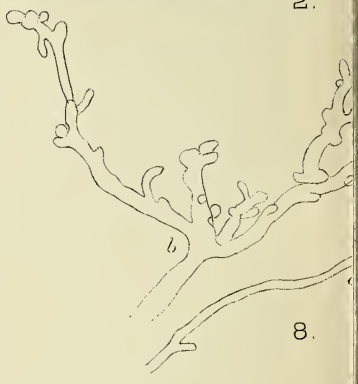

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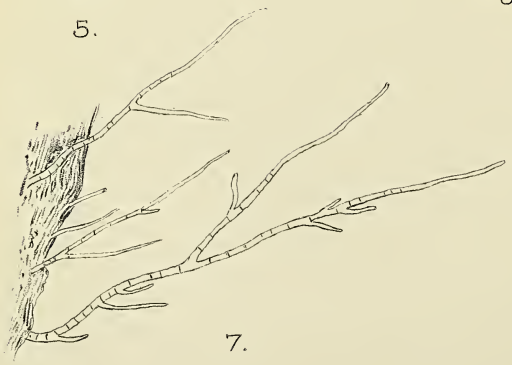

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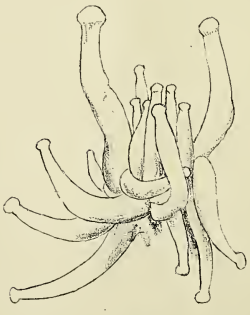

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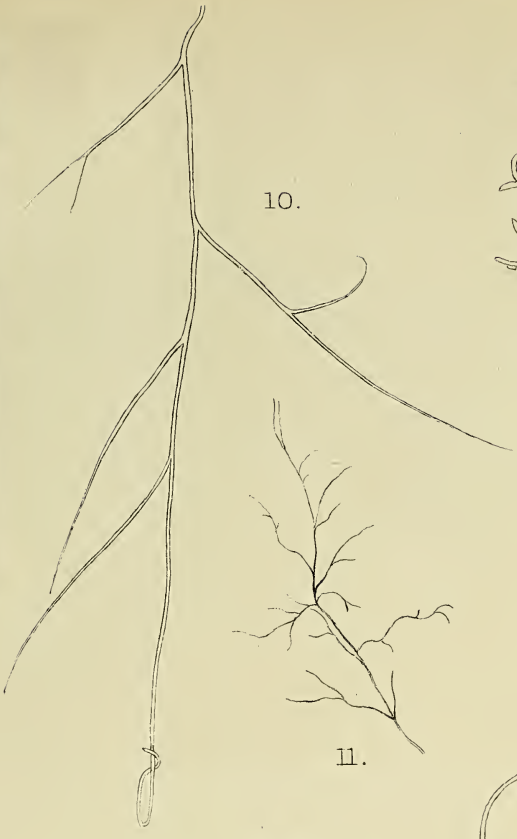

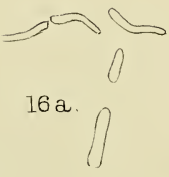

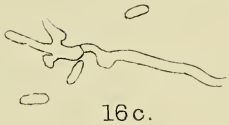

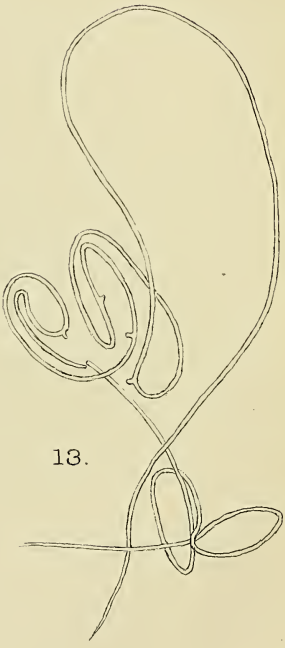

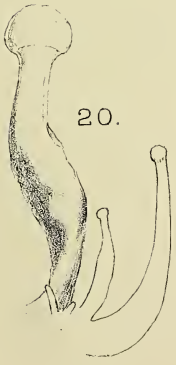

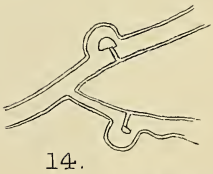

Huth, lith. et imp.

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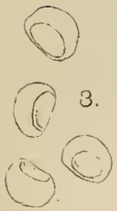

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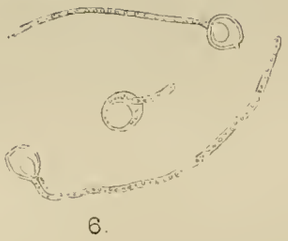

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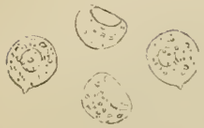

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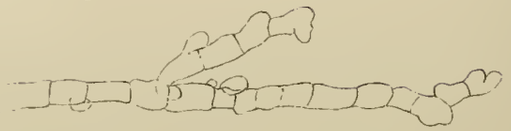

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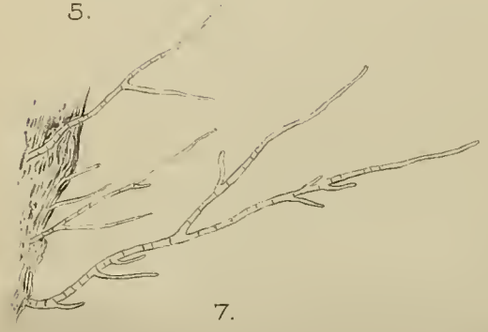

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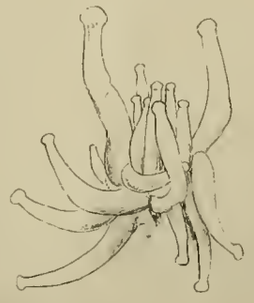

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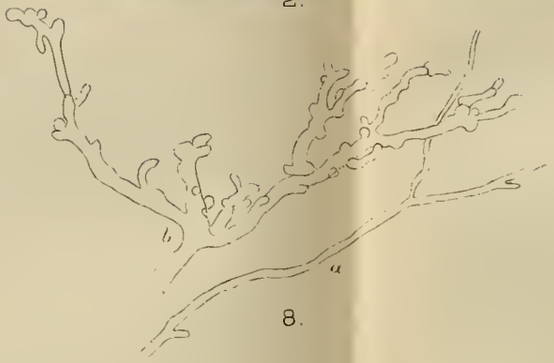

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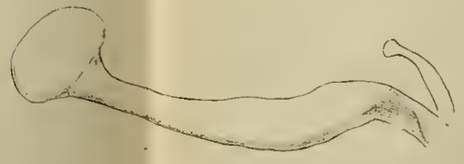

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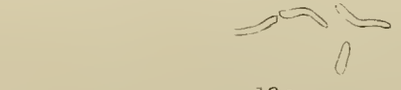

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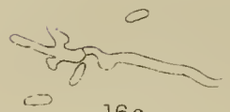

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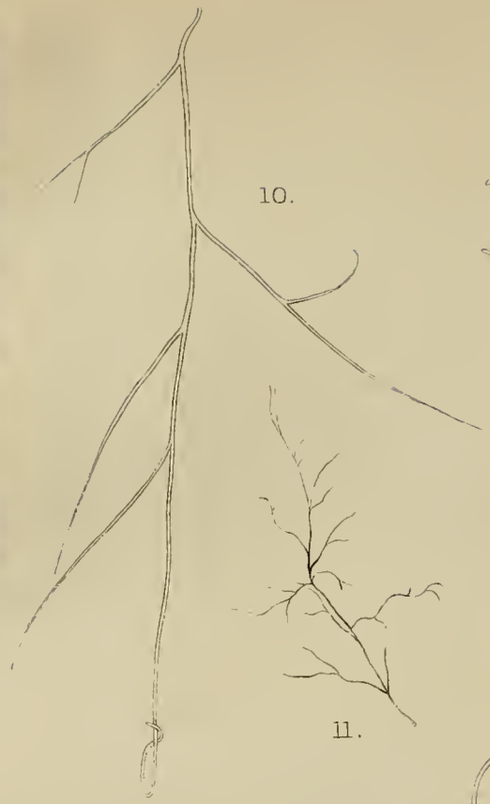

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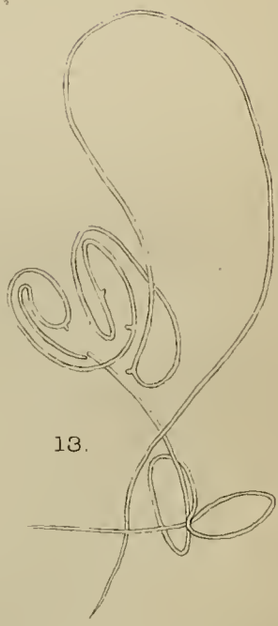

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Huth, lith et imp.

FISCHER — BIOLOGY OF ARMILLARIA MUCIDA.

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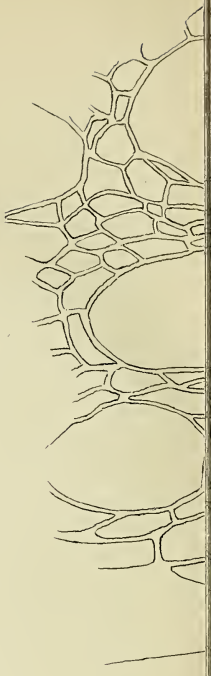

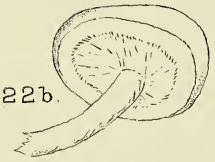

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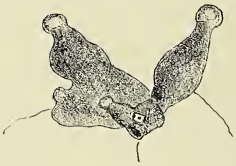

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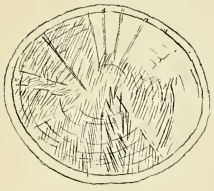

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29c.

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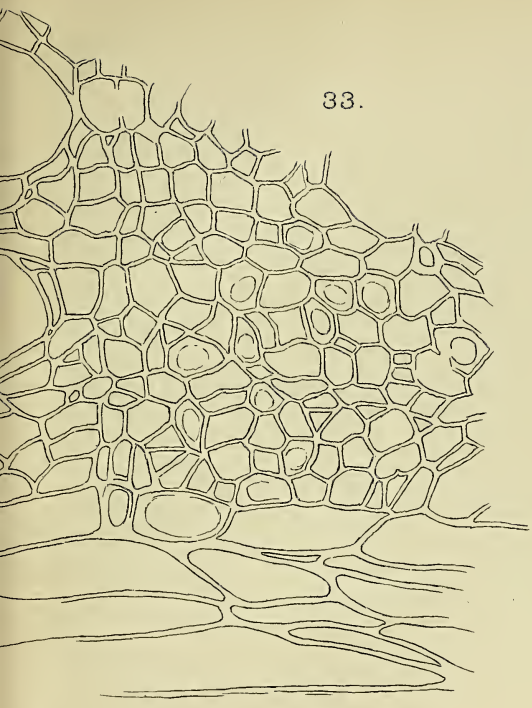
FISCHER — BIOLOGY OF ARMILLARIA MUCIDA.



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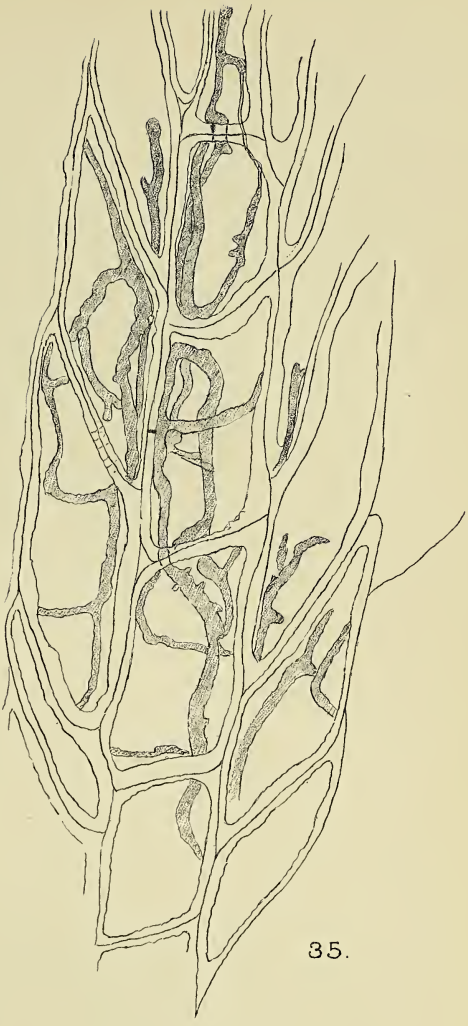

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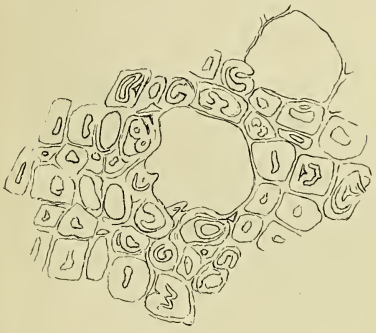

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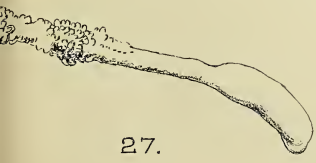

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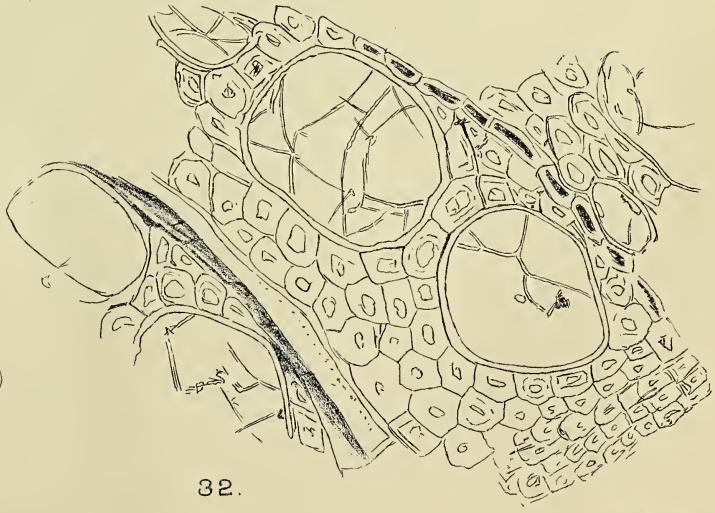

32.

Huth, lith. et imp

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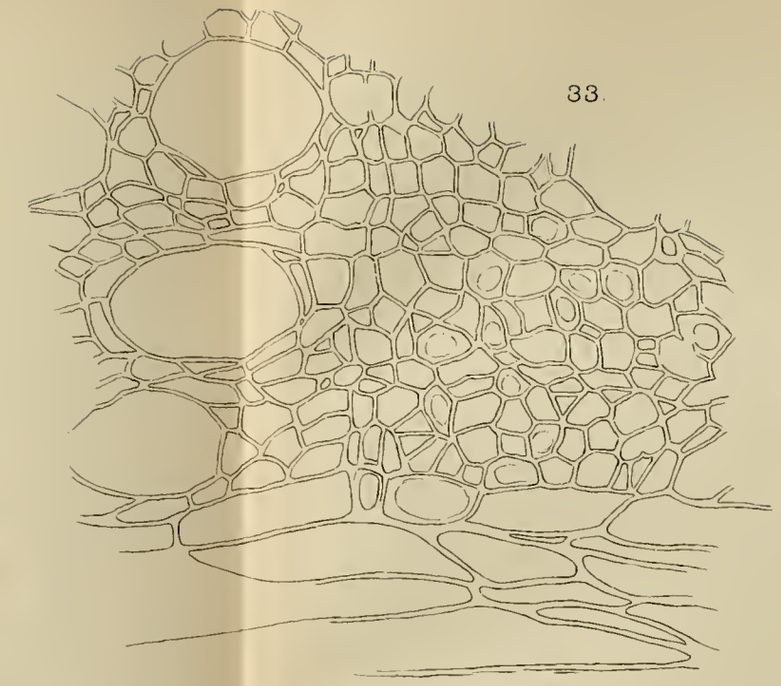

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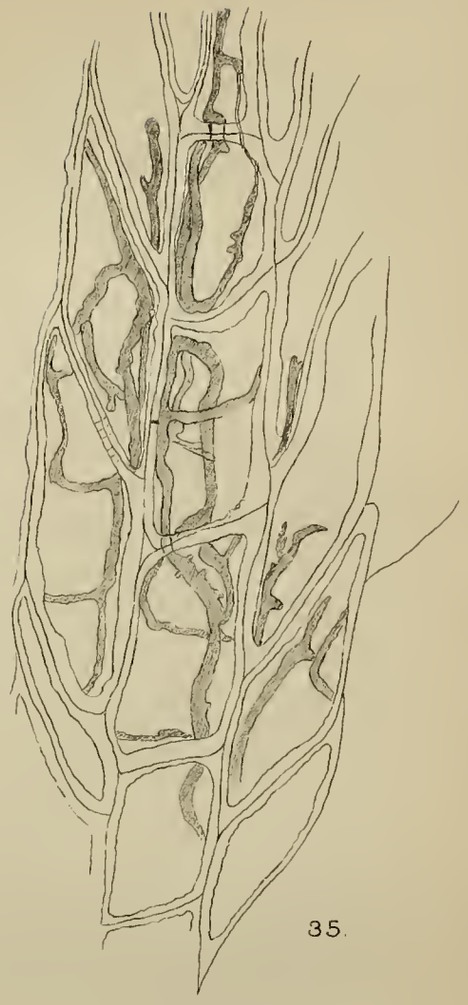

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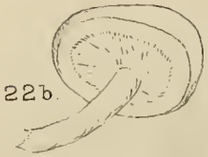

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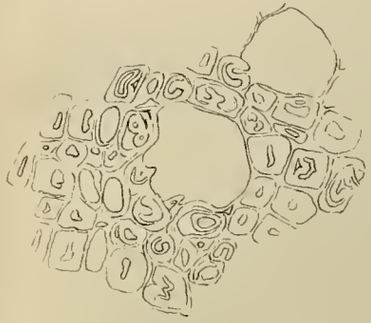

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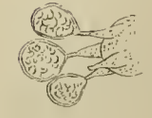

29c.

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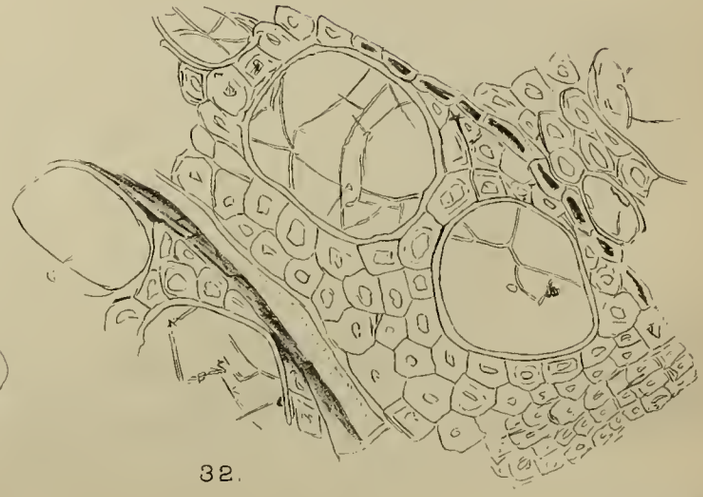

32.

FISCHER — BIOLOGY OF ARMILLARIA MUCIDA.

Huth, lith. et imp.

Further Studies on the Cytology of the Ascus.

BY

H. C. I. FRASER, D.Sc. (Lond.), F.L.S.

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AND

W. E. St. JOHN BROOKS, B.A.

Assistant, Department of Botany, British Museum.

With Plates XXXIX and XL, and a Figure in the Text.

RECENT investigations (8, 9) have shown that the three nuclear divisions in the ascus comprise two processes of chromosome reduction. Such a state of affairs is necessarily correlated with the occurrence of two nuclear fusions, and it has therefore seemed to us worth while to study the ascus divisions in forms in which fusion had already been reported in both the ascogonium and ascus.

For this purpose *Humaria granulata*, Quel., *Ascobolus furfuraceus*, Pers., and *Lachnea stercorea*, Pers., were selected; in each of these reduced fertilization has been observed in the ascogonium, and in each a second and subsequent fusion takes place in the ascus.

Methods.

The apothecia were fixed in various strengths of Flemming's and of Hermann's fluid and were embedded in the usual way. Sections were stained with Flemming's triple stain or with the iron haematoxylin of Heidenhain. In the latter case material was counter-stained with saturated solution of erythrosin in clove oil, or with Licht Grün similarly prepared. The latter substance, though unsatisfactory in aqueous solution, forms a very delicate cellulose stain when dissolved in clove oil; in phanerogamic material it differentiates the cell-wall, leaving the middle lamella unstained.

We are indebted to the Government Grant Committee of the Royal Society for the use of a Zeiss 2 mm. 1·40 apochromatic oil-immersion objective, a Swift panaplanatic condenser, and various other lenses.

Humaria granulata.

Fertilization in *Humaria granulata* was studied by Professor V. H. Blackman and one of ourselves (2) in 1906; no antheridium was observed, but the female nuclei were seen to fuse in pairs in the ascogonium.

The first division in the ascus is characterized by synaptic stages (Pl. XXXIX, Figs. 4, 5), and the chromosomes assume the various forms (V's, Y's, X's, &c.) which indicate the occurrence of meiotic reduction (Figs. 6, 7); the material, however, did not lend itself to the discrimination of the finer details of this process. The number of chromosomes on the spindle in the metaphase is eight (Fig. 8), and eight travel to each pole (Fig. 9).

In the second division four chromosomes appear (Fig. 10), and the same number is distributed to each of the daughter-nuclei (Fig. 11). The third division closely resembles the second, and the number of chromosomes is the same (Figs. 12, 13). A brachymeiotic reduction is thus accomplished, the number of chromosomes present in the heterotype telophase is reduced to half, and the gametophytic number becomes evident. We did not satisfy ourselves of the occurrence of contraction phases in connexion with either the second or the third mitosis, for in *Humaria granulata* no definite spireme was demonstrated at this stage, but the stainable material was aggregated in an irregular mass occupying part, usually the centre, of the nuclear area.

In *Humaria granulata* the divisions in the ascogenous hypha are very clear, and we succeeded in counting the number of chromosomes here also. *Four* are present in the early metaphase (Fig. 1), and in the anaphase four are found travelling to each pole. Four is the gametophytic number in this fungus, and we therefore at first inferred that we were dealing with a specimen in which fertilization had not occurred, or in which, as Claussen (3) suggests, mere nuclear approximation and not fusion had taken place in the ascogonium.

Not only, however, has the fusion of sexual nuclei been already observed in a number of ascogonia (Blackman and Fraser (2)), but the ascus itself shows two reduction stages: first, the synapses and gemini which are generally accepted as characterizing meiosis, and, secondly, in a subsequent division, the numerical change from eight chromosomes to four. Consequently, as the number of our examples of division in the ascogenous hyphae increased, we were led to the conclusion that in *Humaria granulata*, as in *Phyllactinia* (Harper (12)), and in certain other organisms the chromosomes become associated in pairs at the time of fertilization, and the sporophyte therefore shows four bivalent instead of eight univalent chromosomes.[1]

[1] It is perhaps worth noting that the only other possible interpretation, that which denies the fusion in the ascogonium, must also discard the meiotic reduction, for the change in the number

In several of the divisions observed in *Humaria granulata* an elongated, deeply staining body is present (Figs. 8, 10) extending from the group of chromosomes to the nucleolus or to one of the larger of the granules which lie around the nuclear area. The appearance suggests that stainable material is passing towards the chromosomes from the nucleolus, and the irregular or vacuolate character of the latter (Fig. 8) is frequently in keeping with such an inference. Spore-formation was not studied in detail in *Humaria granulata*, but such observations as we have made accord well with the description given of this process in *Lachnea stercorea*.

Ascobolus furfuraceus.

Nuclear fusion was observed by E. J. Welsford (19) in 1907 in the ascogenous cell of this species. In the same year the ascus was studied by Dangeard (4), who described eight chromosomes in the first mitosis, and four in the two which succeed it. When the latter account came to our notice our work on *Ascobolus furfuraceus* was already begun, and we are now able to confirm Dangeard's observations.

The heterotype prophases are here well marked, and it is possible to recognize the synaptic stage (Fig. 15), the double spireme (Fig. 17), and the characteristic forms of the gemini (Fig. 19). Chromosome-formation is preceded by an arrangement of the spireme into loops (Fig. 18) corresponding to the second contraction phase of other forms.

Eight chromosomes are present in the first mitosis (Figs. 20, 22) and four throughout the second and third (Figs. 25, 26, 29, 30). The pairing of the chromosomes which are to pass to different nuclei in brachymeiosis thus takes place, as in *Humaria granulata*, at an early stage. In *Peziza vesiculosa* (8), another species in which the reduced number of chromosomes is apparent on the homotype spindle, a contraction stage was recognized both in the second and third prophase. This was regarded as representing the moment of association of the allelomorphs, and it was suggested that their union did not endure through the resting-stage, since a contraction (and presumably a pairing of the chromosomes) took place in the third as well as in the second prophase. In *Ascobolus furfuraceus* the prophases of the third division are readily studied, and it appears to us that here also the stages represented in Figs. 27 and 28 are best compared to the so-called first contraction of meiosis.

In the majority of Discomycetes examined, either in the course of this investigation or previously, the nuclear area is well marked, and is bounded,

of chromosomes in the later divisions in the ascus is at least as well authenticated as the chromosome number in the smaller nuclei of the ascogenous hyphae. There thus remains a fusion in the ascus and a brachymeiotic reduction, the latter a process nowhere associated with sexual fusion. If this be the case our present specimens of *H. granulata* have progressed a stage further than other investigated forms in the loss of a sexual process.

even at a late stage of mitosis, by a more or less definite line on which the centrosomes lie. In *Ascobolus furfuraceus*, however, the nuclear area, especially in the third division, is ill-defined and shades off gradually into the cytoplasm, and the spindle often lies to one side of or partially outside it (Fig. 30). This arrangement brings the centrosome nearer the centre of the dense mass of cytoplasm which constitutes the aster (cf. Fig. 22), and it also affects the relative distribution of the vacuoles and nuclei.

After the third division the cytoplasm is traversed by irregular series of vacuoles (Fig. 32), which may be termed lines of cleavage. These play an important part in the delimitation of the spore, or rather in the segregation of the masses of spore-plasm. During spore-formation, as during karyokinesis, the cytoplasm near the centrosome takes at first a deeper stain than that which is more remote (Fig. 31). As development proceeds this dense area extends, and the impression is given of an outward flow of some substance which emanates from the centrosome and is capable of producing alterations in the cytoplasm. It has already been suggested (9) that this substance is not improbably an enzyme. The difference between *Ascobolus* and the forms hitherto investigated lies in the much more important part here played by the vacuolate areas which we have termed lines of cleavage. Such lines no doubt originate at the nuclear areas, but we think it not unlikely that their further development is the result of new tensions set up in the cytoplasm by the changes going on around the centrosome. In the forms we have studied the astral rays and the limiting layer of the spore in its early stages are alike less definite than those illustrated by Faull (6).

As development proceeds the dense mass around the centrosome and nucleus becomes more regular, and is bounded by a definite line (Figs. 35, 36), while a similar membrane limits the neighbouring epiplasm. Between the two is a clear space related to the old lines of cleavage, and its boundaries both towards the spore and towards the epiplasm may well be such ectoplasmic layers as delimit an ordinary vacuole. This interpretation is somewhat remote from that given by Harper, though we indorse his conclusion that the aster plays an essential part in spore-formation. In *Ascobolus* the wall of the mature spore is of great thickness, and this fact may be related to the wide vacuolate area which separates the two limiting membranes at an earlier stage.

Divisions in the ascogenous hypha (Fig. 14) were several times observed in *Ascobolus*, but the cytoplasm is dense and contains various granules, and examples clear enough to allow a satisfactory determination of the chromosome number were not obtained. Our observations point to the occurrence of *four* chromosomes at this stage. This would indicate that pairing of chromosomes in fertilization takes place here as in *Humaria granulata*.

Lachnea stercorea.

The development of *Lachnea stercorea* was investigated by one of us (7) in 1907. A trichogyne and antheridium are present, but no longer functional, and, as in *Humaria granulata*, the female nuclei fuse in pairs in the ascogonium.

The nuclei of the ascus are not of great size, but the heterotype prophases are nevertheless remarkable for their clearness and delicacy. The first contraction takes place after the fusion in the ascus (Fig. 38), and is succeeded by the loosening out of a well-marked double spireme (Fig. 39); this subsequently draws itself up into about four loops (Figs. 40, 41) in which the duplication of the chromatin thread may still be observed. Eventually four gemini separate (Fig. 42), contract (Fig. 43), and pass on to the spindle (Figs. 43, 44), where they undergo the usual separation into univalent chromosomes, and four of these pass to each pole (Fig. 45).

It seems to us very clear that in *Lachnea stercorea* the loops of the heterotype prophase each represent a bivalent chromosome, and that the separation on the spindle takes place transversely.

The homotype division is of the ordinary type; the chromosomes are still four in number (Figs. 46, 47), and no 'contraction' phase has been observed to precede the appearance of the spindle. In the third metaphase four chromosomes are present (Fig. 48); they do not divide, and two only pass to each daughter-nucleus (Fig. 49). In these particulars brachymeiosis in *Lachnea stercorea* corresponds to the same process in *H. rutilans* (8), where also the double number of chromosomes is present throughout the second division and in the prophases of the third.

In studying the ascus divisions of this species we frequently observed the occurrence of two long and two short loops in the early meiotic stages (Figs. 40, 41), and of two long and two short chromosomes in the later prophases (Figs. 42, 43), and in the heterotype spindle (Fig. 44). At this time the gemini, since the fusion in the ascus is complete, represent *two* sets of paired allelomorphs. Separation of those which came together in fertilization takes place in the first division, while the distribution of the allelomorphs of the asexual fusion is accomplished in brachymeiosis. In the telophase of the latter division (Fig. 49) a long and a short chromosome can, in favourable cases, be distinguished at the pole.

We were not prepared to attach importance to any of these cases separately, but the frequent recurrence of the long and short chromosomes, and of the long and short loops in the earlier meiotic stages seems tous not without significance. Moore and Arnold (14), investigating the meiotic phase in man and other animals, have found that the forms of the gemini are constant in all spermatocytes of a given species. Baumgärtner (1) also in two species of cricket finds recurrent differences in

the forms of the chromosomes. These differences he regards as indications of the fact that each type of chromosome forms the physical basis of a different set of characters. *Lachnea stercorea* is an instance among plants of a corresponding arrangement.

The ascogenous hyphae (Fig. 37) in *Lachnea* are small, and we have not been able to count the chromosomes.

In spore-formation in *Lachnea* the lines of cleavage are not so much in evidence as in *Ascobolus*. The usual dense and presumably altered mass of cytoplasm is present around the centrosome (Fig. 50), and, as usual, it increases in size (Fig. 51), and constitutes the spore-plasm. It is traversed and defined in *Lachnea* by astral rays which are finer but more numerous and better marked than in *Ascobolus*. Vacuoles corresponding to the lines of cleavage are, however, not without effect, and in the stage shown in Fig. 52 a clear space surrounds the aster and perhaps helps to cut out the lower part of the spore.

Discussion.

The observations detailed above show that in each of the forms investigated two reduction-divisions take place. The first mitosis in the ascus has in every case the familiar characters of the heterotype division, and reasons have been elsewhere adduced (Fraser and Welsford (9)) for regarding this type of reduction as associated with fertilization or its equivalent. The second division follows rapidly on the first, and—at least in *Humaria rutilans* and *Lachnea stercorea*—is without special features; it seems justifiable therefore to accept it as homotype, and as completing the longitudinal fission of the spireme initiated in the heterotype prophase.

The third division differs little from a vegetative mitosis, but in its telophase the number of chromosomes is seen to be half that present on the heterotype spindle, consequently the third division has been recognized as bringing about a second reduction, and has been termed brachymeiosis. This standpoint has recently been criticized by Strasburger (18); he thinks it unlikely that two reductions should take place in the ascus, holding it more probable that, as Claussen [1] (3) suggests, there is only one fusion in the life-history of Ascomycetes. He doubts that reduction would take place in one division or without a contraction phase. It is, therefore, of interest that in some of the forms described both at this time and in a previous paper (9), the third division is initiated by a stage comparable to the 'first contraction' of meiosis. Where this occurs, the reduced number

[1] Claussen's view has recently received confirmation from Schikorra (17), who has seen male nuclei enter the ascogonium of *Monascus* but considers that their association with the female nuclei is of the nature of an approximation and not a fusion. Like Claussen he considers that the associated nuclei travel in pairs up the ascogenous hyphae and fuse in the ascus. He brings forward no evidence as to the reducing divisions in the ascus and his evidence with regard to the absence of fusions in the ascogonium is of course entirely negative.

of chromosomes is apparent in the prophase as well as in the anaphase, the chromosomes having become paired at the beginning of the third division. In yet another species the pairing takes place in the second prophase, and when this happens, two divisions are concerned in brachymeiosis. In such cases the possibility is not excluded that the second reduction is accomplished in the homotype, and that the chromosomes of the second telophase as well as those of the third are actually univalent. Nevertheless such a state of affairs appears to us improbable, since the ordinary rôle of the homotype is clearly to separate the products of a longitudinal fission occurring in the heterotype spireme. If, in certain cases, the second division is brachymeiotic, this separation must be delayed till the third division, and there seems no sufficient reason to suppose so radical a divergence in the reduction processes of closely related forms.

A yet more essential difference between meiosis and brachymeiosis lies in the occurrence of a second contraction in meiotic reduction and its absence in the simpler process. If this phase be indeed the moment of interchange of material between the allelomorphs (Fraser and Welsford (9)) its absence in brachymeiosis may well indicate that this type of reduction and the corresponding asexual fusion have little effect on the forms in which they occur. Where the chromosomes are paired throughout the second division and in the prophases of the third, as in *Ascobolus furfuraceus*, another opportunity for the transfer of material may exist, but even this is absent in *Lachnea stercorea* and *Humaria rutilans*, where after meiosis the allelomorphs are at no time visibly associated.

The fact that reduction in the number of chromosomes occurs after meiosis is complete has received confirmation not only from the work of Maire (13) and Guillermond (11), but more recently from Dangeard (4), who can scarcely be regarded as biassed in favour of a series of events which implies two fusions. He has recorded the numerical changes in question in *Ascobolus furfuraceus*, and is of opinion that they take place also in *Pyronema confluens*. In neither form, it must be added, has Dangeard found a sexual union in the ascogonium, but he has confined his investigations to the occurrence of a male organ, and has ignored the possibility of such a fusion in pairs of ascogonial nuclei as takes place in *Humaria granulata*.

The evidence on this question has been discussed in earlier papers ; it seems unnecessary to enter again into an enumeration of the species in which two successive fusions have been seen.

The Pairing of the Allelomorphs.

Recent investigation has dealt extensively with the method in which the premeiotic chromosomes conjugate to form the gemini of the heterotype

prophase. According to a considerable body of workers the chromosomes become joined end to end, and the early duplication of the spireme is due to fission; this view was elaborated by Farmer and Moore (5) in 1905, and has again lately received confirmation in the work of R. R. Gates (10). Gates finds the premeiotic chromosomes of *Oenothera rubrinervis* arranged in linear series and separated by delicate threads of linin. From this spireme the chromosomes break off in pairs, each pair forming one of the gemini which are thus half as numerous as the chromatin masses on the spireme.

Other investigators, including Strasburger, Grégoire, and their pupils have regarded the paternal and maternal spiremes as arranged side by side in parallel lines; the chromosomes are held to conjugate laterally, and to undergo a more or less intimate union during the so-called synaptic or first contraction. Notably Overton (15), studying the pollen mother-cells of *Thalictrum purpurascens* and some other species, has described a series of double bodies lying along the spireme; he regards these as gemini each made up of two premeiotic chromosomes lying side by side.

It is irresistible to compare with such bodies the chromosomes figured by Gates, and at first sight they appear to be strictly comparable, but it is important that while Gates's structures are said to be twice as numerous as the chromosomes of the homotype telophase, Overton's are described as equal to these in number. In other words, the heterotype prophase of *Oenothera* shows $2n$ chromosomes arranged in linear series and separating two and two, while in *Thalictrum* there appear to be two spiremes with n chromosomes each, and these lie side by side. If both these accounts be correct it must be inferred that the chromosomes become associated in the one case end to end, and in the other laterally. Gates has further shown that in *Oenothera* hybrids the union of fourteen and seven chromosomes in fertilization gives rise to ten or eleven 'gemini' $\left(\frac{14+7}{2}\right)$ in the heterotype prophase. He points out that this could scarcely occur if the paternal and maternal spiremes lay side by side. On the other hand in *Drosera* (Rosenberg (16)) a form with twenty chromosomes crossed by one with ten gives thirty in the sporophyte, and twenty, ten large and ten small $\left(\frac{10+10}{2}+10'\right)$, in meiosis. This is not inconsistent with lateral union, but there seems no reason why it should not equally be the result of association end to end, and it is suggestive in this connexion that the bivalent chromosomes are of greater length than the others.

In the Discomycetes hitherto investigated the chromosomes were found to become paired end to end, and our present studies confirm this conclusion. In Mildews, on the other hand, Harper (12) has shown that the chromosomes are recognizable throughout the resting-stages, and remain

attached by one end to the centrosome. On nuclear fusion the centrosomes unite, and the chromosomes lie parallel for a time, and in that position fuse in pairs. Harper's studies relate primarily to the fusion in the ascus.

It is remarkable that in Mildews nuclear fusion, whether sexual or asexual, appears to be followed directly by the pairing of the chromosomes; thus the gametophytic number of chromosomes in *Phyllactinia* (Harper 12)) is eight, yet eight appear in the ascogenous hyphae after fertilization and eight again in the ascus between the subsequent asexual fusion and meiosis.

Similarly in *Humaria granulata* union of the chromosomes takes place after fertilization; we have unfortunately no evidence to bring forward as to the method by which this is accomplished, but the association is so intimate that on the spindle of the ascogenous hypha the double nature of the pairs cannot be detected. In *Phyllactinia* a pairing of the already bivalent chromosomes to form quadrivalent bodies follows the fusion in the ascus; in *H. granulata* this does not occur, but the chromosomes of the asexual fusion remain apart till the heterotype division is complete: they then pair in the prophases of the homotype.

Union of the chromosomes brought together by the fusion in the ascus takes place at a corresponding stage in *Ascobolus furfuraceus* and *Peziza vesiculosa*, but in *Otidea aurantia* it is delayed till the beginning of the third division. In *Lachnea stercorea*, as in *Humaria rutilans*, no visible pairing of the chromosomes occurs in connexion with brachymeiosis.

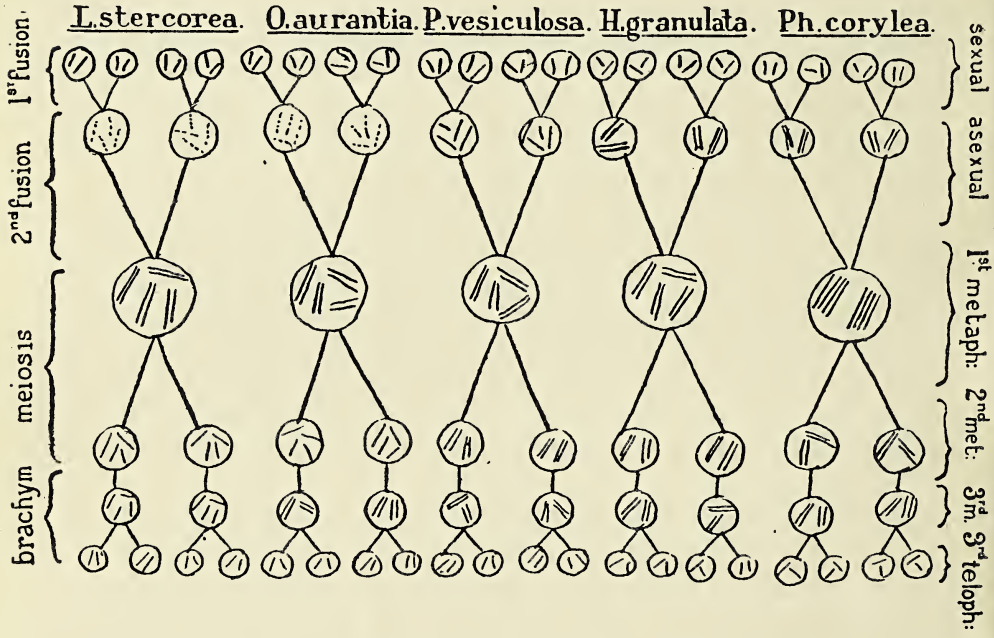
The accompanying Text-figure shows diagrammatically the relation between the forms described in this paper and some of those previously studied. For the sake of uniformity we have represented the minimum number of chromosomes as two throughout.

There is thus a remarkable lack of uniformity in the extent of chromosome union which precedes brachymeiosis. This may be due to the fact that the two nuclei which fuse in the ascus are always the products of development of a single spore—or at most, if a functional antheridium develop on a different mycelium from the ascogonium, of a single pair of spores—and they are often much more closely related. Moreover, they have been subjected to the same environment, and it appears therefore justifiable to suppose them identical. Under these conditions interchange of material between the chromosomes can have little effect, and the intimacy or duration of their union seems to be without importance.

Spore-Formation.

The divergent views held on the method of spore-formation have been enumerated in earlier papers (8, 9). In this connexion the chief interest of our present investigation lies in the important part played by the vacuolate

areas or line of cleavage in *Ascobolus*. We find here a differentiation into dense and vacuolate areas such as is described by Faull (6), though we find also that the centrosome and aster are essential to the development of at least the denser portions. For *Neotiella albocincta*, Faull describes the spore as delimited by a 'curved hyaline line' which may well correspond to our line of cleavage in Fig. 38 or 34. Later he distinguishes two plasma membranes, one of which bounds the spore-plasm, and the other the epiplasm in which the spore lies. This also accords with our observations. But while Faull does not figure an aster after spore-formation has begun, our investigations lead to the conclusion that the changes observed in the cytoplasm are due to a reaction which is set up in the neighbourhood of the



centrosome, and which, by producing new tensions in the ascus, eventually gives rise to the lines of cleavage themselves.

In *Ascobolus furfuraceus* it may be suggested that the altered substance streaming out from the centrosome runs through or forms part of the hyaloplasm, and leaves the cytoplasmic reticulum comparatively unaltered; the same is perhaps the case in Faull's species. In *Lachnea stercorea*, on the other hand, the astral radiations are well marked, and this species, which appears to us quite comparable to *Ascobolus*, thus approach the forms studied by Harper. We have, however, obtained no evidence of a lateral fusion of astral rays to form a membrane such as he describes, but we hold rather that the rays indicate the direction of flow of altered

substance from the centrosome. It was suggested in an earlier paper (9) that such changes might be due to the action of an enzyme.

There appears to exist a somewhat complete gradation of forms from those in which the activity of the centrosome is the only force at work to those where vacuoles delimit—or help to delimit—the spore. In the latter cases we are inclined to regard the changes around the centrosome as ultimately responsible also for the vacuoles.

Summary.

1. The divisions in the ascus have been investigated in *Humaria granulata*, *Ascobolus furfuraceus*, and *Lachnea stercorea*; in each of these species previous investigations showed that a pseudapogamous fusion occurs in the ascogonium, and a subsequent asexual fusion in the ascus.

2. In each the first division in the ascus is heterotype, the second homotype, and the third brachymeiotic. Two reductions in the chromosome number thus occur in the ascus of each species, and afford independent confirmation of the two successive fusions previously described.

3. The number of chromosomes in the first division in the ascus is eight in *Humaria* and *Ascobolus*, and four in *Lachnea*. After brachymeiosis is complete there are four chromosomes in *Humaria* and *Ascobolus*, and in *Lachnea* two.

4. Pairing of the chromosomes which are to separate in brachymeiosis takes place in the second prophase in *Humaria* and *Ascobolus*, but does not occur in *Lachnea*.

5. In *Humaria* the chromosomes brought together in fertilization are found to be paired in the division preceding meiosis.

6. The meiotic prophases were studied in some detail in *Lachnea*; the parts of the bivalent chromosome are united end to end, and separate transversely.

7. In the first ascus division in *Lachnea* two long chromosomes and two short ones may be constantly recognized, and in the third telophase, after brachymeiosis is complete, one long and one short chromosome.

8. The delimitation of the spore in *Lachnea* depends on altered substance flowing out from the centrosome. This is less evident in *Ascobolus* where vacuoles play an important part in spore-formation.

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19. Welsford, E. J.: Fertilization in *Ascobolus furfuraceus*. New Phyt., vi, 1907, p. 136.

EXPLANATION OF PLATES XXXIX AND XL.

Illustrating Dr. Fraser's and Mr. Brooks's Paper on the Cytology of the Ascus.

Humaria granulata.

Fig. 1. Metaphase in ascogenous hypha. × 1900.
Fig. 2. Early anaphase in ascogenous hypha. × 1900.
Fig. 3. Telophase in ascogenous hypha. × 1900.
Fig. 4. Definitive nucleus of ascus, first 'contraction'. × 1900.
Fig. 5. Definitive nucleus of ascus, second 'contraction'. × 1900.
Fig. 6. Gemini of heterotype prophase. × 1900.
Fig. 7. Gemini at time of spindle formation. × 1900.

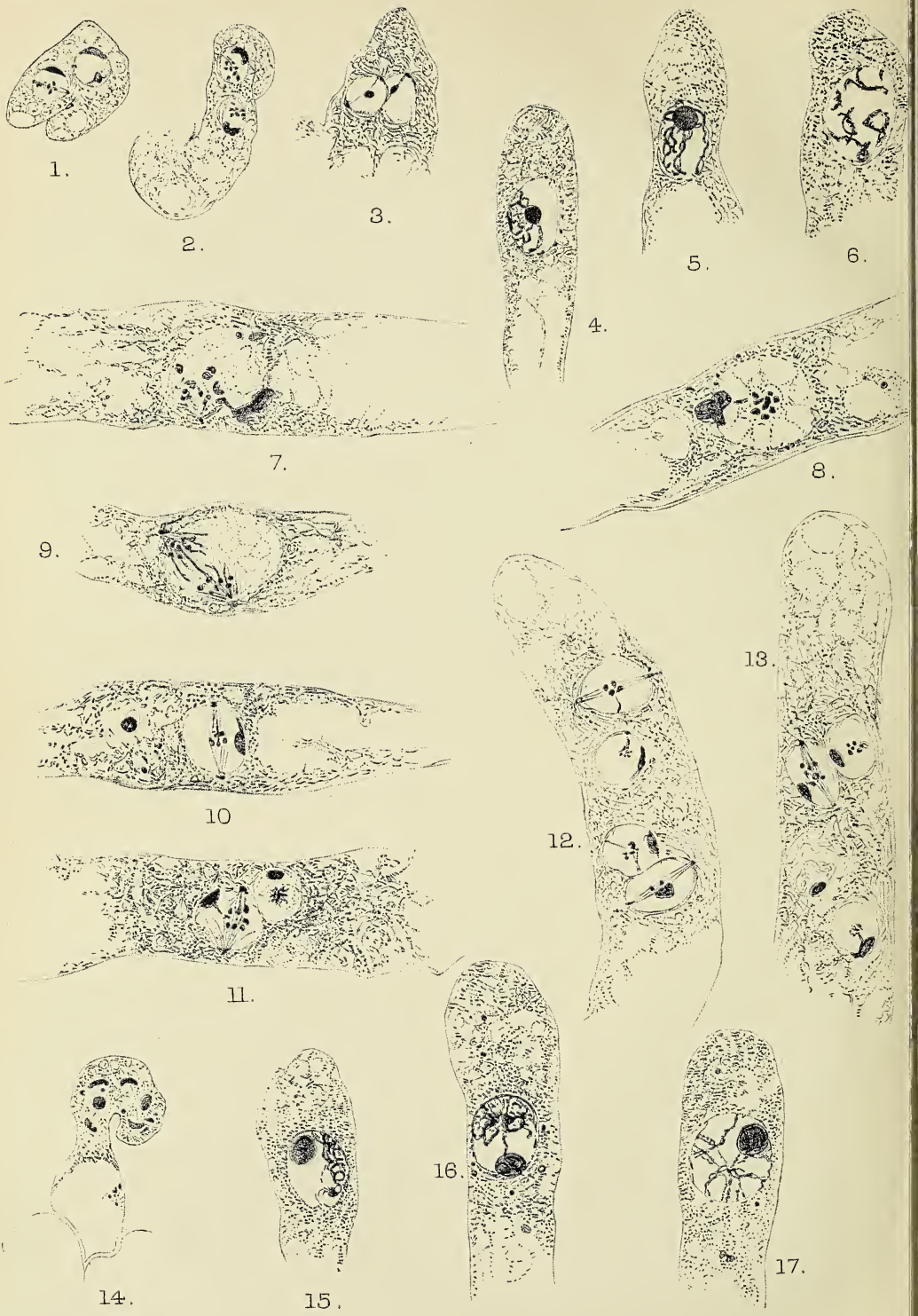
Fig. 8. First division in ascus, equatorial plate showing eight chromosomes. × 1900.
Fig. 9. First anaphase. × 1900.
Fig. 10. Second metaphase. × 1900.
Fig. 11. Second anaphase. × 1900.
Fig. 12. Third metaphase. × 1900.
Fig. 13. Third anaphase, four chromosomes passing to each pole. × 1900.

Ascobolus furfuraceus.

Fig. 14. Mitosis in ascogenous hypha. × 1900.
Fig. 15. Definitive nucleus of ascus, stage of first 'contraction'. × 1900.
Fig. 16. Later stage of same; spireme beginning to spread through nuclear area. × 1900.
Fig. 17. Definitive nucleus of ascus; duplication of spireme. × 1900.
Fig. 18. Definitive nucleus of ascus; formation of loops of second contraction phase. × 1900.
Fig. 19. Gemini of heterotype prophase. × 1900.
Fig. 20. Later stage in development of spindle of first mitosis; eight chromosomes. × 1900.
Fig. 21. First metaphase; gemini on spindle. × 1900.
Fig. 22. First anaphase. × 1900.
Fig. 23. First telophase; around the centrosome is a dense area separated by a vacuolate space from the rest of the cytoplasm. × 1900.
Fig. 24. Second prophase; chromatin lying to one side of the nuclear area; an unusually large ascus. × 1900.
Fig. 25. Second metaphase. × 1900.
Fig. 26. Second anaphase. × 1900.
Fig. 27. Third prophase; lateral aggregation of chromatin. × 1900.
Fig. 28. Later stage of same; from ascus visible in same field of microscope as that of Fig. 27. × 1900.
Fig. 29. Third metaphase. × 1900.
Fig. 30. Third anaphase; four chromosomes passing to each plate. × 1900.
Fig. 31. Third mitosis; late telophase showing nuclear beak, centrosome, and aster. × 1900.
Fig. 32. Later stage; development of lines of cleavage in connexion with vacuoles in cytoplasm. × 1900.
Fig. 33. Same; later stage. × 1900.
Figs. 34, 35. Same; still later stages. × 1900.
Fig. 36. Young spores in ascus; inner spore wall is nearly complete; outer wall is defined by limiting layer of cytoplasm beyond old line of cleavage. × 1900.

Lachnea stercorea.

Fig. 37. Division in ascogenous hypha. × 1900.
Fig. 38. Definitive nucleus of ascus; stage of first 'contraction'. × 1900.
Fig. 39. Definitive nucleus of ascus; longitudinally split spireme. × 1900.
Fig. 40. Second contraction; the four loops show longitudinal fission in places. × 1900.
Fig. 41. Later stage of same; two loops appear long and two short. × 1900.
Fig. 42. First prophase; four gemini. × 1900.
Fig. 43. First prophase; passage of contracted gemini on to spindle. × 1900.
Fig. 44. First metaphase. × 1900.
Fig. 45. First anaphase; four chromosomes travelling to each pole. × 1900.
Fig. 46. Second metaphase; section parallel to long axis of ascus. × 1900.
Fig. 47. Second anaphase; ascus is cut obliquely so that nuclear area is viewed at about right angles to that of Fig. 46. × 1900.
Fig. 48. Third metaphase; one of the nuclei is shown in polar view. × 1900.
Fig. 49. Third telophase; in the second nucleus from the top one of the chromosomes appears shorter than the other. × 1900.
Fig. 50. Early stage of spore formation, showing nuclear beak centrosome, radiations, and near one of the asters, a vacuolate area. × 1900.
Fig. 51. Same; more advanced stage. × 1900.
Fig. 52. Delimitation of spores; vacuolate lines, comparable to the lines of cleavage in *Ascobolus*, are present between the aster and the epiplasm. × 1900.



FRASER AND BROOKS — HUMARIA AND ASCOBOLUS.

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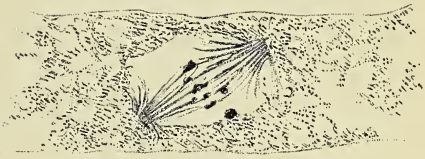

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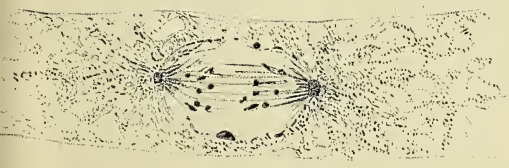

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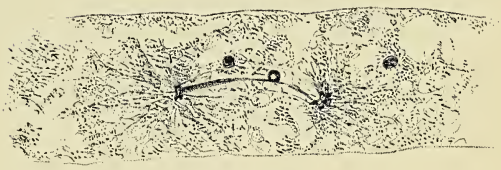

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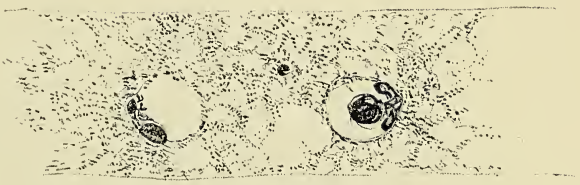

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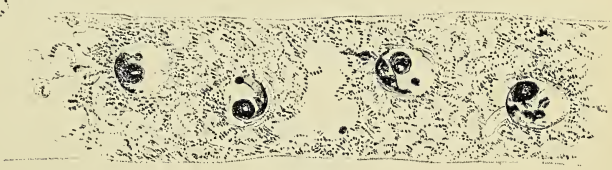

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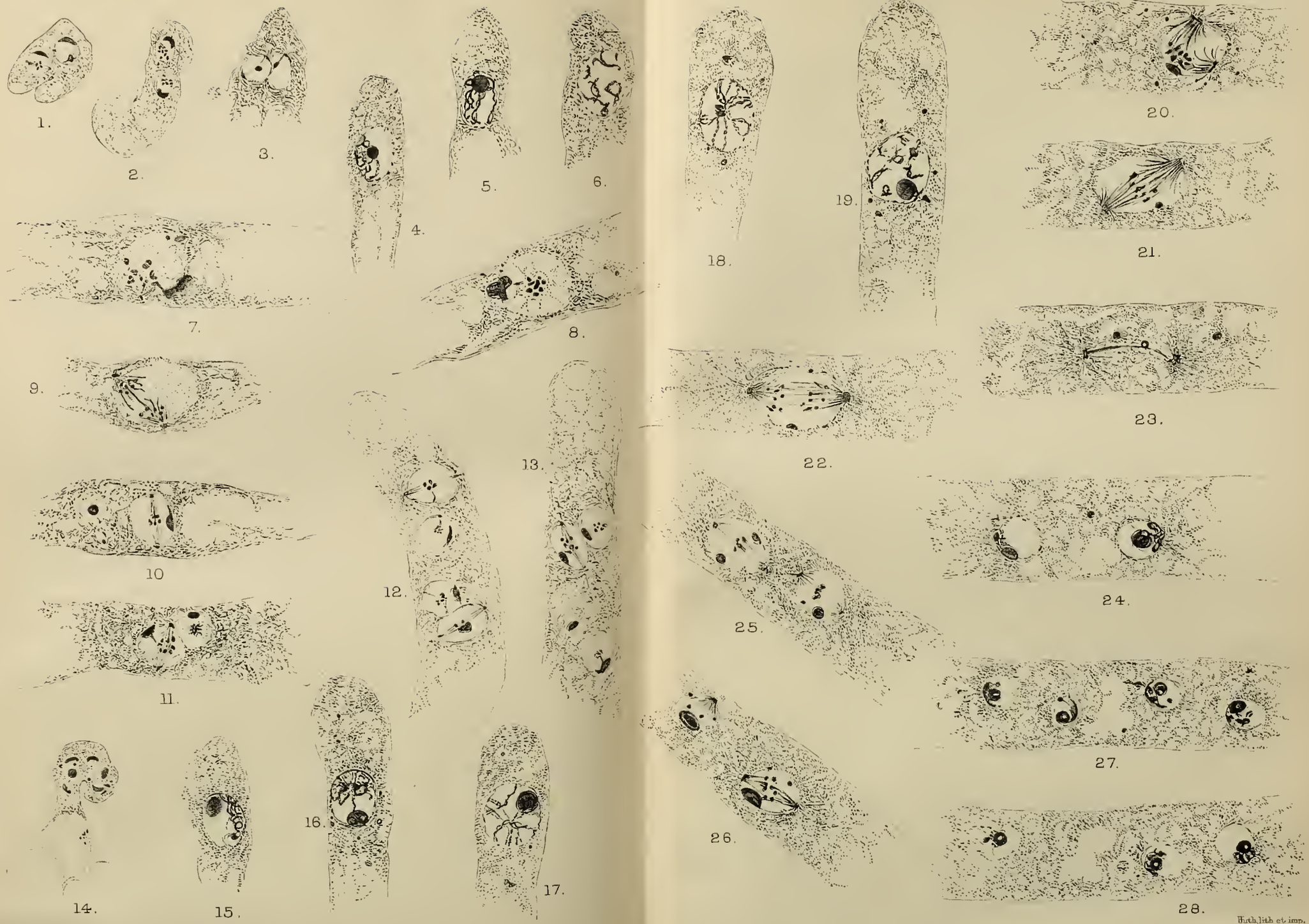
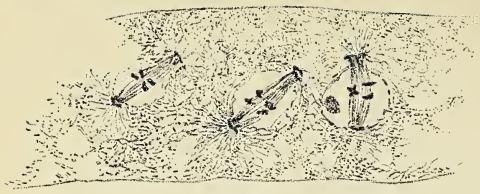


FRASER AND BROOKS — HUMARIA AND ASCOBOLUS.



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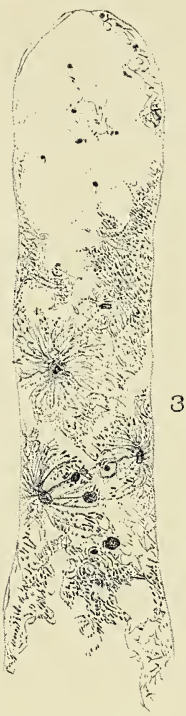

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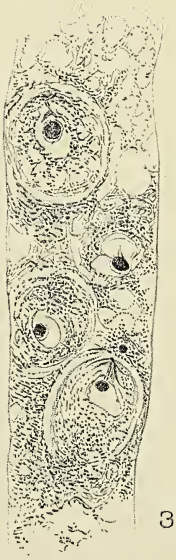

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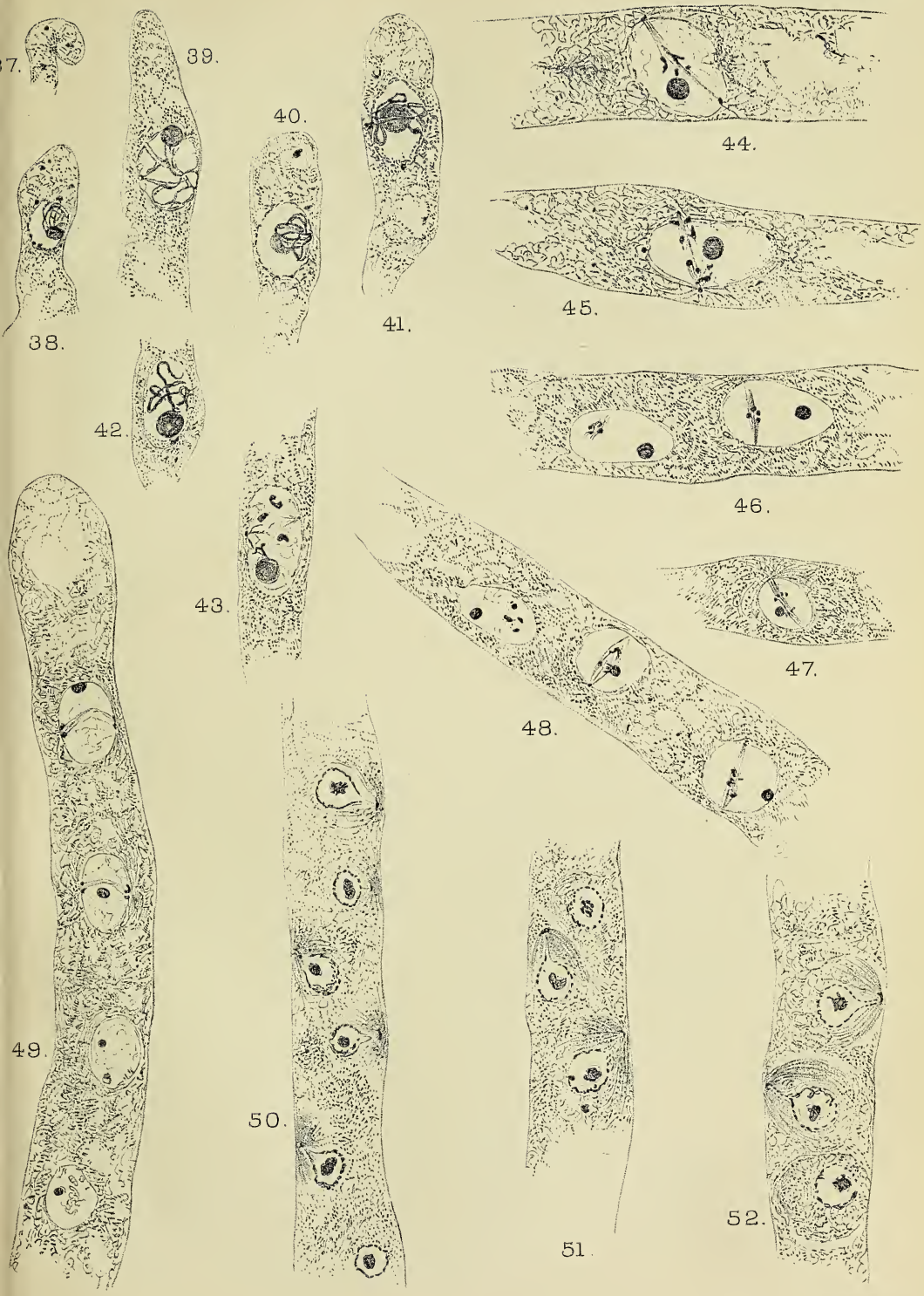
FRASER AND BROOKS—ASCOBOLUS AND LACHNEA.



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FRASER AND BROOKS—ASCOBOLUS AND LACHNEA.

Huth, lith. et imp.

Cytological Studies on Oenothera. I.

Pollen Development of Oenothera grandiflora.

BY

BRADLEY MOORE DAVIS.

With Plates XLI and XLII.

THE interest which attaches to the *Oenotheras* on account of their intimate relation to the mutation theory of De Vries has already led to their cytological study by three investigators. These authors have, however, approached the problems either through *Oenothera Lamarckiana* itself or through some of its derivatives. They have consequently dealt not with old or well-established wild species, but with forms of recent or uncertain origin, for the relation of *Oenothera Lamarckiana* to the American flora is certainly problematical. The present study will treat of a characteristic native species which is perhaps as true to its type as any American form. Further investigation may show that there are certain advantages in approaching the cytological study of the *Oenotheras* through species of established position.

Oenothera grandiflora, Ait., is probably one of the most favourable of the American types for cytological research on the pollen mother-cells, since the remarkably large and showy flowers have anthers which are about twice the size of those of *O. biennis* and almost as large and robust as the anthers of *O. gigas*. The plants which furnished the material for this investigation were grown from seed collected by S. M. Tracy at Dixie Landing, near Tensaw, Alabama, September 3, 1907. The seedlings were started during the winter in the hot-houses of the Harvard botanic garden and planted at Woods Hole, Massachusetts, in the spring of 1908. They developed during the summer into sturdy plants more than two metres high, which flowered profusely in September.

I am greatly indebted to the Bureau of Fisheries for the hospitality of its laboratory at Woods Hole during several months, and to Miss Sarah B. Fay for garden facilities.

Methods.

It is difficult to fix the anthers of *Oenothera* satisfactorily, and great care and patience are necessary in such a manipulation as will assist the killing fluid to wet quickly the surface of the anther, otherwise the

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penetration from the tips is so slow that fixation is generally imperfect. A fixing fluid of the following formula was chiefly employed: 1 per cent. chromic acid 25 cc., 10 per cent. glacial acetic acid 20 cc., 1 per cent. osmic acid 10 cc., water 45 cc. The material was left in this solution 2–4 hours and then transferred for about 20 hours to a similar solution without osmic acid. The good effects of the osmic acid were in this way obtained without excessive blackening, and this may be readily removed with a very short immersion of the sections in a dilute alcoholic (70 per cent.) solution of hydrogen peroxide. A stronger chrom-acetic fixing fluid with osmic acid omitted also gave good results: 1 per cent. chromic acid 40 cc., 10 per cent. glacial acetic acid 40 cc., water 20 cc. Iron-alum haematoxylin proved to be the most satisfactory stain for the nuclei, which are relatively small.

The Vegetative Mitoses in the Anther.

It is important, before considering the events of synapsis and the two mitoses (heterotypic and homotypic) in the pollen mother-cell, to describe the characteristics of the vegetative divisions of the sporophyte. These may be studied in the root tips, as Miss Lutz has already done for a number of forms of *Oenothera*. There are, however, obvious advantages, because of the close relationships of the nuclei concerned, in selecting sporophytic mitoses close to or immediately preceding the reduction divisions of pollen formation.

The archesporium in the anthers of *Oenothera* is differentiated at a very early period as a single row of cells running lengthwise in the middle region of each of the four portions that become pollen chambers. As the anther grows there are cell-divisions in the archesporium both crosswise and lengthwise, and thus the single row of archesporial cells becomes transformed into a somewhat irregular double row throughout most of its length. There are also numerous nuclear divisions in the cells surrounding the archesporium (which cells form in the mature anther a well-defined tapetal tissue) and in other regions of the anther as well. Consequently the anther, as well as other parts of the developing flower, presents excellent subjects for the study of vegetative sporophytic mitoses.

This section of the paper will be concerned chiefly with the final mitoses in the archesporium which convert the primary single row of cells into the double row that become the pollen mother-cells. These mitoses appear in no way different from those in the developing tapetum and in other parts of the anther, but are more easily studied because the archesporial cells are somewhat larger.

The resting nuclei in the archesporium and throughout meristematic regions of the developing flower are similar in structure. Each contains, as a rule, a single large nucleolus, usually accompanied by

one or more small nucleoli, and a variable number of deeply staining chromatic bodies of different forms and sizes (Pl. XLI, Figs. 1 and 8). The chromatic bodies are distributed rather uniformly around the periphery, closely pressed against the nuclear membrane, and frequently traces of a very open, delicate, connecting network may be observed.

The nucleoli vary in size and in numbers. Instead of one large structure there are occasionally two or three nucleoli of approximately equal size. The smaller nucleoli may be so nearly the size of the chromatic bodies as to be scarcely distinguishable from the latter except by their more spherical form and generally more central position in the nucleus. Small nucleoli frequently lie beside the largest, and stages indicating that they are absorbed by the latter are not uncommon, as has been reported by Gates ('08) for *O. rubrinervis*.

The chromatic bodies are of especial interest in view of Rosenberg's ('04) conclusion that structures, apparently similar to these, in the resting nuclei of *Capsella* and some other forms, represent the chromosomes. It is frequently possible to make counts of these bodies that approximate the sporophytic number of chromosomes, which is fourteen, but one finds other nuclei in which the number of evident bodies is less, and also nuclei with more numerous and smaller granules. The investigations of Overton ('05, '09), which appear to have established the presence in the nucleus of prochromosomes representing the chromosomes of mitosis, make it probable that similar conditions are present in *Oenothera*, but I have not as yet been able to follow the history of the chromatic bodies with sufficient exactness to be certain that they correspond to prochromosomes.

The first indication of approaching mitosis is the appearance of a network distributed throughout the interior of the nucleus (Fig. 2), but denser in the peripheral regions. As it develops, the chromatic bodies decrease in size, apparently contributing their substance to the reticulum, and finally they can no longer be differentiated. The smaller nucleoli are then more easily recognized, since there are no longer chromatic bodies with which they may be confused.

Certain strands of the chromatic network gradually thicken and become sharply defined as a coiled and looped thread, portions of which in early stages of development are frequently united to form a very open network (Fig. 3). This thread becomes the spirem which, when fully developed, is clearly continuous for a great length, although portions of the thread in places may appear to fuse at points of crossing. It is, of course, possible that all points of union during the development of the spirem, as described above, are merely those of adherence through the medium of accessory substance, and that the true chromatic spirem is at all times a single thread.

The spirem at an early stage of development is composed of a single

row of granules resembling a string of beads (Fig. 4). These granules are apparently the chromomeres of various authors. They later become no longer recognizable as the spirem thickens and grows shorter. The fully developed spirem is a deeply staining thread wound around the periphery of the nucleus, and at this stage generally appears continuous, although the structure is so small as to make the determination of the latter point very difficult.

Fourteen chromosomes are formed by the cross-segmentation of the spirem. These may sometimes be counted within the nucleus before the formation of the spindle (Fig. 5). They have the form of long rods variously bent, as would be expected of segments derived from a coiled spirem thread.

The count of the chromosomes is, however, most readily made from polar views of the equatorial plate (Fig. 6), where they lie in approximately the same plane. The chromosomes at this time are usually V or U-shaped, but the arms may be bent at various angles. The daughter-chromosomes following the metaphase of the mitosis have a more regular form, which is clearly shown in stages of anaphase (Fig. 7). They lie with the points of the V's directed to the poles of the spindle, and in appearance are quite indistinguishable from one another. The nucleoli disappear during the development of the spindle, which is too small to be a satisfactory subject for study.

Immediately following the organization of the daughter-nuclei the chromatic material takes the form of a loose reticulum, in the meshes of which the new nucleoli appear. As the nuclei enlarge, the strands of the reticulum become thinner throughout most of the structure, and the chromatin becomes distributed around the periphery in the form of deeply staining bodies of various forms and sizes (Fig. 8). These are the chromatic bodies so characteristic of the resting nuclei in meristematic regions of the flower.

The nuclei shown in Fig. 8 are in young pollen mother-cells of the fully differentiated archesporium which, as described before, generally consists of two rows of cells, with, however, frequent irregularities of placement probably due to the adjustment of the cells to mutual pressure. There follows now a long period of growth, during which the nuclei double their diameter and the pollen mother-cells become three or four times larger in diameter. An excellent idea of the extent of these changes may be obtained by comparing Fig. 8 with Figs. 36–39, all drawn under the same magnification (2,000 diameters), the latter illustrating mature pollen mother-cells.

Synapsis.

The events of synapsis and the formation of the bivalent chromosomes are of all phases in the process of pollen formation very much the most difficult to understand. The changes of structure are subtle and hard to interpret, and the correct seriation of stages requires the greatest care. There is, moreover, in the nature of the synaptic processes, which are chiefly concerned with coiled threads, a degree of variation in the form and arrangement of the structures concerned that is frequently very puzzling. Great assistance, however, is afforded by the fact that at no time are all of the anthers of an *Oenothera* flower at exactly the same period of development, and the differences are sufficiently great to be very helpful in determining which of a series of synaptic stages are older and which younger. Indeed, there are at certain periods of synapsis marked differences between the stages of development in the four pollen chambers of the same anther, and even in different portions of the same pollen chamber. As is always true, as far as the writer is aware, the period of synapsis is very much longer than that of any other phase in the history of pollen and spore formation.

The structure of the resting nucleus following the last mitosis in the archesporium is shown in Fig. 8, where it will be seen that the chromatic material is distributed as a number of deeply staining bodies around the periphery. The nuclei remain for some time in this condition. The first indication of approaching synapsis is the appearance of extremely delicate strands connecting these bodies (Fig. 9), and forming a very open network. The strands then rapidly thicken and become more numerous (Fig. 10) until finally the nucleus is filled with a relatively close reticulum (Fig. 11). During its development the chromatic bodies become smaller, apparently contributing their substance to the strands composing the network, and the nucleoli are left as the only large structures lying freely within the nucleus. The smaller nucleoli appear more conspicuous because there are no longer chromatic bodies with which they may be confused.

The advent of synapsis is marked by the beginning of a general slow contraction of the reticulum away from the nuclear membrane, a contraction which carries most of the strands towards the centre of the nucleus (Fig. 12). During this process of contraction there is a marked change in the structure of the reticulum, which results in the differentiation of very numerous threads which are coiled in an intricate manner. These are at first united to one another at many points, but as synapsis proceeds the thread structure becomes more and more evident until it becomes clear that a spirem is to be developed.

The synaptic contraction generally draws inward with it all of the smaller nucleoli which, together with the coiled and crowded threads, form a ball or knot so dense and compact that it is impossible to differentiate

clearly all of its parts. Sometimes, however, an occasional small nucleolus will be left free in the nuclear cavity (Fig. 13). There are usually at the edge of the synaptic knot a number of threads in the form of loops that extend often to the periphery of the nucleus (Fig. 14), but more frequently lie at some distance from the nuclear membrane (Fig. 15). It is clear that the process of contraction is a gradual one, and that some of the threads are drawn in more slowly than others and consequently remain free from the bulk of the contracted structure. Even in advanced stages of synapsis there are frequently present some extremely delicate threads, which later disappear, lying close to the nuclear membrane (Figs. 13, 15, and 19).

Differential staining of the chromatic threads, especially of such free loops as are favourable for study, shows them at certain stages to be composed of denser, deeply staining regions arranged like a string of beads (Fig. 14). These are apparently the oft-described chromomeres comparable to similar structures on the early spirem of the vegetative nucleus (Fig. 4).

The total length of the thread system in the early stages of synapsis is certainly very much greater than that of the early spirem of the vegetative nucleus, and apparently even greater than might be indicated by the proportionate increase in the size of the nucleus (compare Figs. 3 and 4 with Figs. 13–15). It is impossible to say whether the coiled and twisted elements of the synaptic knot are organically parts of a continuous spirem. They are certainly united to one another at many points, but this union may be merely an adherence which does not signify the branching of a true chromatic thread.

As would be expected of such complex structures as the various stages of the contracted synaptic knot it is not difficult to find threads that run closely parallel with one another for considerable distances. Furthermore, as stated above, the threads are certainly united at points (Figs. 14, 15, 16, and 17), but they form such various angles and lie in such complicated coils that a clear understanding of their intimate relations to one another was impossible with the technique employed. Further study employing methods of fixation suggested by Overton ('09) may give clearer information on the conditions. The writer has, however, so far seen no evidence that these parallel threads ever fuse for considerable lengths, or that there is present in the nucleus two independent spirems (maternal and paternal) which might be assumed to unite with one another side by side. The further history of the threads in relation to the bivalent chromosomes also gives no support to such an interpretation. Neither has the writer observed any stages that would establish the splitting of a spirem, such as might indicate a premature fission related to the lengthwise division of the chromosomes in the homotypic mitosis.

The contraction of the chromatic material during synapsis is apparently caused by the gradual shortening and thickening of the threads which later

become the spirem. This process may be readily followed and is illustrated by Figs. 12–20, which show that the threads of the older stages are more than twice as thick as those of the younger, and that the total length of the threads (so far as they may be traced) becomes very much less as synapsis proceeds.

The forms of the fully contracted synaptic knot are exceedingly varied. In the most extreme cases the threads are drawn tightly together into an irregular lumpy mass (Figs. 21 and 23). More often, however, there are several well-defined loops extending into the nuclear cavity from the dense contracted centre of the synaptic knot, as illustrated in Figs. 19, 20, and 23. The number of loops is not fixed, and they are distributed irregularly and are of various lengths. The loops that extend to the periphery of the nucleus are attached to the nuclear membrane, which is frequently drawn inward at such points probably because of the pull exerted by the contracted threads. Even when the contracted material is in the form of a dense mass (Figs. 21 and 22) it is always possible to distinguish definite lobes that in earlier stages were undoubtedly loops, but are now tightly drawn against the synaptic knot.

The mid-phase of synapsis, or the period of greatest contraction, is illustrated by Figs. 21, 22, and 23, which are fairly typical of various conditions. It should be noted that the nuclear cavity contains only the chromatic mass, the single large nucleolus (rarely two large nucleoli), occasionally smaller nucleoli (Fig. 21), and perhaps remnants of the extremely delicate threads (Fig. 23) which are present close to the periphery in earlier stages of synapsis (Figs. 13, 15, and 19). The chromatic mass may lie near the centre of the nucleus (Fig. 22), but it is usually somewhat pressed against the large flattened nucleolus and consequently at one side. The latter situation suggests a polar organization of the nucleus that is frequently emphasized by the radiating arrangement of the loops from the chromatic mass as a centre (Figs. 20 and 23). The chromatic loops are very important structures, for some of the ring-shaped bivalent chromosomes arise directly from them.

The Formation of the Bivalent Chromosomes.

As stated before, the structure of the synaptic knot when fully contracted is very difficult of analysis. The chromatin frequently has the appearance of a lumpy mass (Figs. 21 and 22), the surface being broken by irregular globular projections, some of which were formerly the loops that extended into the nuclear cavity. In other cases these loops may remain clearly defined, although much shorter than in earlier stages of synapsis (Fig. 23). Differential staining clearly indicates that the contracted synaptic chromatin has regions of greater density, some of which

are loops of the now very much shortened and thickened spirem, and there seems no reason to doubt that a structural organization is present no matter how compact and homogeneous the synaptic knot may appear.

The chromatic material emerges from synapsis by a general loosening up of the elements which compose the contracted knot, and then for the first time it becomes evident that the chromatin has taken the form of a group of rings. Later the rings may be easily counted, the number being seven, which is half the number of chromosomes present in the vegetative or sporophytic mitoses. These become the seven bivalent chromosomes characteristic of the heterotypic mitosis.

The origin of certain of the rings from chromatin loops which extend from the synaptic knot appears very clear in such preparations as have a synaptic knot of the looser type, such as is illustrated in Figs. 20 and 23. In these cases each loop is transformed directly into a chromatic ring, and frequently three or four of the seven rings composing the group arise from such loops (Figs. 24, 25, and 26). The remainder of the rings in such nuclei are derived from the contracted portion of the synaptic chromatin, where the shortened and thickened coils of the spirem are so closely pressed together that the elements cannot be distinguished. Rings that are formed from loops are generally at first exceptionally large (Figs. 25, 26, and 29), but they rapidly grow smaller by evident condensation as the nucleus prepares for the heterotypic mitosis.

It was impossible with the technique employed to trace with exactness the development of the rings from synaptic knots of the dense much-contracted type, such as are shown in Figs. 21 and 22. These rings are organized when the chromatin is in the contracted state, and appear fully formed with the loosening up of the synaptic knot as that structure emerges from synapsis (Fig. 27). Such rings are readily distinguished from those formed from the free loops because of their small size and more regular and compact form (Figs. 27 and 28). They are at first closely grouped around the nucleolus (Fig. 27), but later become more generally distributed in the nuclear cavity (Fig. 28), illustrating the phase termed diakinesis. Frequently two or three of the rings become separated early from the others, and consequently lie freely in the nuclear cavity, while the remainder are still so closely massed in a group that their outlines cannot be clearly followed. Such free rings are very conspicuous and may be readily studied (Fig. 28).

It is certain that the fully developed rings are generally completely closed. It is exceptional to find a ring open on one side, but such are sometimes present. The rings are, however, not uniform in density, but are generally thinner at one or two points. These conditions become much more evident as a nucleus approaches metaphase of the heterotypic

mitosis, when two much thickened halves of a ring (each a sporophytic chromosome of this bivalent structure) become clearly defined, connected at two points by very delicate strands (Pl. XLII, Fig. 37).

When groups of chromosomes are viewed in favourable positions, two, three, or more rings may sometimes be seen actually linked together, thus forming short chains or clusters of rings. The appearance of these linked chromosomes is shown in Figs. 29 and 30, and there seems to be no doubt of the condition, since careful focussing enables one to follow the parts of the links above and below one another.

An explanation of the groups of linked rings seems to require their origin from an involved tangle of loops, united at certain points, which finally segment in such a manner that the fused loops (now rings) are linked together. Such an arrangement may be constructed from a piece of string (representing a spirem), loops of which are passed through one another and then tied together by thread; finally, if the string be cut at the proper points the loops will be found to separate as linked rings.

The groups of linked rings remain together until the metaphase of the heterotypic mitosis, when they become separated as the halves of the rings (sporophytic chromosomes) are drawn apart. Such complicated arrangements of the rings on the equatorial plate are probably sometimes responsible for a confused cluster of chromosomes that is difficult to interpret, as illustrated by Fig. 35.

In concluding the account of synapsis and of the formation of the bivalent chromosomes, certain striking features should be noted that are of importance in relation to the hypotheses that have been offered in discussions of the structure and origin of heterotypic chromosomes :—

(1) The spirem at no period lies freely in the nucleus in such a form that it may be followed for any considerable portion of its length. It is from the beginning an involved coiled structure, the loops of which adhere to one another at points especially in contracted portions of the synaptic knot.

(2) The spirem does not segment into fourteen chromosomes, lying freely in the nuclear cavity or later becoming associated in pairs.

(3) The chromosomes appear at the outset as seven pairs (bivalent chromosomes) which have the form of generally closed rings, and remain so united in pairs until metaphase of the heterotypic mitosis.

(4) The origin of some of the rings (bivalent chromosomes) from loops of the spirem extending freely into the nuclear cavity indicates that the halves (sporophytic chromosomes) are segments of the spirem arranged end to end, which are brought together in ring-shaped pairs by the coiled arrangement of the spirem.

(5) The occasional presence of linked rings indicates that loops of the spirem sometimes become interlaced in such a manner that after the

segmentation of the spirem the loops (now rings) are joined together like links in a chain.

(6) It seems impossible in this form to divide the phases of synapsis into periods of a first and second contraction as has been done in some other studies. The changes of structure during synapsis are so gradual as to make sharp divisions between the periods very difficult to define. Furthermore, the degree of variation in the form of the chromatic material at the same phase of synapsis makes it unsafe to assume that looser types of synaptic contraction indicate periods of expansion to be followed later by further contractions.

The Heterotypic Mitosis.

The prophases of spindle formation of the first, or heterotypic, mitosis in the pollen mother-cell appear shortly after the seven chromatic rings, or bivalent chromosomes, emerge from the synaptic knot and are distributed through the nuclear cavity presenting the stage called diakinesis (Figs. 28 and 31). The rings by this time have grown smaller, that is, their substance has become more condensed, as may be seen by comparing Figs. 31, 32, and 33 with Figs. 25, 26, and 29. The halves of the rings soon become more sharply defined and gradually take on the bent or V-shaped forms, characteristic of the sporophytic chromosomes with which they are identical. These half-rings, or sporophytic chromosomes, however, remain connected in pairs so that the rings are still intact. It is clear that the process of condensation and differentiation continues through the stages of prophase up to the period of metaphase when the two sets of sporophytic chromosomes are ready to be distributed by this, the heterotypic mitosis.

The process of spindle formation in its main features follows a history similar to that described for the heterotypic mitosis in a number of higher plants (e. g. *Equisetum*, *Larix*, *Lilium*, &c.). The nucleus becomes surrounded by a web of fibrillae which enter the cavity of the nucleus with the breaking down of its membrane (Figs. 31, 32, and 33). The large nucleolus at this time stains faintly and soon disappears. The developing fibrillae gradually fill the nuclear cavity, and at the same time push their way out into the cytoplasm in several directions, giving the well-known multipolar stages of spindle formation. Sections of prophases showing three or four poles are common (Figs. 35 and 36).

The chromatic rings are carried towards the centre of the developing mass of fibrillae, where at first they frequently lie in a group so closely massed that their arrangement can be made out only with difficulty (Fig. 35). However, one or more of the rings are generally apart from the main group and their forms may be clearly seen (Figs. 34 and 36).

The spindle at metaphase of mitosis (Plate XLII, Figs. 37 and 38) is a rather broad bipolar structure developed from the multipolar conditions

of prophase by the gathering of the fibrillae into two opposite sheaves. No centres were observed at the poles of the bipolar spindle, the fibrillae ending in a granular area which merged into the surrounding alveolar cytoplasm.

The chromosomes become generally arranged in a very symmetrical manner to form the equatorial plate at the metaphase of mitosis. The condition illustrated by Fig. 37 is thoroughly typical. It will be seen that the sporophytic chromosomes are still joined together in pairs forming the rings, but they are now mostly V-shaped instead of semi-circular, the points of the V's being directed towards the spindle poles. The sporophytic chromosomes may be readily counted at this stage of mitosis, and the number is fourteen, grouped of course in seven pairs derived from the seven rings characteristic of the prophases. The only observed irregularities of this arrangement (in well cut sections) were instances where one or more rings lagged behind the others in taking their positions in the equatorial plate and in completing the separation of the chromosomes comprising such pairs (Fig. 38).

The V-shaped form of the chromosomes becomes even more evident during anaphase, as the two sets of chromosomes move away from one another towards the poles of the spindle (Fig. 39). Sometimes the arms of the V's are brought so closely together that the line of contact might be mistaken for a line of premature fission in preparation for the second or homotypic mitosis, but this is not the case.

The fission of the chromosomes in preparation for the succeeding homotypic mitosis takes place in the latter part of anaphase of the heterotypic division when the chromosomes are congregated at the poles of the spindle (Fig. 40). The split is lengthwise of each V-shaped chromosome in the plane of the page on which the above letter (V) is printed. However, at this time the arms of the V's generally separate somewhat, so that most of the chromosomes are not so sharply bent as in earlier stages of this mitosis. This premature fission, peculiar to late anaphase, is most readily observed when the chromosome group at a pole is cut obliquely (Fig. 41) so that the chromosomes are viewed at various angles.

There is a well-defined period of rest between the heterotypic and homotypic mitoses in the pollen mother-cell that is especially interesting for the history of the chromatin, which may be very easily followed in this form. The seven split chromosomes that gather at the poles of the heterotypic spindle are almost always arranged so that six of them lie in a circle around the seventh (Fig. 42). At this time the line of fission is clearly marked, and the chromosomes look like seven pairs of rods when the group is viewed from above the pole of the heterotypic spindle, as shown in Fig. 42. In reality, however, the chromosomes are not rods but are V or U-shaped, the bent ends pointing downward or away from the pole of the spindle.

There then develops around each group of chromosomes at the pole of

a heterotypic spindle a vacuole-like region, the boundary of which becomes the membrane of the daughter-nucleus. The seven split chromosomes, which were at first massed closely together, separate as the daughter-nucleus gradually increases in size, and become distributed rather symmetrically around its interior just under the nuclear membrane. A change in the form of the split chromosomes becomes then at once apparent. The ends of one chromosome of the pair swing away from the ends of the other until they lie in approximately the same plane, when the structure becomes that of two U's joined together at the bent middle regions. This condition is illustrated in Figs. 44 and 45, which also show the development of the nucleoli that are conspicuous in the full-sized resting nucleus.

A careful study of stages makes it perfectly clear that the halves of the split chromosomes are not crossed, even though the form of the double structure is sometimes like that of an X, but that they lie side by side joined at the bent middle regions. This striking association of chromosomes is very characteristic of these resting nuclei. There is sometimes a tendency on the part of the free ends of the chromosomes to extend and to branch, and even to become united with one another, thus forming a loose and imperfect network (Fig. 43). However, I have never seen a resting nucleus in which the outlines of one or more chromosomes could not be accurately followed. Fig. 45 shows a pair of nuclei that are fully at rest; in the lower one of the two the seven split chromosomes are entirely distinct from one another and may be counted, and in the upper one (cut by the knife) four of the split chromosomes may be readily distinguished.

There can be no doubt that in this type the chromosomes maintain their individuality in the resting nucleus between the heterotypic and homotypic mitoses. This conclusion is further supported by the structure of the chromosomes as they appear in the early stages of the homotypic mitosis to be described below. The point is one of considerable importance because of the views held by some plant cytologists that the chromosomes may entirely lose their individuality in the resting nucleus.

In conclusion, it is evident that the heterotypic mitosis distributes the fourteen sporophytic chromosomes in two groups of seven each, and consequently is a reduction-division. These sporophytic chromosomes are grouped in pairs as ring-shaped bivalent chromosomes which appear after synapsis, and are characteristic of diakinesis and the prophases of spindle formation. The sporophytic chromosomes are V-shaped at the metaphase of mitosis and appear essentially similar to one another. There seems to be as great uniformity in their shape and size as is common for the heterotypic mitoses of plants.

The Homotypic Mitosis.

The spindle of the homotypic mitosis is formed as in the heterotypic division from a mesh of fibrillae that develop around the resting nucleus and enter the nuclear cavity with the dissolution of its bounding membrane (Fig. 46). The prophases likewise frequently show multipolar spindles (Figs. 47 and 48) that become bipolar at the metaphase of mitosis. The two spindles may lie parallel in the pollen mother-cell or at right angles to one another.

The chromosome group emerges from the resting nucleus at the beginning of the homotypic mitosis in very much the same form as it entered that structure at the end of the heterotypic. There are the same seven split chromosomes, the halves of which are bent outward so that they are joined to one another only in the bent middle region. This peculiar chromosome group presents an interesting contrast to the seven rings characteristic of the heterotypic mitosis (compare Figs. 46–48 with Figs. 34 and 36).

The seven split chromosomes are gathered in the usual manner at the equatorial plate, where they are arranged so that the halves separate into two sets at the metaphase of mitosis (Figs. 49 and 50). A comparison of this stage with the metaphase of the heterotypic mitosis (Fig. 37) shows that the daughter chromosomes are not so large as those of the latter mitosis and have the form of short and sometimes slightly bent rods rather than of V's. One or both ends of the chromosomes may become somewhat enlarged, giving them an irregular appearance that is frequently quite pronounced during anaphase of this mitosis (Fig. 51).

Each daughter-nucleus following the homotypic mitosis thus receives one set of the halves of the seven split chromosomes. These halves may be readily counted in favourable preparations of anaphase (Fig. 52), and especially when the chromosome group at the pole of the spindle is viewed from above (Fig. 53). The arrangement of the chromosomes in late anaphase and early telophase is similar to that in the heterotypic mitosis (i. e. six chromosomes are grouped in a circle around the seventh), but in this group there is not present of course any trace of a premature fission in preparation for a succeeding mitosis.

As the four daughter-nuclei following the homotypic mitosis increase in size, the chromosomes which enter into their construction begin to elongate and finally to branch. Although they may be distinguished in the younger nuclei, such as are shown in Fig. 54, their forms become irregular in the older (Fig. 55), where a network is finally developed. The nucleus of the young pollen-grain (Fig. 56) generally has several medium-sized nucleoli, and the chromatin is distributed in the form of small deeply staining bodies connected with one another by a delicate open reticulum.

It is clear that the homotypic mitosis is an equation division distributing the halves of the seven sporophytic chromosomes that are separated from a similar set by the heterotypic mitosis. These seven sporophytic chromosomes become divided lengthwise during anaphase of the heterotypic mitosis, and the halves remain closely associated to form seven split chromosomes, which may be followed through the period of interkinesis between the heterotypic and homotypic mitoses.

Cytological Discussion.

As stated before, three investigators have within recent months published the results of cytological studies on *Oenothera Lamarckiana* or certain of its derivatives.

Miss Anne M. Lutz has made extensive chromosome counts chiefly from studies of root tips, and reports some interesting variations from the numbers typical of the genus, which all authors agree are fourteen chromosomes for the sporophyte and seven for the gametophyte. The most remarkable exception is found in *O. gigas* which presents twenty-eight chromosomes in the sporophyte. It is to be hoped that her papers, largely in the nature of preliminary announcements,[1] will soon be followed by detailed accounts with figures.

Geerts has published preliminary notes on a lengthy study of *O. Lamarckiana*,[2] which include an investigation of the embryo sac as well as of pollen formation. A full account of his conclusions, to be accompanied by figures may be expected in the Recueil des Travaux Botaniques Néerlandais. The absence of figures in his preliminary papers makes a close comparison of his results and those of the present paper difficult, and I shall postpone for the present their consideration.

Gates has studied the processes of pollen formation in *O. Lamarckiana* and several of its derivatives, and reported the results in a number of notes[3] and in two illustrated papers (Gates, '07 and '08). The last of the two illustrated papers (Gates, '08) gives his cytological conclusions based chiefly on an investigation of *O. rubrinervis*. I shall not, however, discuss these in relation to the present study of *O. grandiflora* until I have had an opportunity myself to study *O. Lamarckiana* or some of its relatives. The results of Gates's investigation of *O. rubrinervis*, as presented in his last paper, differ from those here recorded in a number of important particulars, the chief of which are as follows:—

1. Following synapsis, Gates describes and figures a thick spirem which may be traced continuously for considerable lengths in the nuclear

[1] Science, 1907, p. 151; 1908, p. 335; 1909, p. 363.
[2] Ber. deut. bot. Gesellsch., 1907, p. 191; 1908, p. 608.
[3] Science, 1907, p. 259; 1908, p. 193 and p. 335; 1909, p. 269.

cavity and which segments crosswise to form a chain of fourteen chromosomes.

2. Pairs of chromosomes may be precociously cut off from the spirem, the members of the pairs bending around to form loops but never becoming united to form rings.

3. The chromosome segments are at first several times longer than broad, but by a process of condensation become nearly globular or pear-shaped.

4. The chromosomes are at first arranged irregularly on the heterotypic spindle, many of them frequently remaining separate and unpaired.

5. Such a distribution allows irregularities of distribution in the heterotypic mitosis, so that both (unpaired) chromosomes belonging to one pair will occasionally enter the same daughter-nucleus.

It seems to the writer best to defer a detailed discussion of the cytological problems touched by the present study until further investigations have been made on this interesting genus, but a few important considerations may be briefly outlined.

It is clear for *Oenothera grandiflora* that the sporophytic chromosomes become associated in ring-shaped pairs, which first appear as the chromatin emerges from synapsis, and are characteristic of the period termed diakinesis and of the prophases leading to the heterotypic mitosis. These ring-shaped bivalent chromosomes are assembled in a very orderly manner at the equatorial plate of the heterotypic mitosis, where they remain intact as rings until the separation of their halves into two numerically equivalent sets of sporophytic chromosomes. There is little or no opportunity for irregularities in the distribution of the numbers when the normal processes of the mitosis follow such a history.

The V-shaped sporophytic chromosomes of the heterotypic mitosis are in general similar to one another in form and size. There is apparently no more variation in these respects than is shown in the heterotypic mitosis of plants in general. The condition as illustrated in Fig. 37 is the normal one for this form, and variations from this as shown in Fig. 38 indicate only that certain rings (bivalent chromosomes) occasionally lag behind the others in completing the separation of the sporophytic chromosomes.

The chromosomes may readily be followed between the telophases of the heterotypic and the prophases of the homotypic mitoses, maintaining their individuality with very little change of form throughout this period of interkinesis. This history has also been noted by Gates and Geerts in *O. Lamarckiana* and *O. rubrinervis*. It adds further evidence against the views of Mottier ('03 and '07), Lewis ('08), and others that chromosomes lose their individuality in the resting nucleus between the heterotypic and homotypic mitoses.

The interpretation of synaptic and presynaptic conditions presents far

greater difficulties than the post-synaptic events considered above. Not only must one deal with more intricate structure but also with conditions where the necessities of the best possible technique are far more exacting than in the later stages of the reduction processes. The last contribution of Overton ('09) offers such important evidence on the structure and arrangement of the chromosomes during presynaptic phases in the history of the pollen mother-cells that the writer prefers to defer an expression of opinion concerning the conditions in *Oenothera* at these periods until further studies have been concluded.

Overton ('09), from studies on *Thalictrum*, *Calycanthus*, and *Richardia*, believes that the chromosomes are represented in the vegetative (somatic) nuclei by prochromosomes arranged in parallel pairs separated by linin intervals indicating the presence of parental sets of chromosomes associated together to form heterogeneous spirems. The synaptic contraction is a gradual process during which parental spirems become more closely associated but still remain distinct, and the chromosomes of each pair are in their most intimate relation during post-synaptic stages. Each pair becomes a bivalent chromosome composed of two sporophytic chromosomes, the bivalent structures being characteristic of diakinesis. No folding process to form these bivalent chromosomes seems possible. The sporophytic chromosomes of the bivalent diakinetic pairs undergo lengthwise divisions, thus forming apparent tetrads. The heterotypic mitosis distributes entire sporophytic chromosomes, which remain distinct during the following interkinesis. The homotypic mitosis separates the split halves of the sporophytic chromosomes, and these may be followed in the pollen-grain as prochromosomes arranged in a single row.

It will be noted at once that Overton's conclusions offer strong support to the theory of chromosome reduction held by Grégoire ('04, '07), Allen ('05), Rosenberg ('05), and certain later writers. This group believe that two sets of sporophytic chromosomes of maternal and paternal origin are represented in the nucleus by two distinct spirems or sets of threads which become closely associated in synapsis, so that by a parallel arrangement of the spirems the chromosomes of one set are paired with those of the other to form the bivalent chromosomes of the heterotypic mitosis. Another group of investigators, represented by Farmer and Moore ('05), Mottier ('07), Strasburger ('04), and others, hold that the sporophytic chromosomes are arranged end to end on a single chromatic thread and that the bivalent chromosomes of the heterotypic mitosis are formed from loops of this single spirem that include a pair of sporophytic chromosomes which thus come to lie side by side.

The writer, on theoretical grounds, has been very strongly inclined towards the views of the first-named group represented by Allen, Grégoire, Overton, and Rosenberg. It must, however, be pointed out that the present

study of *Oenothera grandiflora* presents conditions that the writer cannot bring into harmony with their conclusions. Our present information for this type strongly indicates a pairing of the sporophytic chromosomes according to the method held by Farmer and Moore. The writer is disinclined to believe that so important a process in the life-history of plants as that of chromosome reduction is likely to be effected by two methods so diverse as those held by the opposing groups of investigators mentioned above, but there is at present a conflict of such apparently good evidence that one may well hesitate before taking a positive position. The conditions in *Oenothera grandiflora* that seem to require the explanation of chromosome association advanced by Farmer and Moore may be summarized as follows:—

1. The arrangement of the chromatic material during synapsis is characterized by the presence of several loops extending freely into the nuclear cavity from the synaptic knot as a centre (Figs. 19–23).

2. These free loops thicken as synapsis proceeds, and it seems clear that some of the ring-shaped bivalent chromosomes are formed directly from them.

3. The fact that the ring-shaped bivalent chromosomes are sometimes linked together is difficult to understand except on the theory that they have become so associated by the segmentation of a much-coiled spirem the loops of which were interlaced.

Summary.

1. The vegetative (somatic) nucleus contains chromatic bodies that frequently approximate the number of the sporophytic chromosomes, which is fourteen. It seems probable that they are prochromosomes. These bodies in the resting nucleus are connected by a delicate open network which becomes much more conspicuous and denser before the development of the spirem of the vegetative mitoses.

2. The spirem arises from this reticulum which later disappears, after which the spirem may be followed for great lengths as a single thread. Fourteen chromosomes in the form of long rods variously bent are formed by the cross-segmentation of the spirem.

3. Following the vegetative mitoses, the chromosomes become distributed through the nuclear cavity of the resting nuclei, where they take on the form of chromatic bodies.

4. Previous to synapsis the nucleus becomes filled with a close reticulum during the formation of which the chromatic bodies lose their rounded form and could not be followed.

5. Synapsis consists of a general slow contraction of the reticulum away from the nuclear membrane, a contraction that carries most of the

strands towards the centre of the nucleus. During the process of contraction numerous threads are differentiated from the reticulum which become coiled in a very intricate manner.

6. The synaptic contraction draws the coils of threads into a dense knot close to the large nucleolus, which generally lies at one side of the nucleus. Loops of the threads extend into the nuclear cavity from the synaptic knot as a centre. The threads gradually thicken as synapsis proceeds, and the length of the thread system is very much shortened.

7. When fully contracted, the synaptic knot consists of much-thickened threads (constituting the spirem) which are drawn so tightly together that their arrangement cannot be traced. The loops extending from the contracted mass are at this stage thick and very conspicuous.

8. The contracted material emerges from synapsis by a general loosening up of the elements of the contracted knot. It then becomes at once apparent that the chromatin is in the form of a group of seven rings, some of which are derived from the loops referred to in paragraph 7. The rings are generally closed, but consist of semicircular halves, which are the fourteen chromosomes. The rings are therefore bivalent chromosomes, very characteristic structures of diakinesis and the prophases of the heterotypic mitosis.

9. The seven ring-shaped bivalent chromosomes become much condensed during the prophases of the heterotypic mitosis. They then become arranged at metaphase to form the equatorial plate, after which the halves separate as two sets of V-shaped sporophytic chromosomes (seven in each set) that pass to the poles of the spindle. The heterotypic mitosis is therefore a reduction division. A lengthwise fission of each chromosome which takes place during anaphase is the premature division of these elements in preparation for the succeeding homotypic mitosis.

10. The sporophytic chromosomes of the heterotypic mitosis are V-shaped and appear essentially similar to one another. There seems to be as great uniformity in their shape and size as is common for the heterotypic mitoses of plants.

11. A resting nucleus is organized between the heterotypic and homotypic mitoses, and throughout this period of interkinesis the seven split chromosomes may be readily followed.

12. The homotypic mitosis distributes the halves of the split chromosomes into two sets (seven in each), and is consequently an equation division.

Woods Hole, Massachusetts.
April, 1909.

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EXPLANATION OF FIGURES IN PLATES XLI AND XLII.

Illustrating Prof. Davis's paper on pollen development of *Oenothera grandiflora*.

All figures were sketched with the aid of a camera lucida under the Zeiss apochromatic objective 1·5 mm. (aper. 1·30) in combination with the compensating ocular No. 12, giving a magnification of 2,000 diameters. Figures 1–10 from sections 4 μ thick; 11–18, 21, 28, 30, 32–42, 44, 46, 52–56 from sections 5 μ thick; 22, 24–27, 43, 45, 47–51 from sections 7 μ thick; 19, 20, 23, 29, and 31 from sections 10 μ thick. Sections stained with iron-alum haematoxylin.

PLATE XLI.

Oenothera grandiflora.

Fig. 1. Adjacent sections from nucleus in young archesporium, showing chromatic bodies and large nucleolus.

Fig. 2. Nucleus of archesporium, reticulum formed preparatory to the development of the spirem.

Fig. 3. Nucleus of archesporium, early stages of the spirem showing the threads united at points.

Fig. 4. Nucleus of archesporium spirem with granules (chromomeres?) arranged in a single row.

Fig. 5. Segmented spirem in archesporial nucleus resulting in fourteen sporophytic chromosomes.

Fig. 6. Metaphase of mitosis in archesporial cell viewed from the pole of the spindle, fourteen chromosomes.

Fig. 7. Anaphase of mitosis in archesporial cell, showing V-shaped chromosomes.

Fig. 8. Pollen mother-cells after the final mitosis in the archesporium, nuclei with chromatic bodies and large nucleoli.

Fig. 9. Nucleus before synapsis, chromatic bodies connected by a delicate reticulum.

Fig. 10. Later stage, the reticulum more prominent in preparation for synapsis.

Fig. 11. Nucleus just before the beginning of synapsis, a close reticulum present.

Fig. 12. Early stage of synapsis, system of threads developing from the reticulum shown in Fig. 11.

Fig. 13. Synaptic contraction well under way. Most of the threads are in closely twisted coils near the large nucleolus, certain very delicate threads lie close to the nuclear membrane.

Fig. 14. Synapsis, the loops extending from the synaptic knot consist of rows of granules (chromomeres ?).

Fig. 15. Synapsis, coiled and looped threads around the large nucleolus, very delicate threads close to the nuclear membrane.

Figs. 16–18. Synapsis, the threads thickening.

Fig. 19. Synaptic contraction almost complete, showing the dense knot, the loops extending into the nuclear cavity, and some delicate threads lying close to the nuclear membrane.

Fig. 20. Synaptic contraction probably complete, thickened loops extending from the synaptic knot.

Figs. 21 and 22. Complete synaptic contraction of the dense type, the loops represented by protrusions from the synaptic knot.

Fig. 23. Contracted synaptic mass ready to loosen up, the loops and prominent protrusions probably now organized into ring-shaped bivalent chromosomes.

Fig. 24. A stage in the loosening of the synaptic knot. The loop-like structures are chromatic rings.

Figs. 25 and 26. The ring-shaped chromosomes shortly after their emergence from the synaptic knot. The rings are large at this stage.

Fig. 27. Approaching the phase of diakinesis. Most of the rings are still clustered around the large nucleolus.

Fig. 28. Diakinesis, the rings now much more condensed than when first organized (Figs. 24–26).

Fig. 29. A group of rings, certain of which are linked together—a stage in the emergence of the rings from the synaptic knot similar to Figs. 24–26.

Fig. 30. A group of linked rings.

Fig. 31. Early prophase of the heterotypic spindle, large nucleolus still present. The nuclear membrane has disappeared, chromatic rings distributed in the nuclear cavity, which is surrounded by a web of fibrillae.

Fig. 32. Multipolar spindle, chromatic rings gathered in the centre of the nuclear cavity.

Fig. 33. Certain chromatic rings show clearly their bivalent nature, being composed of two thickened semicircular halves.

Fig. 34. The seven bivalent ring-shaped chromosomes gathering at the equatorial plate.

Fig. 35. Some of the chromosome rings densely clustered at the equatorial plate as if they might be linked together.

Fig. 36. Pollen mother-cell in prophase of the heterotypic mitosis, showing the relation of the multipolar spindle to the surrounding alveolar cytoplasm. Seven bivalent chromosomes are gathered in the centre of the spindle.

PLATE XLII.

Oenothera grandiflora.

Fig. 37. Pollen mother-cell at metaphase of the heterotypic mitosis, the two sets of sporophytic chromosomes still united in pairs forming rings. The similarity of the chromosomes in form and size is clearly shown.

Fig. 38. Another metaphase illustrating the fact that certain rings may lag behind the others in completing the separation of their halves (sporophytic chromosomes).

Fig. 39. Early anaphase of the heterotypic mitosis, showing the V-shaped sporophytic chromosomes, all of similar form and size.

Fig. 40. Late anaphase, chromosomes gathering at the poles of the spindle.

Fig. 41. A group of chromosomes in late anaphase of the heterotypic mitosis (cut obliquely), showing certain of the chromosomes split lengthwise.

Fig. 42. Group of seven split chromosomes gathered at the pole of the heterotypic spindle viewed from above.

Fig. 43. Telophase of the heterotypic mitosis, the halves of the split chromosomes bending away from one another, showing a tendency to branch and to form an open network, small nucleoli appearing.

Fig. 44. The resting nucleus of the interkinesis between the heterotypic and homotypic mitoses. Seven split chromosomes are present, the halves in the form of U's joined together in the bent middle region.

Fig. 45. Pollen mother-cell containing resting nuclei in the interkinesis following the heterotypic mitosis, seven split chromosomes evident in the lower nucleus of the two.

Fig. 46. Prophase of spindle formation of the homotypic mitosis, the seven split chromosomes distributed throughout the nuclear cavity surrounded by a web of fibrillae.

Figs. 47 and 48. Multipolar stages in the formation of the spindle, the split chromosomes being gathered at the equatorial plate.

Fig. 49. Metaphase of the homotypic mitosis, split chromosomes at the equatorial plate.

Fig. 50. The separation of the halves of the split chromosomes.

Fig. 51. Anaphase of the homotypic mitosis, showing certain irregularities in the forms of the chromosomes.

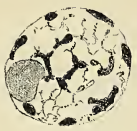
Fig. 52. Anaphase, the two groups of chromosomes, seven in each.

Fig. 53. Late anaphase of the homotypic mitosis, the upper group of seven chromosomes viewed from the pole of the spindle.

Fig. 54. Early telophase following the homotypic mitosis, the chromosomes becoming elongated and irregular in outline.

Fig. 55. Pollen mother-cell shortly before the formation of the pollen tetrad, chromosomes of irregular form connected by a very open reticulum.

Fig. 56. Nucleus of young pollen-grain, chromatic bodies present in an open reticulum.



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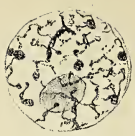

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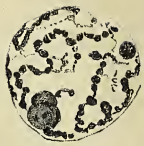

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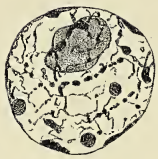

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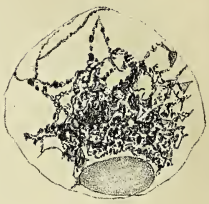

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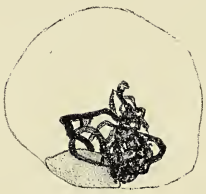

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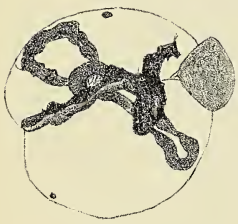

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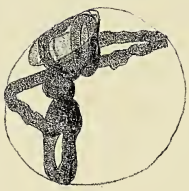

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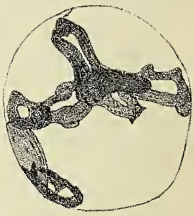

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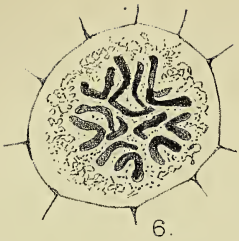

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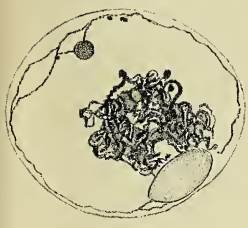

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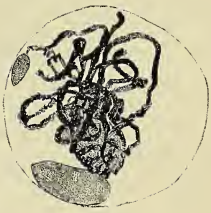

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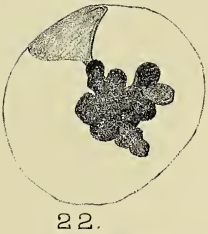

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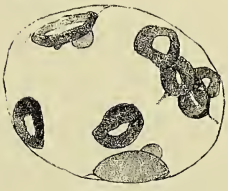

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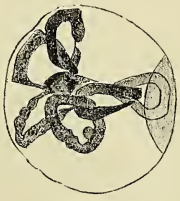

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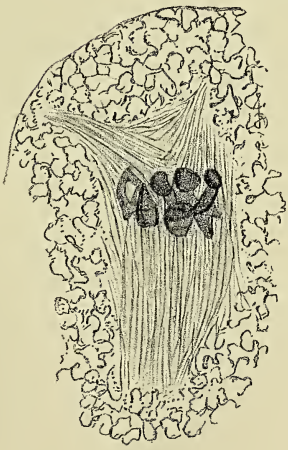

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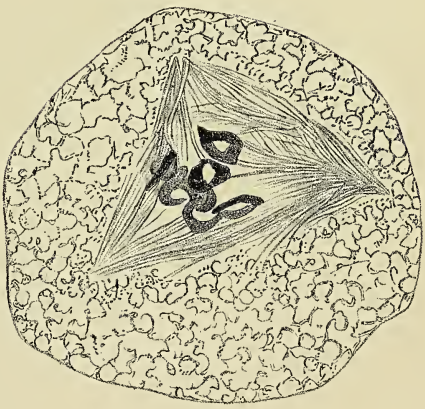

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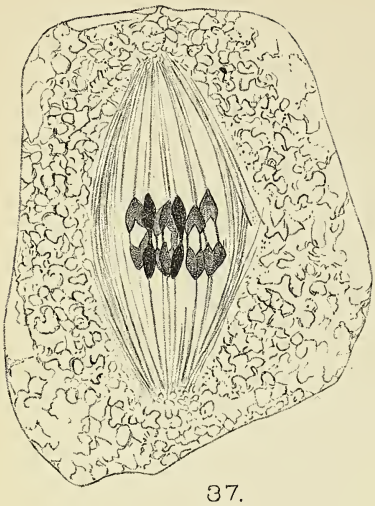

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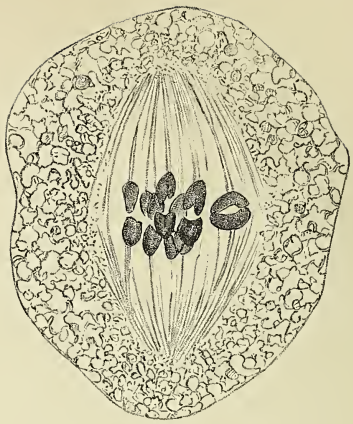

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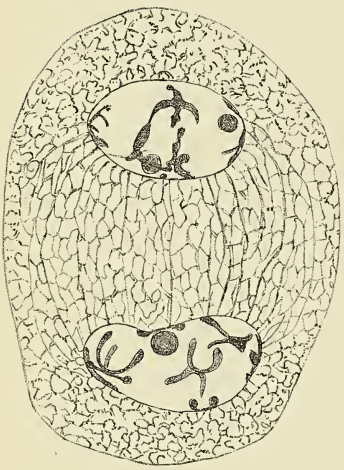

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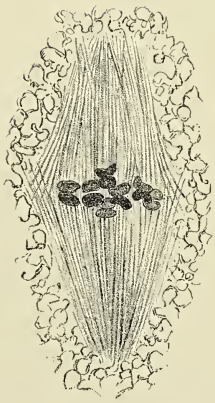

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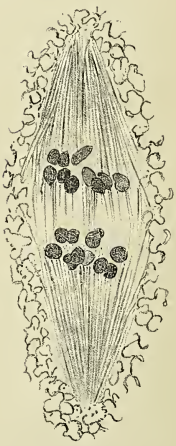

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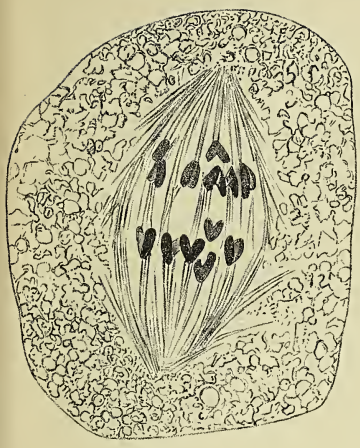
DAVIS — POLLEN DEVELOPMENT OF OENOTHERA GRANDIFLORA.



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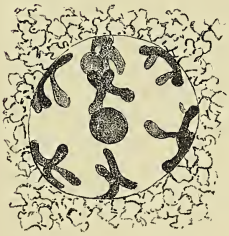

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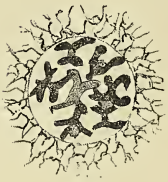

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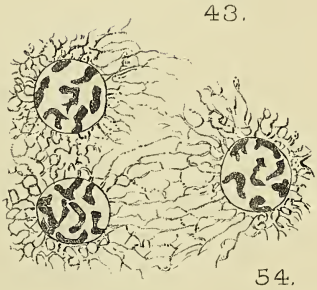

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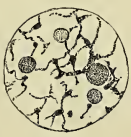

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Huth, lith. et imp.

Coccomyxa subellipsoidea, a new member of the Palmellaceae.

BY

ELIZABETH ACTON, B.Sc.

University Scholar, Birmingham.

With Plate XLIII.

THE Alga which forms the subject of this paper has long required thorough investigation. It is widely distributed in all parts of the British Islands, occurring only in subaerial habitats, generally on damp rocks and stones. It forms a thin mucous stratum of a dark green colour, which when dry becomes almost black and peels off the stone. The same Alga is not infrequently found in cold greenhouses, the green mucous stratum occurring both on the glass and woodwork, if sufficiently damp.

The stratum consists of large numbers of thin-walled cells embedded in a colourless mucilage. The cells are not all of precisely the same form, but for the most part they could be described as somewhat irregularly or obliquely ellipsoid.[1] Among these ellipsoid cells are some in which the ends are more pointed, and others which are almost spherical. Sometimes one end of the cell is much more pointed than the other, especially after division has just taken place. The cells are 6–10 μ in length by 4–6 μ in breadth. Each cell has a thin inner cellulose wall and an outer gelatinous coat. The chloroplast is parietal, and of variable shape, seldom covering more than half the cell-wall (Pl. XLIII, Fig. 1). It sometimes forms a U-shaped band and sometimes a shallow cup; and the margin may be entire, undulated, or drawn out into fine processes, which occasionally meet across the central vacuole (Fig. 1 *a*). In unstained material no pyrenoid is visible, though in some specimens small refractive granules can be distinguished; but on treating with a solution of iodine in potassium iodide a single pyrenoid becomes visible in the centre of the chloroplast, while the refractive granules are seen to be starch granules.

[1] The form of the cell evidently varies slightly under different conditions. In material collected three months later from the same place as that described above, the majority of the cells were nearly spherical.

[Annals of Botany, Vol. XXIII. No. XCII. October, 1909.]

Multiplication takes place by oblique fission, the mother-cell dividing into two, or occasionally four daughter-cells, which are exactly similar to the parent cell (Fig. 2).

Reproduction takes place by the formation within the mother-cells of four, or rarely eight, non-motile gonidia,which are nearly spherical 2–4 μ in diameter, and arranged either in the form of a tetrad or all in the same plane (Fig. 3). These may be formed in rapid succession, and as the gonidia do not at once separate, subspherical aggregates consisting of a large number of cells sometimes occur (Fig. 4). Also by the formation of macro- and microzoogonidia, 2, 4, 8, or 16 of which are formed within the mother-cells.

Cultures.

In order to obtain, if possible, other stages in the life-history, cultures of the Alga were tried in 0·25 per cent., 0·5 per cent., and 0·75 per cent. Knop's solution. In the stronger solution development was slow, the cells were small, and the culture assumed a brownish tinge. In the 0·25 per cent. solution the Alga reproduced actively by the formation of non-motile gonidia (Fig. 5), and the culture appeared to be a healthy one, although the cells showed some modification in structure. In this solution the cells increased very much in size and gradually became spherical, frequently attaining a diameter of 14 μ (Fig. 6). The chloroplast, which was crowded with large granules of starch, was no longer small, and in extreme cases appeared to cover the whole interior of the cell-wall. A large pyrenoid with a distinct starch sheath was present in each cell, and was quite visible without treatment with iodine solution. Sometimes two, and rarely three pyrenoids were present (Fig. 7).

This would indicate that the number of pyrenoids present is not constant, but is dependent on nutritive conditions. Further, in the culture in 0·75 per cent. Knop's solution, where development was slow, a pyrenoid could only be detected in a few cases, even after treatment with iodine solution. Thus it would appear that the presence or absence of pyrenoids, or the number present in the chloroplast are characters of no value from a systematic standpoint.[1]

Formation of Zoogonidia.

At the end of six months, material from the culture in 0·25 per cent. Knop's solution was transferred to a block of sterilized sandstone, standing in a culture dish with a layer of water at the bottom : in order to see whether

[1] These and many other observations relating to the occurrence of pyrenoids in chloroplasts of the Protococcoideae are entirely opposed to the views advocated by Schmitz, Schmidle (in Ber. Deutsch. Botan. Ges., Bd. xix, 1901, p. 24), and others, that the presence or absence of pyrenoids is sufficiently constant in the chromotophores of the lower types of Green Algae, to be utilized for specific and even generic distinctions.

the Alga would return to its normal state when placed in suitable surroundings. This culture was kept under constant observation. In about ten days zoogonidia began to appear, and in fourteen days zoogonidia were being actively formed, the culture rapidly spreading over the sandstone.[1]

The cells from the old culture continued at first to form non-motile gonidia (Figs. 8 *a*, 9 *a*, *b*). The zoogonidia were formed from these non-motile gonidia, often while the latter were still within the parent cell, cases being frequent in which some only of the daughter-cells had formed zoogonidia (Figs. 8 *b*, 9 *c*). Four, eight, or even sixteen zoogonidia were formed within a single gonidium (Fig. 10). If four only are formed they are 9 μ long by 5 μ broad, while if eight or sixteen are formed they are 7 μ long by 3 μ broad, so that macrozoogonidia and microzoogonidia occur, though the distinction is one of size only, as in other respects they are identical. The zoogonidia vary slightly in shape, being either cylindrical or more or less pointed at one end. They are biciliated, with a parietal chloroplast, which is granular and has a distinct pyrenoid (Fig. 12).

When fully formed they escape from the mother-cell, the process occupying from ten to twenty minutes. Material examined in water at this stage shows at first zoogonidia closely packed within the mother-cell and lying close to the cell-wall. In a few minutes they begin to vibrate slowly, forcing themselves apart and thus distending the cell-wall. As the wall is gradually distended they begin to rotate in different directions, at first slowly, and then with increasing speed, straining the cell-wall more and more until finally it is ruptured, and the zoogonidia escape. These swim actively for some time with the ciliated pole forwards, after which the movements gradually cease and the cilia are withdrawn. On coming to rest, the cell becomes rounded, develops a wall, and at once forms two or four zoogonidia; or it may increase in size and form eight zoogonidia (Fig 13). After repeated generations have been produced in this way, the zoogonidia finally settle down and divide by oblique fission, forming the typical vegetative cells (Fig. 15).

Systematic Position of the Alga.

The Alga just described belongs without doubt to the family Palmellaceae. The form and size of the cells suggest comparison with those palmellaceous Algae which possess elongated cells.

In the first place it is necessary to consider the Alga described by Harvey,[2] and subsequently by Cooke,[3] as *Palmella Mooreana*. This Alga has occupied a doubtful systematic position since its original description.

[1] Klebs, Bedingungen der Fortpflanzung bei einigen Algen und Pilzen, 1896, p. 60, states that *Vaucheria repens* produces zoospores when transferred from a solution of inorganic salts to water.

[2] Harvey: Manual British Algae, 1851, p. 178.

[3] Cooke: Brit. Freshw. Alg., 1882–84, vol. i, p. 14.

The colonies were described as irregularly globose, of a dark-green colour, about one inch in diameter, and to the naked eye resembling *Rivularia*. This leads one to the conclusion that Harvey's plant was *Aphanothece prasina*, a Blue-green Alga which occurs in precisely such colonies as he described. Rabenhorst [1] was also of the same opinion. Both Hassall,[2] and De Toni,[3] have likewise referred *Palmella Mooreana* to the Blue-green Algae.

Thus the Alga under consideration cannot be referred to *Palmella Mooreana*, as that Alga is without doubt a blue-green one.

The only other Alga with elongated cells which is definitely known to belong to the Palmellaceae is *Dactylothece Braunii*, Lagerh.[4] In this species, however, the cells are symmetrical, and the cell-division is transverse. The mucous envelopes of the cells are also lamellose.

In 1901 Schmidle [5] described the genus *Coccomyxa* to include an Alga with symmetrical cells, which formed a green gelatinous stratum. The present Alga agrees with this genus in its general habit, its elongated and asymmetrical cells, and in its oblique cell-division. It differs, however, from Schmidle's *Coccomyxa dispar* in the greater regularity in the form of the cells, and in the presence of pyrenoids.

It would appear that the genus *Coccomyxa* occupies an intermediate position between the Palmellaceae and the sub-family Selenastreae of the Protococcaceae, and would thus include certain Algae which could not be placed with certainty in either of these groups.

The present Alga can be diagnosed as follows:—*Coccomyxa subellipsoidea*. Stratum mucous and expanded, of a dark-green colour. Cells commonly obliquely ellipsoid (rarely subspherical), with a single chloroplast in the form of a parietal plate occupying about half the cell-wall and containing one pyrenoid; commonly occurring in pairs after division or scattered in a structureless mucus.

Multiplication by oblique division of the cell. Reproduction by 4 (rarely 8) non-motile gonidia, or by the formation of 2, 4, or 8 macrozoogonidia, or 8 or 16 microzoogonidia within a mother-cell. Length of cells 6–10 μ; breadth of cells 4–6 μ *Hab.* Widely distributed in British Islands, occurring in subaerial situations as a dark-green stratum on damp rocks and stones.

In the oblique division and in the form of the cell and of the chloroplast, this Alga shows a great resemblance to *Oocystis submarina*, Lagerh., as described and figured by Wille.[6]

[1] Rabenhorst: Flor. Eur. Alg. iii, 1868, p. 34.
[2] Hassall: Brit. Freshw. Alg., 1845, p. 316, t. 78, Fig. 1.
[3] De Toni: Sylloge Algarum, 1889, vol. i, p. 683.
[4] Lagerheim in Öfvers. af K. Sv. Vet. Akad. Forh., 1883, No. 2, t. 1, f. 22–4.
[5] Schmidle: loc. cit., p. 20.
[6] Wille in Ber. Deutsch. Botan. Ges., Bd. xxvi, 1908, p. 812, t. 15.

This investigation was carried out under the direction of Dr. G. S. West, to whom I wish to express my sincere thanks for his kindness in supplying me with material, and for many helpful suggestions in the course of the work. I also wish to thank Professor Hillhouse for his kindly advice and for revising the manuscript.

DESCRIPTION OF PLATE XLIII.

Illustrating Miss Acton's paper on *Coccomyxa.*

Figs. 1–15 × 1000. *p* = pyrenoid.

Fig. 1. Isolated cells. The shaded portion represents the chloroplast.

Fig. 2. Cells with mucous investment (indicated by dotted line) showing oblique fission.

Fig. 3. Mother-cells containing four non-motile gonidia.

Fig. 4. Subspherical aggregate of cells formed by development of non-motile gonidia in rapid succession; (*a*) shows an early stage.

Fig. 5. Formation of non-motile gonidia in a culture in 0·25 per cent. Knop's solution.

Fig. 6. Cells from a culture in 0·25 per cent. Knop's solution, showing increase in size.

Fig. 7. Cell from the same culture with two pyrenoids.

Figs. 8–15. Material from a culture on damp sandstone.

Fig. 8. *a*, Formation of four non-motile gonidia; *b*, Three of these have formed zoogonidia.

Fig. 9. *a*, *b*, and *c* show three stages in the formation of zoogonidia.

Fig. 10. Cells containing four, eight, and sixteen zoogonidia respectively.

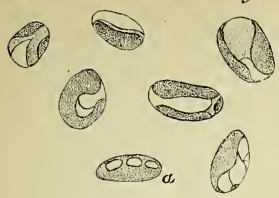
Fig. 11. Fully formed zoogonidia on the point of escaping.

Fig. 12. Zoogonidia. *a*, *b*, *c*, are macrozoogonidia; *d*, *e*, microzoogonidia.

Fig. 13. Generation of zoogonidia formed immediately from other zoogonidia without any period of rest.

Fig. 14. (*a*) zoogonidium with cilia withdrawn; (*b*) shows a later stage, the pyrenoid has increased in size and the cell has become more rounded; in (*c*) the pyrenoid has divided.

Fig. 15. Typical vegetative cell which appeared in the culture after a period of two months.



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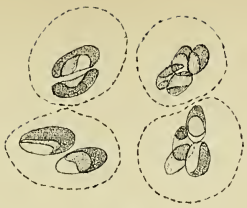

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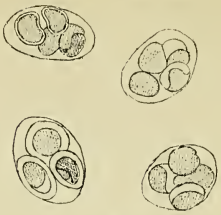

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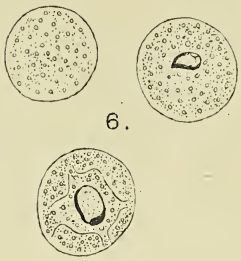

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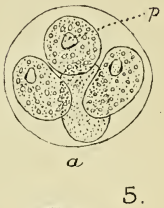

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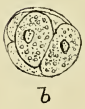

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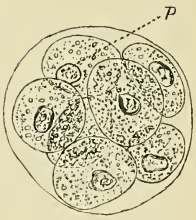

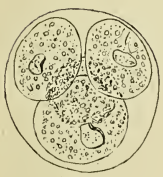

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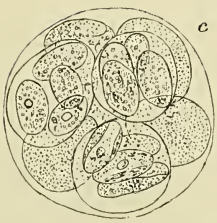

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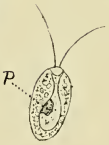

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E. Acton del.

Huth lith. et imp.

ACTON — COCCOMYXA SUBELLIPSOIDEA.

Botrydina vulgaris, Brébisson, a primitive Lichen.

BY

ELIZABETH ACTON, B.Sc.

University Scholar, Birmingham.

With Plate XLIV, and a Figure in the Text.

IN the more recent systematic accounts of the Thallophyta, *Botrydina vulgaris* has almost invariably been placed as a green alga, the nature of which was not thoroughly understood. In order to clear up this uncertainty it was suggested by Dr. G. S. West that I should thoroughly investigate some living material of this plant which he had obtained amongst *Mylia Taylorii*, *Campylopus flexuosus*, and other Bryophytes, in the damp woods in the vicinity of Capel Curig, Carnarvonshire.

The earliest description of *Botrydina vulgaris* is that given by Brébisson in Meneghini's 'Nostochineae' ('41),[1] in which publication it is placed as a member of the Nostocoideae. It is included by Kützing ('49)[2] in his 'Species Algarum' in the Palmellae, and he gives as his reference 'Bréb. Nouv. genr. d'Alg. ('39)'. I can only find, however, one paper by Brébisson with the title referred to,[3] and in this there is no mention of *Botrydina*, nor can I find any reference to it earlier than Brébisson's communication to Meneghini ('41).[4]

It is figured by Kützing ('49),[5] and for the first time short unseptate filaments are shown appended to the more solid portions of the thallus.

Rabenhorst ('68)[6] and Kirchner ('78)[7] have each given a brief diagnosis of it, and placed it in the Palmellaceae, but these descriptions do not strictly correspond with the original one given by Brébisson.

[1] Meneghini, 'Monogr. Nostoch. Ital.,' Atti R. Accad. Sci. Torino, ser. ii, tom. v, 1841, p. 98, Tab. XIII, Fig. 2. (Hassall gives a translation of this in his Brit. Freshw. Alg., 1845, p. 320, and an extract from this translation is given by Cooke, Brit. Freshw. Alg., 1884, vol. i, p. 14.)

[2] Kützing, 'Species Algarum,' 1849, p. 210.

[3] Brébisson, 'Description de deux nouveaux genres d'Algues fluviatiles, avec 2 pl. col.' (Ann. Sci. nat., 1844, vol. i.)

[4] Meneghini, loc. cit. ('*Botrydina vulgaris*, Bréb. in litt.')

[5] Kützing, Tabul. Phycolog., 1845–9, vol. i, Tab. 10.

[6] Rabenhorst, Flor. Europ. Algar., iii, 1868, p. 37.

[7] Kirchner, Alg. Schles., 1878, p. 111.

[Annals of Botany, Vol. XXIII. No. XCII. October, 1909.]

De Toni ('89)[1] did not consider the plant as an alga, and only refers to it as ' Sistit Hepaticarum propagula '.

West ('04)[2] states that the genus requires further investigation.

The specimens of *Botrydina* examined were growing indiscriminately amongst a Moss and an Hepatic, covering the shoots with a layer of dark green globular structures just visible to the naked eye. So freely was it growing that it was often difficult to distinguish the individual leaves of the Bryophyte. The *Botrydina* was firmly attached in some way to the shoots, and appeared to bind the leaves together by its growth (see Pl. XLIV, Fig. 1).

A microscopic examination showed the dark green bodies (varying in diameter from 20 to 300 μ) embedded in mucilage, by which they were attached to the shoots. The mucilage was traversed by fungal hyphae. Each globular structure consisted of a central mass of green cells invested by a colourless envelope of considerable thickness, which was apparently cellular. This was present in all cases, and showed little variation in thickness, being almost as thick in the smallest specimens as in the largest ones. The smaller specimens were subspherical, but the older ones showed a tendency to become irregular in shape as they increased in size, and in some cases two or three had become confluent, forming an irregular mass about 500 μ in diameter.

Cultures.

The apparent cellular structure of the colourless envelope formed a striking feature of the plant, and, with a view to ascertaining how this was formed, cultures of *Botrydina* were started early in July, 1908. The cultures were made in Petri dishes containing peptonized agar, and in culture dishes containing Knop's solution of various strengths. These were all overgrown by moulds and bacteria, and at the end of July a fresh series of cultures was made, taking every possible precaution to obtain them pure.

Hanging-drop cultures were made in water and Knop's solution. In the majority of these the *Botrydina* turned brown and died in a few days, evidently killed by the active development of fungal hyphae. In other cases the cover-glass soon swarmed with a small unicellular green alga, the active growth of this preventing the development of *Botrydina.*

Two cultures were made in 0·25 per cent. Knop's solution. In both cases there was at first a slight development of a fungus, but this soon ceased and a green alga appeared. One culture consisted mainly of *Stichococcus flaccidus*, a subaerial alga which was evidently present among the original tufts of moss; but the other, after a period of fourteen days, was practically a pure culture of the unicellular green alga which had

[1] De Toni, Sylloge Algarum, 1889, vol. i, p. 667.

[2] G. S. West, Treatise Brit. Freshw. Alg., Cambridge, 1904, p. 247.

appeared in the hanging-drop cultures. There appeared to be no development of *Botrydina* in either.

Cultures were made on sterilized sandstone and on a sterilized porous plate, but these also were unsuccessful, the *Botrydina* showing no development.

Finally, cultures were made in Petri dishes on 3 per cent. agar dissolved in 0·25 per cent. Knop's solution. In these the *Botrydina* lived for some time seemingly unchanged, though as before some cultures developed a fungus, and others a unicellular green alga. In one case only were there any signs of multiplication of the *Botrydina*. In this culture the globular structures had been broken up before infecting, and examination, after several weeks, showed the presence of very small, but fully-formed, globules. There was no indication how these had appeared, and so, in spite of all precautions, the repeated attempts at procuring a pure culture of *Botrydina* seemed to have failed, while no light had been thrown upon the formation of the envelope.

The Envelope.

At the same time that the culture experiments were being carried out, attempts were made to get some clear idea as to the structure of the globular bodies and the nature of the envelope, but here again results were at first unsatisfactory. Various stains were tried, but no clear preparation could be obtained. Stains penetrated with great difficulty, and when once they had done so, stained so deeply that the structure could not be observed. The extreme resistance of the envelope to chemical reagents was further shown by the fact that in material soaked for three days in Eau de Javelle, washed in water, and placed in 70 per cent. alcohol for three days, the green colour of the cells in the interior was still retained. Moreover, the envelope was not dissolved by the action of concentrated caustic potash for 24 hours, and was only dissolved by concentrated hydrochloric acid after a period of two days.

On testing for the presence of cellulose, both with iodine and sulphuric acid, and with a solution of chlor-zinc-iodine, no trace of it could be detected. Neither is it likely that the envelope is of a gelatinous nature, as stated by both Rabenhorst and Kirchner ;[1] for it does not swell in water, neither is it dissolved by water even on boiling, and it only stained with great difficulty in methylene blue.

As the envelope was thus shown to be neither cellulose nor a gelatinous one, the question arose of what does it consist? The absence of true cellulose and the great resistance of the envelope to the action of acids and alkalies led to the suggestion that it was of the nature of fungus-cellulose.

[1] These authors both state that the pseudo-parenchymatous investment is formed by the increase in size of the gelatinous walls of the original green cells.

Working on this hypothesis, the material was carefully examined to see if any connexion could be traced between the *Botrydina* and the fungal hyphae occurring in the mucilage in which it was embedded. After much material had been examined, two or three specimens were found (40–50 μ in diameter) in which a hypha could be seen in direct continuity with the envelope (Pl. XLIV, Figs. 2 and 3). Very few specimens were seen showing this connexion, so that if the envelope were formed by a proliferation of a fungus hypha it must lose all connexion with it at an early stage. A re-examination of the material brought to light a number of early stages in which the connexion of the globular bodies with the fungus could clearly be seen (Figs. 4 and 5). There is thus no doubt that the colourless envelope is formed by investing fungal hyphae.

The Structure and Arrangement of the Green Cells.

On gently crushing the globular structures which had been mounted in water, numerous green cells escaped into the surrounding fluid. These proved to be a unicellular green alga. The cells were usually elliptical in form, about 6–8 μ in length by 3–4 μ in breadth, though many of them were flattened and angular, and others curiously twisted (Fig. 6 *b*). The chloroplast was parietal, but did not extend over the whole cell, one side of the cell being colourless. There was a single pyrenoid in the centre of the chloroplast. In the colourless portion of the cell, several highly refractive granules were frequently present (Fig. 6 *c*). The nature of these was not determined. They gave no reaction for starch, oil, or sulphur.

The alga multiplied by oblique fission, freshly liberated cells often showing this condition (Fig. 6 *a*).

Sections were then cut in paraffin to determine the morphological relationships of the alga and fungus. Those sections stained in a one per cent. solution of acid-fuchsin gave the best results. The inner structure of the thallus varied in different specimens, and depended on whether the alga or the fungus was predominant, and not on the size or age of the thallus. In some specimens the interior was entirely filled with algal cells embedded in mucilage (Fig. 7), the fungus forming an investing sheath ; while in others the interior was filled with cells similar to those of the outer envelope, but with intercellular spaces, so that here the fungus did not form a sheath, but a pseudo-parenchymatous tissue, to the intercellular spaces of which the alga was restricted (Fig. 8). The crowding of the alga into the intercellular spaces would account for the flattened and distorted forms mentioned earlier. Stages intermediate between these conditions were present. Possibly a condition where a tissue is present would result from an earlier condition similar to that shown in Fig. 5, where the envelopes from a group of young thalli are confluent and the fungus seems to be predominant.

Multiplication.

Both the alga and the fungus seem to be able to develop quite well apart, and multiplication of the *Botrydina* is probably due to this. Here and there among the thalli specimens may be seen in which the fungal part has a brownish tinge and appears to be disintegrating, while the alga appears to be growing actively. It seems likely in these cases that the fungal membrane finally ruptures and the alga is set free, though this has not been observed. In other cases it is the alga which dies. A specimen was seen in which the fungal tissue was quite intact, yet no green cells could be seen. From two adjacent cells of the fungal tissue hypha had been sent out with septa at short intervals (Fig. 9). The alga when set free divides and multiplies until the cells come in contact with the fungal hyphae, by which they are enveloped with subsequent formation of a new thallus.

The Origin and Nature of Botrydina.

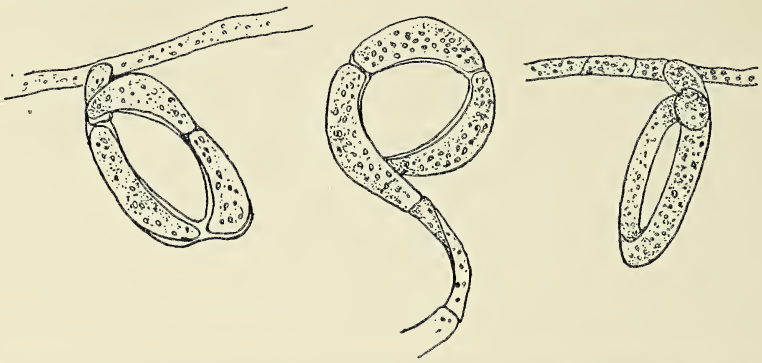
The manner in which the cellular structure of the fungal tissue has arisen is not quite clear. From Fig. 4 it would appear that on coming into contact with an algal cell, a single hypha applies itself and swells up enormously round the algal cell until it has completely covered it, and then septa are formed. In Fig. 4 it will be seen that, though the algae have been completely invested by the fungus, no trace of septa appears. A somewhat later stage is shown in Fig. 5, where a few septa are present. When it has reached this stage connexion with the hyphae appears to be lost, for Figs. 10 and 11 show specimens only slightly larger in which there is no connexion. The envelope must then increase in size and form new septa independently of this hypha, while the alga continues to multiply within.

The nature of *Botrydina* is fairly obvious from the foregoing description. *It consists of an alga and a fungus growing symbiotically, and is therefore a lichen.* It is certainly a primitive type of lichen; yet the union at an early stage of several smaller thalli shows a tendency towards the formation of a large thallus, though the diameter never exceeds 500 μ. It differs from the soredial stage of a typical lichen in the complete absence of any trace of hyphae in many adult specimens, and in the cellular nature of the envelope. The structure of the envelope and the inner pseudo-parenchymatous tissue formed from it shows a striking resemblance to the structure of the perithecial envelope of an Ascomycete such as *Eurotium*.

The assumption that *Botrydina vulgaris* is a lichen at once explains the difficulty experienced earlier in the work in obtaining pure cultures. The fungus would naturally develop in the nutrient agar, while the Knop's solution would favour the growth of the algal constituent; so that the

Botrydina, while apparently dying, was developing one or other of its two constituents, the conditions being unsuitable for the existence of both.

There is some evidence that the fungus belongs to the section Helicosporae of the family Mucedineae, for hyphae were observed bearing curious ring-shaped structures. These, on closer examination, proved to be incomplete rings, and suggested the first coil of a spiral conidium (Text-fig.).



The three figures represent the beginnings of conidial stages of the fungus which forms one of the constituents of *Botrydina*. The fungus belongs to the Mucedineae.

The alga appears to be identical with the unicellular alga which appeared so frequently in cultures of *Botrydina* in Knop's solution, and which occurs commonly in damp subaerial situations. This alga I have recently investigated and have given it the name of *Coccomyxa subellipsoidea*.

The cells are similar in size and form. Both have a parietal chloroplast of similar shape, with a single pyrenoid, and both multiply by oblique fission.

It is interesting to note that a culture of *Coccomyxa* on damp sandstone developed subspherical aggregates of cells which only wanted the fungal investment to resemble *Botrydina*. In this same culture fungal hyphae were present, but not the *Botrydina*-fungus, as the hyphae were much thinner and unseptate ; yet it is an interesting fact that this fungus was combining with the algal cells, and seemed to be forming a lichen in some respects analogous to *Botrydina*. Each algal cell was completely invested by the fungus, though up to the present no further stage has been noted, and no septa have appeared in the investing sheath.

Conclusions.

Botrydina vulgaris, Bréb., is a primitive lichen with a more or less spherical thallus of small size (15–300 μ). It shows no resemblance to the soredial stage of a typical lichen, and is therefore possibly one of the most primitive types of existing lichens. It consists of an envelope of fungus

cells, often proliferating inwardly, including the green algal cells, the latter forming either a central green mass or occurring more sparingly scattered in the interspaces between the fungus cells.

The alga is *Coccomyxa subellipsoidea*, one of the Palmellaceae. The fungus belongs to the section Helicosporae of the family Mucedineae.

Its natural habitat is in damp shady situations among various Bryophytes, generally on rocks, but sometimes on damp ground. It is not uncommon in the mountainous districts of the British Islands, and occurs among a variety of Mosses and Hepatics.[1]

DESCRIPTION OF PLATE XLIV.

Illustrating Miss Acton's paper on *Botrydina*.

Fig. 1. Shoot of *Campylopus flexuosus* covered with *Botrydina*. × 30.

Fig. 2. Thallus of *Botrydina* showing envelope with attached hyphae. × 1000.

Fig. 3. Thallus showing arrangement of green cells in interior, and attached fungal hypha. × 1000.

Fig. 4. An early stage. The algal cells have been invested by the fungus, but no septa have appeared. × 1000.

Fig. 5. A later stage showing septa. × 1000.

Fig. 6. Algal cells. *a*, shows oblique division; *b*, distorted form; *c*, typical cell with refractive granules. × 1000.

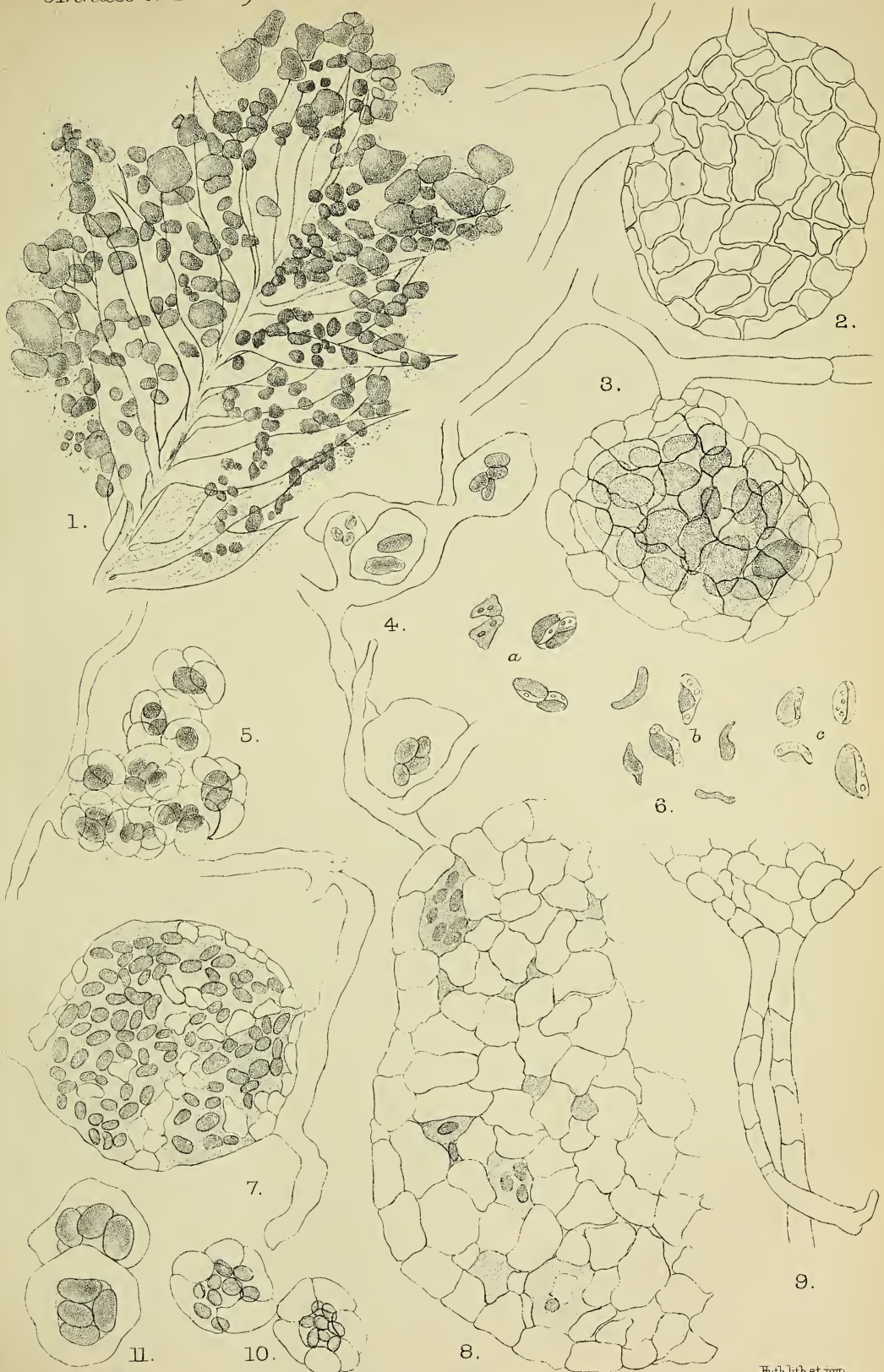
Fig. 7. Section through a thallus. The interior is filled with algal cells lying in mucilage. × 1000.

Fig. 8. Portion of a large thallus. The interior consists almost entirely of fungal cells. A few algal cells are shown. × 1000.

Fig. 9. Portion of a thallus from which the green cells had disappeared, with hyphae growing out from two adjacent cells. × 1000.

Figs. 10–11. Young specimens with no attached hyphae. × 1000.

[1] It occurs in many parts of West and North-West Yorkshire, and has been noticed more particularly among *Tetraphis pellucida* and *Leucobryum glaucum* (vide W. and G. S. West, 'The Alga-flora of Yorkshire,' Trans. Yorks. Nat. Union, vol. v, 1901, p. 129).



E. Acton, del.

Huth, lith. et imp.

ACTON — BOTRYDINA VULGARIS.

On the Occurrence of Centripetal Xylem in Equisetum.[1]

BY

ARTHUR J. EAMES.

With Plate XLV.

AMONG living Vascular Cryptogams perhaps no group more readily attracts attention than the Horse-tails. These plants, with their odd appearance and peculiar organization, clearly proclaim themselves a stock distinct from other recent Pteridophytes. The few species, about twenty-five in all, do not vary greatly among themselves, and constitute a single genus, *Equisetum*. Although reduced in numbers in the present epoch, the Equisetales formed a large and important part of the flora of the earth during Paleozoic time. In the Devonian period well-developed and specialized Equisetaceous forms already existed; through the succeeding Paleozoic periods, especially during the Carboniferous, flourished the tree-like Horse-tails with secondary thickening, called Calamites. After the Permian age there lived other members of the Equisetales, resembling always more and more in succeeding time the existing genus. In spite of continued reduction, the living representatives of the Equisetales resemble the Calamites very strongly: form, structure, and anatomy, both of stem and reproductive branches, are in many important features very similar. From these ancient, arborescent Horse-tails, our present genus has without question descended, and of that great and flourishing group its few wide-spread species are the only remaining representatives. *Equisetum*, because of this isolated position among existent plants, and because of its unusual structure, has been the subject of much study. Particularly has its organization in comparison with that of Calamitean fossil remains been of interest since the latter have become better known from abundant specimens, and the cryptogamic rank of all has been firmly established. For, in the anatomical study of plants, the object, as in similar investigation in the animal kingdom, is not merely to reveal the relationship of living genera, but especially to fix phylogenetic lines. For such determinations, the preserved remains of forms that existed in long-past time must hold great interest; a knowledge of their organization becomes a necessity to a clear understanding of their evolution.

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 19.

[Annals of Botany, Vol. XXIII. No. XCII. October, 1909.]

Most Calamitean fossils consist of fragments of stems, or of casts of these, or of their internal cavities. Where the structure of tissues can be determined, the vascular system has naturally been best preserved. This system in the Calamites has been the subject of considerable investigation, and until recently its structure, in the stem, at least, was thought to be pretty well understood. But one of the older genera has lately been shown to possess a vascular structure previously unknown in this group, and of very great importance. The plant in question is from Lower Carboniferous strata, and has been named *Calamites pettycurensis* by its discoverer, Dr. Scott.[1]

The suggestion that a new genus, *Protocalamites*, be founded upon it has been provisionally accepted by Dr. Scott, although he believes it may belong to the already established genus, *Archaeocalamites*. The vascular structure of the stem is like that of other Calamites, with one important exception—the primary wood developed both outwardly and inwardly from the protoxylem lacuna. We have in this plant the most ancient of the Equisetales showing distinct *centripetal* wood in the stem.

In its anatomy *Equisetum* differs strikingly from the Calamites only in the lack of secondary wood. The secondary growth largely disappeared in the Equisetal stock at the end of the Permian period, although the Mesozoic Equisetales may have possessed it to some extent. A few even of the living species show remnants of such structure. In these cases, it occurs as a continuation in the growth of the most 'woody' portion of the stem, the 'nodal ring', best called the supranodal wood. Cormack[2] has shown such development in *Equisetum maximum*, Lamarck (*E. Telmateia*, Ehrh.), and Queva[3] confirms its presence there, noting it also in *E. arvense*, L. In *E. hyemale*, L. and its variety *robustum*, A. A. Eaton (*E. robustum*, A. Br.), as well as in the two above-mentioned species, the writer has seen evidences of this ancestral character. Often it appears most definitely in the rhizome. Doubtless other large and well-grown terrestrial species will show such conditions. In the much-reduced or semi-aquatic species, (*E. scirpoides*, Michx., *E. fluviatile*, L. (*E. limosum*, L.), and *E. sylvaticum*, L., no clear indications of cambial activity were seen.

Since, then, our recent species possess so little secondary growth, comparison of their vascular system with that of their Paleozoic ancestors concerns chiefly the primary wood. Even here great reduction has occurred. In the internodes of *Equisetum* we find the fibro-vascular system to consist of a circle of separated bundles lying opposite the external ridges of the stem, just outside the large central cavity that occupies most of the pith.

[1] Scott, D. H.: On a Primitive Type of Structure in Calamites, Ann. Bot., vol. xv, p. 773; 1901. Studies in Fossil Botany, 2nd edition, p. 36, Fig. 11; 1908.

[2] Cormack, B. G.: On a Cambial Development in *Equisetum*. Ann. Bot., vol. vii, p. 63; 1893.

[3] Queva, C.: Histogenèse et structure du stipe et de la fronde des *Equisetum*. Mém. de la Soc. d'Hist. Nat. d'Autun, t. xx, p. 21; 1907.

In structure these bundles are unusual: the xylem of each is in three parts, a median inner and two lateral external portions. Among these groups of elements lies the phloem. The innermost xylem-group consists of the protoxylem, the first-formed tracheides, spiral or loosely ringed in sculpture. In a mature internode the position of this wood is occupied by a cavity, the so-called 'carinal canal', formed by the breaking-up and tearing-apart of these early-formed tracheides during elongation of the internode, and by the enlargement of the so-formed lacuna during radial and tangential growth of the surrounding cells. The more or less scattered and disarranged remnants of the disrupted cells are found in this opening in the tissues. The two other xylem-groups lie farther out, on the flanks of the canal, separated therefrom by one or more parenchyma cells. They consist of rows, or in some species of groups, of a few elements which develop considerably later than the protoxylem. In general these tracheides are scalariform, reticulate, or pitted, but occasionally annular and even spiral thickenings are found. These form the metaxylem of the bundle. Over their order of development, and the significance of the complexity of this bundle, considerable controversy has arisen. Owing to this and to the intimate relation of the leaf-trace to this bundle, the writer has made some study of the order in which the tracheides arise and the relationship of the different parts of the bundle to the whole.

In the nodal region the primary wood of *Equisetum* is much increased. The internodal strands alternate in the well-known manner. At the node they fork; each metaxylem group passes to the bundle nearest in the internode above, and, after passing the next node above, returns to the same bundle. The carinal canal disappears as the bundle approaches the node. The protoxylem, there filling the position of this lacuna, behaves in much the same manner as the metaxylem. The forking and fusion of these portions, with some enlargement or additional growth, forms a solid ring of wood just above the node. This band, the supranodal wood, projects inwardly somewhat, and externally to a considerable extent, the extension in the latter direction becoming greater when secondary growth occurs.

In structure the primary bundle of the Calamitean stem was collateral. It possessed near its inner margin a carinal canal, which, according to Williamson and Scott,[1] is identical in nature, origin, and position with that in *Equisetum*. Extending outwardly in a wedge-shaped mass from the external periphery of this lacuna is the metaxylem. As seen in radial sections its development was centrifugal. Beyond this lay the phloem, or in large or mature stems, secondary wood. The supranodal primary wood of these old plants also much resembled that of *Equisetum*. Its develop-

[1] Williamson, W. C., and Scott, D. H.: The Organization of Fossil Plants of the Coal Measures. Phil. Trans. of the Royal Soc. of London, vol. clxxxv B; 1894.

ment, as shown by the same authors, is likewise centrifugal; the protoxylem of the internodal bundles is continuous with the innermost elements of this xylem-band. In *Equisetum*, too, there has been no question raised, so far as is known to the writer, as to the centrifugal development of this supra-nodal wood. Certainly its innermost elements are protoxylem; many radial sections show this condition clearly.

Although it is generally agreed that the supranodal wood and the carinal strand in *Equisetum* are homologous with the similar tissues in Calamites, there has been some difference of opinion in regard to the interpretation of the remaining parts of the bundle.

The two metaxylem-groups, in the internode distinct from the clearly endarch protoxylem, have the appearance of being exarch, for the tracheides constituting them are often smallest at the outer extremity, and increase in size with some constancy inwards. Mr. Gwynne-Vaughan [1] emphasizes this fact, and bases thereon his suggestion 'that the lateral xylem strands in the vascular bundles of the existing Equisetums may perhaps be taken to represent the last remnants of a primitive central mass, and that this would be entirely in agreement with their apparently centripetal development, and in particular with their cauline course'. Poirault [2] also considers the development of the metaxylem centripetal, and Lignier [3] regards it as at least partially so. The first-mentioned author at the same time doubts the integral nature of the Equisetal fibro-vascular bundle, stating that of the three groups of tracheides in each internodal bundle the carinal alone leaves the node, passing out as the leaf-trace, and 'the two lateral strands join on to the xylem of the nodal ring, and in certain species may be traced as externally projecting ridges over the nodal xylem into the internode above'. The fact that the metaxylem-groups, so definitely separated from the protoxylem, develop much later than the latter may be said also to point to the composite character of this bundle.

The study of the vascular system of *Equisetum*, both nodal and internodal, developing and mature, and of that of the reproductive axis, together with a comparison with the stele of the Calamites, has led the present writer to the conclusion that the bundle of the Horse-tails is essentially centrifugal in development and, without doubt, simple, or integral, in nature.

Let us grant for the moment the centripetal development of the flanking metaxylem-groups in the bundle. The supra-nodal wood is centrifugal. And *into* or *over* this, fusing therewith, pass these strands. Adopting the first supposition, we find centripetal wood passing directly,

[1] Gwynne-Vaughan, D. T.: Remarks upon the Nature of the Stele in *Equisetum*. Ann. Bot., vol. xv, p. 776; 1901.

[2] Poirault, G.: Développement des Tissus dans les organes végétatifs des Cryptogames vasculaires. Mém. de l'Acad. Imp. des Sc. de Saint-Pétersbourg, 7e série, t. xxxvii, p. 13; 1890.

[3] Lignier, O.: Équisétales et Sphénophyllales; leur origine filicinéenne commune. Bull. Soc. Linn. de Normandie, 5e série, vol. vii, p. 114, note. Caen, 1903.

through continuous lines of tracheides, into centrifugal, as the node is reached, and *vice versa* as the node is left in passing upward along the bundle. Such a condition is difficult to imagine, and is unknown elsewhere in the anatomy of stems. Assuming the alternative view, that these 'centripetal' groups pass *over* the supra-nodal wood, an absurd condition is likewise presented, for we have *centripetal* wood, the suggested possible 'remnants of a primitive central mass' of such wood, passing *external* to *centrifugal* wood, the remains of a mass to which it formerly lay internally. Such relation obviously cannot hold. Moreover, in the two species, *E. hyemale* and *E. maximum*, in which the statement is made that such a condition exists, there has been shown to be at the point in question some cambial growth. Hence the centripetal wood there passes *outside secondary wood*! Yet, according to Gwynne-Vaughan (p. 779 of the above-mentioned paper), the view that these bundles are the vestiges of an ancestral, solid, internal stelar mass is '*entirely in agreement* with their apparent centripetal development and in particular *with their cauline course*'. (The italics are the present writer's.) Again, the metaxylem-groups lie externally to the protoxylem, which is granted, apparently by all, to be endarch. Can the former then, *if exarch*, be the vestiges of tissues central in position, and thus necessarily internal to the carinal canals, as is the centripetal wood in *C. pettycurensis*? The carinal canals of *Equisetum* and of the Calamites are indisputably homologous.

It is further stated that the gradation in size and the sculpture of the walls of the tracheides of the lateral groups indicate a centripetal development. Size does not necessarily indicate the order of development; and the sculpture of the tracheide-walls in metaxylem is, at least to a certain extent, independent of order of development. In his 'Observations on the Anatomy of Solenostelic Ferns', Mr. Gwynne-Vaughan[1] mentions cases in the stems of which the first-formed tracheides 'appear without order here and there throughout the whole xylem-mass, so that the differentiation is quite irregular'. Under these conditions, the sculpture is always scalariform, and 'there is no difference whatever between the first-formed elements of the xylem and those formed later on'. Plate XLV, Fig. 1, shows a somewhat similar condition in *Lycopodium*. Here, though, a definite protoxylem has first formed; lignification proceeds in general centripetally, but with much irregularity. Even in secondary wood completion of lignification does not proceed in perfect centrifugal order. This was seen by the writer, for example, in the maturing wood of *Betula*. The loss of protoplasm, indicating the completion of lignification in tracheides, is decidedly irregular.[2]

[1] Gwynne-Vaughan, D. T.: Observations on the Anatomy of Solenostelic Ferns. Ann. Bot., vol. xvii, p. 727; 1903.

[2] Special precautions were taken to assure the existence of this condition. Longitudinal sections of the wood were studied. The length of the tracheides and the shrinkage of their protoplasm in length were observed. These elements are formed in rather distinct tiers, and practically no

The development of a bundle in *E. hyemale* var. *robustum* is shown in Fig. 2. In this case, as in that of the birch wood, necessary precautions were taken in the preparation of the section. The preparation photographed came from near the centre of a good-sized piece of stem embedded in celloidin, which was not removed. Here is seen a condition which is apparently most significant. For in the rows of metaxylem-cells, which can be clearly made out flanking the carinal canal on either side, differentiation, as shown by loss of protoplasm, is seen to be centrifugal upon the right, and centripetal upon the left. The study of many sections, moreover, shows that other bundles lose protoplasm from the median tracheides first. Thus considerable irregularity of development of this tissue is established. Other phyla of living Pteridophytes show, as stated above, the same irregularity of xylem-development. Its occurrence, therefore, appears to be of no special importance in the Equisetales, the less so because a similar irregularity of development appears even in tracheides laid down by a cambium, as in the case of *Betula* cited above.

The structure of the tracheary wall of these portions of the bundle was also studied. As the conditions of disappearance of protoplasm would cause one to suspect, there is some irregularity in structure; reticulate and pitted tracheides occupy various positions in relation to scalariform elements in *E. maximum* and *E. hyemale* var. *robustum*. The examination of a considerable number of sections is clearly necessary, for, as Fig. 2 suggests, one may show apparently clear endarch, and another as clear exarch arrangement. *In the majority of cases* in these two species, however, development toward the outside was manifest. In *E. arvense* an unusual condition is found. The metaxylem-tracheides of some bundles are essentially alike, only very slight differences being noticeable; those of other bundles are in part spiral and annular. Wherever this latter, not uncommon, condition occurs, the order of development is distinctly centrifugal. A possibly parallel case has been noted in *E. maximum* by Queva.[1] He figures (p. 16) a cross-section of a bundle in a long internode of the sterile stem. The metaxylem-groups are in part invaded by lacunae very similar in nature to that of the protoxylem. In the same investigation, Queva seems to have seen a rather regular development in this species. He says (p. 35), '*La différentiation est exclusivement centrifuge*, mais le bois se forme en deux temps.' His Fig. 11 shows this order. Thus, though lignification does not occur invariably in an outward direction, irregularities

longitudinal shrinkage of their contents had occurred. Transverse sections were then cut so far down into the block as to be from a region the tracheides of which could not have been torn open and their contents in any way lost during the cutting-up of the material for killing and fixing. The infiltrating material, celloidin, was not removed from the sections, thus guarding against any loss of protoplasm by 'dropping-out.'

[1] Queva, C.: Histogenèse et structure du stipe et de la fronde des *Equisetum*. Mém. de la Soc. d'Hist. Nat. d'Autun, t. xx; 1907.

in its order having in the Filicales and Lycopodiales no observable effect upon the sculpture of the cell-wall, most stress in deciding the morphological order of development must be laid upon the latter. Judged by this standard the flanking xylem-groups of *Equisetum* are centrifugal in their development.

It is consequently evident that all parts of the stem-bundle develop in the same direction. Had there been no apparent proof that the respective portions of this much-disputed bundle were of varying differentiation, doubt would not, in all probability, have been cast upon its simple nature. Gwynne-Vaughan,[1] in his statement that only the protoxylem-strand is concerned in the formation of the vegetative leaf-trace, has brought forward evidence for the complex nature of the internodal bundle. Lignier (see above-mentioned article, p. 114, note) is of the same opinion in regard to the origin of the leaf-trace. On the other hand, Campbell[2] and De Bary[3] seem to understand the leaf-trace as connected with the entire internodal bundle. Dr. Scott's position on this point is clear. In his 'Studies in Fossil Botany' (1908), he says (p. 25): 'A single vascular bundle enters the stem from each leaf and passes straight down through one internode only. At the node below it forks.' The bundle in its entirety is clearly indicated.

Fig. 3 appears to settle this point at once. The photograph is of a portion of a radial section through the node of *E. hyemale* var. *robustum*. In this species the metaxylem is very well developed, and its share in the formation of the leaf-trace can be easily seen. In other species it is less easy to observe. Differences of opinion on this point are not hard to explain. Even though the tracheides of the metaxylem are related to those of the protoxylem at the point of origin of the leaf-trace, they are still situated upon its flanks and a radial section through the very centre, showing the departure of the protoxylem, does not usually also show well the relation of the metaxylem to the trace. And a section passing through the metaxylem might readily be cast aside as not strictly radial; especially would this be likely to occur where the metaxylem is small in amount, and the section contains none of the protoxylem. The metaxylem unquestionably takes part in the formation of the leaf-trace. In Fig. 4, which illustrates another feature of the leaf-trace, further evidence for the centrifugal development of the metaxylem is presented. The tracheides upon the right of the protoxylem are the direct continuation of the elements given to the leaf-trace by a lateral metaxylem-strand in Fig. 3. Their order of development is definitely outward.

The unit nature of the cauline internodal bundle becomes further

[1] Gwynne-Vaughan, D. T.: Remarks upon the Nature of the Stele in *Equisetum*. Ann. Bot., vol. xv, p. 775; 1901.

[2] Campbell, D. H.: Mosses and Ferns, p. 462; 1905.

[3] De Bary, A.: Comparative Anatomy of the Phanerogams and Ferns (trans.), p. 279; 1884.

evident from the structure of the leaf-trace itself. The latter is nearly divided in its proximal portion into three parts, in position and in structure corresponding to those of the complete, cauline, fibro-vascular bundle. A connexion with all parts of the stem-bundle is here indicated. For, if the protoxylem alone passed into the leaf, we should not expect to find this simple structure dividing in such a case into three new bundles, or taking on a form indicating such a condition; especially is it difficult to conceive its forming two new bundles centripetal in development, when it, itself, is centrifugal. Whereas, if all parts of the internodal bundle unite in the formation of the leaf-trace, they would undoubtedly assume their normal position and relations, forming there a bundle of the same general structure as that of the internode.

The internodal bundle of the Calamites has been briefly described above (p. 589). From this has come, by reduction of the metaxylem, the fibro-vascular bundle of *Equisetum*. In the reproductive axis of the latter genus the bundle-structure points to this relation. The cone-bundles of *E. hyemale* and of *E. fluviatile*, when seen in cross-section, show a condition intermediate between that of the internodal bundles of the Calamites and of the living genus. In many of them there is found the typical protoxylem-lacuna. Spreading externally from this is a considerable body of elements that are clearly seen to be metaxylem in radial sections. They are not solidly packed, but have many parenchyma-cells intermingled with them. There is a marked tendency for these metaxylem-tracheides to form groups outwardly flanking the protoxylem. In the cone of *Equisetum* we have accordingly repeated the Calamitean primary bundle well started on its way in reduction toward the condition found in the vegetative stem of the living descendants of the group.

An analogy of some value can be drawn between aspects of the vascular system of two groups of plants which are believed to be much reduced, the Horse-tails and the Monocotyledons. The ancestors of both undoubtedly possessed secondary growth; in the living members this is found only as a remnant. It is a striking fact that this growth, as observed by Dr. Chrysler [1] in the Gramineae, is in exactly the same situation as it appears in *Equisetum*—slightly above the node. The resemblance of the primary wood in the bundles of these two widely separated groups has been noted by many writers. It is, indeed, close; in both are found a canal containing broken-down protoxylem and two externally flanking metaxylem-groups, to a greater or less extent separated therefrom.

The internodal bundle, then, of *Equisetum* is, without doubt, centrifugal throughout in its development, and is structurally a fibro-vascular unit, a much reduced Calamitean bundle.

Paleobotanists have recognized a close relationship between the

[1] Chrysler, M. A.: The Nodes of Grasses. Bot. Gazette, vol. xli; 1906.

Equisetales and the Sphenophyllales. The latter possess a stolid stele of centripetal wood. *Calamites pettycurensis* has a considerable amount of centripetal xylem; and even the axial bundles of *Equisetum* show clear centripetal wood in some cases.

Cross-sections of the cones of *E. hyemale* and *E. fluviatile* show bundles as described above (p. 594) with this addition: upon the inner side of the lacuna are often found, adjacent to the canal, or separated therefrom by a few parenchyma cells, from one to four or five tracheides. Radial sections (Fig. 6 of *E. hyemale*) show these to be metaxylem, scalariform or reticulate elements, situated on the inner side of the broken-down spiral and ringed elements of the protoxylem. With its considerable body of metaxylem upon the external side of the lacuna, the reproductive branch possesses fibro-vascular bundles of the type that has been called 'mesarch'.[1]

The reproductive axis of *Equisetum* gives accordingly some evidence of the derivation of the genus from Calamitean forms similar to *C. pettycurensis*. Still stronger evidence is provided, however, in the foliar traces emanating from this region. The strobilus consists of a continuation of the stem, with much shortened internodes bearing, at the nodes, whorls of modified leaves, the sporophylls. The bundles of the sporophylls were studied in transverse and longitudinal sections in the following species:—*E. maximum*, *E. hyemale*, *E. fluviatile*, *E. arvense*, *E. palustre*, *E. sylvaticum*, and *E. scirpoides*. In all save the last two strongly-marked mesarch strands appear. In external appearance the sporophylls vary but slightly. Their fibro-vascular system, however, shows considerable difference in development, due to the great range of habitat found in the genus—from xerophily in *E. scirpoides* and *E. arvense* to hydrophily in *E. fluviatile*. But even in the latter the cone is not weak in vascular elements. In nearly all species a lateral fusion of sporophyll-peduncles occasionally occurs, and the union of their flattened distal ends is often found. Figs. 7 and 8 show cross-sections of sporophylls of *E. maximum* and *E. fluviatile* respectively, the former near the middle of the stalk, and the latter through the trace as it leaves the cortex of the strobilus. Both show well-defined lacunae in the centre of the bundle. Above and below, and to some extent also upon the sides, are tracheides. The longitudinal sections were cut in the dorsiventral plane of the sporophyll. Looking now at such a section (Fig. 9) from the fertile leaf of *E. fluviatile*, the structure seen in transverse section is explained. The elements upon the left are ventral, corresponding to the upper group in the cross-section; those upon the right are dorsal tracheides. Between, is a partially open space, the

[1] The term 'mesarch' was first applied to such structure by Count Solms-Laubach. This type of arrangement in bundles has since been used to a considerable extent in the determination of relationships among the groups of Vascular Cryptogams.

lacuna of the cross-sections. The origin of the latter is the same as that of the carinal canal of the stem. The elements outside the disarranged spiral and annular tracheides of the lacuna are closely ringed or scalariform. This is, then, a mesarch bundle. The dorsal elements are of course centrifugal, the ventral, centripetal. It might, perhaps, be objected that this arrangement of elements in the sporophyll-bundle is due to the concentric arrangement of the sporangia. But this is not the case. The strand continues in the general form shown in Fig. 8 until it approaches the base of its flattened end. There occurs a division into a number of bundles corresponding to the number of sporangia. Fig. 10 shows this condition in *E. maximum*. In the lower portion the undivided bundle is seen. Its resemblance to that of *E. fluviatile* is close. There is a central mass of distorted protoxylem bounded by unbroken tracheides. As the bundle passes outward division is made into several parts, only two of which, of course, lie in the plane of section. The separation is made from the very centre, a portion of the protoxylem passing to each branch-strand. The latter immediately forms metaxylem upon its inner face. At the very top of the figure this can be seen. And this mesarch structure in each of these small bundles can often be traced clear to the base of the sporangium. The arrangement of the sporangia upon the tip of the sporophyll cannot, then, control the form of the main bundle, causing it to simulate mesarch structure. The protoxylem of the two species discussed above consists chiefly of loose-ringed tracheides. Two other species are interesting in comparison: *E. arvense* (Fig. 11) shows portions of a spiral tracheide in the protoxylem; the central elements of *E. palustre* (Fig. 12) are spiral throughout.

The sporophyll fibro-vascular bundles appear not only to be mesarch but concentric, a condition which may also be regarded as a relic of ancestral structure.

Jeffrey[1] has shown that 'the Equisetaceous strobilus perpetuates both the non-alternating strands and the complete absence of foliar-gaps of the oldest Calamitean forms'. These figures make it probable that it also retains, so far as the sporophylls are concerned, the bundle-structure of ancestors contemporary with *C. pettycurensis*.

In the trace of the vegetative leaf the mesarch condition is also found. Investigations were made by means of radial sections of complete nodes with leaves attached, by cross-sections of the stem just above the origin of the traces, and by tangential sections at the outer limit of the supranodal wood. Well-marked centripetal wood was found in *E. maximum*, *E. hyemale*, *E. hyemale* var. *robustum*, *E. arvense*, and *E. scirpoides*. In *E. sylvaticum* and *E. fluviatile*, species of shaded and aquatic situations, the

[1] Jeffrey, E. C.: Are there foliar Gaps in the Lycopsida? Bot. Gazette, vol. xlvi, p. 254. 1908.

xylem-system is much reduced, particularly in the leaves. Yet indications of the mesarch structure of the leaf-trace were seen even in these two last-named species.

A brief description of the course of the trace will aid in understanding the conditions presented. As shown in the first part of this paper, the leaf-trace is formed by a continuation of the various elements of the internodal bundle. The carinal canal becomes discontinuous as it approaches the node. The protoxylem occupying that space enlarges, extending radially, then passes upward and forms the innermost tracheides of the supranodal wood. Just before that xylem-mass is reached, a 'branch' to the leaf is sent off more or less horizontally. Each of the metaxylem-groups enlarges somewhat, and gives off a small strand which passes upward and slightly outward, joining the protoxylem of the leaf-trace a short distance outside the point where the latter is first seen clearly. These three strands fuse more or less, and form the leaf-trace. Fig. 4 shows the structure of such a trace in *E. hyemale* var. *robustum* as it passes through the cortex. (The leaf lies above to the right.) Centrally are seen the scattered rings of protoxylem; to the right is metaxylem that is clearly formed centrifugally, its outermost elements being pitted. Another group of metaxylem is seen upon the inner side, centripetally developed. Thus the trace is mesarch. Parenchyma cells occur scattered through the xylem. Fig. 3 of the same plant does not show centripetal wood for the reason that it is not strictly median as to the bundle, but passes to one side of the centre, so as to show the attachment of the metaxylem. *E. maximum* (Fig. 5) shows very similar conditions—disorganized protoxylem with metaxylem both external and internal thereto. The strongly sclerified cells are those in the axil of the leaf-sheath. Mesarch structure in these two species appears close to the origin of the trace, and continues nearly to the end of its xylem. *E. hyemale* resembles its variety *robustum* very closely in the organization of the leaf-trace. In *E. arvense* no distinction was noticed among the tracheides until the trace had left the stem, but throughout the leaf proper mesarch conditions are well defined. Even in that most reduced species, *E. scirpoides*, with stems only a few millimetres in diameter, the foliar bundle is distinctly mesarch.

The study of transverse sections of these bundles in the larger species completes the understanding of their structure. The leaf-trace of *E. hyemale* var. *robustum* just before it leaves the cortex has a strong resemblance to the axial bundle of the cone. It is made up as follows: centrally, or often a little to one side, is a protoxylem-lacuna; external to this are the centrifugal tracheides arranged largely in two groups upon its flanks; directly inward from the centre are the centripetal elements, from three to seven in number. *E. maximum* presents much the same conditions. The protoxylem-lacuna is well developed in the proximal portion of the

trace. Queva, in his study of leaf-development in this species, however, did not find it.

Equisetum shows, therefore, centripetal wood, not only in the traces of the sporophylls, but also in those of the vegetative leaves. The descent of this genus from ancient Calamites of the type of *C. pettycurensis* is thus made highly probable. The centripetal wood in the leaf-trace of the Equisetales, together with many other characters, seems to indicate a clear relation of these plants to the Sphenophyllales, and through them, perhaps, to the Lycopodiales, which the latter resemble in anatomy.

The Lycopodiales and the Sphenophyllales possess centripetal wood in their stem structure. In *C. pettycurensis*, alone among the Calamites, small arcs of centripetal xylem remain in the stem-bundles. The stem proper of all other known Equisetales has completely lost it, and it has persisted only in the more conservative organs, the reproductive and vegetative leaves. The structure of the foliar traces of the Tertiary, and especially of the Mesozoic, Equisetales should prove very interesting. Some of the Equisetaceous fossils of the latter era have recently been shown [1] to be interesting in other respects—in the number and course of the stem-bundles and in the markings of the spores. Doubtless, when well-preserved material is available, they will show considerable centripetal xylem in their foliar traces. In the stems of the living members of this class secondary growth has nearly disappeared; the centrifugal primary wood has largely become parenchymatous, and the centripetal has completely vanished.

Through the countless generations of Equisetaceous plants, from the Lower Carboniferous Period to the present day, the vegetative leaf has continued to form centripetal wood. This places new and strong emphasis upon the conservatism of that portion of the vascular system which supplies the leaves. Its value in this respect has often been noted, but has perhaps not yet received the attention it deserves. A few instances in different groups of plants may be cited. The Cycads present a case parallel in many respects to that of *Equisetum*. Mettenius, in 1860, discovered mesarch structure in their leaf-bundles. Dr. Scott [2] has shown that the peduncles of several genera have mesarch bundles; also that the traces of the vegetative leaves and, to a less extent, of the sporophylls have centripetal growth. The vegetative stems of the Cycadaceae, however, have only centrifugal wood. Yet their probable ancestors among the Pteridospermeae had mesarch bundles in the stem. Among the Cordaitales the most ancient forms had mesarch stem and mesarch leaf-bundles; later forms had the same leaf-structure, but endarch structure had become the

[1] Halle, T. G.: Zur Kenntnis der mesozoischen Equisetales Schwedens. Uppsala und Stockholm; 1908.

[2] Scott, D. H.: The Anatomical Characters presented by the Peduncle of the Cycadaceae. Ann. Bot., vol. xi; 1897.

rule in the stem. Still another example occurs in the lower Gymnosperms. The bundles of the cotyledons of *Ginkgo*, and also of the vegetative leaves to a slight extent, have been shown by Worsdell [1] to be mesarch. Its ancestors were probably allied to the primitive Gymnosperms and possessed mesarch stem-structure. Further, Jeffrey [2] has recently shown that the leaves of a primitive pine-like Gymnosperm, named by him *Prepinus*, also had well-marked centripetal xylem. Thus in many places we find foliar organs retaining the typical xylem-structure of the older vascular plants.

Lately there has been some objection to the division of Vascular Cryptogams into the Pteropsida and the Lycopsida, on a basis, in part, of the presence or absence, respectively in the two groups, of foliar gaps. *Equisetum* among the Lycopsida has been regarded by some writers as varying from other members of the series in possessing leaf-gaps. In the examination of leaf-trace structure in this genus, a large number of sections, both transverse and radial, through the points of origin of traces, both of vegetative and reproductive leaves of several species, has been studied by the writer. In no case did a gap exist. The strands of the cone continue upward without sign of interruption after giving off the sporophyll-trace. Immediately over the origin of the vegetative leaf-trace, and connected with all portions of the internodal bundle, lies the unbroken ring of supranodal wood. Even in the much reduced vascular system of *E. fluviatile*, this xylem-mass is unbroken; in the tiny *E. scirpoides* it likewise completely shuts off the leaf-trace from the so-called 'gap' above.

Summary and Conclusions.

In *Equisetum* the development of the xylem of the vegetative stem is *centrifugal throughout.*

The internodal bundle does not consist of three united bundles, representing different portions of an ancestral stele: it is a *unit* in structure, and represents the much-reduced internodal primary bundle of the Calamites. The strobilus shows in its axial bundles conditions intermediate between those in the vegetative stems of the Calamites and of *Equisetum.*

The vegetative leaf-trace does not arise solely from the protoxylem-strand of the internodal bundle; all three parts of the xylem of the latter contribute to its formation.

Through Dr. Scott's discovery of an ancient Calamite with centripetal wood in the stem-bundle, a link has been formed between the Equisetales and the Sphenophyllales, and possibly through the latter with the Lycopodiales. The most primitive Equisetaceous forms doubtless possessed well-developed centripetal wood. Early in the history of the series this

[1] Worsdell, W. C.: On Transfusion Tissue. Trans. Linn. Soc. of London. Series 2, vol. v; 1897.

[2] Jeffrey, E. C.: On the Structure of the Leaf in Cretaceous Pines. Ann. Bot., vol. xxii; 1908.

disappeared. *Equisetum* shows signs of relationship to these ancient plants in the possession of centripetal wood in regions recognized as conservative of ancestral characters. The axial bundles of the strobilus are weakly mesarch; those of the sporophylls, strongly so. This condition in the sporophyll-trace is not due to the concentric arrangement of the sporangia upon the scutes, for the subdivisions of the trace, one of which passes to each sporangium, are themselves equally mesarch throughout their course. The traces of these fertile leaves are likewise concentric. The traces of the vegetative leaves show well-developed centripetal wood, in some species throughout their course. In no case does the passage of a foliar trace from the stele leave a gap.

Equisetum presents additional and very strong evidence of the conservatism of the leaf-trace, thus emphasizing its value in phylogeny.

Centripetal wood is now known to exist in all the large groups of Vascular Cryptogams. In all those in which it is well developed it is continuous with the protoxylem; where it no longer appears at all, as in the higher plants, or where only vestiges are found, as in *Equisetum*, the protoxylem usually becomes continuous with the centrifugal xylem. The formation of xylem adaxially from the protoxylem is, therefore, a Cryptogamic character. Moreover, bundles in which both centripetal and centrifugal xylem are present cannot be said to be characteristic of any one group of the Vascular Cryptogams, as, for example, of the Ferns, nor can such bundles in higher plants be of other phylogenetic value than as indicating general Cryptogamic affinities.

This work has been carried on in the Phanerogamic Laboratories of Harvard University, and to Dr. E. C. Jeffrey the writer is much indebted, both for abundant material and for advice and guidance throughout the investigation.

DESCRIPTION OF PLATE XLV.

Illustrating Mr. Eames's paper on Centripetal Xylem in *Equisetum*.

Fig. 1. *Lycopodium*, sp.; transverse section of part of the central cylinder during lignification of the xylem. × 180.

Fig. 2. *Equisetum hyemale*, var. *robustum*; transverse section of a developing internodal bundle. × 500.

Fig. 3. The same; radial section through the origin of the vegetative leaf-trace, showing connexion with both protoxylem and metaxylem. × 100.

Fig. 4. The same; radial section through vegetative leaf-trace passing through cortex, showing mesarch structure. × 500.

Fig. 5. *Equisetum maximum*; radial section through vegetative leaf-trace as it leaves the cortex. × 500.

Fig. 6. *Equisetum hyemale*; radial section through an axial bundle of the cone. × 500.

Fig. 7. *Equisetum maximum*; transverse section of sporophyll-trace. × 1100.

Fig. 8. *Equisetum fluviatile*; transverse section of sporophyll-trace. × 1100.

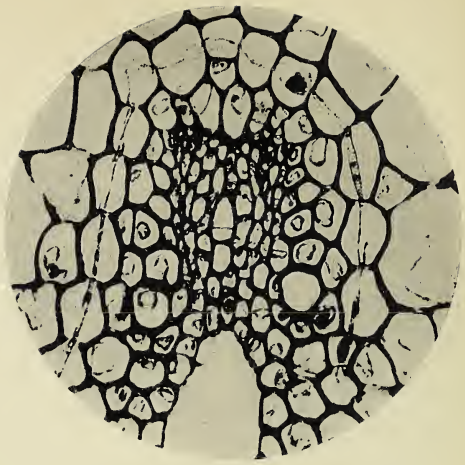
Fig. 9. The same; longitudinal section of sporophyll-trace. × 500.

Fig. 10. *Equisetum maximum*; longitudinal section of the sporophyll-trace, showing forking at base of scute and the mesarch structure of the branch-bundles. × 180.

Fig. 11. *Equisetum arvense*; longitudinal section of sporophyll-trace. × 500.

Fig. 12. *Equisetum palustre*; longitudinal section of sporophyll-trace. × 500.

1.



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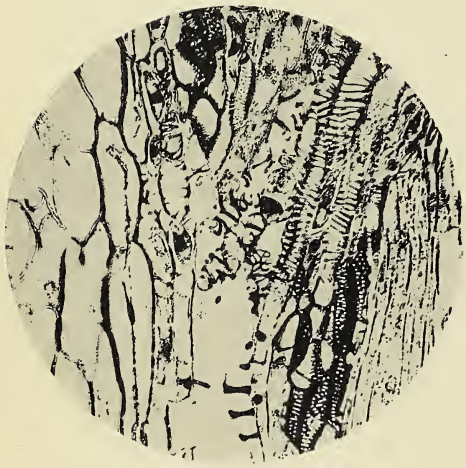

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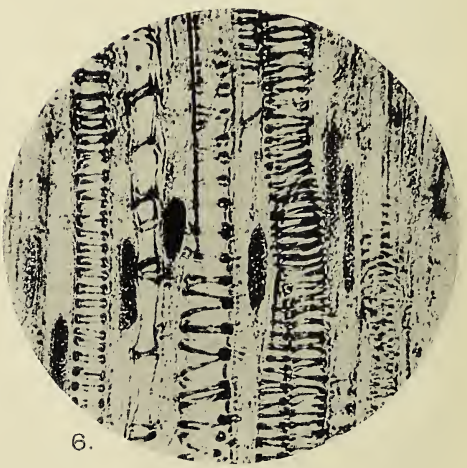

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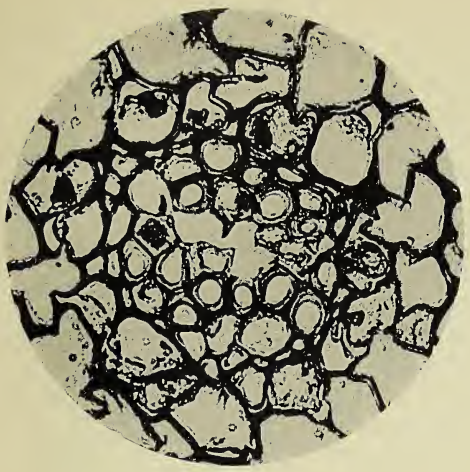

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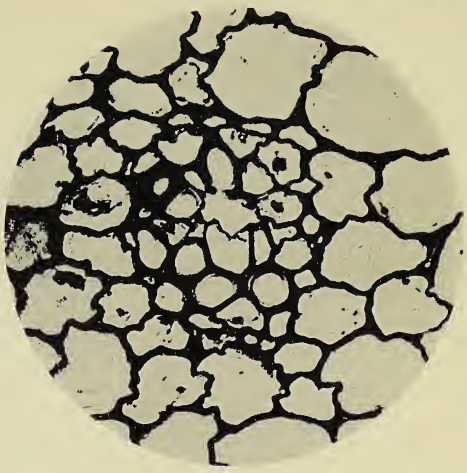

8.

9.

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11.

12.

The Lateral Roots of Amyelon radicans, Will., and their Mycorhiza.

BY

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With Plates XLVI and XLVII.

AMYELON RADICANS was described by Professor Williamson (22) in his fifth memoir, 'On the Structure and Organization of the Fossil Plants of the Coal-Measures' (1874), as the root of *Sphenophyllum*, but has since been shown to be the root of *Cordaites*. In his description, which is mainly concerned with the big roots that have undergone secondary thickening, he refers to groups of lateral rootlets. His Fig. 46, Pl. VII, shows two such bunches of much-branched roots. These are, he says, arranged irregularly on the periphery, and not definitely as in *Stigmaria*.

As far as I am aware this is the only description of the small roots. Solms-Laubach (16) gives a short account of *Amyelon*, based on that of Williamson. The root is given amongst those of uncertain affinity.

Renault (11) has described the roots of *Cordaites*, together with other parts of the plant from Autun. The species described by him have a diarch root, and the periderm forms in the outer cortex. Another difference from *Amyelon radicans* is that in his specimens the phloem is not preserved.

Scott's 'Studies' (13) also contain a short account of *Amyelon* as the root of *Cordaites*, but no mention is made by him of the lateral rootlets. The periderm is stated by him to be deep-seated in its origin.

At the suggestion of Mr. D. M. S. Watson, I examined some slides of *Amyelon*, with a view of seeing if these remarkable bunches of rootlets might correspond in any way with the 'root-tubercles' of recent plants, in which he knew me to be interested. I have found evidence that this supposition is probably correct, and that the tufts of short, much-branched roots were probably of the nature of 'coralline roots'. I wish here to express my deep indebtedness to Mr. Watson for calling my attention to this point, and for most generously placing his slides, some of which he had made for the purpose, at my disposal. The slides on which this

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investigation is principally based are: A 275, 281, 282, 283*a* and ser., in Mr. Watson's collection, and R 707, 716 in the Manchester Museum. I have also to thank Dr. Smith Woodward, Keeper of the Geological Department, British Museum (Nat. Hist.) for facilities to examine the Williamson Collection, in which I found numbers 931 and 932 to be specially useful.

In spite of the common occurrence of mycorhiza amongst recent plants, only one case has been recorded from the Coal Measures. In 1902 Professor Weiss (21) described from this laboratory a small rhizome that appears to have had an undoubted mycorhizic habit. The material in this case was a petrifaction from the Halifax Hard Bed, which is renowned for the beauty of its preservations. In this respect he was more fortunate than I have been in the material here used. All the slides examined in this investigation have been cut from material from Shore or Oldham, at which places *Amyelon* is particularly abundant. Though the preservation of cell-structure is excellent, the detail in many cases is necessarily not so clear, owing to the contents being of the same brownish colour as the cell-walls. In spite of this difficulty it has been possible to make out an abundant occurrence of hyphae in some of the cells.

An examination of an unthickened and uncrushed rootlet (Pl. XLVI, Fig. 2), which may be as much as 1·5 mm. in diameter, shows a wide cortex, consisting of two well-defined zones. The outer cortex is of parenchymatous cells, often of small radial diameter. In some specimens an epidermis appears to be distinguishable by its more distinct outer wall, and the more regular shape of the cells. The cells of this outer region are elongate in longitudinal section, and are almost without exception void of contents, so that it is clearly distinguishable from the succeeding zone. In the inner cortex the cells are also of thin-walled parenchyma, larger than those of the preceding region, and irregularly hexagonal in transverse section. They are somewhat elongate when seen longitudinally. In contra-distinction to the cells of the outer cortex, the cells here frequently have a dark cell contents. In some cases this occupies the whole cell, though in others it appears to have contracted towards the walls, or to form a more or less central mass with connecting strands to the walls. These masses of cell contents are apparently tangled knots of fungal hyphae filling the entire cell. In those cases where the contents are contracted, the hyphae appear to have been absorbed by the host plant, the indigestible residue being left. Such is usually the case with recent plants, and has been noted by Weiss for *Mycorhizonium*.

Some cells appear to be perfectly empty, though well preserved, as if the fungus had not affected them in any way. This invasion of certain cells and omission of others by the symbiotic fungus has been commented upon by Groom (3) in *Thismia Aseroe*.

There is a well-marked endodermis in the cells of which no fungus is apparent. This encloses a phloem of elongate, thin-walled cells, which are usually in a good state of preservation. The transverse walls are slightly oblique.

The rootlets are usually diarch. This point was not known to Williamson when he described them originally, but the following observations can be seen in his manuscript catalogue of slides in the British Museum. 'There is *obvious evidence that the vascular bundle originated in a bipolar manner* and may be centripetal in its further development; this fact was not observed when Memoir 5 was written.' The diarch origin is very well shown in some of the younger rootlets in Mr. Watson's slides, two groups of protoxylem being visible separated by a few thin-walled elements not yet lignified. At the same time triarch rootlets are not uncommon. I have not seen any tetrarch or pentarch *rootlets*, though in full-grown roots with secondary thickening this condition is to be met with not infrequently. It may have been that only the primary roots had the larger numbers of protoxylem-groups, and the chance of finding these is, of course, not great. In longitudinal section the tracheids are seen to be spiral in the protoxylem. The rest of the centripetal wood has scalariform tracheids with oblique ends. The tracheids of secondary growth are of typical Cordaitean structure with bordered pits.

In slide A 281 I have been fortunate enough to come across a longitudinal section of a root apex (Pl. XLVI, Fig. 3). There is every reason to believe that this belongs to *Amyelon*, though there is no actual connexion. There are a number of unmistakable young rootlets near; these are the only well-preserved roots on the slide, and it agrees with them in every way that I have been able to compare them.

A well-marked root-cap, triangular in shape, and of presumably corky cells (measured across which, the root is about ·4 mm. in diameter), protects the meristematic zone. Unfortunately the section is not absolutely median, so I am unable to trace the full extent of this tissue. The few cells that are visible are quite small (8 μ) and very thin walled; the dermatogen, periblem, and calyptrogen are not distinguishable as is usual in Gymnosperms. Petrifaction appears to have occurred at a period of rest, for behind this region rapid differentiation occurs, the elements derived from the periblem and pleurome being easily distinguishable. Only one spiral tracheid is to be seen against the latter cells, which otherwise are elongate and parenchymatous. The fungus is absent from the growing region. About 1 mm. from the apex (the bit of root is but 1·5 mm. long) traces of hyphae are to be seen running longitudinally in the inner cortex. Infection of the rootlets appears to have been from behind forwards as they elongated.

The rootlets appear to have had a typical endogenous origin. Before secondary thickening began in the roots, before periderm had begun to

form, the rootlet that was to become the main one of the group pierced the cortex. It seems to have had its origin in the pericycle opposite one of the protoxylem groups. Pl. XLVI, Fig. 1, shows the root-trace passing through the secondary wood from opposite one of the protoxylems of a triarch root. This rootlet, which may be regarded as the main one of a group, appears to have developed a very wide cortex zone, which is seen in the section; and, in some cases, the periderm of the root is continued into the rootlet with probably one cork cambium.

Branching of this rootlet was very frequent, but there is no sign of definite arrangement of the branches. As the rootlet bearing them was diarch they were probably arranged in two rows, as in the case of the tubercles of *Podocarpus* (Van Tieghem, 19) though this is not apparent from the sections. These branches of the main rootlet divided again almost immediately (Pl. XLVI, Fig. 1); possibly more than once, for there is always a large number of rootlets near the point of origin of a group. Once the tuft was produced, the individual rootlets seem to have run some distance (certainly some 2 or 3 cms.) without branching again. In the last part of their course they appear to have run in and out of the mass of débris with which they are found in the manner of Stigmarian rootlets. They may constantly be seen burrowing through fragments of stems and even older *Amyelons*. In general appearance it will have been seen that the rootlets are not unlike those of *Stigmaria*; which resemblance Williamson commented on, both in his memoir and in his catalogue.

The origin of the periderm is stated by Williamson to have been in a peculiar manner—namely, by radial elongation of the cells of the outer cortex, which subsequently formed numerous radial walls. This appears at first sight to be possible, if not probable, when an old *Amyelon* is examined. It is not, however, borne out by the younger stage. The periderm has been stated by Renault to have been superficial in its origin (loc. cit.). Scott, however, states it to be deep-seated in *Amyelon radicans*, and my observations confirm this. The rootlet, when the metaxylem was formed, produced a cork cambium immediately outside the endodermis, as is usual in Gymnosperms. The whole of the cortex would then be sloughed, the inner mycorhizal zone included (Pl. XLVI, Fig. 4). This is exactly what occurs in *Podocarpus*.

The fungus is apparent in the cortex as hyphae, with a diameter of about 4 μ. Septa are either absent or very rare. In the outer cortex the hyphae are to be seen best in longitudinal section, when they are noticed running apparently from the exterior to the interior of the cell (Pl. XLVII, Fig. 5). The inner cells may be divided, as Werner Magnus (6) has shown, into host cells (*Pilzwirthzellen*), and digestive cells (*Verdauungszellen*). In the former the hyphae are noticed to coil around the exterior of the cell (Pl. XLVII, Fig. 6). In places the walls are dilated to about 1½ times

their former diameter ; in the latter cells the hyphae appear to have entered the cell and grown around a central nucleus. Pl. XLVII, Fig. 7, shows the beginning of such a stage, of which Figs. 8 and 9 may be later stages in the absorption of the hyphae. Dilations of the hyphae are apparent here also ; but the large bladder-like growths, which Groom has described, are not to be seen. In the cortex of the rootlets that are apparently full-grown, and are about to form a periderm, there are to be observed large knob-like growths with thickened walls that are apparently terminal on the hyphae, and are about ·05 mm. in diameter (Pl. XLVII, Fig. 6). These I take to be a form of resting-body which the fungus forms to carry it over the period during which the cortex will be sloughed, and before it has an opportunity of infecting a fresh rootlet. They are not unlike those swollen hyphae figured by Seward, but they are formed in definite connexion with the rest of the mycelium, and in roots which, there is every reason to believe, were healthily growing ones, and not detached fragments of decaying vegetable matter. Quite recently, while examining roots of *Podocarpus cupressina*, I have found exactly similar dilations terminal on the hyphae, in the cortex of the roots at the time that cork-formation had set in. This parallel I consider particularly useful, as it meets the possible objection that the bladder formations are sporangia of a parasitic fungus.

All sign of spore-formation is lacking, though some sign might be expected to be present in either a saprophyte or a parasite.

It is manifestly impossible to offer any suggestion as to the relationship or systematic position of the fungus ; and it is quite unsafe to formulate any theory of Phycomycetous relationship based on the general absence of septa. To quote Shibata, 'Das Fehlen der Querwände in den Hyphen beweist hier nichts, da die Septirung oftmals in intracellular-lebenden Mycelien ausbleibt.'

The relationship of the fungus to the lateral roots of *Amyelon* is a matter on which there may reasonably be some uncertainty. It is well known that the saprophytic fungi occur quite commonly with the remains of higher Palaeozoic plants. Seward (14) has given a collected account of them in the first volume of his 'Fossil Plants', and there is also the large monograph by Meschinelli (7), though this is not confined to Palaeozoic specimens. They are omnipresent amongst decaying vegetation to-day, so that there is nothing to cause surprise that similar destructive agencies should have existed in the Coal Measure Period.

The fungus in question, however, hardly gives the impression of being a saprophyte. In the first place the preservation of the material is against this, for it is equally good for both fungus and roots. These latter are in many cases admirably preserved, e. g. the root apex, and do not give the impression of being decaying pieces of vegetable matter on which the fungus was feeding, but rather sound and living rootlets, at the time that infection

occurred (cf. Weiss). Again, the hyphae are confined to one region of the roots, namely to the inner cortex. Traces of the hyphae are, it is true, to be found in the epidermis and the outer cortex, but they are comparatively rare. The fungal filaments, when seen there, occur singly, and appear to be running from the exterior inwards, or longitudinally with the root. There are, as it has been pointed out, no dilations or branchings in this region. Moreover, I have never observed them in the phloem region or in the wood. It should also be remembered that they are absent from all the tissues of the main roots that have undergone secondary thickening.

The possibility of parasitism has also to be considered. Oliver (10) has described parasitic fungi from the Palaeozoic era. The argument of restriction of infection, cited against saprophytism, also holds here. A parasitic fungus might be expected either to ramify over the whole of the tissues, or to attack such a region of soft tissue as the bast, as in the case of *Armellaria melleus,* to take a common instance. This, however, is not the case.

The constancy with which the fungus appears is also against its being of the nature of a disease. In all cases where the preservation is sufficiently good in material, from both different blocks and different localities, hyphae are to be observed. It is hardly to be expected that a fungus would have so widespread an occurrence, unless there were a great epidemic, or else some vital reason for its presence.

This last explanation, that of a symbiosis, seems to be the most probable relationship. First the fact, that the root has the tendency to branch so frequently forming definite tubercles, is strongly reminiscent of *Myrica*, *Alnus*, &c. The division of the cortex into infected and non-infected regions, and the occurrence of knots of hyphae in some only of the cells, reminds one of similar cases described by Frank (2), Warlich (20), Groom (3), Werner Magnus (6), Shibata (15), &c. Weiss, in describing *Mycorhizonium*, has commented upon the frequency with which some form of symbiotic union is to be met with among recent plants; instancing Janse's paper (5) in the Buitenzorg 'Annales'. The structure described by Weiss is, however, the only recorded case among Palaeozoic plants.

In the case of recent Gymnosperms some union of the spermophyte with a thallophyte is of common occurrence. The case of *Cycas* is well known. *Dacrydium* and *Podocarpus* have tubercles, the Coniferae generally have an ectotropic mycorhiza. It need not, therefore, be a matter of surprise that their Palaeozoic ancestors should have had a similar relationship.

The very interesting question of the biologic conditions under which *Cordaites* lived appears to be somewhat elucidated by these observations. It has been pointed out that in some respects the fungus has a resemblance to that found in *Podocarpus*. There is, however, no formation of nodules, as in the latter plant, but rather a production of numerous short and much-

branched lateral roots at intervals along the main ones. Whether this frequent branching is a direct outcome of the presence of the fungus, which would exert a stimulus on the tissues, or not, is a point that cannot, of course, be decided. From analogy with recent plants, this seems highly probable.

Recent work on the physiology of mycorhizic plants seems to show that the relationship of the fungus to the host is a very vital one. And whether it is one in which the host benefits by obtaining a more abundant supply of mineral salts, from a soil rich in humus, which it is unable to do efficiently without the aid of the fungus, according to Stahl (17), or whether the endophyte has the property of fixing atmospheric nitrogen, so giving its host a larger quantity of nitrates than it could otherwise obtain (Nobbe and Hiltner, 9, and Hiltner, 4) the general result seems fairly established.

Myrica (when it grows in swampy soil), *Alnus* and *Elaeagnus* all produce short fleshy roots caused by either a fungus or a bacterium and inhabited by it, and the benefit accruing to the spermophyte in at least one of these cases has been strikingly demonstrated (Möller, 8). It is not unreasonable to suppose in that case that the rootlets in *Amyelon*, which in many respects seem to have had a similar structure to recent mycorhizal roots, may have had a similar function.

Stopes and Watson (18), in their paper on 'Coal Balls', have given cogent reasons to prove that these concretions were formed *in situ* by gradual petrifaction of vegetation at the bottom of a brackish swamp. The conditions of life in such a locality are exactly of the kind that would lead one to expect the production of root-tubercles on some of the plants growing there. The soil would be deficient in nitrates, while it would be rich in humic acid. Mycorhiza are frequently associated with plants liable to suffer from drought, or growing in a soil abundant in humus. These are the conditions that we might expect in a saline marsh, the drought being physiological rather than physical (Schimper, 12). Indications are not lacking that the leaves of some species of *Cordaites* (*C. crassus* and *angulostriatus*) were possessed of xerophyllous characters (Renault and Scott).

It has been pointed out that these rootlets are found mingled with, and growing amongst, a mass of débris of other plants of all kinds. While the *Amyelon* roots are frequently well preserved, the fragments of vegetation around are often much decayed. It is quite easy to see that such would be the appearance in a petrifaction formed *in situ*, if the *Cordaites* roots were growing there, the other plants being mere débris.

These, indeed, are very much the conditions in which *Myrica Gale* is to be found growing around the Cheshire meres to-day, only the water is fresh and not salt, as it probably was in a Coal Measure swamp. An examination of these rootlets has led me to the conclusion that they were probably borne by plants adapted to such conditions as would exist in the kind of marsh that Stopes and Watson describe.

Summary.

1. A redescription is given of the tufts of short much-branched roots borne at intervals on *Amyelon radicans*, and originally described by Williamson in 1874.

2. The lateral roots are found to have a wide cortex divisible into two regions.

3. The inner cortex of all specimens examined is found to contain, more or less abundantly, dark cells, many of which on examination show evident fungal hyphae.

4. The fungus occurs in knots of apparently non-septate hyphae. These bear in some cases terminal vesicles. There is no trace of any spore-formation.

5. The relationship of the fungus to *Amyelon* is discussed, and the conclusion arrived at that it was of the nature of a symbiosis.

6. *Cordaites* was probably a tree, inhabiting saline swamps, and having bunches of coralline rootlets on its roots, such as are known to occur in many recent plants growing under similar conditions.

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EXPLANATION OF PLATES XLVI AND XLVII.

Illustrating Mr. Osborn's paper on *Amyelon radicans*.

PLATE XLVI.

Fig. 1. Root surrounded by numerous rootlets. *px.* = protoxylem; *pd.* = periderm; *t.r.* = bundle trace running to main rootlet of a bunch; *m.r.* = main rootlet; *m'.r'.* = main rootlet of a bunch that left root (*r.t.*) higher than plane of section; *rr.* = rootlets, lateral branches of *m'.r'.* showing the way that lateral rootlets branch immediately on leaving the main root.

Fig. 2. Transverse section of rootlet. *x.* = diarch xylem; *ph.* = phloem; *end.* = endoderms; *i.c.* = inner cortex, cells with dark contents; *o.c.* = outer cortex.

Fig. 3. Longitudinal section of root apex. *r.c.* = root cap; *m.c.* = meristemmatic cells; *pb.c.* = periblem elements; *pl.c.* = pleurome elements; *sp.v.* = spiral vessel; *f.c.* = cells with hyphae.

Fig. 4. Transverse section of tetrarch root showing deep-seated origin of periderm. *px.* = protoxylem; *x.* = xylem; *ph.* = phloem and pericycle; *c.c.* = cork cambium; *pd.* = periderm; *c.* = cortex cut off by periderm.

PLATE XLVII.

All figures drawn with camera lucida in outline. Reichert, 7a. obj., x8 comps. occ. reduced $\frac{2}{3}$.

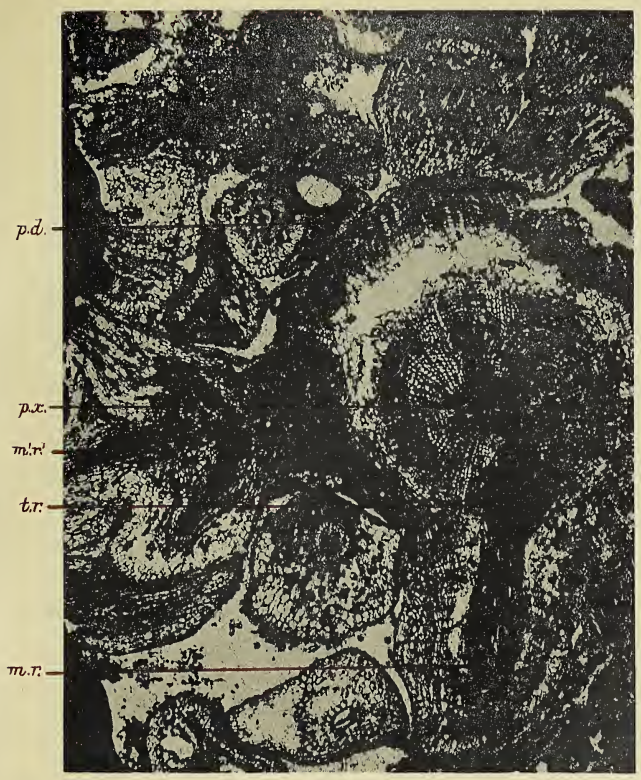
Fig. 5. Part of longitudinal tangential section of a rootlet, showing epidermis (*epi.*) and outer cortex (*o.c.*) with hyphae cut transversely. *i.c.* = inner cortex with host cells containing hyphae. A. 282.

Fig. 6. Cells of inner cortex in transverse section, just before periderm formation. *h.c.* = host cells; *r.b.* resting bodies. A. 275.

Fig. 7. Transverse section of rootlet. Young stage in formation of digestive cells. R. 716.

Fig. 8. Digestive cell in longitudinal section, hyphae disorganizing, a central knot of indigestible matter is forming. A. 282.

Fig. 9. Transverse section of cells in inner cortex showing late stages in disorganization of digestive cells. A. 282.



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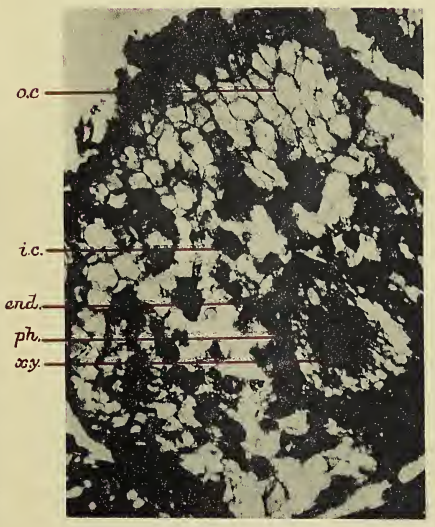

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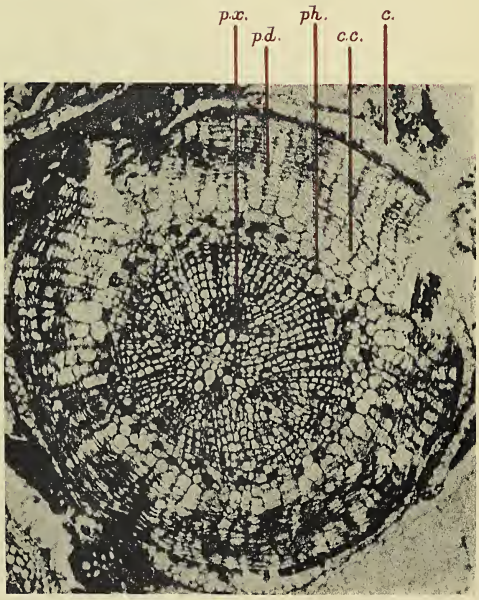

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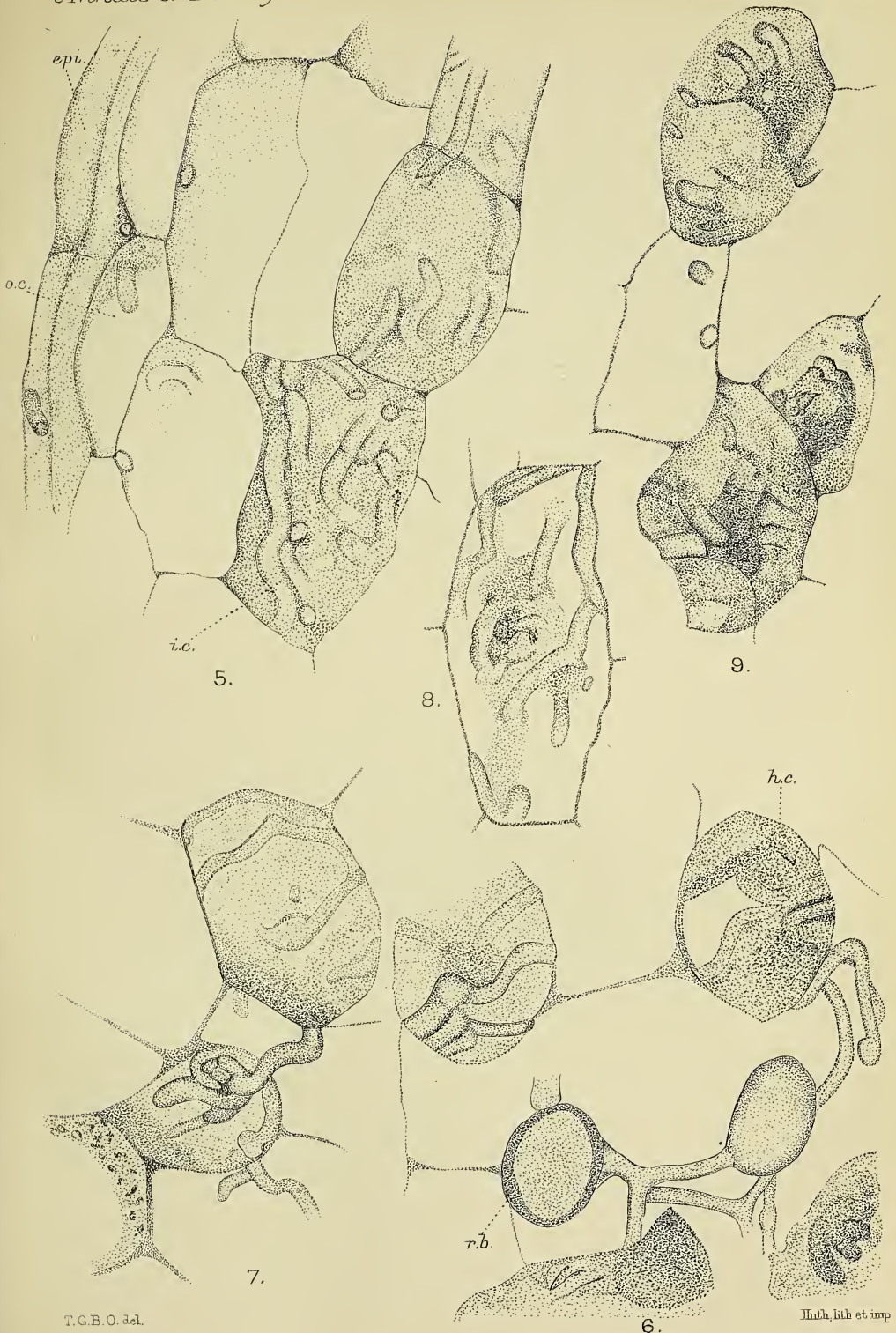
OSBORN — AMYELON

Huth, coll.



OSBORN — AMYELON

ERRATUM

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Annals of Botany
No. XCII.

On the Mucilage Glands of Undaria.

BY

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With Plate XLVIII.

IN describing *Undariopsis* (*Laminaria*) *Peterseniana*, Miy. et Okam., Dr. Okamura[1] has noted a peculiar structure in the subepidermal layers of the lamina. As the original paper seems to have but a narrow circulation, it will be desirable to quote here the lines which relate to the subject :—

'Over both the surfaces ot the lamina, both younger and older, minute dark dots are thickly scattered, which are easily visible to the naked eye. In a cross section of the lamina we find beneath the cortical layer a roundish-triangular or depressed-conical, deep reddish brown mass enclosed in a hyaline bag, situated beneath a minute hole left in the epidermal layer. On the surface-view of the lamina, epidermal cells appear to converge towards the hole. The chemical nature of these masses I did not study. It may perhaps be an excretion. I mention this here especially, for the presence of dots in this species is so characteristic that it is sufficient in itself to distinguish the species from all others.'

A similar body has been found by Prof. Miyabe in *Undaria pinnatifida*, Sur., and its variety *distans*, Miy. et Okam., and by the present writer in *Hirome undarioides*, Yendo. I have compared the 'dots' of these species and made it clear that they have similar characters in various respects, however much the species may differ. It is very probable that the problematic bodies found in the three genera may be identical in their properties, functions, and mode of formation. But the material studied was all from dried specimens, and, as regards the nature and function of the 'dots', nothing could have been added to the discoverer's remarks. A stay of two weeks at the Oshoro Marine Station in December last

[1] Okamura: Enumeration of Algae of Japan (in Japanese), p. 128. 1902. (Diagnosis of the genus not yet published). Ibid.: On Laminariaceae of Japan. Bot. Mag. Tokyo, vol. x, p. 99. 1896. Yendo: Three New Algae from Japan. Ibid.: vol. xvii, p. 102. 1903.

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gave me a favourable opportunity to observe the problematic structure on fresh specimens of *Undaria pinnatifida*, Sur., var. *distans*, Miy. et Okam. The material was fixed in picric acid as well as in sublimate solution, both saturated in filtered sea-water. It was brought back to the laboratory of the Agricultural College of Sapporo, and further examination has been carried on under various methods of treatment. I have arrived at the conclusion that the 'hyaline body' cannot be anything but a mucilage gland. As such a gland has never been known to occur in other members of the Laminariaceae, and as the species concerned are all confined to Japan, the results obtained in the present study, incomplete as they are, may not be unworthy of publication.

The glands found in the species under consideration vary in their size and shape according to their stage of development and position in a pinnule. At an early stage they are ovate, with the sharper end towards the surface of the frond. They grow gradually into roundish, pyramidal or conical bodies, and may often elongate horizontally through the medullary tissue. Details of their development will be given afterwards.

In the fresh specimens the substance contained in a mucilage gland is a colourless and highly refractive mass. In the material preserved in alcohol, after being fixed in the picric acid solution directly from the fresh specimens, the contents are also colourless. Okamura does not mention whether the specimens he studied had been preserved by any method of treatment, or were fresh ones. But in the dried specimens or in those preserved in formalin, or in those preserved in alcohol after being fixed in the sublimate solution, the contents are always brown as has been described by him. In the older parts which are nearly going to decay, in the formalin specimens, the contents become of a dark brownish colour approaching almost to black. As a 'minute hole' is situated just above each gland, a fresh frond in surface view shows numerous colourless spots, instead of the dark brownish dots, scattered in the brown epidermal layer. These spots are irregularly distributed all over the blade, but very few, if any, in the stem and rachis. I could not detect any in the rhizines and sporophylls.

The number of glands in a blade varies considerably according to the part and to the age of the plant, as well as to the individual. In general, however, it may be safely remarked that they are proportionally most rich in the young pinnules of a frond at a post-embryonal stage, and that they are more numerous at the marginal regions than in the middle of a pinnule. When a plant has grown to its full size they occur in comparatively less numbers in the pinnules at the transitional region. The following table gives the manner of distribution. The figures show the number of glands in a square mm. :—

Number of Glands in a Pinnule at the Transitional Region.

Of a plant about 11 cm. high.	I.	II.	III.
Middle part	30	25	26
Marginal part	59	56	70
Of a plant about 30 cm. high.			
Very small ligule, hence middle and marginal together . . .	21	16	20
Of an adult sporophyllous plant, about 43 cm. high.			
Middle part	6	0	4
Marginal part	13	14	21

Number of Glands in Young and Old Pinnules of a Sporophyllous Plant about 43 cm. high.

	Young Pinnule.			*Old Pinnule.*		
	I.	II.	III.	I.	II.	III.
Middle Part	0	11	7	10	7	5
Marginal part	20	16	21	13	19	27

These spots are narrow spaces, mostly circular in surface view, with a diameter varying from 10 to 15 μ. Some are elliptical or ovate, measuring 10–15 μ in the minor axis and 20–40 μ in the major axis. In the younger pinnules which are found near the transitional region of a frond they are much smaller, often not exceeding twice the size of an epidermal cell. Those found in the rachis, though rather rarely, are narrow and longitudinally stretched. Before going further, a brief description of the structure of a young part of the lamina of *Undaria* will be necessary, as nothing has ever been reported concerning the matter. There are many interesting points to be considered in the morphology of *Undaria*. Description in detail may undoubtedly contribute various new facts to the knowledge of Laminariaceae. But I have to confine myself in this paper to remarking on the points which directly concern the subject under consideration.

In the essential characters the structure of a young part of the lamina of *Undaria* is similar to that of the rest of Laminariaceae.[1] The epidermal layers are built up of a single stratum of elliptical cells with the major axes perpendicular to the surface of the frond. The cortex is built up of parenchymatous cells disposed in one or several irregular layers. The cortical cells, which are directly in contact with the epidermal layer, are angulate-polygonal and closely fit on to the inner side of the epidermal layer. In the transitional region the epidermal cells are nearly isodiametric and have a similar appearance to the contiguous cortical cells (Pl. XLVIII, Fig. 8). Both are, however, easily distinguished one from the other by the contents and future mode of development (Fig. 12). The inner cortical cells vary in shape from spherical to elongated-elliptical, and are arranged loosely and irregularly. The medullary portion between the two strata of the cortical

[1] Cf. Wille: Bidrag til Algernes Physiologiske Anatomi. K. Svenska Vet. Akad. Handlingar 21. No. 12. 1885. Rosenthal: Zur Kenntnis von *Macrocystis* und *Thalassiophyllum*, Flora, 1890, and other papers.

tissue consists of separate hyphal cells, often ending in a cortical cell. They are very loosely arranged, and the space between them is filled up with a gelatinous matrix.

The chromatophores are also found in the cortical cells. Large numbers of the hyphal cells lack the chromoplasts; but one or two of the grains may often be found in the form of a ring, closely fitted to the inner surface of the cell-wall (Pl. XLVIII, Fig. 2). Here and there in the epidermal layer an embryonal stage of the hairs may be met with in a depressed circular area. In the well-grown sporophyllous fronds numerous hyaline corpuscles are found in the cortical cells. These corpuscles turn yellowish brown in the dried or formalin specimens.

To understand the relative position of a gland and the space above it, or supraglandular space as it may be called, it was found best to apply a special staining matter to the former. The glandular contents stain much deeper than the surrounding tissue in aniline blue or in haematoxylin. A part of a frond is dipped in either of the colouring materials for a few minutes. It is then washed thoroughly in a diluted acid alcohol. In the case of anilin blue almost all parts of the tissues except the glandular contents are decolorized; in haematoxylin, the glandular contents are stained a purplish colour and the nuclei of other cells a bluish violet, while the cell-walls remain almost unstained. When a piece thus treated is observed from the surface under a moderate power, the position of the space above a gland is satisfactorily recognized (Fig. 1).

In the earlier stages, each supraglandular space is situated just above the centre of the gland. But as the latter grows larger, the space also increases in size, elongating, at the same time, into an elliptical or linear-oblong area. The longer axis is always parallel to the margin of the pinnule. In the elongated old glands, the supraglandular spaces are in many cases stretched in the direction following the glands and are situated above their middle or terminal point. It has been ascertained that the most elongated glands lie near the margins of the pinnules, in such a degree as often to join with an adjacent one. There is little doubt that the direction of the elongation of both the supraglandular spaces and the glands is a consequence of the growth of the pinnule.

As soon as a ligule has appeared at the transitional region, the formation of glands begins to take place in it. Numerous completed glands are already to be found mingled with younger ones, when a ligule has attained to the length of 1 cm. The table given above reveals at once that the glands are formed in large numbers in the pinnules of a young plant and that they become rarer in the adult pinnules, as the pinnules increase in area, with few additional glands. The present writer obtained the best results by the following method of treatment, among various others, in searching after the mode of development of the glands.

A piece of frond fixed beforehand in the picric acid and preserved in a strong alcohol is imbedded in paraffin. The sections are stained first in aniline blue. They are treated with 70 per cent. and 90 per cent. alcohol successively until the colouring matter is washed out without any shade of blue in the tissue except in the glands. The sections are then stained in fuchsin, then sealed in Canada balsam after the usual process. The glands are now stained a deep violet and the other parts of the tissue a beautiful red. Delafield's haematoxylin also answers well. In it the glands are stained a deep purple, while the remaining parts take a bluish violet colour. The former method of staining gives a sharper contrast of colours, while the latter is better for the purpose of studying the plasmic contents of the tissues.

The glands have their origin in the cortical cells which are situated directly beneath the epidermal layer (Fig. 8). These cortical cells are so closely in contact with the overlying cells as to give a distromatic appearance to the epidermal layer. That the epidermal layer is never distromatic may be proved by comparing the cell-contents and the future development of both layers. As the first step of the gland formation, one of the cortical cells begins to swell up gradually, undoubtedly gaining its nourishment from its contiguous cells. As the glandular cell swells up, the epidermal cell situated just above it is gradually compressed upwards. The surface of the latter retains its former position in the same level with the neighbouring ones. The consequence is the diminishing of the cell-cavity. The cell-contents are dissipated by degrees, but the nucleus is limited to the narrow space now allowed for it (Fig. 10). The glandular contents are at this stage already differentiated, so as to assume a deeper staining than the others, and change into a hyaline plastic mass filling up the whole cavity of the swollen cell. The epidermal cell is finally flattened into a thick hyaline membrane, roofing over the gland. Hence it may be clearly understood that there is not any actual perforation upon a gland. Thus the primary supraglandular space is formed.

In the surface view of the epidermal layer we often find two or three contiguous cells in course of degeneration (Fig. 4). This may be interpreted in two ways. In the first case two or more cells are sharing in the formation of one supraglandular space, as shown in Fig. 9. In the second, two or more supraglandular spaces may be in course of formation, each with a gland below, as shown in Fig. 10. Practically, however, the former case seems to be more frequent than the latter. As an abnormal case it was found that the epidermal cell upon the embryonal glandular cell did not degenerate, but both fused together into a long cylindrical cell (Fig. 13). In a rare case three cells, the one epidermal and the others cortical, serially disposed perpendicularly to the surface of the frond, have swollen up as shown in Fig. 14. The innermost cell stained deepest, in

the same degree as the other glandular cells, and the epidermal one hardly at all. I can only explain this arrangement of cells as an aberrant mode of formation of the gland. The two septa would probably decompose subsequently, resulting in an oblong glandular cell.

After the epidermal cell has completely atrophied and the supra-glandular space has been first formed, the glandular cell ceases to grow towards the surface of the lamina. The addition to the size of the gland takes place mainly at the inner end of the cell (Fig. 11). As the glandular cell increases in size, the cells around it are evidently pressed aside. But the epidermal cells seem to have been disturbed in a very little degree by the pressure, and their division and growth continue as the lamina extends. This will be treated further in connexion with the growth of the supra-glandular spaces.

The glands add to their size at their inner ends, displacing the loosely arranged surrounding cells. Hence their shape, which was first ovate or pyriform, with the sharper end towards the surface of the lamina, becomes roundish-pyramidal or compressed-conical. In the next stage, most of them, if not all, grow horizontally, elongating in the direction of the longer axis of the pinnule. In the cross-section of the lamina such glands appear as flattened cones with the apices protruding towards the supraglandular spaces. But in the surface view it is clearly to be seen that the glands have their largest diameter at the point below the supraglandular space, and that the horizontal elongations are outgrowths from such principal part (Fig. 1).

It was not ascertained whether any neighbouring cortical or hyphal cell has or has not amalgamated with the glandular cell during its growth. Judging from the arrangement of the cortical and hyphal cells around a completed glandular cell, I am inclined to believe that none has fused to add to its size. The direction of the outgrowth is due to the fact that the hyphal cells are largely running parallel to the margin of the pinnule—the direction of the growth of the pinnule—and thus it has fewest obstacles to its elongation in that direction.

As the primary epidermal cells in the transitional regions are cubical, the supraglandular spaces, when they are first formed by the normal process above referred to, are naturally square in surface view. They are hardly larger than the size of an epidermal cell. As the pinnule grows larger the spaces are also extended in area, retaining their former shape or elongating into a rectangular form. This is due to the fact that an epidermal cell divides into four successively by partition walls perpendicular to one another. In the fresh specimens, as well as in those fixed in picric acid, this mode of cell multiplication is not clearly seen, for the cells are closely compressed together. But when the material is fixed in the sublimate solution, the boundaries between the groups of the cells are vividly shown by the hyaline middle lamella. Each group consists of four cells cruciately

arranged. It may be geometrically proved that the primary supraglandular space extends four times in area as each surrounding cell divides into two; and that, when the cells on two opposite sides divide quicker than those in the other two, the results will be rectangular spaces. This is practically proved in the early stages of the formation of the spaces (Figs. 5, 6).

As a frond grows further, the epidermal cells increase in number and size. As a fact, the cells surrounding a space multiply less quickly than those in a more remote position. The result is, as the geometrical principle may well prove, the radial arrangement of the cells with the space as the centre. Eventually the space gradually approaches to a round or elliptical area as it widens. Moreover, the cells bordering a space tend to elongate towards its centre as the mutual compression leaves that side entirely free. As a consequence, the cells surrounding a supraglandular space apparently converge towards its centre, as has been described by Okamura (Fig. 7).

The contents of a primitive gland are a comparatively large nucleus and many colourless hyaline grains enclosed in a thin coating. As the gland grows larger the grains increase in number and remind one of a potato cell full of starch grains (Fig. 2). In some glands, during observation under the microscope, the contents have suddenly turned into a homogeneous mass, resembling, in a manner, the action of a strong potash solution upon the starch grains in a potato cell. The mass contained in the gland then made its way through the supraglandular space, changing into a mucilaginous matter, undoubtedly by the turgor-pressure of the gland. The semi-fluid which is ejected from the surface dissolves away by degrees in the surrounding medium. I was not able to detect any sort of opening in the external covering of the gland. But judging by the speed of the emission, the roofing membrane over the gland must have burst open to form a passage at that moment.

At the moment when the glandular contents have been freed from the gland, the mass forms a globular body upon the supraglandular space and rests there for a considerable time. This is not recognizable, even under a high power of the microscope, before treating with the colouring matter, as the refracting power of the mass is nearly equal to that of water. Staining by aniline blue, however, shows the mass very clearly; at the same time the mass remaining in the gland assumes the same colour. Hence in such cases, one globular body is seen on each side of an epidermal layer, constricted at the newly opened supraglandular passage. It is very interesting to note that the aniline blue can do nothing to the glandular contents before it has diffused into the homogeneous mass. In the microtome sections the glandular contents, in both young and old, are seen as a reticulated or areolar mass adhering to the inner surface of the wall, and there is hardly any difference in the degree of staining.

Okamura suspected that the 'bag' might be a sort of excretory organ.

It is not very clear to me how he came to think of finding an excretory organ in an alga. It is now established that the mass contained in the 'hyaline bag' is not a coloured solid substance while in the living state, but a colourless, plastic semi-fluid. The chemical properties, however, of this mass are not yet satisfactorily known to me. But I have ample reason to believe that the problematic cell is a gland secreting a mucilaginous substance.

It is rather doubtful to me whether the mucilaginous substance actually flows out in nature through the supraglandular space in the manner just alluded to above. What I have observed might have been due to an unusual turgescent state of the glandular cells during the preparation. The thick membrane roofing over the gland seems to be strong enough to hinder the free emission of the substance. But judging from the fact that the compressed matter in the gland seeks its way through the thick membrane and not by bursting the thin coating, it is more legitimate to believe that the weakest part of the membrane is at the roof, and that the contents pass out slowly by osmotic action through this part.

The fronds of *Undaria*, *Hirome*, and *Undariopsis* have been described as lacking the mucilage canals. Yet the plants belonging to these genera are highly gelatinous to the touch while in the fresh state. That it is due to the mucilage thinly coating the surface of the frond may be proved by the reaction of an aniline blue solution. A fresh frond is dipped *in toto* in the solution: the mucilage is stained, and at the same time coagulates into a flaky matter. This coagulated matter may be easily brushed away, leaving the surface of the frond highly resistant to friction.

So far as researches on the mucilage ducts of the other Laminariaceous plants extend, there is no positive proof that the ducts have free openings in the surface of the frond. The canals may terminate at the epidermal layer, but are always closed at the end by a single layer of cells. We are led to believe that the mucilage is squeezed out through this layer. This suggests the probability of the above hypothesis relating to the gland under consideration.

It was remarked above that the glandular contents stain very well in the fresh material after the granular structure has disappeared. This may lead us to suppose that the contents had changed their chemical properties before and after the transformation. It must be borne in mind, however, that aniline blue stains plasmolyzed cell-contents much deeper than those in a turgescent condition. This is true for all living cells of Algae, so far as my experience extends. The exceptions are met with in special absorption-organs such as the hairs of the Phaeophyceae. So also in many phanerogamic cells. The glands whose contents have changed into a hyaline homogeneous mass might lose some of their contents through the supraglandular coating. The plasmic membrane coating the inner

surface of the glandular cell-wall will have been relaxed and pressure released. In other words, the glandular cell must have been plasmolyzed unless it had a thin dilatable cell-wall. The difference in the degree of staining might have been due to the degree of turgescence rather than the chemical properties. But this is merely my supposition and needs further proof.

Summary.

1. *Undaria* has numerous glandular cells scattered in the lamina.

2. As a rule, each glandular cell originates from a single cortical cell which is in contact with the epidermal layer.

3. The epidermal cell upon a glandular cell degenerates as the latter develops, leaving a membranous coating over the gland.

4. The function of the gland is possibly to secrete a mucilaginous substance.

5. The glands found in the lamina of *Hirome* and *Undariopsis* will probably prove to be similar to those of *Undaria* in their mode of development and function.

EXPLANATION OF PLATE XLVIII.

Illustrating Prof. Yendo's paper on the mucilage glands of *Undaria*.

Fig. 1. Surface view of a portion of a pinnule stained *in toto* with aniline blue. The left side, shown in thick outline, indicates the margin; the lower end is toward the rachis. *a*, *b*, *c*, various stages of development of the cryptostomata. × 54.

Fig. 2. Cross-section of a pinnule, showing a gland with granular contents; from fresh material. × 450.

Fig. 3. Surface view of a supraglandular space; from fresh material. × 450.

Fig. 4. The same, with the three contiguous cells in course of degeneration. × 450.

Figs. 5–7. Surface view of the supraglandular spaces, showing the stages of development. × 250.

Fig. 8. Cross-section of a frond with two young glands. × 1050.

Fig. 9. A gland at a stage a little more advanced. Two epidermal cells above the same gland. × 1050.

Fig. 10. Two glands side by side. × 1050.

Fig. 11. A glandular cell nearly completed; typical form. × 1050.

Fig. 12. Cross-section of one of the youngest pinnules of a sporophyllous frond, preserved in formalin. × 450.

Fig. 13. Various stages of development of the glands. In the middle one the epidermal cell seems to have fused with the glandular cell. × 1050.

Fig. 14. An abnormal gland. × 1050.

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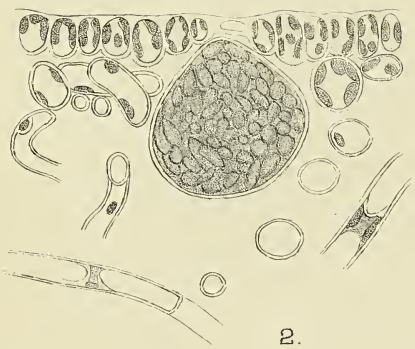

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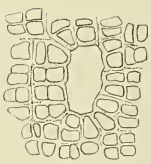

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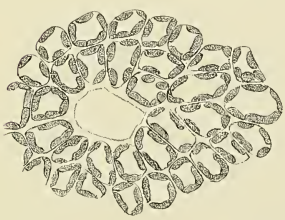

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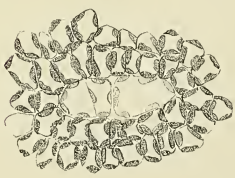

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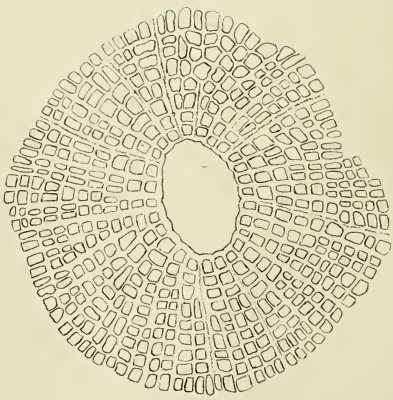

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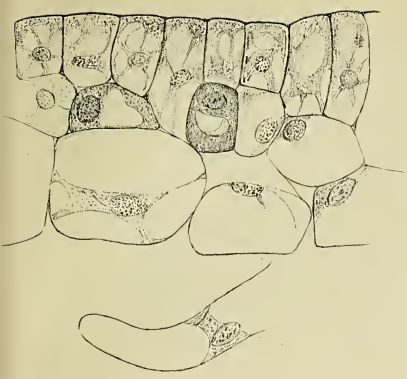
YENDO — UNDARIA



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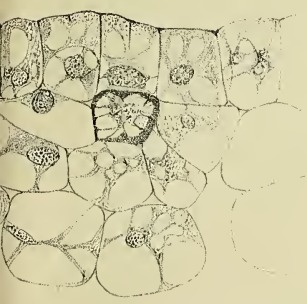

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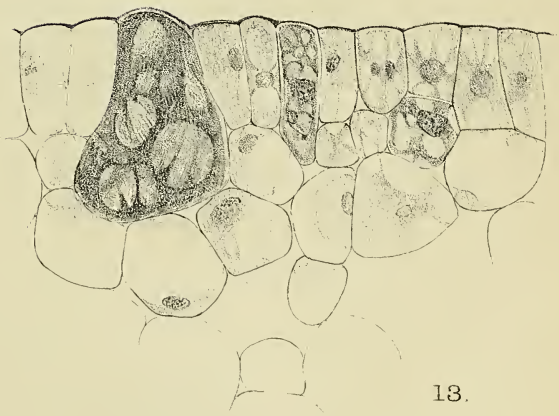

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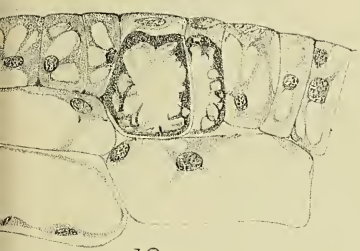

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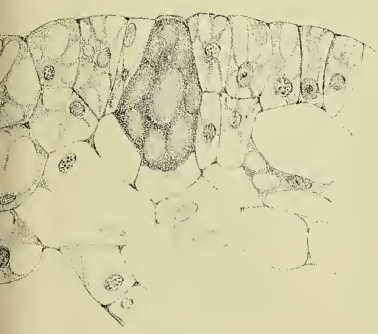

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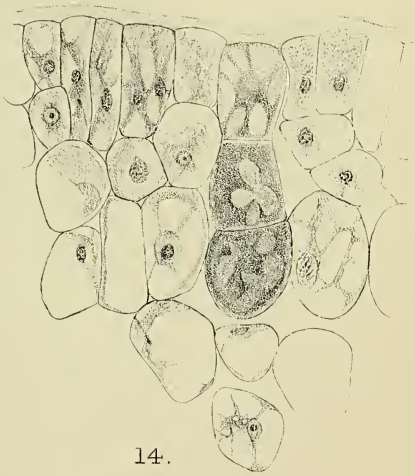

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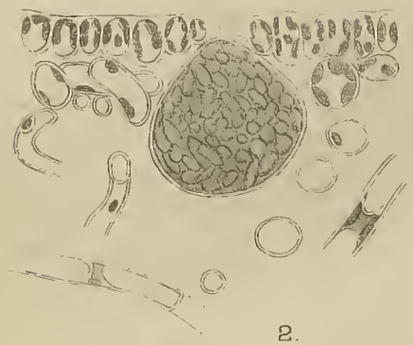

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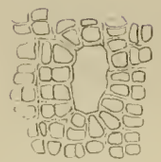

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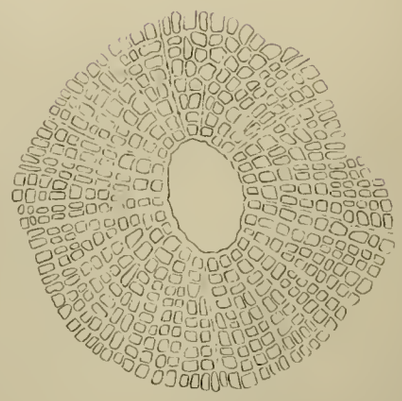

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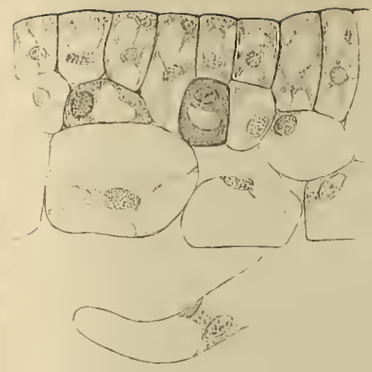

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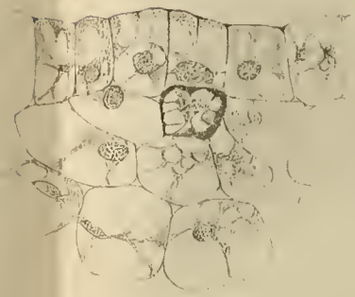

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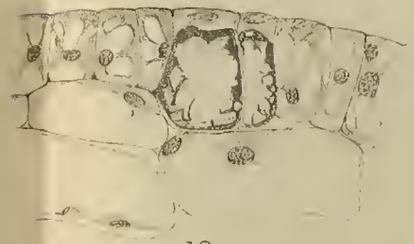

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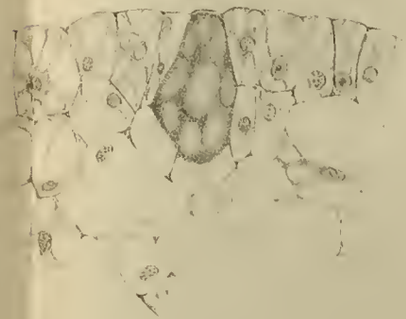

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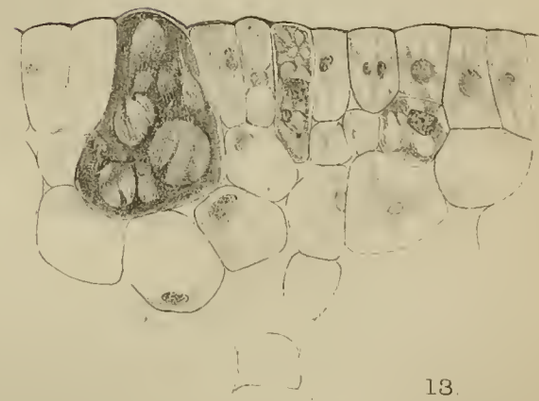

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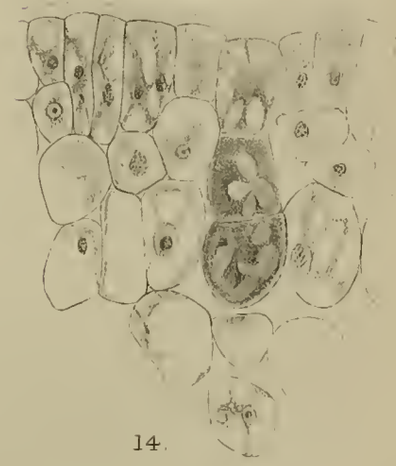

14.

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YENDO — UNDARIA

The Morphology of the Ovule and Female Flower of Juglans regia and of a few allied Genera.

BY

M. BENSON, D.Sc., F.L.S.

AND

E. J. WELSFORD, F.L.S.

With eight Figures in the Text.

THE morphology of the walnut was worked out by Van Tieghem as long ago as 1869 but his results were unfortunately published without any figures.[1] In 1905 appeared an account by Nicoloff,[2] differing in many important points from that of Van Tieghem. Numerous figures were given but they do not demonstrate the points on which he differs from Van Tieghem. We determined therefore to investigate this problem and can now state that our results confirm in all particulars those of Van Tieghem. Nicoloff, though ostensibly working out the placentation of the ovule, has not once figured the dorsal bundles of the carpellary leaves. He insists that the placental bundles are a direct continuation of those of the stem but does not figure them in continuity with the bundles of the stem. We have been able to show that they are part of the carpellary leaf-traces and that hence the ovule is appendicular.

Those who wish to clearly understand the structure should consult Van Tieghem's paper. We only propose to state so much of our results as will render the diagrams intelligible and bring the facts elicited by Van Tieghem and others into relation with more modern views on the nature of the Angiospermic flower.

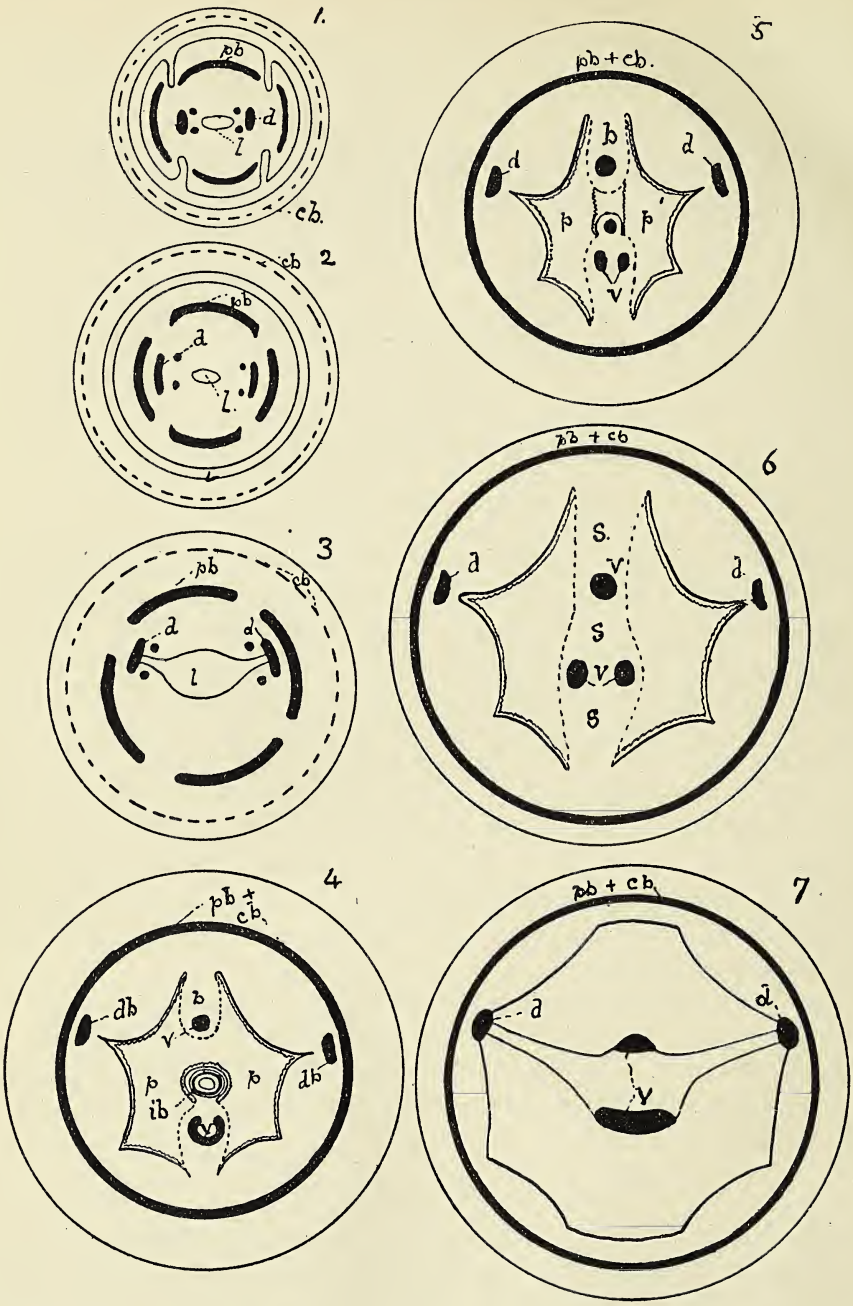
Juglans regia.

The female flower of *Juglans regia* is generally composed of 2–3 carpels surrounded by four green 'perianth' leaves, distichously arranged, and a cupule constructed probably of the subtending bract and two lateral bracteoles. All these nine leaves are connate in such a way that only their

[1] Van Tieghem, Anatomie de la fleur femelle du noyer. Bull. Soc. Bot., t. xvi, p. 412.

[2] Nicoloff, Sur le type floral etc. des Juglandées. Journ. de Bot., t. xxviii, xxix.

[Annals of Botany, Vol. XXIII. No. XCII. October, 1909.]



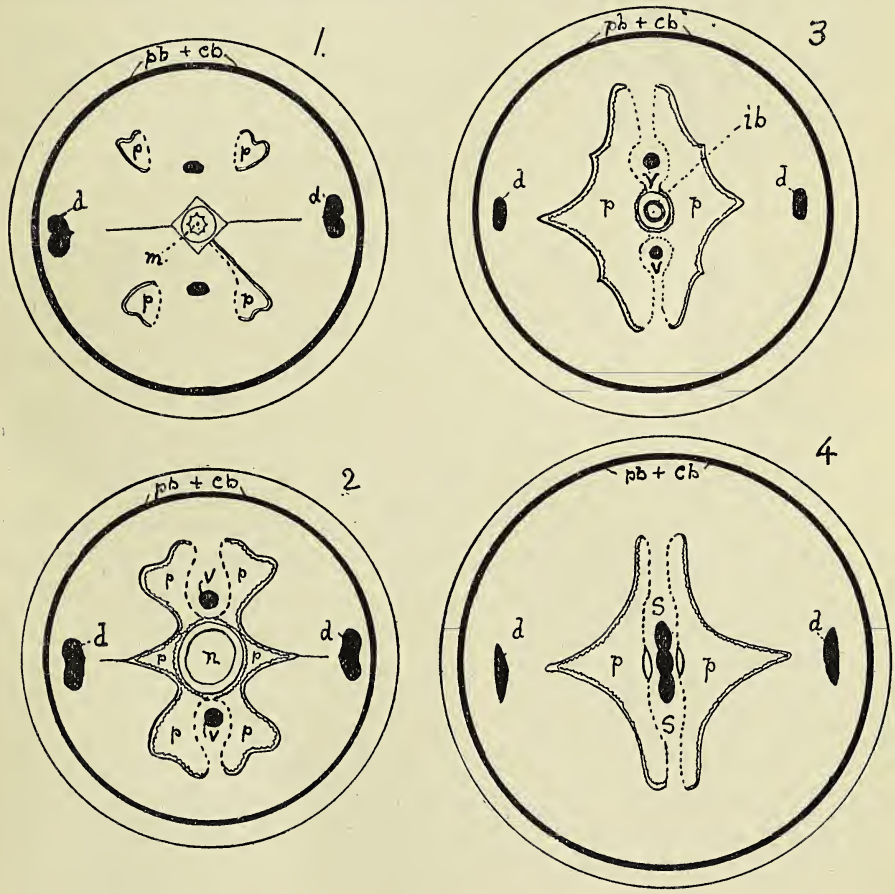
TEXT-FIG. I. 1–7. *Juglans regia.* Seven diagrams of transverse sections through the female flower. 1. In region near apex where the cupule and perianth are becoming free. 2. The cupule rim is still free, but the four perianth leaves are adherent to the ovary. 3–7 show the greater development of one carpel with its fertile placenta.

b = barren placenta. *cb* = cupule bundles. *d* = dorsal bundles of carpel. *ib* = integumentary bundle. *l* = loculus. *p* = packing tissue. *pb* = perianth bundles. *s* = septum. *v* = ventral bundle of carpel or placental bundle.

upper portions are free. The bract varies in the degree of adhesion, the cupule shows only a free rim and the perianth four free tips surrounding the two- or three-lobed styles. Nevertheless the vascular supply for each of the leaves is given off from the stem stele at the base of the flower, which, as Van Tieghem points out, is erroneously called 'an inferior ovary'. These leaf bundles are entered in Text-fig. I. 1–3, but in the other figures of *Juglans*, Text-figs. I. 4–7, III, and IV, the outer bundles are merely represented by a single line, since it is with the gynoecium we are mainly concerned.

Gynoecium.

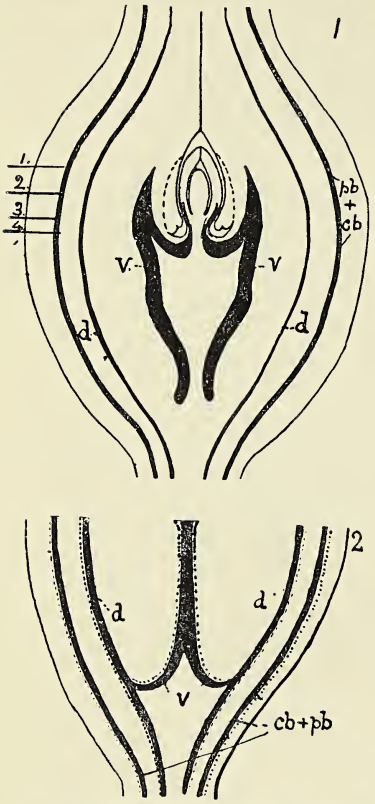
The trimerous condition is relatively rare but interesting as showing a resemblance with the gynoecia of the Fagaceae.



TEXT-FIG. II. 1–4. *Juglans regia.* Four diagrams from transverse sections in the planes indicated on Text-fig. III. 1. These represent the ordinary case in which both placentae contribute to the support of the single ovule.

cb = cupule bundles. *d* = dorsal bundles of carpel. *ib* = integumentary bundle. *m* = micropyle. *n* = nucellus. *p* = packing tissue. *pb* = perianth bundles. *s* = septum. *v* = ventral bundle of carpel or placental bundle.

By far the commoner condition is that of dimery. In such flowers both Van Tieghem and ourselves have found that the placentation may be obviously parietal. Diagrams from some of the sections of a complete transverse series are given in Text-fig. I. The two dorsal bundles which run straight up into the styles are obliquely placed and the placental bundles (*v*) are of unequal size. It is seen that only one placenta (i. e. the larger) bears an ovule (Text-fig. I. 4–5), the other is barren. Van Tieghem points out that the existence of a barren placenta indicates that the ancestors of *Juglans* had produced more than one ovule per flower.



TEXT-FIG. III. 1 and 2. *Juglans regia.* Two diagrams from longitudinal sections of the female flower. 1. This is at right angles to the plane of the stigmas, i.e. it is in the antero-posterior plane of the flower. The horizontal lines 1, 2, 3, 4 indicate the planes of the diagrams Text-fig. II. 1–4. 2. This is in the lateral plane of the flower slightly tangential, to show one of the placental bundles formed by the union of lateral veins of the connate carpels.

cb = cupule bundles. *d* = dorsal bundles of carpel. *pb* = perianth bundles. *v* = ventral bundle of carpel or placental bundle.

By far the commonest case is that illustrated by Text-figs. II and III.

In Text-fig. II we have four transverse sections at planes indicated on Text-fig. III. 1. These four diagrams (II. 1–4) show clearly that both placentae here contribute to the vascular supply of the ovule. In fact, the ovule appears to have become the common property of two placentae. This is the more curious as each placenta is itself supplied with a dual bundle (i. e. one from each of the two abutting margins of the carpels (Text-fig. III. 2).

Another interesting point is elucidated by these sections, namely, the distribution and character of the packing tissue (Text-figs. II and IV *p*). This tissue is highly transparent and is constructed of series of cells running at right angles to the surface of the placentae. The tissue is apparently composed of a system of concrescent trichomes which are uniseriate and grow on until they abut upon the carpellary walls and more or less fuse with them. Nawaschin [1] describes it as follows :—' Dieselbe füllt die ganze Fruchtknotenhöhlung so vollständig aus, dass die Oberfläche der Placenta und die Fruchtknotenwandung sich gegenseitig berühren und sogar stellenweise mit einander verschmelzen.'

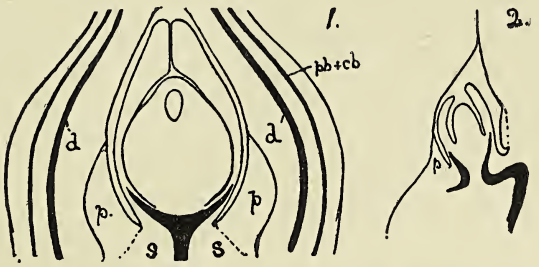
[1] Nawaschin, Ein neues Beispiel der Chalazogamie. Bot. Centralbl., 1895.

It is through this tissue that he succeeded in showing that the pollen-tube made its way to the chalaza of the ovule.

The upwardly running series of cells of this packing tissue are those referred to by E. M. Kershaw [1] as 'an obturator'. In all the specimens we have seen they are so closely attached to the ovarian wall that they appear as represented in Text-fig. IV. 1 and 2.

They are attached to the middle region of the low septum made by the junction of the two placentae, and are seen in transverse section in Text-fig. II. 2.

Their form and height in longtitudinal sections depend on the plane in



TEXT-FIG. IV. 1, 2. *Juglans regia.* Two diagrams rom longitudinal sections to explain the relation of the packing tissue (*p*) and the ovule. 1. Lies in the median plane through the midribs of the two carpels. 2. Is oblique, to illustrate the limited extent of the upwardly running packing tissue (*p*). These two diagrams should be compared with Text-fig. II. 2 and Text-fig. III. 1.
cb = cupule bundles. *d* = dorsal bundles of carpel. *p* = packing tissue. *pb* = perianth bundles. *s* = septum.

which they are cut. They disappear in radial sections in the antero-posterior plane of the flower (Text-fig. III. 1).

The packing tissue in the ovary of *Juglans* appears to be comparable morphologically with the hairs borne on the placentae and funicles of the ovules of *Populus* and *Salix*, although in these genera the growth subserves a different function.

If we turn now to the diagrams from radial sections of the flower we see in Text-fig. III. 2 the source of the placental bundles. They have no direct representatives in the axis, as Nicoloff claims, but are built up from the leaf-traces given off at the base of the flower to the carpels. This fact is demonstrated by radial sections in the plane of the two styles. The bundles in question travel horizontally until they reach the centre of the flower. Their subsequent course is shown in Text-fig. III. 1, which is a diagram from an almost radial section in the lateral plane of the flower. Van Tieghem [2] explains this course as follows:—'Le faisceau descendant destiné à l'ovule forme d'abord un assez long funicule qui demeure compris dans le parenchyme du bord fertile.' He thus claims that not only is the ovule

[1] E. M. Kershaw, Annals of Botany, April, 1909, p. 337.
[2] Van Tieghem, loc. cit.

appendicular but parietal and anatropous. With this conclusion we entirely concur—even though for purely descriptive purposes it may be convenient to call it basal and orthotropous.

Possibly if we knew more of the phylogenetic history of the basal ovule in other families it might be equally clearly demonstrated that it admitted of the same interpretation.

The Ovule.

The placentation has already been dealt with in the previous paragraph. We wish now to discuss the integumentary structures, as they somewhat resemble those of *Myrica Gale*, on which E. M. Kershaw [1] has made some interesting observations. In the first place we note that the nucellus is free from the integument, a character which, contrary to E. M. Kershaw, we regard as almost universal among Angiosperms. In the second place, although the integument appears single, there are indications of a dual origin. Judging from the almost universal presence of two integuments in Angiosperms [2] and from the fact that among the Amentiferae we find all stages from the two completely free integuments (e.g. in Fagaceae) [3] to two more or less fused integuments (e. g. in Casuarinaceae and Corylaceae) [4] we feel justified in regarding the ovule of *Juglans* as bitegumentary. The integuments are however very nearly completely merged.

Vascular Supply of the Ovule.

Van Tieghem [5] not only accepts the dual nature of the integument but makes the following statement with respect to the distribution of the vascular supply :—' Parvenu sous la base du corps de l'ovule, ce faisceau pénètre dans la membrane externe sans envoyer aucune branche à la membrane interne ; le siège exclusif, en profondeur, du système vasculaire du corps de l'ovule est donc la membrane externe.'

As in *Myrica Gale*, the placental strand on entering the ovule of *Juglans* gives rise to a number of bundles which lie symmetrically about the ovule, but the number is much larger than in *Myrica*. The number is approximately twenty and they appear to be as numerous in the rarer case where only one placenta feeds the ovule as where two placentae contribute.

Crenate Form of Transverse Section of Micropyle.

This phenomenon, which is not uncommon among the more primitive Angiospermic ovules, e. g. *Rheum*, reaches a remarkable development in *Juglans regia*. It occurs in young ovules before fertilization and is well

[1] E. M. Kershaw, The Structure and Development of the Ovule of *Myrica Gale*. Annals of Botany, 1909, p. 357

[2] V. Payer, Organogénie de la fleur (*undique*).

[3] Benson, Contribution to the Embryology of the Amentiferae, part i, Trans. Linn. Soc., 1894.

[4] Benson, Sanday, Berridge, Contribution to the Embryology of the Amentiferae, part ii, Trans. Linn. Soc., 1905, pp. 68 and 69, pp. 48, Plate 6.

[5] Van Tieghem, loc. cit., pp. 415–16.

marked, while the peripheral outline is strictly circular. This is shown in Text-fig. II. 1.

It is tempting but probably at present unsafe to see in this structure a vestige of the canopy which is so universally present in the 'Lagenostoma' series of Palaeozoic ovules and persists clearly in *Bennettites Morierei*.

Summary of results in Julgans regia.

1. The female flowers of *Julgans regia* exhibit interesting phases of reduction as follows:—

(*a*) The origin of a dimerous condition from a trimerous.

(*b*) Barren placentae with a vascular supply.

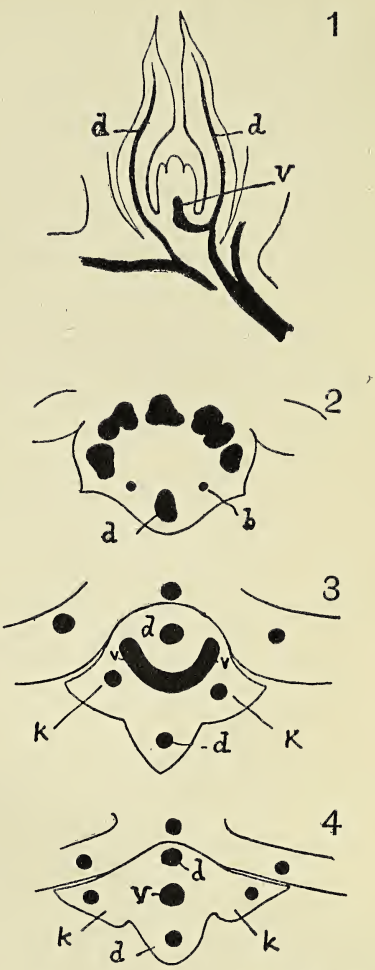
(*c*) One mode of the phylogenetic origin of the orthotropous basal ovule from an anatropous, parietal type.

2. The above explanation of the female flower of *Juglans* tends to reduce the importance of the divergent characters of Juglandaceae and Salicaceae. We refer to the following characters:—

(*a*) The non-contribution of the axis to the ovary.

(*b*) The presence of packing tissue originating from the funicle and placenta.

(*c*) The parietal placentation of the anatropous ovule.



TEXT-FIG. V. 1–4. *Myrica Gale.* Four diagrams, of which 1 is from a longitudinal section in the antero-posterior plane. This shows the origin of the placental bundle from the anterior carpel. 2–4 are obliquely transverse to the peduncle. They show the region where the traces to all the floral leaves are given off. The lateral strands of the barren carpel which can be seen in 2 *b* soon die out, but those of the anterior fertile carpel converge (3 *v*) and form the placental strand (4 *v*).

b = barren placenta. *d* = dorsal bundles of carpel. *k* = bracteole. *v* = ventral bundle of carpel or placental bundle.

MYRICA GALE.

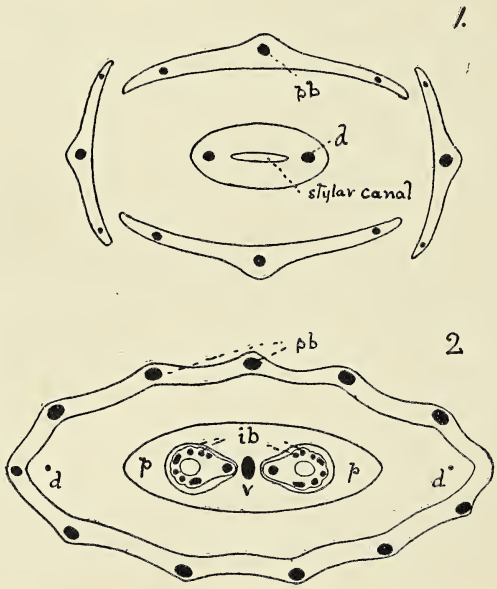
As the account given by E. M. Kershaw[1] of the vascular supply of the so-called 'basal' ovule of *Myrica Gale* did not appear to us in harmony with the view that it was appendicular, we have made a careful investigation of the vascular supply of the whole flower, both in transverse and longitudinal micro-

[1] E. M. Kershaw, loc. cit., p. 355.

tome series. E. M. Kershaw[1] appears to have overlooked the sections in the planes of Text-fig. V. 1–3, which show clearly that the placental supply comes from the carpellary leaf-trace and is not a direct continuation upwards of the stem stele. Each carpel receives a triple leaf-trace but each bracteole only one, Text-fig. V. 2, 3. The two lateral veins of the posterior carpel abort, while those of the anterior carpel are well developed and converge to form the placental supply. We thus regard the ovule as appendicular. The subsequent intercalary development of the outer walls of the ovary above the placenta gives rise to the appearance of basal origin in the ovule and lifts up the two bracteoles like wings.

Carpinus Betulus.

Here the right-hand margin of one carpel supplies the strand for one ovule and the left-hand margin of the other carpel the other ovule. These



Text-fig. VI. 1 and 2. *Carpinus Betulus.* Two diagrams from transverse sections of the female flower. 1 shows the four distichously arranged perianth leaves now free. 2 shows them united with ovary. The dorsal bundles of the two carpels are very small. Here may be seen the bundles of the outer integuments, *ib*.

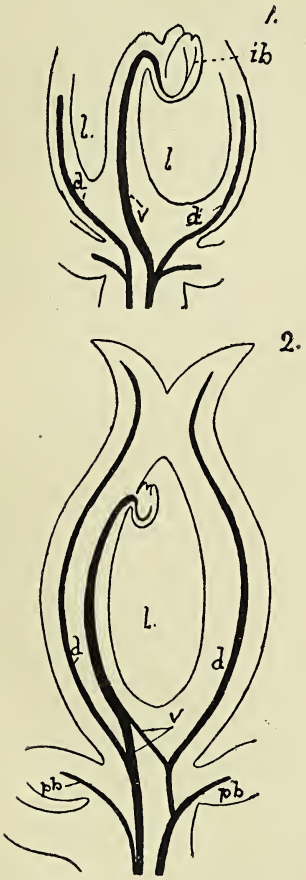
d = dorsal bundles of carpel. *ib* = integumentary bundle. *p* = packing tissue. *pb* = perianth bundles. *v* = ventral bundle of carpel or placental bundle.

two strands run together up the placenta, which is developed basipetally, and separate to pass to their respective ovules in the upper region of the two loculi. The subtending bract and two bracteoles are coherent together to form the familiar wing of the fruit, but this is only slightly adherent to the flower. The perianth receives its vascular supply from the axis below

[1] E. M. Kershaw, loc. cit., p. 355.

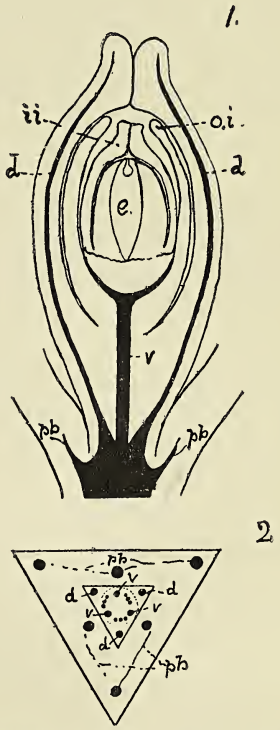
the region of insertion of the carpels. These bundles can be traced up the whole surface of the flower and pass off, three running to each of the four distichously arranged tips of the perianth leaves, as in *Juglans* (Text-fig. VI. 1 and 2).

The ovule is bitegumentary, appendicular, and anatropous, and in these



TEXT-FIG. VII. 1 and 2. *Morus nigra.* Flower. Two diagrams from longitudinal sections of the ovaries of different flowers. 1. From a bilocular, uniovular ovary. 2. From a unilocular ovary.

d = dorsal bundles of carpel. *ib* = integumentary bundle. *l* = loculus. *pb* = perianth bundles. *v* = ventral bundle of carpel or placental bundle.



TEXT-FIG. VIII. 1 and 2. *Rheum undulatum.* 1. Diagram from a longitudinal section of a female flower; the diagram is drawn as if the flower were dimerous. 2. Diagram from a transverse section just below the separation of the perianth. Two whorls of three bundles go to the perianth. The triangular area within these represents the base of the trimerous ovary.

d = dorsal bundles of carpel. *e* = embryo-sac. *ii* = inner integument. *oi* = outer integument. *pb* = perianth bundles. *v* = ventral bundle of carpel or placental bundle.

particulars resembles those of *Castanea*, *Quercus*, and *Fagus*, where, however, the outer and inner integuments are quite free from one another.

In all the Amentiferae so far investigated by us the integuments are free from the nucellus.

With a view to comparing our results among the Amentiferae with those from nearly allied forms we selected for investigation species of *Morus*, *Urtica*, and *Rheum*.

Morus nigra.

The ovary here is trimerous or dimerous as in *Juglans*. It may be bilocular with one ovule in each loculus,[1] or bilocular with a single ovule (Text-fig. VII. 1), or unilocular also with one ovule (Text-fig. VII. 2). We find that in the latter form the ovule is supplied by a strand from each carpel (Text-fig. VII. 2, *v*). The outer integument is supplied with vascular strands which bifurcate once before they terminate (Text-fig. VII. 1, *ib*).

Urtica dioica.

With the exception of the dimery of the *Urtica* gynoecium the relation of the ovule and carpel in this genus seems to resemble that of *Rheum undulatum*. Hence no separate account will be given.

Rheum undulatum.

In this flower we have a very advanced type of the basal orthotropous ovule. As is well known the ovary is trimerous and unilocular.

In a complete microtome series of transverse sections through the attachment of the flower we cannot detect any horizontal placental supply. This is undoubtedly to be explained by the tapering form of the base of the superior ovary. All the area enclosed within the base of the three carpels is occupied by the placenta up which runs the lateral bundles contributed equally by the three carpels. These relations are shown in Text-fig. VIII. 1 and 2. As the series of transverse sections is continued upwards the concentration of the tracheides increases. We do not think therefore that even in Polygonaceae the ovule should be conceived of as axial.

General Discussion.

I. *Epigyny.*

A consultation of the works of the leading authorities on the morphology of the flower shows a general recognition of the difficulty involved in the use of term ' epigyny '.

Asa Gray[2] defines epigynous as ' on the ovary or seemingly so '.

Pax,[3] after defining the inferior ovary as consisting of axis *plus* floral leaves, continues:—' Freilich wird immer zu untersuchen sein, ob nicht der

[1] Payer, Organogénie de la fleur. Atlas, Plate 61, Fig. 27.
[2] Asa Gray, Structural Botany, Glossary, p. 409.
[3] Pax, Morphologie der Pflanzen, p. 206.

unterständige Fruchtknoten durch Anwachsen der Blüthenhülle entstanden ist.'

In the genera under consideration in this paper we find no trace of that form of epigyny which is brought about by the concavity of the axis and sinking and inclusion of the ovary within it. The female flower of *Morus* bears a striking resemblance to that of *Juglans*, and the Urticales as a whole are brought more into line with the Amentiferae if this fact is borne in mind. The coalescence of the floral leaves is found more or less in such relatively unspecialized families as Nymphaeaceae and Calycanthaceae and hence the so-called epigyny of the Amentiferae need not be regarded as an advanced character. In describing such flowers it would be preferable to avoid the use of the term epigynous.

II. *The Ovule in Angiosperms.*

(*a*) *The ovule is appendicular.* The present investigation explains some of the more difficult cases. Eichler[1] came to the same conclusion, and adds :— 'Treten mehrere Fruchtblätter zu einem Ovar zusammen, so kann diese Verarmung noch weiter gehen, bis zu dem Extrem, dass von sämmtlichen Carpellen des Ovars nur ein einziges fruchtbar ist und bloss ein einziges Ovulum entwickelt. Je nachdem dies nun höher oder tiefer an der betreffenden Carpellsutur entspringt, erscheint es bald in halber Höhe, bald im Grunde desselben, im letztern Falle oft so tief, dass es den Gipfel der Blüthenaxe zu bilden scheint.'

(*b*) *The ovule is phylogenetically provided with a dual integument.* This appears very probable from the investigations of Payer. Such cases as *Carpinus* afford an interesting link between the less specialized Cupuliferae and the Juglandaceae and Myricaceae which in the reduction of their floral organs seem in advanec of both former families.

(*c*) *Vascular supply of the ovule.* We have shown that there are several cases showing a vascular supply similar to that described for *Myrica Gale* by E. M. Kershaw.[2] There is no adequate reason to doubt that a wider investigation would reveal numerous instances.

We do not as yet know of a case among Angiosperms with a *double* vascular supply such as is familiar to us in *Lagenostoma Lomaxii.*

E. M. Kershaw compares the vascular supply of the ovule of *Myrica Gale* with the outer series of vascular strands in the ovule Trigonocarpon. There is still some doubt as to the homologies of the complicated integument of the latter. It might therefore be preferable to compare the vascular supply of the outer integument of *Carpinus* and *Morus* (and hence probably that of *Myrica Gale*) with the vascular supply of the outer integument or 'cupule' of *Lagenostoma Lomaxii* which is far better understood.

[1] Eichler, Blüthendiagramme, vol. ii, p. xvi.

[2] E. M. Kershaw, loc. cit., p. 357.

Preliminary Observations on the Transpiration Current in Submerged Water-plants.

BY

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THE growing tendency to doubt whether known physical forces are sufficient to bring about the ascent of water in tall trees, and to look to the agency of living cells adjacent to the conducting channels to supplement them, has suggested to us that the water current in submerged water-plants might be worth investigation. In this case evaporation from the leaves, an essential link in the chain of physical factors in the transpiration of land plants, is eliminated.

Experimental evidence for the existence of a transpiration current in water-plants has been brought forward by Sauvageau,[1] Hochreutiner,[2] Pond,[3] and others. Hochreutiner, by using a solution of eosin, obtained values for the rate of the current in cut branches of *Potamogeton pectinatus*, *P. crispus*, *P. densus*, and *Ranunculus aquatilis*, but his experiments were of many hours' duration, and the eosin travelled not more than half a centimetre per hour.

A similar eosin method was employed in some experiments with *Potamogeton lucens*, which were made by a class of students superintended by one of us during a course of demonstrations on the physiology of plants given in the laboratory of the Royal Holloway College. A glass cylinder was divided into two portions by a closely fitting flat cork covered with a soft wax mixture. The lower chamber was filled with a dilute solution

[1] Sauvageau: Feuilles des Monocotylédones aquatiques. Ann. des Sc. Nat., Bot., sér. 7, xiii, 1891, p. 103.

[2] Hochreutiner, G.: Études sur les Phanérogames aquatiques du Rhône et du Port de Genève. Rev. Gén. de Bot., viii, 1896, p. 158.

[3] Pond: The biological relation of aquatic plants to the substratum. Contributions to the biology of the Great Lakes, 1905.

[Annals of Botany, Vol. XXIII. No. XCII. October, 1909.]

of eosin, the plant stem firmly fixed by means of cotton wool in a hole bored in the cork, and the rest of the cylinder filled with water so that the branch was completely submerged. The eosin was observed to penetrate at rates considerably greater than those recorded by Hochreutiner, the maximum being 10 cm. in $4\frac{1}{2}$ hours. This suggested to us that experiments *in situ* might be interesting, for although the material was handled as carefully as possible it could not, from the nature of the method, be kept submerged during the short interval occupied in setting up the experiment.

Experiments were therefore made with plants of *P. lucens*, growing in the river Cam, during July and August of this year. The method adopted was to attach a small glass bulb of eosin to the cut end of a submerged branch. A good leafy stem was chosen, cut under water, and left submerged for a short time. A little cotton wool was then wrapped round the stem near the cut end, a small bulb of eosin brought down to the surface of the water, and the cut end lifted for a moment above the surface and inserted in the bulb. When the experiment was of short duration the stem was merely held under water, but when a longer time was required it was attached at two or three points to a bamboo float and kept beneath the water by one or two small strips of lead bent round it. At the end of the experiment the bulb was removed and the stem at once examined.

The rate of transmission of the eosin solution was found to be surprisingly rapid. The earlier experiments lasted from fifteen to five minutes, and in each case the eosin was found to have travelled up to the apex, a distance of 20 to 30 cm. The times of experiment were then reduced; the appended table gives a number of the results so obtained for the rate of flow of the eosin.

Time.	*Duration of experiment in minutes.*	*Number of internodes traversed by eosin.*	*Total length traversed by eosin, in cm.*	*Rate of flow in centimetres per minute.*
8.15 p.m.	1	$2\frac{1}{3}$	5·7	5·7
8.28 p.m.	1	1	7·5	7·5
11.0 a.m.	2	4	19·0	9·5
7·53 p.m.	2	$2\frac{1}{2}$	14·0	7·0
4.52 p.m.	3	7	17·5	5·8
12.23 p.m.	3	4	18.2	6·1

The results are found to depend largely on the state of the material employed, and such high results as those given in the table were only obtained with healthy branches. Probably external conditions also affect the results; this point we hope to investigate later. It was not found that the length of time which elapsed between the cutting of the stem and the immersion of its cut end in eosin had any appreciable effect on the result of the experiment.

We also hope by further experiments to obtain some light on the mechanism of the rapid water current in *P. lucens*, and in other submerged water-plants. Some preliminary experiments have indicated that it depends principally on the leaves:

1. When the apex of a detached stem was removed the eosin entered from below more slowly than before, in one case 4·5 cm. in three minutes. When the bulb of eosin was affixed to the upper cut end a very slow current was observed in the reverse direction. In one experiment it was found that after eight hours the eosin had travelled backwards throughout the length of the detached stem employed. In two other experiments, lasting three minutes the eosin had traversed a distance of about 2 cm. When bulbs of eosin were attached to both cut ends, the eosin penetrated slowly from each end.

The possibility of a reverse current suggests that the normal current depends at most only to a very small extent on the stem itself.

2. That, on the other hand, the leaves are the determining factors is indicated by experiments in which some or all of the leaves were removed. When all the leaves and the apical bud were cut off the current in the upward direction was extremely slow, e. g. in one experiment, 3 cm. in thirty-two minutes. In the backward direction the eosin had not penetrated at all at the end of an experiment lasting eight hours. When *some* of the leaves were removed, the current was diminished roughly in proportion to the number removed. For instance, in an experiment in which about half the leaves were removed the rate of transmission of the eosin was 3 cm. per minute as compared with 6 cm. in a control experiment with a similar branch.

On anatomical and experimental grounds, Sauvageau,[1] Weinrowsky,[2] and Max v. Minden[3] point to special apical 'pores' as organs of exudation. From experiments in which the tips of the leaves were removed these do not appear to be *active*, as the results showed no appreciable diminution in the current; but we do not regard these experiments as conclusive.

In conclusion, our experiments have shown

1. An unexpectedly rapid water current in detached rootless stems of *Potamogeton lucens*;

2. That this current is at any rate largely dependent on the leaves.

Botany School, Cambridge,
August, 1909.

[1] Sauvageau, l. c.

[2] Weinrowsky, P.: Untersuchungen über die Scheitelöffnungen bei Wasserpflanzen. Bot. Centralbl., Beihefte, ix, 1900, p. 176.

[3] Minden, Max v.: Beiträge zur anatomischen und physiologischen Kenntniss wassersecernierender Organe. Inaug.-Diss., Bonn, 1898.

The Life History of Griffithsia Bornetiana.[1]

BY

I. F. LEWIS.

With Plates XLIX–LIII, and two Figures in the Text.

THE red alga, *Griffithsia Bornetiana*, was first described by W. G. Farlow (28). It has been reported as occurring commonly from northern Massachusetts (Collins, 18, p. 50) south to Long Island Sound, and has been recorded from New Jersey (Britton, 13).[2] It forms rosy tufts of branching filaments (Pl. XLIX, Fig. 1), 2·5 to 15 centimetres high, on rocks, 'wharves, sponges, shells, and occasionally on Zostera' (Farlow, 29, p. 131). South of Cape Cod it is found growing from one to four feet below low water mark, in protected situations such as the Little Harbor at Wood's Hole, Mass., and on rocks in more exposed localities.

The present investigation was begun in 1905 on material collected at Cold Spring Harbor, New York, by D. S. Johnson in 1902, and has been continued during 1906 and 1907 at Wood's Hole and at the Johns Hopkins University.

In all plants examined, with two exceptions noted below, the antheridia cystocarps, and tetraspores are borne on separate individuals, which may be readily distinguished with the aid of a hand lens. The male plant is smaller and more compact than either the female or tetrasporic plant, and may be identified by the abrupt terminations of the filaments (Fig. 2). It rarely becomes more than 4 centimetres high. The female plant is more loosely tufted than the male, and reaches a much larger size, becoming 12 to 15 centimetres high. The cystocarps form deep red dots at the sides of the nodes (Fig. 3). The filaments of the female plant do not end abruptly, but become gradually smaller toward their tips. The tetrasporic plant is more slender than the female, and to the eye more nearly like it than the male. It may be distinguished by the whorls of tetraspores, which form complete rings at the nodes (Fig. 4). The tetrasporic plant,

[1] Contribution from the Botanical Laboratory of the Johns Hopkins University, No. 9.

[2] The form reported from as far south as the Barbadoes by Mlle. Vickers (86) is believed by Dr. Farlow not to be identical with *G. Bornetiana*.

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like the sexual individuals, sometimes produces reproductive organs when consisting of but 10–20 cells and having a height of less than half a centimetre.

One plant was seen in which a few antheridial branches occurred, while the majority of the filaments bore numerous procarps and cystocarps. In another case, most of the branches produced antheridia, but a considerable number bore at the nodes rings of cells resembling in all particulars tetraspore-mother-cells, with the involucral rays characteristic of the tetrasporic sorus. These tetraspore-like structures are described in detail on p. 671.

Griffithsia Bornetiana becomes conspicuous at Wood's Hole in the first half of July, and grows rapidly until it reaches its maximum development about the first week in August. Towards the middle of August the plants of all ages cease to produce new branches, and slowly become disorganized, losing their rich pink colour, and becoming easily detached from the substratum. At this season great quantities are washed up on exposed points like Nobska Point at Wood's Hole. After being torn loose from their fastenings, the plants float about in the water for days or even weeks, continuing to produce spores which were shown by experiment to be capable of germination. At this season, the tetrasporic plants frequently show a very robust habit, forming spherical masses upwards of 15 centimetres in diameter.

The spores develop quite rapidly in the open. Bits of cotton cloth, tied to piles near mature plants, showed in two weeks' time sexual plants with ripe antheridia and carpospores, and tetrasporic plants with mature spores. The largest of these plants showed eight orders of branching, and consisted of as many as 500 cells.

A noteworthy fact about the occurrence of the various forms is that tetrasporic plants are always more abundant, as well as on an average larger than sexual plants. During the first two weeks of August, 1907, more than 500 plants were collected, care being taken to collect every plant seen, and not to select the larger specimens. On one occasion 352 individuals were brought into the laboratory and sorted carefully. Of these 321 were found to be tetrasporic, 15 cystocarpic, and 16 antheridial. At another time more than 200 plants showed about the same relative proportions. In other words, there is on an average an equal number of antheridial and cystocarpic plants, and for each sexual plant about ten tetrasporic ones. An exact count was not kept of plants collected earlier in the season, but there seems to be no doubt that tetrasporic plants greatly predominate in number at all seasons.

The same relations are shown quite strikingly by *Champia parvula* and *Chondria tenuissima*, at Wood's Hole, and will probably be found to obtain in many other red algae. Similar numerical preponderance of tetrasporic

plants has been noted in *Laurencia* by Phillips (61), in *Polysiphonia* at Naples by Oltmanns (59, 1, p. 650), and in *Corallina* by Solms-Laubach (74). Professor Farlow states that among the red algae, 'tetrasporic plants are a good deal more common than sexual plants, and, in decidedly the majority of species which I have examined, I have had to look through a mass of tetrasporic plants before coming to any bearing sexual organs. In the great majority of Florideae the chances are decidedly in favour of finding tetraspores rather than sexual organs.'[1]

Methods.

Of various fixing fluids employed, the weak chrom-acetic-osmic acid mixture was found to be best for cytological details (Yamanouchi, 92, p. 425). The time of fixation varied from one to ten hours. Paraffin sections 3 and 5 μ thick were used almost exclusively for the finer details of cell structure; grosser anatomical features were found to be best made out from mounts *in toto*. The most successful stain employed was Heidenhain's iron alum haematoxylin (2 hours in the alum solution, 4 hours in the stain), followed by eosin in clove oil, as recommended by Miss Fraser (31).

Difficulty was experienced in obtaining material showing abundant nuclear figures. Plants brought into the laboratory and fixed at all hours after having been kept in running water showed almost no mitoses. Finally favourable material was obtained by fixing 'in the field' at eleven or twelve o'clock at night.

Vegetative Characters.

The thallus forms a hemispherical tuft, and is composed of much-branched filaments, which are made up of large swollen cells placed end to end in series. The filaments radiate from a common point of attachment, the holdfast. In a plant of average size, from the base to the apex of a single filament, exclusive of the branches, there are twenty to thirty cells; in large specimens the number of cells in a single filament may be twice as great.

The cells differ greatly in shape and size in different parts of the filaments (Fig. 4). Toward the base of the plant they are approximately cylindrical below and much swollen toward the upper end. The cells nearer the tip of the filament become shorter and relatively thicker, of an obovate shape, and of a deeper colour. Those cells of the female plant which bear the older cystocarps become very much swollen toward their upper ends. In the male plants the terminal cells bearing the antheridia are almost globose. The following table gives a general idea of the sizes of the various cells in a filament composed of twenty cells.

[1] From a personal letter.

No. of cell.	*Tetrasporic and cystocarpic plants.*			*Antheridial plants.*		
Apex.	*max. diam.*	*min. diam.*	*length.*	*max. diam.*	*min. diam.*	*length.*
1	·060 mm.		·060 mm.	·65 mm.		·65 mm.
2	·130		·190	·50	·25 mm.	1·25
3	·250		·400	·40	·25	1·25
4	·320		·600	·40	·20	1·25
5	·320	·160 mm.	1·000	·45	·20	1·50
6	·400	·200	1·15	·45	·20	2·00
7	·400	·200	1·30	·45	·20	2·00
8	·400	·200	1·60	·50	·18	2·25
9	·450	·160	2·00	·55	·16	2·50
10	·520	·150	2·50	·60	·15	3·00
15	·580	·160	3·20	·60	·15	3·00
20	·640	·200	2·50	·65	·20	2·50

The cell-wall responds to the usual tests for cellulose. After the death of the cell, the wall swells greatly in aqueous fluids. When so swollen, it shows a plainly lamellate structure (Fig. 5), similar to that described for *Bornetia* by Correns (19).

The cytoplasm forms a thin layer over the inner face of the free portion of the cell-wall, averaging ·6 μ in thickness in the older cells. On the cross-wall it forms a thickened circular pad ; adjoining pads are in communication through the intercellular pores. The cytoplasmic pad over the upper cross-wall averages in the larger cells 10 μ in thickness in the centre, becoming thinner toward the edges. On the lower cross-wall the pad is usually thinner, averaging about 3 μ in thickness. The pads are about evenly divided into a granular layer adjoining the sap-vacuole, and a denser homogeneous layer next the cross-wall (Fig. 6). Nuclei are quite abundant in the granular layer but are not of usual occurrence in the homogeneous portion of the pad. Spherical bodies of various sizes, probably of a proteid nature, occur commonly in both layers of the cytoplasm over the cross-walls.

In *Griffithsia barbata*, Berthold (7) found the cytoplasm divided into a clear outer layer and a granular inner layer, the latter containing the nuclei and the chromatophores. This seems to be the usual arrangement of the protoplasmic elements in the coenocytes of algae. In *Griffithsia Bornetiana* the cytoplasm becomes plainly differentiated into two layers only where it reaches a considerable thickness, as in the thickened pads mentioned above, and in very young cells in which the sap-vacuole is still small.

Intercellular connexions are conspicuous in living as in stained specimens by reason of the peculiar plugs which close the otherwise open pit between the cells. *Griffithsia* is an unusually favourable form in which to observe the intercellular connexions because of their large size. Evidence presented below is believed to be strongly in favour of the view, doubted by many workers, that even the older cells are actually in physical and organic connexion through the large open pores in the cross-walls.

A typical intercellular connexion is shown in Fig. 6. The pore in the cross-walls is closed on each side by a disk, which is the 'stopper,' or 'plug' of Archer (3). This disk is in direct contact with the thickened pad of cytoplasm lying on the cross-wall. Connecting the disks is a broad strand of thin clear cytoplasm, or, in some cases, several smaller strands (Fig. 8). In several instances, bits of the proteid substance normally present in the pad have been found in the cytoplasmic strand which connects neighbouring disks, apparently having been fixed in transit from one cell to another (Fig. 6). The middle lamella mentioned later as being formed in some cases in cell-divisions has not been demonstrated in the older intercellular connexions.

The size of the pore varies with the size of the cells which it connects. The average diameter of the disks, which is the same as that of the pores, is about 11 μ in the large cells at some distance from the apex.

In living and in unstained fixed material the disks are refractive colourless bodies. They stain heavily with nuclear dyes, particularly with Heidenhain's haematoxylin. Cytoplasmic stains, such as eosin, colour them much less intensely. They are soluble in Javelle water, as was pointed out by Kienitz-Gerloff (46). This fact, coupled with the fact that the disks are continuous on both sides with unaltered cytoplasm, gives support to the view, first expressed by Schmitz (70) that they are protoplasmic in nature.

The results obtained by various workers on intercellular connexions in the red algae are conflicting. Archer (3) described in *Ballia* pits which were at first open and later became closed by a plug, or 'stopper.' Schmitz (70) came to the conclusion that the pit is closed by a delicate membrane, which is pierced by many or several protoplasmic strands. Hick (42) thought he had demonstrated a simple protoplasmic strand passing through the open pit. Moore (55) found that a pit-closing membrane is pierced by one or several protoplasmic strands. Wille (88) described and figured a sort of sieve tube in *Cystoclonium*. Harvey-Gibson (39) found that in *Polysiphonia fastigiata* an actual protoplasmic connexion is present only in young stages and that later a plug closes the pore-canal. However, he mentions that from the edges of the plug fibrillar thickenings connect the neighbouring protoplasts. Kohl (49) regarded the matter of protoplasmic continuity in the Florideae as still unsettled. Kienitz-Gerloff (46) found that the pit is closed by a delicate membrane, and reached the conclusion that an unbroken connexion cannot be said with certainty to exist in the form studied (*Polysiphonia*).

The number of nuclei in a single vegetative cell is always large. Since the nuclei are approximately equidistant in each cell below the apex, it is evident that the number in a cell varies directly with the size of the cell. Estimates made from several preparations show that the large cells near the base of the plant contain, on an average, 3,000–4,000 nuclei. As the

cells become smaller toward the apex of the filament, the number of nuclei becomes correspondingly less. A subterminal cell of average size contains about 100 nuclei; an exceptionally large subterminal cell may contain as many as 500 nuclei. In the newly formed terminal cell the number is much less, varying from 12 or 15 to 50, or even 75. The terminal cells, however, like the other vegetative cells, are always multinucleate.

The occurrence of multinucleate cells is rather general in the older portions of the thallus of other Florideae, while the terminal cell is usually uninucleate (Davis, 22; Oltmanns, 59, ii, p. 89). Schmitz, who first called attention to this fact (67), showed also that in the different species of a single genus the number of nuclei in the cells varies greatly. For example, in the genus *Callithamnion*, all the cells in the thallus of *C. plumula* are uninucleate; in *C. corymbosum* the older cells are multinucleate; in *C. Borreri* even the youngest cells have two or more nuclei. Obviously, then, the number of nuclei in the cell is no index of relationship in the red algae.

The nuclei of *Griffithsia Bornetiana* are pretty uniformly distributed through the cytoplasm. While the distance separating them varies somewhat with the age and condition of the cell, usually it is 25–30 μ. Not infrequently several nuclei, with the cytoplasm immediately surrounding them, form small clumps which project into the central vacuole (Fig. 7). In the cytoplasmic pad on the cross-wall 10–15 nuclei usually form a ring around the intercellular pore (Fig. 8).

The size of the nuclei varies considerably with the age of the cells, as has been shown by Berthold to be the case in the coenocytes of *Codium* (6). In the young cells the resting nucleus is, on an average, about 4 μ in diameter just before nuclear division and less than half that just after mitosis. In the older cells the average diameter of the nucleus is 2–3 μ. In the young sporelings the nuclei are very small, measuring 1–2 μ in diameter.

The resting nucleus is nearly spherical or somewhat flattened against the cell-wall. It shows a large, densely staining chromatin-nucleolus in the centre. The size of the nucleolus varies from one-fifth to two-thirds the diameter of the nucleus. It is smallest at the time of complete rest of the nucleus, and grows larger as the time for mitosis draws near. Around the periphery of the nucleus a faint linin network is visible. This is connected with the nucleolus by faint radiating strands (Fig. 10). Immediately enveloping each nucleus is a zone of cytoplasm, which appears denser than the cytoplasm elsewhere, and which is probably to be considered of kinoplasmic nature. The thickness of this zone is quite variable. It often becomes about one-third the diameter of the nucleus.

Nuclear division occurs regularly by mitosis, being found most frequently in the terminal cell. It occurs also commonly in the subterminal cell, less

commonly in the third cell from the apex, and rather infrequently in cells older than this.

The divisions of the nuclei of a single cell near the apex are almost, though not quite, simultaneous (Fig. 9). In general, the nuclei near the apex of the cell are at a slightly more advanced stage of division than those near the base. For instance, the nuclei near the apex may show stages of anaphase, or even of telophase, while those in the middle region of the cell are at metaphase, when the nuclei near the base have reached only the condition of prophase (Fig. 9). When there is an accumulation of protoplasm in the apex of the terminal cell preparatory to cell-division, the nuclei in this protoplasmic mass may divide considerably before the nuclei of the lower part of the cell. In the older cells the nuclei do not show the same simultaneity of division. Here small groups of nuclei may undergo mitosis while the majority of nuclei are in the resting condition. In the younger cells, however, when one nucleus divides, all divide, though not exactly synchronously.

In this connexion, it is interesting to note the behaviour of the nuclei in the multinucleate cells of other plants. In the sexual organs of various Phycomycetes, the numerous nuclei divide at the same time, as in the oögonium of *Saprolegnia* (Davis, 24), in the oögonia and antheridia of *Pythium* (Miyake, 54), *Albugo* (Stevens, 76), and *Peronospora* (Wager, 87). Simultaneous nuclear division is reported also in the plasmodia of *Fuligo* (Harper, 38), in *Plasmodiophora* (Nawaschin, 56), in the 'ascus' of *Hemiasci* (Juel, 44, Popta, 62), in the ascus of *Ascomycetes* (Harper, 37), in the basidium of *Basidiomycetes* (Maire, 53), and in the binucleate cells of Uredineae (Sappin-Trouffy, 66). Among algae, approximately simultaneous nuclear division is known in the germinating zygotes of desmids (Klebahn, 47), in the young colonies of *Volvox* (Overton, 60), in *Sphaeroplea* (Klebahn, 48), in *Hydrodictyon* (Timberlake, 80), in the antheridia of *Fucus* (Guignard, 36). In the vegetative cells of *Cladophora*, Strasburger found that nuclear division is not simultaneous, though he reports that several stages of mitosis are to be found in a cell at the same time, which seems to indicate that the stimulus to division affects more than one nucleus at a time. Among the Archegoniates, simultaneous nuclear division is figured by Miss Lyon for *Selaginella* (52), and seems to be the rule in the developing endosperm and in the early divisions in the fertilized egg of Gymnosperms (Coulter and Chamberlain, 20, pp. 20, 31, 41, 83, 98); in the free cell formation of the endosperm of many Angiosperms (Coulter and Chamberlain, 21, pp. 165–6, 172), and in the developing embryo sac (ibid., p. 87). In certain Leguminosae, Guignard (35) reports simultaneous nuclear division in the cells of the suspensor. The second mitosis in the gonotokonts of Archegoniates is simultaneous in the two nuclei.

Schmitz, in his studies on the nuclei of Siphonocladiaceae (67, 68) does

not discuss the question of simultaneity of nuclear division, but leaves the reader to infer that the mitoses in a cell do not occur at the same time. The same is true of Berthold's work on *Codium* (6), and of Fairchild's account of *Valonia* (27).

Approximate simultaneity of nuclear division may be said to be a very general phenomenon in multinucleate plant cells.

The small size of the nuclei renders *Griffithsia* a rather unfavourable object for the study of the details of mitosis. The following account is based on observation of the nuclei in vegetative cells of the tetrasporic plants.

The nuclei are throughout their history very poor in linin. The chromatin of the resting nucleus is not, therefore, distributed on a linin reticulum, but is contained in a centrally placed, homogeneous nucleolus, or karyosome (Fig. 10). It seems possible that a small amount of chromatin is distributed on the peripheral linin network, but the bulk of it is certainly in the nucleolus. As the nucleus prepares for mitosis, it increases somewhat in size, becoming about 4·5 μ in diameter; the nucleolus also enlarges. Chromatin from the nucleolus, in the form of rather large granules, passes out to the periphery of the nucleus along faint linin strands (Figs. 14, 15), very much as was described by Wolfe in *Nemalion* (90). At the same time, the nucleolus becomes differentiated into faintly and darkly staining areas, the latter probably representing chromatin. The chromatin continues to pass out of the nucleolus until the whole chromatin content is distributed through the nuclear cavity in the form of granules, some of which are connected with one another by linin threads (Figs. 11, 12, 13, 14, 15, 16). The number of these granules seems in every case examined to be more than twice the number of chromosomes, and in some instances the granules become much more numerous. The granules now approach the centre of the nucleus, at the same time becoming fewer in number, probably by the fusion of separate granules (Fig. 17). As they move toward the centre, they become arranged roughly in a flat plate, though all the granules do not lie in exactly one plane (Fig. 18). While this is going on, a faint spindle is formed, apparently by the rearrangement of the linin threads (Figs. 18, 19). The spindle fibres are connected with small, darkly staining kinoplasmic caps, which lie on the nuclear membrane at opposite poles of the nucleus (Fig. 19).

At metaphase the spindle is seen to be short and broad and more or less truncated at the ends (Fig. 21). The nucleus is flattened at right angles to the axis of the spindle, so that it is broader than long. The chromosomes are closely packed on the equatorial plate, which is nearly as broad as the nuclear cavity. The nuclear membrane is intact, so that the whole spindle is intranuclear.

In addition to being closely packed, the chromosomes do not lie in

exactly the same plane, and it has been difficult to count them with certainty. Between the chromosomes lies a darkly staining substance that renders counting still more uncertain. Numerous estimates, made from polar views of the equatorial plates, vary from 11 to 14. The normal number of chromosomes in the nucleus of the vegetative cell of the tetrasporic plant seems pretty certainly to be 14 (Fig. 20).

The nuclear cavity is largest at the time of prophase, measuring as much as 5·5 μ in diameter. At metaphase it is considerably smaller, averaging 5·5 μ broad by 3 μ long. A similar decrease in the content of the nuclear cavity has been noted by Yamanouchi in *Polysiphonia* (93).

At metaphase the group of chromosomes splits into two, which withdraw toward the opposite poles of the spindle (Figs. 22–23). In anaphase the daughter-chromosomes of each group are seen to be arranged somewhat in the shape of a watch crystal, with the concave surface toward the pole of the spindle (Fig. 23), as was figured in certain nuclear divisions in *Nemalion* by Wolfe (90). The two groups of chromosomes are connected by a few spindle fibres.

As the daughter-groups of chromosomes approach the kinoplasmic caps, the outlines of the individual chromosomes become lost in a dense mass of chromatin, which is to give rise to the nucleolus of the daughter-nucleus (Fig. 24). At telophase the mass of chromatin is in immediate proximity with the kinoplasmic cap. In the meantime the nuclear membrane, which becomes fainter during the course of mitosis, disappears, the original nuclear cavity becoming filled with cytoplasm, only a few faint striae remaining of the spindle (Fig. 25). The mass of chromatin resulting from each group of daughter-chromosomes becomes surrounded by a clear area, which is bounded by a faint nuclear membrane (Fig. 26). The kinoplasmic cap grows around the daughter-nucleus, whose organization is now complete.

The axes of the mitotic figures seem to bear no relation to the axis of the cell nor to the position of the cell-wall (Fig. 9). When the axis of the spindle is at right angles to the cell-wall, however, the daughter-nuclei shift their position at telophase so that a line connecting them is parallel to the cell-wall.

In the vegetative cells of the sexual individuals the behaviour of the nuclei in mitosis is in general similar to that in tetrasporic individuals. The number of chromatin granules which pass out from the nucleolus and become distributed through the nuclear cavity, while variable, is always much less than in the nuclei of the tetrasporic plant (Pl. L, Figs. 27, 28). The size of the nucleus at prophase is about the same in the two cases. At the time of metaphase, however, the cavity of the nucleus of the sexual plant is somewhat smaller than that of the tetrasporic plant. The number of chromosomes on the equatorial plate in the sexual plant is about seven,

though here, too, the counting is made difficult by the presence of a darkly staining substance between the chromosomes (Figs. 29, 30, 31).

Mitoses of the type described above have been observed in vegetative cells of various ages, in the hair-cells of the procarp and cystocarp, in the primary tetrasporic cells, in the stalk-cells of the tetrasporangia, in the involucral cells of the tetraspore-sorus, in the sporelings from tetraspores, and in the sporelings from carpospores.

No undoubted cases of amitosis have been observed. An appearance suggesting amitosis has been noted in the stalk-cells of the tetrasporangium and in the cells of the sporogenous lobes, and may possibly occur in the vegetative cells; but the small size of the nuclei renders exact observation on this point very difficult. It may be said with certainty, however, that the usual mode of nuclear division is by mitosis.

It may be well to compare at this point the behaviour of the nuclei in division with those of *Polysiphonia* (Yamanouchi) and *Nemalion* (Wolfe), the two other red algae which have been carefully studied from the cytological standpoint.

	Resting nucleus.	*Origin of Chromosomes.*	*No. of chroms.*	*Poles of spindle.*	*Nucleolus.*
Nemalion	Chromatin in central nucleolus	Chromatin passes to periphery	8 (16)	centrosomes	karyosome
Polysiphonia	Chromatin in peripheral network	Derived from chromatin network	20 (40)	centrosphere like bodies	plasmosome
Griffithsia	Chromatin in central nucleolus	Chromatin passes into nuclear cavity	7 (14)	kinoplasmic caps	karyosome

The chromatophores are numerous, small, oval or round, flattened bodies of rosy pink colour lying in the granular cytoplasm next the cell-wall. They vary considerably in size; on an average, each is about 3·5 μ long, 2·5 μ broad, and 1·2 μ thick. Usually the outline of the chromatophore is smooth (Fig. 33), but occasionally it is toothed, as is true in other species of *Griffithsia*. In the younger cells the chromatophores are crowded together without definite arrangement. In the older cells they often occur in curved rows, which are arranged in the form of an irregular network (Fig. 32), as was described for other species of *Griffithsia* by Berthold (7), and for many genera of the Siphonocladiaceae by Schmitz (67).

The number of chromatophores in a cell is very large. In an older cell of average size, about 400,000 were estimated to be present.

The chromatophores in the protoplasmic pads lying on the cross-walls are much fewer in number and smaller than in other portions of the cytoplasm.

Chromatophores apparently are absent from some lateral cells when first cut off; nor have they been seen in the young procarps, in the hair-cells, in the stalk-cells of the tetrasporangia, or in the young tetrasporangia. While no leucoplasts have been demonstrated in these cells, it is possible that they are present.

In dividing, the chromatophores simply pull apart. They first elongate and the pigment collects in each end; then they assume approximately a dumb-bell shape; and finally either separate completely, or more usually remain connected by a fine strand, as though the division were not quite complete (Fig. 33).

Starch is normally present in the vegetative cells, as has been found to be true of Florideae generally by Bütschli (15), Bruns (14), Kolkwitz (50), and others. It occurs as very small granules in circular groups, or as larger granules lying in the cytoplasm between the chromatophores. Each starch grain is rounded or oval, usually with a dark centre; no signs of lamination have been observed. Starch is especially abundant in sporelings, and in the cells of the attaching organ.

Besides the starch grains, there are normally present in the cytoplasm rounded masses of various sizes of what seems to be proteid material. These spheres usually occur in small groups, each group being surrounded by a clear area. The groups seem to be specially abundant in the cells at the time of nuclear division, and often simulate nuclei (Fig. 9). Spheres of what seems to be the same material are usually present in the pads of protoplasm lying on the cross-walls, and small bits have been observed lying in the cytoplasmic strands connecting neighbouring cells (Fig. 6).

Cell-division in *Griffithsia* is remarkable for the disparity in the size of the daughter-cells. It was first described by Wright (91), whose account was supplemented by the observations of Berthold (7).

In the vegetative cell, division occurs (1) by the cutting off of daughter-cells from the terminal cell of the filament, (2) by the cutting off of small dome-shaped segments from the upper borders of cells below the apex. The first type of division simply increases the length of the filament, the second results in the formation of a new branch.

There appear to be two methods of cell-division. The first occurs most commonly in the larger cells, and is always preceded by an accumulation of cytoplasm, nuclei, and to a less extent of chromatophores, which forms a dense, more or less homogeneous mass in the terminal portion of the apical cell (Fig. 34). A thin dome-shaped membrane is now laid down, with its convexity towards the apex, cutting a solid accumulation of protoplasm from the tip of the cell (Fig. 35). This membrane is formed simultaneously over its whole extent. There is no trace of cleavage in connexion with its formation, the protoplasm being in contact with it on each side. The nuclei appear to have nothing to do with its formation,

nor is its formation visibly associated in any way with nuclear division. The membrane is, however, never formed until there is an accumulation of nuclei and cytoplasm in the tip of the cell. The young cell, at first a solid cap over the tip of the apical cell, grows rapidly, soon acquiring a central vacuole (Fig. 36), and forming a typical vegetative cell. During the growth of the young cell, the cross-partition loses its convex appearance and becomes flattened. It becomes overlaid on each side by a cellulose wall, which does not cover the membrane completely (Fig. 37). There is left in the centre a circular area or pit, which is noticeable because of the early development of the cytoplasmic plugs described on page 642.

This is a very unusual method of cell-division in coenocytes (Davis, 25, pp. 452–3). So far as I know, it has been described in detail in no other form, though a somewhat similar process appears to take place in the large vesicles of *Valonia* (Schmitz, 67).

The details of the division which gives rise to a lateral branch are very similar to those of the division of the apical cell. The daughter-segments of the subterminal cells are formed on the side of the upper border of the cell, usually four or five cells from the apex. There is first a solid accumulation of protoplasm, then the adjoining cell-wall bulges outward, and a dome-shaped membrane cuts the outer part of the protoplasmic mass from the inner, precisely as in the division of the apical cell. The young segment pushes out and becomes cylindrical, a vacuole early appearing in its middle, and forms the apical cell of a new branch (Fig. 38). The branch thus initiated grows for a time more rapidly than the main filament, until it about equals it in size. Thus an apparent, or false, dichotomy results. True dichotomy appears never to occur, as in no case has a branch been found to divide longitudinally. Frequently more than one branch is laid down at a node, so that trichotomy results, and in the larger cells near the base of the plant, four branches have been observed to proceed from the summit of the same cell.

A second method of cell-division occurs commonly in the apical cells of the smaller branches, and sometimes in the division of the larger cells below the apex. A ring of cellulose projects inward from the cell-wall a short distance from the apex, very much as was described for *Cladophora* by Strasburger (77) (Figs. 39, 40, 41). The ring grows inward, but not so as to cut off the new cell completely. An open circular pore is left in the centre, across which the protoplasmic plugs are soon formed in the usual way. No pit-closing membrane is formed between cells separated in this manner.

The second method of division, which is the usual one in coenocytes, differs from the first in that the daughter-cell is from the beginning much more nearly equal in size to the cell from which it is cut than is the case in the first method of division.

Wherever the second method of cell-division occurs, the partition is in one plane, never arched. In all the cases examined in which division occurred by the ingrowth of a cellulose ring, the partition cut into the central vacuole, so as to cut off a segment containing part of the vacuole of the mother-cell; in the first method of division, however, the segment cut off is at first solid. The relation of the cleavage plane to the vacuole seems to determine the method of cell-division; in the division of the vegetative cells, where the cleavage plane occurs so as to cut into the solid protoplasmic accumulation in the apex of the cell, division takes place by the first method. Where the plane of division is sufficiently removed from the apex to allow the partition to cut into the central vacuole, division is by the second method.

As mentioned above, the nuclei appear to take no part in cell-division. This seems to be the rule in coenocytic cells (Strasburger, 77), though Wille has noted an apparent exception in *Acrosiphonia* (89).

The number of nuclei in the smaller daughter-cell just after its formation is various. The average number is between 12 and 20, but in some cases, and always following the second method of cell-division, the number is considerably greater. The cell next below the apex may show 30–250 nuclei.

Branched hairs are frequently borne on the upper borders of the younger cells. There are usually six or seven of these around each node on which they occur.

Their mode of origin is very similar to that of the tetrasporic filaments to be described later. Small papillae arise nearly simultaneously around the upper border of a cell near the cross-partition, each papilla containing a single nucleus and dense, homogeneous cytoplasm. The papillae are cut off from the protoplast by an arched membrane, similar to that formed in the division of some of the vegetative cells. The nucleus now divides, and one of the daughter-nuclei wanders into a bud from the papilla, the bud, with its nucleus, now becoming cut off. A second, a third, and sometimes a fourth bud are formed and cut off like the first. Each of these daughter-cells behaves in the same way, cutting off three or more buds, and in this way a thrice compound hair is formed (Fig. 42). Each of the terminal cells divides into two. The basal cell of a hair becomes multinucleate, as do the cells of the first order of branching; the cells of the second order of branching remain uninucleate. The basal cell is connected with the cell on which it is borne by an intercellular connexion of the same type as that which occurs between neighbouring vegetative cells.

After they are fully formed, the hairs elongate greatly and become hyaline. Each cell takes part in the elongation. A vacuole is formed in the cytoplasm which increases in size as the cells elongate. A very thin layer of cytoplasm lies between this vacuole and the cell-walls; in the outer

ends of the long cells are seen accumulations of cytoplasm, in which most of the nuclei lie.

The fully formed hairs may remain a considerable time before elongating. Elongation occurs in all the hairs of a single node at the same time, and seems to take place rather suddenly. The total length of a hair of average size before elongation is about 40 μ, and after elongation about 350 μ. Such great increase in size in a short time seems to be rendered possible by the fact that the cells of a hair do not secrete a cellulose wall until after elongation has taken place.

After elongating, the hairs remain for a while on the plant, but finally the connexion between the basal cells and the vegetative cell breaks, and the hairs fall off as a whole. Not infrequently a second crop of hairs is formed before the first crop falls off, so that there appear to be 'two sets of hair-like organs' (Farlow, 29, p. 132). By the time the second set is formed, the first set is carried by the growth of the vegetative cell to some distance from the cross-partition between the vegetative cells, and the second set of hairs is always formed between the cross-partition and the first set (Figs. 42, 43).

Individuals vary greatly in the number of hairs produced. In some specimens, hairs are found on almost every node in the younger portion of the plant. Again, one may look over a great many shoots before encountering a single set of hairs. What external conditions are favourable to the production of hairs in *Griffithsia* is not known.

In the material examined by Miss Smith (73), hairs occurred on the female plant only on nodes bearing cystocarps. Such a restricted distribution is not general. Any of the young vegetative cells seems to be capable of producing hairs, and while hairs occur usually in the vicinity of reproductive organs, there seems to be no necessary connexion between the two.

The function of the hairs is quite unknown. They undoubtedly increase markedly the surface exposed to the water, and inasmuch as they occur especially abundantly in the neighbourhood of the reproductive organs, where the processes of metabolism may be assumed to be most active, and are usually absent on the sterile portions of the plant, it seems likely that they perform the functions of absorption and respiration, as is believed by Rosenvinge (65) to be the functions of similar organs in the Rhodomelaceae.

Rhizoids are frequently formed from the older vegetative cells. Protoplasm accumulates at a spot on the lower half, or near the middle, or even at times on the upper half of the cell, and pushes out as a hollow tube with a plug of protoplasm at its tip (Fig. 44). In the cytoplasm of a rhizoid the chromatophores are rather few in number, and the nuclei are smaller than usual in vegetative cells. The average diameter of a rhizoid is about 80 μ, the length 2 mm. or more. The rhizoid secretes a rather thick cellulose wall. The longer rhizoids become divided into two or three long cylindrical

cells by the centripetal growth of a cellulose ring such as occurs in the division of certain vegetative cells.

The rhizoids so formed attach themselves to any neighbouring object, curving around it in the manner of a tendril (Fig. 45). In this way the plant is more securely anchored than it would be by a holdfast alone. Further, rhizoids frequently become entangled among neighbouring filaments of the same plant, thus binding the lower parts of the filaments more closely together and rendering them less easily torn apart (Fig. 46). This is especially true of the antheridial plants, in which the rhizoids are very richly developed.

After the rhizoids become attached, new shoots may arise from them in the same way that lateral branches arise from the vegetative cells.

Tendrils have been known in the red algae since Agardh (1) first described them in *Hypnea*, *Mychodea*, *Rhabdonia*, and other genera. Setchell (71) described tendrils in *Laurencia* and *Cystoclonium*, and stated that they may also serve for vegetative propagation. Nordhausen (57) described tendrils in *Hypnea*, *Spyridia*, and *Nitophyllum* and showed that new plants may arise from root-tendrils in *Hypnea*.

A species of regeneration occurs in the filament when, as is often the case, one of the old cells perishes. Continuity of the filament is re-established in the following way: An outgrowth from the cell next above pushes through the intercellular pore, and grows down into the cavity of the dead cell. The outgrowth is a tube, similar in appearance and mode of formation to a rhizoid. The cavity of the outgrowth is perfectly continuous with the cavity of the cell from which it originates. A similar tube grows up more slowly from the cell below, and the two meet near the centre of the old cell cavity (Figs. 47, 48). They fuse at their tips (Fig. 49) to form a continuous hollow cylinder; the cylinder increases in size and comes to replace the dead cell exactly. The usual intercellular connexion is formed at the junction of the new cell with each of the two old cells which contributed to its formation. A similar process of regeneration was described for *Griffithsia Corallina* by Janczewski (43), with this difference, however, that in *G. Corallina* only the cell above the dead cell plays a part in the formation of the new cell. Tobler (83) has shown that a similar process takes place in other species of *Griffithsia* and in *Bornetia*.

This process leads at times to the production of a cell of very peculiar appearance. When the cell next below two branches perishes, the lowest member of each branch puts out a tube (Fig. 47) which meets the tube from the cell below. The three fuse at the point of contact and a Y-shaped cell results, which is a product of the fusion of three distinct cells (Fig. 50).

Griffithsia may be anchored to the substratum either by a special attaching disk, or more usually by a tangled mass of rhizoids. An attaching disk has been noted in plants growing on *Zostera* and smooth rocks, but

when, as is often the case, *Griffithsia* is attached to other algae of cylindrical habit the disk is replaced by a tangled mass of rhizoids, which are short, thick-walled, and filled with starch. Inspection of a number of specimens shows all stages of transition from a mass of rhizoids to a well-developed attaching disk. The disk may be said to be formed of rhizoids in contact laterally. The development of the attaching organ is described on page 676.

The attaching disk, when present, is formed of a single layer of heavy-walled cells, bright pink in colour owing to the presence of numerous chromatophores, densely filled with protoplasm, and packed with large starch grains (Figs. 51, 52). From it new shoots may arise.

Judging from analogy with other forms (see Oltmanns, 59, i, p. 648; and ii, p. 212) we may assume that the plant winters over by means of the attaching disks or the mass of rhizoids at its base. In the spring these give rise to the plants which reach perfection in the summer. The evidence for this is rather negative: 1. The first plants found in 1907 possessed the attaching disk already well developed, whereas we know that in the sporelings the basal disk is developed quite slowly. 2. From the time *Griffithsia* was first found in July development was extremely rapid, and this seems to point to the conclusion that the plants drew on some reserve food-supply.

Sexual Reproduction.

The antheridia are distributed as caps over the upper ends of the somewhat globose terminal cells of the male plants (Fig. 2). They are formed as the terminal cells of short, much branched antheridial filaments. On a cell of average size there were found to be about 500 of these filaments, each of which produce about 50–75 antheridia, a total of 25,000–37,500 antheridia for each fertile cell of the male plant. The number of antheridia on a single antheridial filament, as well as the number of filaments produced on a single cell, varies greatly.

The mode of origin of the antheridial filaments is as follows: While the terminal cell of the male plant is still small and not much swollen (measuring on an average 0·2 mm. long and 0·15 mm. broad at this stage) about 100–200 protuberances arise simultaneously on its apical surface (Fig. 53). Each protuberance is at first hemispherical. There is in each a single nucleus, surrounded by dense, clear cytoplasm, which is in free communication with that of the mother-cell (Fig. 54). Their formation is not connected with nuclear division, but takes place while the nuclei are in the resting condition. The withdrawal of so many nuclei from the upper portion of the parent-cell leaves this region almost free of nuclei. As growth proceeds, however, nuclei wander up from the basal region, and become again evenly distributed in the cytoplasm.

Each primary protuberance is soon cut off from the mother-cell by a delicate partition, which is laid down by the protoplasm in the same way as

in the first-mentioned cell-division described on page 649 (Pl. LI, Fig. 55). When its formation is complete, the primary protuberance divides several times vertically. A lateral cytoplasmic process is formed, then the nucleus divides by mitosis (Figs. 56, 57). One daughter-nucleus remains in the body of the primary protuberance, the other passes into the cytoplasmic process. The two protoplasts now become separated by a constriction of the *Hautschicht*, whose ingrowth appears to be aided by the formation of a vacuole at the point of constriction (Fig. 57). This process of division continues until there are about 200–500 secondary protuberances on the apical portion of the terminal cell.

The protuberances appear not to develop cellulose cell-walls of their own, but lie in the swollen wall of the mother-cell (Figs. 58, 59).

Each of these secondary protuberances gives rise to a branched antheridial filament. The single nucleus in each divides by mitosis, and a partition is formed separating the two nuclei, and cutting the protuberances into an upper and a lower cell. The lower or basal cell buds off other cells above, each uninucleate, to the number of five or six (Fig. 59). These, along with the upper of the two cells first formed, in turn bud off groups of uninucleate cells, which become the spermatia directly (Fig. 60). Thus the antheridial filament is a twice compound structure like a small bush, the terminal twigs of which become the spermatia.

The basal cell of the antheridial filament is somewhat cubical is shape and may contain ultimately several nuclei. The cells of the first and second order of branching are uninucleate and are remarkable for their shape, each resembling a pear with a very long stem. These cells early become filled with a large vacuole, the cytoplasm forming a very thin film next the *hautschicht*, and the nucleus lying in the apical portion. None of the cells of the antheridial filament appear to form a cellulose wall. The whole filament is covered by the swollen wall of the mother-cell (Fig. 59). When the spermatia are mature, they simply break loose from the cells on which they are borne, and float freely out into the water (Fig. 63). As they become free, the long neck which attached them to the cell next below becomes drawn into the body of the spermatium, which assumes an oval shape.

The mature spermatium is about $3\,\mu$ long and $2\,\mu$ in diameter. Its bulk is occupied by a large vacuole, which is bounded by a thin film of cytoplasm. The single nucleus lies in the end which pointed away from the antheridial filament. No chromatophores have been discovered in any of the antheridial cells. The living spermatium is quite clear and somewhat refractive.

It seems of interest to note the fact that the living spermatia appear not to be extruded unless a slight pressure is exerted on the cells of the thallus. If branches of an antheridial plant are transferred carefully from

sea-water to a slide and left undisturbed, few antheridia become extruded. However, the pressure of a cover-glass, or even a mere touch with a needle, causes the extrusion of hundreds of antheridia from each large antheridial cap. This, coupled with the fact that the tufts of *Griffithsia* are feeding-grounds for several species of minute crustacea, especially of a species of *Caprella*, seems to lend probability to the suggestion that the antheridia of the red algae are sometimes translocated through the agency of animals.

No evidence was secured as to whether the antheridial filaments produce successive crops of spermatia. It is certain, however, that after a time the antheridial cap ceases to produce spermatia, and the antheridial filaments become disorganized and break away, leaving the globose terminal cell of the thallus free of any antheridial cells. When this occurs, it is usual for two or more side-branches to arise from the subterminal cell and to begin to produce antheridia when three or four cells long (Fig. 64).

The globose terminal cell which has produced one crop of spermatia frequently produces one or more new branches from its summit, so that this cell becomes again a functional apical cell (Figs. 64, 65).

The procarps occur laterally on the nodes near the tips of the filaments of the female plants (Fig. 3). They are produced successively, so that on a fertile branch a procarp is formed on nearly every node. Their origin and development has been described in detail by Miss Smith (73), whose account supplements that of Farlow (29) and Spalding (75).

The procarps are formed from the small terminal vegetative cells. When a procarp is to be initiated, the terminal cell, instead of dividing in the way usual in terminal vegetative cells, becomes pushed to one side by a lateral branch of the subterminal cell (Fig. 66, 67) which becomes the main axis of the filament. The terminal cell which is to give rise to the procarp contains several nuclei; it divides into two cells in such a way that one cell lies partly over the other. The plane of division is oblique, the inner edge of the partition being somewhat lower than the outer (Fig. 67). The lower of the two cells so formed is the basal cell of the procarp. The upper cell divides again by a transverse wall to form the central cell of the procarp and the first peripheral cell (Fig. 68). The first peripheral cell is cut off from the central cell on the axial side. A second and third peripheral cell becomes cut off from the upper border of the central cell, with no discernible regularity of position (Figs. 69, 70). The fourth peripheral cell mentioned by Farlow has not been seen, and must occur only occasionally. Of the peripheral cells only one has any further part in the production of carpospores. Often, though not always, the peripheral cells cut off terminally a small sterile cell (Fig. 71).

Up to this point the number of nuclei in each of the cells of the procarp varies from 4 or 5 to 10 or 12 or more; there appear to be always

more than one. As the procarp develops, the number of nuclei increases by mitosis, until in the cells of the mature procarp the nuclei become quite numerous. The average numbers are about as follows: basal cell, 50; central cell, 45; peripheral cells, 8–30; each sterile cell, 4.

The cytoplasm in the cells of the young procarp is homogonous and rather dense. A vacuole of considerable size occupies the centre of the basal cell and of the central cell. No chromatophores or leucoplasts have been seen in the cells of the procarp, though chromatophores are developed in the cells of the cystocarp.

It sometimes happens that after the first three cells of the procarp are formed the procarpic branch becomes metamorphosed into a vegetative shoot of the usual type, but distinguishable from other vegetative shoots by the fact that the cell at its base remains broad and flat, retaining the appearance of a basal cell of a procarp (Fig. 72). In such cases the first peripheral cell, which is really a terminal cell, functions as an apical cell of a vegetative branch.

From the second or third peripheral cell the carpogenic branch is formed laterally. The peripheral cell from which the carpogenic branch is produced is the 'supporting cell' of Miss Smith, and is equivalent to the 'auxiliary cell' of Hassencamp (40). In this account I shall adopt the term auxiliary cell.[1] From it a small uninucleate cell is produced laterally, which is the basal cell of the carpogenic branch. This cuts off a terminal cell, which in turn divides (Fig. 71). The upper of the three cells so formed divides again, thus forming a carpogenic branch of four cells (Fig. 73). The carpogenic branch is bent at right angles in such a way that the terminal cell, from which the carpogonium and trichogyne are formed, is usually in contact with the auxiliary cell. Each cell of the carpogenic branch is at first uninucleate. From the free border of the terminal cell the trichogyne is produced as a club-shaped projection (Figs. 73, 74). Whether a division of the nucleus of the carpogonium accompanies the formation of the trichogyne, as has been found to be the case in *Batrachospermum* (Davis, 22), *Nemalion* (Wolfe, 90), and *Polysiphonia* (Yamanouchi, 93), has not been determined. The trichogyne increases in length, becoming 35–40 μ long, and becomes quite slender, with a diameter of about 1 μ. In the mature trichogyne several granules, which stain like chromatin, are to be observed (Fig. 78a).

The mature trichogyne is straight or somewhat curved and sometimes, though not always, slightly swollen at the free end.

Before and during the formation of the carpogenic branch, noteworthy changes take place in the auxiliary cell. The cytoplasm becomes denser and the nucleus nearest the centre of the cell increases very greatly in size.

[1] The terminology used in the present account of the procarp and cystocarp is essentially that employed by Miss Smith, with the exception noted.

Before the differentiation of the auxiliary cell, the nuclei may average 1·5 μ in diameter; when the carpogenic branch is fully formed, the central nucleus of the auxiliary cell may reach a diameter of 6·5 μ (Fig. 76). A similar change may take place in one of the nuclei of the other peripheral cells.

The structure of the mature procarp is as follows (Figs. 73, 75): The broad, flat basal cell bears on its upper border the central cell, which is also broad and rather flat. The central cell bears usually three peripheral cells which may or may not cut off sterile cells at their tips. One of the peripheral cells bears laterally the carpogenic branch, which consists of a basal cell, two intermediate cells, and the carpogonium with its trichogyne. The intermediate cells and the carpogonium are disposed in a straight line, which lies at right angles to a line passing through the basal and the auxiliary cells.

All the cells of the procarp are multinucleate except those of the carpogenic branch. Of these the terminal cell and the two intermediate cells are uninucleate, and the basal cell of the carpogenic branch is usually binucleate. The connexions between the cells of the procarp appear to be similar in general to the connexions between neighbouring vegetative cells.

Mention has been made above of the hairs which usually occur in the vicinity of procarps.

The small size of the trichogyne and of the carpogonium renders *Griffithsia Bornetiana* a rather unfavourable object for the study of the details of fertilization, but it has been possible to make out the essential facts. A spermatium becomes attached to the trichogyne near its tip (Figs. 75, 77, 78). The spermatium is either applied directly to the surface of the trichogyne, or there may be a short tube connecting the two. Whether the nucleus of the spermatium divides at this stage, as is the case in *Nemalion* (Wolfe, 90) was not determined, though such an appearance as is presented in Fig. 78 suggests that division may occur. A sufficient number of stages was not obtained to enable me to speak with certainty on the subject but the stages that were obtained seem to render it highly probable that the nucleus from the spermatium passes down the trichogyne, enters the carpogonium, and there fuses with the nucleus of the carpogonium (Figs. 77, 79).

Immediately after fertilization the trichogyne becomes much twisted and falls off, leaving a short stump on the carpogonium (Fig. 80). Since the fusion nucleus stains very heavily, the details of its structure were not made out. However, because of this very capacity for taking up dyes, it is easily distinguished from the other nuclei of the procarp.

Very soon after fertilization, the carpogenic branch begins to be withered, and the fusion nucleus is seen to be present in the auxiliary cell

(Fig. 81). The actual passage of the fusion nucleus into the auxiliary cell was not observed. In no case has a fusion nucleus been seen in any of the cells of the carpogenic branch other than the carpogonium, and it seems unlikely that it passes into any of these other cells. The position of the carpogonium in contact with the auxiliary cell renders it possible that the two become connected by resorption of the walls at the point of contact, and that the fusion nucleus passes directly into the auxiliary cell, as was suggested by Schmitz for other species of *Griffithsia*, and has been demonstrated in *Thuretella* and *Chylocladia* by Hassencamp (40). In *Polysiphonia violacea* the communication between the carpogonium and the auxiliary cell is transient (Yamanouchi, 93), so that it might well be difficult to demonstrate in such a form as *Griffithsia*.

The cells of the carpogenic branch after fertilization and the passage of the fusion nucleus into the auxiliary cell usually degenerate simultaneously, and often the whole carpogenic branch breaks away from its attachment to the auxiliary cell, and lies free among the cells of the procarp (Fig. 82). In one case the lower cells of the carpogenic branch were seen to have withered before the passage of the fusion into the auxiliary cell, which lends support to the view that the fusion nucleus passes directly into the auxiliary cell and not through the cells below the carpogonium. Miss Smith's account (73, p. 41) of the withering of the carpogenic branch was not corroborated in the present study. She states that the carpogonium first becomes disorganized, 'the adjacent cell at the same time apparently increases in size, but it also soon loses its contents, and in some cases appears to become disorganized, while the two lower cells take a deeper stain than before'. As stated above, the carpogenic branch usually withers as a whole, and not cell by cell.

At the time of the passage of the fusion nucleus into the auxiliary cell there is in the centre of the latter a very large clear nucleus. This is one of the nuclei originally present in the auxiliary cell. Besides this, two or three small nuclei are frequently seen in the peripheral portion of the cell (Fig. 81); these are the remaining nuclei present at the time of the organization of the auxiliary cell. They seem to disappear during the course of the further development of the auxiliary cell.

The fusion nucleus in the auxiliary cell is of very characteristic appearance. It differs from the usual type of nucleus in possessing two chromatin-nucleoli instead of one. It would seem as if the chromatin from the male and the female parent does not fuse completely, and that the nucleoli of different origin remain distinct for some time after nuclear fusion. The behaviour of the chromosomes in the early divisions of the fusion nucleus was not observed, though it would be of considerable interest to know whether two distinct groups of chromosomes are formed at this stage.

The fusion nucleus divides once in the auxiliary cell, and the two nuclei come to lie in the opposite ends of the now somewhat elongated cell (Fig. 83). Between them lies the greatly enlarged central nucleus originally present. Each of the nuclei resulting from the division of the fusion nucleus usually shows the characteristic double nucleolus. The auxiliary cell now divides, one daughter-cell containing the enlarged central nucleus and a single fusion nucleus, and the other containing only a fusion nucleus (Fig. 84). The latter may be called the placental cell; from it the sporogenous lobes usually arise. Fig. 86 shows clearly that sporogenous lobes may also be formed from the auxiliary cell after the placental cell has been formed; the nuclei entering these lobes are derived from the fusion nucleus. Very similar behaviour has been observed by Hassencamp (40) in the auxiliary cells of *Thuretella* and *Chylocladia*.

During these changes, the large nucleus of the auxiliary cell continues to increase in size. It becomes almost empty of contents, the nuclear outline becoming less and less distinct, and finally the nucleus disappears in the cytoplasm.

Changes also take place in the other elements of the young cystocarp. The central cell, which at first contains a large central vacuole, becomes filled with homogeneous cytoplasm and with numerous nuclei formed by the multiplication of those originally present (Fig. 81). From the sides of the basal cell of the procarp soon after fertilization several small cells are cut off successively. These in turn divide, and the outer cell becomes an involucral ray (Fig. 85). Three to seven involucral rays are formed; they are of various sizes and ages, and curve up over the cystocarp so as to cover it almost completely, except at the top. The structure of the involucral rays offers nothing especially remarkable. They are distended sacs, pale pink in colour owing to the presence of a small number of chromatophores. A thin layer of protoplasm bounds a very large vacuole. The nuclei may become quite numerous, 87 having been counted in a ray of average size. The rays do not form a definite pericarp, as they are not united at the sides. None are produced between the cystocarp and the vegetative cell which bears it.

The placental cell formed at the division of the auxiliary cell increases in size and in number of nuclei, all of which are the product of the division of the fusion nucleus. Small protuberances are formed on its free border, each of which contains a single nucleus (Figs. 85, 86). Each protuberance is cut off from the mother-cell by an arched membrane, and the cells so formed give rise by repeated division to the sporogenous cells from which the carpospores are formed.

While this is taking place, there is a general fusion of cells in the centre of the cystocarp. The following cells take part in this fusion: The placental cell, the auxiliary cell, the central cell, and sometimes the

peripheral cells. The result of the fusion is the production of a very large, irregularly shaped placenta, on the upper surface of which the sporogenous lobes are formed (Figs. 85, 87).

The placenta contains nuclei from three sources: (1) the original nuclei of the peripheral and auxiliary cells, which appear to take no part in spore formation; (2) the numerous nuclei of the central cell, which lie in the base of the placenta, a region from which no sporogenous lobes are formed; and (3) the numerous nuclei resulting from the division of the fusion nucleus. These last lie in the upper region of the placenta, where the sporogenous lobes are being formed, and appear to be the only nuclei to enter these. A similar placenta, with numerous nuclei of diverse origin, has been described in *Chylocladia*, by Hassencamp (40).

At least in some cases, the nuclei from the central cell appear to become abnormal and break down. The chromatin forms a crescent-shaped mass applied to the nuclear membrane on one side (Fig. 88), the nuclei swell, their outlines become faint, and finally their contents mingle with the cytoplasm. Not all of the nuclei from the central cell degenerate, and it is often difficult to distinguish those which remain normal from the sporogenous nuclei, especially in the older cystocarps, except by their position in the cell.

The mode of division of the sporogenous lobes seems to vary considerably in different lobes. The series of figures from 89–93 (Pl. LI and LII) gives a fair idea of what usually takes place. Following the division of the nucleus of one of the protuberances mentioned as being formed on the free surface of the placental cell or on the upper part of the placenta, small curved segments are cut off from the outer surfaces of the protuberance in much the same way as a segment is cut off from the apical vegetative cell, with this difference, however, that the cells of the sporogenous lobes are usually uninucleate. In this way a compact tissue is formed, the cells of which round themselves off, each cell producing a carpospore. As the cells round off, the sporogenous lobe becomes converted into branched chains of oval cells. The links of a chain are free at the sides, but connected with each other above and below by a narrow strand of cytoplasm; midway between connected spores occur callus-like plugs similar to those lying in the pits between adjacent vegetative cells. From the time when they first round off, the spores increase greatly in size. When the spores are ready to be shed, their diameter is about 30–35 μ, their length 40–54 μ, the diameter of the nuclei 8·5–9 μ.

This is the usual history of a sporogenous lobe. In some cases a difference is presented because of the fact that the spore-mother-cells are rounded off at a very early age, so that the sporogenous lobe is not a compact tissue when young, but a group of rounded cells (Figs. 94, 95). There is some evidence that in this case the method of cell-division in the

sporogenous lobe is by constriction, somewhat after the manner of the second method of cell-division described on page 650. The final result is the same in the two cases, i. e., the production of branched chains of carpospores.

The sporogenous lobes of a single cystocarp are of various ages. Lobes with mature spores may be seen by the side of unicellular sporogenous lobes, and usually all stages of development may be seen in a single cystocarp (Fig. 95).

Each sporogenous lobe is covered with a gelatinous envelope, not easily seen until swollen with glycerine or a watery fluid. The individual spores seem to be without a cellulose wall, being enclosed only by the *hautschicht.*

As a rule, all the spores of a single lobe become mature at the same time. The links of the chains break at the point where the callus-like plugs are developed, the connecting strands are drawn into the body of the spores, and the spores slip out of the gelatinous envelope and float away into the water.

The number of spores produced in a cystocarp can hardly be estimated with certainty, because while the mature spores are being shed, new sporogenous lobes are being inaugurated. From a study of several cystocarps of average size, it was found that about 6 lobes are present at one time, with an average of about 40 spores in each lobe, giving a total of about 240 spores in a normal cystocarp. Undoubtedly the number of spores produced during the life of a cystocarp may often be greater than this.

When set free, the spores present much the same appearance as the tetraspores described on page 670. They are oval in shape. Around the periphery is a zone containing rather dense cytoplasm and numerous flattened chromatophores, which sometimes present their edges, but usually their flat surfaces, to the outside. In the centre is the large nucleus, enveloped in a zone of homogeneous cytoplasm. The nucleolus, which contains the chromatin, is usually in the form of 12–14 rounded bodies in the centre of the nuclear cavity. The linin is scanty in amount, being barely visible around the periphery of the nucleus. Between the nucleus and the peripheral zone of chromatophores, the cytoplasm is very coarsely vacuolar (Fig. 96).

In a few cases, spores have been noted which have germinated *in situ*, and which contain two nuclei.

Nuclear divisions in the cystocarp are of the usual type. In the divisions of the sporogenous nuclei, the number of chromosomes is about fourteen (Fig. 97).

Food material appears to be passed into the cystocarp from the vegetative cell on which it is borne. In the cytoplasmic pad occurs a great abundance of the spheres of food material mentioned on page 649, and it

seems probable that this material is passed up into the cystocarp from the cell below.

Asexual Reproduction (Tetraspores).

The tetraspores are formed in a ring around the upper border of any cell below the apex of the filament (Fig. 4). The ring of tetraspores appears to encircle the node, fitting snugly in the constriction between neighbouring vegetative cells. On the outside of the tetraspores a circle of involucral rays grows up around the sorus. The number of these is variable; there are often only six or eight; sometimes there are as many as twenty. They appear rather late in the development of the sorus, in some cases the most advanced tetraspores having already matured before the involucral rays are formed. The rays are expanded curved plates, connected at the base with the vegetative cell, and free laterally and terminally. Usually neighbouring rays are in contact at the sides, so that the circle of tetraspores is well screened from without. Each ray is a single cell similar in appearance and in structure to the outer cell of the involucral ray of the cystocarp. Where the rays are in connexion with the vegetative cell, the plugs characteristic of the intercellular connexions elsewhere are formed.

The tetraspores are formed as follows:—Around the upper border of a young cell below the apex, protoplasm accumulates in small rounded masses, each containing a single nucleus (Fig. 98). The cell-wall near each protoplasmic accumulation becomes gelatinous, which allows the accumulations to protrude as small papillae (Fig. 99). Each of these papillae early becomes cut off from the mother-cell by a delicate dome-shaped membrane (Fig. 100). The cell so formed, with its nucleus, increases in size, and at the same time the membrane loses its convex form and becomes flattened (Fig. 101).

The formation of these primary tetrasporic cells seems to take place entirely independent of nuclear division.

On the upper border of each of these cells a finger-like outgrowth of cytoplasm is protruded (Fig. 102). The nucleus then divides by mitosis in the way described for vegetative nuclei of the tetrasporic plant (Fig. 103). One of the daughter-nuclei remains in the basal portion of the cell, the other passes into the cytoplasmic outgrowth, a membrane appearing between the nuclei (Fig. 104). Thus is formed a small two-celled branch, with a single nucleus in each cell (Fig. 105). The lower may be called the stalk-cell, while the upper is the tetrasporangium or tetraspore-mother-cell.

During the growth of this structure, the stalk-cell pushes out another cytoplasmic projection similar to the one first formed. The nucleus divides by mitosis (Fig. 106), one of the daughter-nuclei remaining in the stalk-cell, and the other passing into the projection, which becomes cut off like the first. Thus a second tetraspore-mother-cell is formed on the stalk-cell.

A third and sometimes a fourth mother-cell may be formed in the same way. The first mother-cell may be regarded as terminal, the other as lateral.

In rare cases, two nuclei occur in the primary tetrasporic cell from its inception, and in one case, at least, two nuclei have been noted in the very young tetraspore-mother-cell. This recalls the suggestion of Heydrich (41) of a possible sexual significance of the tetraspore. Examination of a large series of developing tetraspore-mother-cells convinces me, however, that there is here a purely accidental phenomenon, which has no place in the normal life-history, and which is not to be considered as analogous in any way to the sexual process.

The cells of the tetrasporic branch appear at first not to secrete cellulose walls of their own. The stalk-cell, with its tetraspore-mother-cells, remains surrounded by the gelatinized wall of the vegetative cell on which it is borne. This wall, much swollen, covers the tetrasporic branch completely (Fig. 107). It continues to swell, and by the time the spores are ready to be discharged it seems to dissolve largely or completely in the sea-water.

The stalk-cell increases in size, and its nucleus at the same time divides by successive mitoses until usually sixteen daughter-nuclei are finally produced. With the growth of the cell in size, the cytoplasm becomes less dense, and vacuoles appear in it. There may be a single large central vacuole, or several smaller ones variously disposed. The connexion of the stalk-cell with the vegetative cell, and also with the tetraspore-mother-cells, is of the usual type. On the side toward the stalk-cell the cytoplasm of the mother-cell is produced into a rather narrow strand, which meets a similar strand from the stalk-cell at the point where the callus-like plugs are developed (Fig. 109).

It sometimes happens that the stalk-cell produces laterally a tubular process that curves up around the mother-cells and resembles in appearance an involucral ray (Fig. 110). This recalls the condition in *Griffithsia barbata* and other species, in which the tetraspores are borne laterally on short involucrate ramuli.

The tetraspore-mother-cell increases in size, the nucleus showing corresponding enlargement. The cytoplasm begins to show numerous small vacuoles between the rather dense cytoplasm surrounding the nucleus and that lying in the periphery of the cell.

The behaviour of the nucleolus during the period of enlargement of the nucleus is interesting. As the nucleolus increases in mass, it fragments into several rounded bodies of various sizes (Figs. 107, 108). This process of fragmentation continues until from 12–14 rounded masses of chromatin of about the same size are formed (Fig. 111). These lie in a clump in the centre of the nucleus, staining very heavily with nuclear dyes.

At this stage the tetraspore-mother-cell may be considered to be mature. The length of a mature mother-cell is about 20 μ, the width 15 μ, and the diameter of the nucleus 7 μ. Further changes in the mother-cell are in anticipation of division into tetraspores.

From this time the changes in the cytoplasm occur mainly in connexion with the vacuolar area. The vacuoles become larger and the whole vacuolar area presents a coarse spongy appearance. In the meshes are deposited numerous spheres of substance staining deeply with haematoxylin. There is reason to believe that these bodies are derived from the nucleus. As the time of nuclear division approaches, these granules become larger and fewer in number, so that it is possible, by noting their size and number, to predict in just what stage of mitosis the nucleus will be found. The granules seem to be analogous to the chromidial substance of Protozoa (see Goldschmidt, 33) and of some plants (see Tischler, 81).

The changes in the nucleus are profound. Most striking is the decrease in staining capacity of the nucleolar masses. These become irregular in form, and at the same time fuse with one another, so that their number is reduced by more than half (Fig. 112). At this stage they are in the form of thick, curved rods, in which light and darkly staining areas may be discovered. Often four dark areas may be detected in each rod, which suggests that this stage corresponds to the formation of tetrads in the oocytes and spermatocytes of many animals. Coincidently with these changes, small granules are to be seen in the nucleus near its periphery, which seem to pass out into the cytoplasm (Figs. 112, 113), to form the granules already mentioned as occurring in the vacuolar area.

The stage just described is considered to be the period of synapsis. It is of long duration, it shows a condition which does not occur elsewhere in the life-history, and it immediately precedes the mitoses in which numerical reduction of the chromosomes takes place. It differs from the usual type of synapsis in that no spirem or synaptic thread is formed; but this is not to be wondered at inasmuch as nowhere in the life-history of *Griffithsia Bornetiana* is a spirem produced. Perhaps the worm-like nuclear masses are to be considered as replacing the usual spirem stage. Somewhat similar conditions in the nucleus of the zygote of Spirogyra are interpreted by Karsten (45, p. 6) to represent the period of synapsis in this form.

While the thick, irregular rods continue to lose their capacity for taking up stains, there appear scattered throughout the nuclear cavity, but mainly near the periphery, a number of small spherical or oval bodies (Figs. 114, 115). About fourteen of these bodies are usually present, though the number may vary within narrow limits. This is the stage of prophase, and the small bodies are the chromosomes. At this time traces of the achromatic substance of the nucleolus may be detected near the centre of the nuclear cavity.

Not all the nucleolus goes to form the chromosomes. As already mentioned, part of the nucleolar substance passes out of the nucleus and becomes deposited in the vacuolar cytoplasm, and part may remain in the nuclear cavity, where it forms irregular masses. The part remaining in the nucleus ultimately disappears in the cytoplasm after the nuclear membrane is dissolved.

The details of the organization of the spindle are made out with difficulty. During prophase, kinoplasmic caps are formed at the poles of the nucleus by differentiation of cytoplasm at these points. In most cases, at the centre of each kinoplasmic mass is a darkly staining body (Fig. 116), probably comparable to the 'centrosphere-like-structures' of *Polysiphonia* (Yamanouchi, 93). In some cases these are large and prominent; in others they could not be demonstrated at all. They are certainly not permanent structures; they seem rather to be the expressions of some temporary kinoplasmic activity. To them the spindle fibres are attached. The spindle is entirely intranuclear, and is probably differentiated from materials within the nuclear cavity, as no evidence has been seen to indicate that the fibres grow in from without, as is the case in *Spirogyra* (Berghs, 4). The spindle is truncate at the poles and slightly broader at the equatorial plate (Figs. 116, 117). The chromosomes, which lay scattered in the nuclear cavity before the formation of the spindle, now move in toward the centre of the nucleus (Fig. 118); here they become arranged on the equatorial plate. Some preparations (Fig. 119) seem to indicate that during this process they become associated in pairs, which soon separate; but on this point it is impossible for me to speak with certainty at present. The number of chromosomes in the equatorial plate is approximately fourteen. They are small rounded bodies, not lying exactly in the same plane (Fig. 120).

The axis of the spindle seems to bear no constant relation to the axis of the cell. It is more usual, however, to find the long axis of the spindle coincident with the long axis of the mother-cell. The outline of the nucleus at metaphase is nearly circular, or more often, slightly elongated in the direction of the axis of the spindle (Fig. 116).

At anaphase the chromosomes separate into two groups, probably of seven each (Fig. 121). As the groups of chromosomes approach the poles of the spindle, the nuclear membrane fades away, and the cavity of the nucleus is obliterated by the cytoplasm. In some cases, however, this does not happen; the nuclear membrane persists throughout mitosis. During anaphase, it elongates and then pulls apart in the middle (Fig. 122). Whether this diversity in the behaviour of the nuclear membrane is in any way connected with certain irregularities of development to be described later, is not obvious.

As each group of chromosomes approaches the pole of the spindle, the individual chromosomes unite to form a densely staining spherical mass,

which becomes the nucleolus of the daughter-nucleus. When the original nuclear membrane persists, the organization of the daughter nuclei is complete on the separation of the two halves of the nucleus, by which time no trace of the spindle is seen. When, as is more usual, the nuclear membrane disappears toward telophase, the mass of chromatin in the immediate vicinity of the kinoplasmic cap becomes surrounded by a new nuclear membrane, around which the kinoplasm becomes distributed.

In any event, two daughter-nuclei are formed, which lie at some distance from each other. Each has a large, spherical, uniformly staining nucleolus in the centre, and frequently with two or three smaller bodies of chromatin in the nuclear cavity (Fig. 123). The daughter-nuclei are considerably smaller than the nucleus of the mother-cell. Each is about 5 μ long by 6 μ broad, though the size varies. No trace of any partition has been observed between the daughter-nuclei, which lie quite freely in the cytoplasm.

The daughter-nuclei do not remain long in the resting condition. In each the nucleolus disappears, and seven rounded chromosomes, probably derived from the nucleolus, appear in the nuclear cavity (Fig. 124). At the same time the nucleus elongates further, and there is to be seen a kinoplasmic cap at each end. A spindle is organized as before, and the seven chromosomes arrange themselves in an equatorial plate. The division of the two nuclei is synchronous, their axes of division lying at right angles to each other (Fig. 125). At anaphase, two groups of seven chromosomes pass to the poles of each spindle, the nuclear membranes disappearing (Fig. 126). At telophase the chromosomes of each group which are in close proximity to the kinoplasmic cap, fuse to form the nucleolus of the daughter-nucleus. A new nuclear membrame is formed around each mass of chromatin and the kinoplasm again becomes distributed around the nucleus.

The four nuclei thus formed lie very near the periphery of the mother-cell, and equidistant from one another (Fig. 107). Each is a definitive tetraspore nucleus. Their arrangement in the cell is determined by the fact that one nucleus always lies at the point from which the cytoplasmic strand passes to meet the stalk-cell. The structure of the nucleolus at this stage is somewhat different from that of the preceding stages. The chromatin mass is usually plainly lobulated. Outside and near this is to be seen a much smaller, regularly spherical body, whose history I have been unable to trace. Probably it is of the same nature as the nucleolus, since, when the nucleolus fragments, as it does a little later, the smaller body is indistinguishable from the other chromatin masses.

An appearance frequently seen at this stage lends support to the view that food material is passed up from below into the tetrasporangium (see Yamanouchi, 93, p. 424). The nucleus which lies near the strand of cytoplasm connecting the tetrasporangium with the stalk-cell is seen to be

surrounded by a mass of food material, which is probably derived from the stalk-cell. The other nuclei at the same time lie in clear cytoplasm in which little stored food is visible (Fig. 127).

During the progress of these changes in the nuclear content of the tetrasporangium, the deeply staining granules in the cytoplasm disappear, so that by the end of the first mitosis they are no longer visible. At the same time, the large vacuoles in the cytoplasm give place to smaller, more regular ones.

Cleavage of the cytoplasm begins always when the four nuclei begin to move toward the centre of the tetrasporangium, which happens soon after their formation. The *hautschicht* folds inwards along planes which, if continued to the centre of the cell, would cut the protoplast into four equal parts, presenting the familiar tripartite arrangement (Fig. 128). However, the partitions are produced inwards only about two-thirds of the distance to the centre of the cell (Pl. LIII, Fig. 129). The central portion of the tetrasporangium is occupied by the four definitive spore nuclei with their envelopes of kinoplasm which are in contact with one another, so that a rather definite nucleo-kinoplasmic mass is formed. In the very centre of the tetrasporangium lies the portion of the undifferentiated cytoplasm enclosed by the nucleo-kinoplasmic mass (Fig. 130).

The nuclei at this stage are either spherical or somewhat biscuit-shaped, the inner surface being less convex than the outer. The nucleolus has by this time fragmented into 12–14 granules, similar in appearance to those in the nucleolus of the tetraspore-mother-cell before synapsis.

At this point of development 5–10 per cent. of the tetrasporangia begin to show degenerative changes and do not develop further. The outer surface of the tetrasporangium becomes wrinkled, and the nuclei become very much flattened, almost wafer-like. The entire contents of the protoplast stain very heavily, owing to the presence in the cytoplasm and in the nuclei of numerous dark granules. These degenerating tetrasporangia are easily distinguishable, even in the living condition, by reason of their dark, opaque appearance, and some are to be found in every tetrasporangial sorus. What causes lead to their degeneration I have not been able to determine.

In the normal tetrasporangia, the cleavage partitions, which represent folds of the *hautschicht*, but which appear in section as a single line except near the periphery (Fig. 129), split so as to reveal clearly their double nature. At the same time the cytoplasm, which lay in close contact with the partitions, separates along the line of the partitions so that the cleavage furrows become wide as well as deep (Fig. 130). Even at this stage, however, they extend no further in than to the edge of the nucleo-kinoplasmic mass. Coincident with these changes in the partitions, the nuclei which were flattened become again approximately spherical.

Vacuoles develop in the cytoplasm in the centre of the nucleo-kinoplasmic mass. At the same time, small chromatophores begin to appear in the cytoplasm along the outer border of the tetrasporangium. These increase in size, and a few extend along the partitions into the body of the protoplast. In this condition the tetrasporangium remains for a long time, increasing in size and in vacuolization of the cytoplasm. The significance of this incomplete separation of the spores probably lies in the fact that food material seems to pass up through the basal cell. If the spores were completely separated before maturity, only one of the four would be in communication with the stalk cell, the source of supplies. However, inasmuch as the chromatophores at this stage are well developed, it seems probable that the tetrasporangium is capable of elaborating at least part of its food material for itself.

Berthold (7) seems to have been the first to point out this incomplete separation of the tetraspores of red algae after the division of the nucleus of the mother-cell, though Schmitz (69) had given an account of two successive nuclear divisions in the tetraspore-mother-cell.

The tetrasporic branches are from their inception surrounded by the swollen wall of the vegetative cell on which they are borne. A portion of this wall is carried out by the developing tetrasporic cells. As the cells develop, the portion of the wall surrounding them swells greatly and appears to become gelatinized, ceasing to respond to the tests for cellulose.

The tetrasporangium, with the incompletely separated spores, increases markedly in size. The nuclei also enlarge and show abundant chromatin, in the form of the 12–14 masses already mentioned. Each mass is differentiated into lightly and darkly staining areas. Not infrequently the number of these masses is greater than this, as many as twenty having been counted in some cases; and sometimes the number is considerably less than twelve. This variability in the number of chromatin masses in the resting nucleus serves to show that they do not have the same constancy in numbers that the chromosomes show, and therefore are not to be relied on as an index of the condition of the nucleus, whether haploid or diploid.

As the tetrasporangium enlarges, the cytoplasm becomes more coarsely vacuolate, and the vacuoles in the central protoplasmic mass become conspicuous. The partitions now grow in until they meet in the centre of the tetrasporangium, their ingrowth being apparently aided by the position of the large central vacuoles already mentioned (Fig. 131). The spores are now completely separated, with the nuclei in the inner corners. The nuclei wander toward the centre of the spores, the chromatophores at the same time migrating so as to line the entire periphery, and the spores round off, becoming oval in shape (Fig. 132). The lowest spore, up to this time attached to the stalk-cell by a slender thread of cytoplasm, breaks away at the point of attachment, and the strand is withdrawn into the body of the

spore. The gelatinized cell-wall, now very much swollen, appears to dissolve in the sea-water, and the four spores are set free, almost immediately becoming spherical. Like the carpospores, they are heavier than sea-water, and slowly sink if left undisturbed.

The mature tetraspore resembles the carpospore in appearance. It is approximately spherical. In the centre the large nucleus is conspicuous, with its chromatin segregated into 12–20 small masses. Immediately around the nucleus is a zone of rather dense cytoplasm; outside this the cytoplasm is coarsely vacuolar. In the peripheral cytoplasm is a single layer of chromatophores, outside which is the limiting membrane of the spore. No cellulose cell-wall is visible.

The average size of the tetrasporic structures is shown in the following table:—

	Cell.	*Nucleus.*
Mature mother cell	15—20 μ	7 μ
Mother cell at synapsis	20 × 24 μ	8 μ
Four nuclei peripheral	20 × 24 μ	4—6 μ
Four nuclei central	25—30 μ	4—6 μ
Partitions separate	40—50 μ	8 μ

The tetrasporic structures in a single sorus are of very various ages. While the first-formed tetraspores are developing, new tetraspore-mother-cells are being formed nearer the cross-walls between the vegetative cells, the older tetraspores being carried away from the cross partition by the growth and the stretching of the wall of the vegetative cell. A longitudinal section of a sorus shows primary tetrasporic cells being formed very near the point of junction of the vegetative cells; outside these are the older tetraspore-mother-cells; farther out mature spores are to be seen; while farthest from the centre occur the involucral rays (Fig. 133).

The number of tetraspores produced in a single sorus is quite large. The average number in well developed sori was found to be about 300.

The process of nuclear division in the tetraspore-mother-cell of *Griffithsia* offers many striking points of difference from the same stages in the life-history of *Polysiphonia*; it resembles much more nearly similar stages in *Corallina* (Davis, 23). As these three forms are the only members of the Rhodophyceae in which the behaviour of the tetraspore-mother-cells has been carefully studied from a cytological standpoint, it may be well to summarize here some of the points of resemblance and difference:—

	Griffithsia.	*Corallina.*	*Polysiphonia.*
Resting nucleus: chromatin	In large central granules	In scattered granules of varying number	In reticulum
Resting nucleus: nucleolus	Karyosome with some plasmosome substance	Plasmosome	Plasmosome
Synapsis: chromosomes	Broad irregular bands, free at ends	?	Double spirem
Origin of chromosomes	From central nucleolar bodies	From scattered chromatin granules	Segmentation of spirem
Fate of nucleolus	Achromatic portion disappears at prophase or persists till telophase	Disappears at prophase	Disappears after prophase
Daughter-nuclei	Assume resting condition	Assume resting condition	Not organized
Second mitosis	In daughter-nuclei		Inside membrane of mother-nucleus
Nuclear membrane	Disappears or pulls apart after metaphase of first division	Disappears before metaphase of first division	Persists through both divisions

This comparison serves to emphasize one point particularly. At a critical stage in the life-history of rather closely related members (*Polysiphonia* and *Griffithsia*) of a highly specialized group, the cytological phenomena are of a most varied nature. During the period of synapsis, and up to the time of the formation of the chromosomes, the cytological events in *Polysiphonia* are more like those in *Lilium* than those in *Griffithsia* or *Corallina*. The behaviour of the nucleus in the formation of the tetraspores in *Griffithsia* is much more similar to that in *Corallina* than to that in *Polysiphonia*, a more nearly related genus. The bearing of these facts is obvious; cytological phenomena cannot be considered trustworthy guides to relationships.

Tetraspore-like Structures on Sexual Plants.

As stated above, one individual has been found which produced normal antheridia on the majority of its filaments, but which produced on a considerable number of filaments structures resembling the sori of tetraspores. As this case is of considerable theoretical interest at the present time, I shall now give some of the details of the structure of this plant.

The portion of the plant bearing antheridia was perfectly normal in appearance. The cells were of the usual size and shape, and bore antheridia of the normal type in abundance. The number of chromosomes appearing in mitoses in the antheridial filaments was found to be about seven (the reduced number, characteristic of the gametophyte). Mitosis has as yet

been seen in only a single nucleus of the portion of the plant bearing the tetraspore-like structures. In this case, in the dividing nucleus of the stalk-cell, the number of chromosomes was seen to be seven (see Fig. 135).

The branches bearing tetraspore-like structures are of the same type as those of the normal tetrasporic plant, but are composed of cells that are on an average a good deal smaller.

The early development of the tetraspore-like structures is similar in every detail to corresponding stages in the development of the normal tetraspores. Small uninucleate papillae are cut off from the upper border of the vegetative cells. Each divides to form a short two-celled branch, the lower cell representing the stalk-cell of the tetrasporangium, the upper corresponding to the tetraspore-mother-cell (Figs. 134, 135). The stalk-cell increases in size and becomes vacuolate, the mother-cell also becomes larger, but remains somewhat smaller than the normal tetraspore-mother-cell. The average diameter of the fully formed mature mother-cell is about 20–22 μ as against 24 μ for the mother-cell of the tetraspore. The nucleus in the two cases shows the same configuration, but remains smaller in the mother-cells borne on the sexual plant (Fig. 135). Nuclear material passes out into the cytoplasm, where it forms small darkly staining granules.

Involucral rays are formed in the ways characteristic of the tetraspore-sorus, usually as outgrowths from the vegetative cell outside the ring of spore-mother-cells, exceptionally as lateral outgrowths from the stalk-cell (Fig. 136).

The further development of the mother-cells on the sexual plant differs strikingly from that of the normal tetraspore-mother-cells. In the majority of cases the nucleus divides (whether by mitosis or amitosis I have not yet been able to determine), and cleavage begins at the periphery (Fig. 137). The cleavage furrows do not advance far into the body of the mother-cell. The surface of the cell begins to show irregular wrinkling, and degenerative changes set in similar to those described for certain tetraspore-mother-cells. The number of nuclei in cells in which cleavage furrows begin is usually 4–8, of which some are very much larger than the rest (Fig. 137).

One case deserves special mention. Sixteen nuclei lie scattered in the cell which shows no trace of the formation of cleavage furrows (Fig. 138). The whole cell presents the appearance of a germinating spore. It would seem that here the cell corresponding to the tetraspore-mother-cell behaves as a monospore, though whether such a cell ever produces a normal plant is uncertain.

The chromosome-history of the nuclei of the cells just described has not yet been determined.

Vegetative Multiplication.

Griffithsia Bornetiana may reproduce itself vegetatively in two ways: first, by accidental isolation and subsequent growth of single cells or small pieces of a filament; second, by the production of new plants from tendrils.

The first method of propagation was described for *G. Corallina* by Janczewski (43) and mentioned as occurring in *G. Bornetiana* by Farlow (29). More recently Tobler (82, 83, 84) has called attention to the fact that *Griffithsia, Bornetia, Dasya, Polysiphonia,* and other forms may reproduce themselves under laboratory conditions by a process of fragmentation of the filaments and growth of the resulting portions into new plants. In *G. Bornetiana* this process takes place not rarely in nature. In such cases the isolated cell produces a rhizoid from its base and a new growing point from its apex (Figs. 139, 140). The rhizoid is formed normally in the manner already described. The apical cell is produced by the accumulation of protoplasm at the tip and subsequent unequal division of the parent cell in the usual manner.

Vegetative propagation by means of tendrils has been described on page 653.

Germination of Spores.

The spores germinate readily in the laboratory. If a mature tetrasporic or cystocarpic plant be placed in sea-water over night, young sporelings up to the 3-celled stage will be found abundantly attached to the bottom and sides of the vessel the next morning. Many of the stages of germination here described were collected in the field under natural conditions, but the majority of the figures given, especially of the younger stages, were taken from material cultivated in the laboratory.

The similarity of the structure of the carpospores and the tetraspores has been noted above; the phenomena of germination are also practically the same in the two kinds of spores. On being released, the spores become spherical and settle slowly in the water. They appear to become attached to the surface of any solid body they touch, such as rocks, glass, other algae, and even such soft bodies as the gelatinous substance enclosing chains of diatoms.

During the progress of germination, soon after the spore becomes attached, there is formed around it a cellulose wall of the usual type, which becomes tolerably thick, especially around the basal region of the sporeling. At the same time, numerous starch-grains also become visible in the cytoplasm of the spore. Several hours after the spore is shed the nucleus divides by mitosis. During this time there is no noticeable change of shape in the spore.

Opportunity has not occurred for the examination of a large series of dividing nuclei in the sporelings, but in the cases examined the mitoses were of the type usual in vegetative nuclei. In the dividing nuclei of sporelings from tetraspores, about six or seven chromosomes appear on the equatorial plate (Fig. 143). In the sporelings from carpospores the number of chromosomes is always greater than this, but appears to be less than the number that might be expected (14). There is believed, however, to be sufficient evidence for regarding these nuclei as diploid in character.

The daughter-nuclei withdraw to opposite sides of the sporeling (Fig. 141) and divide again to form four nuclei, which in turn divide to form eight (Fig. 142), then sixteen (Fig. 143). The increase in the number of nuclei is not accompanied by a corresponding increase in the size of the spore ; the size of the nuclei becomes less with each succeeding division. At about this time, the sporeling changes its shape, pushing out, at the point of attachment, a small rounded projection, which later becomes cut off as the basal cell. Immediately after this projection is formed the sporeling elongates, becoming about twice as long as broad, but without undergoing cell-division (Fig. 144).

As these changes take place the cytoplasm migrates more and more to the periphery. The central part of the sporeling is occupied by small regular vacuoles, whose exceedingly thin walls are roughly hexagonal in section (Fig. 141). A single large central vacuole such as is characteristic of the vegetative cells of the older plants is not usually formed until the sporeling reaches the 3-celled stage.

Cell-division occurs usually when about sixteen nuclei are present. A wall at right angles to the long axis of the sporeling cuts off a basal from an apical cell (Fig. 145). Shortly after this, without further elongation of the sporeling, the apical cell divides into two by a wall parallel with the first (Fig. 146). The details of the formation of these walls were not followed out. As the partitions have not been seen to assume the arched shape characteristic of the first type of cell-division at other points in the life-history (see p. 649), it may be inferred that they are formed by the ingrowth of a ring of cellulose (p. 650), such as is formed in the divisions of the cells of *Cladophora*. From the two divisions a small obovate 3-celled sporeling results, which consists of a smaller, somewhat pointed basal cell and two larger rounded cells towards the apex, the three cells lying in a row (Fig. 147). At this stage, the chromatophores and protoplasm are so closely packed in the peripheral portion of the cytoplasm that the sporeling appears dense and opaque. The pointed end of the basal cell is filled only with homogeneous protoplasm, starch-grains, and chromatophores being absent from this region. Intercellular connexions were not demonstrated in the small 3-celled sporelings, though they are apparent after the enlargement of the cells.

The description given above applies to the majority of sporelings examined. Many sporelings, however, show variations from the type described. For instance, it happens rather frequently that cell-division occurs after the formation of only four nuclei (Fig. 145) and before the sporeling assumes the elongated shape represented in Fig. 144.

The 3-celled stage is first attained in about twelve hours from the time the spore is shed. It persists, under laboratory conditions, for several days, during which time the three cells change greatly in size, shape, and appearance. The most striking changes occur from the second to the third day after the spores are shed. The cells increase greatly in size, and a large central vacuole is formed in each. The protoplasm and inclusions being spread over a larger area form a thin film, in which the chromatophores are no longer crowded, but are separated by considerable clear spaces; the sporeling therefore becomes lighter in colour and more transparent. The following comparison of sporelings of the second with those of the third day gives some idea of the great increase in the size of the cells.

	Length in mm.		*Diameter in mm.*	
	Second day	*Third day*	*Second day*	*Third day*
Apical cell	·037	·133	·067	·073
Middle cell	·037	·467	·067	·1
Basal cell	·047	·107	·04	·073
Total	·121	·707		

The number of nuclei in the cells, even after enlargement, is small. Several counts indicated that there are in the apical cell on an average 25–30 nuclei, in the middle cell 20–25, in the basal cell 5–10. These nuclei in the resting condition are very small, averaging, perhaps, 1 μ in diameter. In structure they resemble the nuclei of the older vegetative cells.

The changes in the apical and middle cells consist mainly of (1) a great increase in length, (2) slight increase in breadth, and (3) the distribution of the protoplasm and inclusions over a much larger area.

In the basal cell, besides an increase in size, the most striking changes are those of shape. These changes depend to a large degree on the substratum. In case the sporeling is attached to some soft body, such as another alga, the basal cell remains somewhat top-shaped, with the pointed end applied to, or in some cases wedged into the substratum (Figs. 148, 149, 152, 153). If, however, the sporeling is attached to a hard body, such as glass or stone, the basal cell becomes greatly elongated, in some cases coming to equal in length all the rest of the sporeling (Figs. 150, 151). When this occurs, the basal cell resembles strikingly a rhizoid of the older plant.

When the stage just described is reached there seems to come a natural pause in the life-cycle. In a state of nature a great many more sporelings are found in the 3-celled stage than in any other, indicating that this stage occupies a longer time in the course of development than any other. Under laboratory conditions the 3-celled stage is retained at least several days, and frequently development goes no further. The factor determining further development seems to be, in part at least, the character of the substratum. In the cultures examined it was found that on glass or on clean, though rough stones, the basal cell continued to elongate, though without further division of the apical cell, until the whole sporeling lost its natural colour and died. However, in case the elongating basal cell came in contact with some soft substance, it fastened itself immediately, and normal development proceeded. In a state of nature, young sporelings of *Griffithsia* have been most commonly found at Wood's Hole on *Champia parvula* and on *Lomentaria uncinata*, though they occur on other algae and on *Zostera marina*. Young plants were sometimes found on stones, the surface of which appeared clean, but proved, on careful examination, to bear other sporelings, to which the plantlets of *Griffithsia* were probably at first attached. There is no evidence of parasitism, however, in the early development of *Griffithsia*. Sporelings flourish on any soft substratum, such as bits of cotton cloth. From the observations noted above, it seems clear that *Griffithsia Bornetiana* needs some other substratum than the stones on which the mature plant is often found, to pass through the early stages of its existence.

When the sporeling is growing on some other alga, the basal cell may simply become attached to the surface by some adhesive at the surface of contact (Fig. 152), or may grow in between the cells of the algal substratum (Fig. 153), or may even twine about it in the manner of the rhizoidal tendrils.

Further development of the basal cell results in its division into cells of various sizes and irregular shape. Usually short tubular projections resembling rhizoids become cut off (Fig. 154) by the circular ingrowth of the cell-wall. Somewhat less frequently dome-shaped segments are formed on the sides of the basal cell (Fig. 155). In either case a multinucleate holdfast, or attaching disk is formed, all the cells of which are derived from the division of the basal cell (Fig. 156).

The apical cell cuts off daughter segments in the usual manner (Fig. 157). By the time the sporeling is four or five cells long, lateral branches appear on the upper borders of the cells below the apex (Fig. 158). The rapid growth of the lateral branches gives the characteristic false dichotomy to the young thallus. Branching is profuse near the base of the sporeling. Frequently five or six lateral branches are given off from each of the lower cells, so that the young plant is copiously branched. In this

event the cells bearing numerous branches become thick-walled and almost globose in shape.

It is interesting to note that when numbers of sporelings are found in immediate vicinity in nature, all are often at precisely the same stage of development. For instance, about fifteen sporelings were observed on a single branch of *Lomentaria*; all were at the stage of germination represented in Fig. 158.

Hairs are usually wanting in the young plants, nor are rhizoids developed except from the basal cell.

The phenomena of germination noted above agree in all essentials with the account of *Griffithsia Bornetiana* given by Miss Derick (26), and are in line with the phenomena reported in other species by Tobler (85).

It is of considerable interest that the coenocytic condition characteristic of the cells of the mature plant is attained in the sporeling before any sign of cell-division or differentiation. The recapitulation theory [1] has been shown in many cases to be applicable to other plants, e. g. in the formation of the megaspores of the Hydropteridineae (Strasburger, 79), in the forms of juvenile leaves (Berry, 5), in the post-embryonal stages of the Laminariaceae (Setchell, 72, and Griggs, 34), in the formation of the eggs of the Fucaceae (for the facts, see Oltmanns, 59, ii, pp. 47–8). If this theory is at all applicable to *Griffithsia*, we should expect some evidence of it at the times in the life-history when the plant returns to the unicellular condition. If there is virtue in the conclusions drawn from comparative morphology, the ancestors, and even the comparatively recent ancestors of *Griffithsia*, possessed uninucleate cells. The coenocytic habit was acquired late in the history of the race, and we should expect it, therefore, to appear late in the history of the individual, so that the cells of the early stages would be uninucleate; yet in the germinating spore of *Griffithsia* the first visible change is the attainment of what we must regard as the recently acquired coenocytic habit. In this respect, then, *Griffithsia* does not conform to the recapitulation theory.

Discussion of Results.

From the cytological evidence brought forward in this paper it seems probable that there exists in *Griffithsia Bornetiana* an alternation of generations similar to that which has been suggested for *Polysiphonia violacea* (Yamanouchi, 93). The fusion nucleus, which contains fourteen chromosomes, with the co-operation of the cytoplasm of some of the cells of the procarp, produces the cystocarp, in which are formed carpospores;

[1] 'A highly organized plant, which begins its development with the simplest stages and gradually advances to a state of higher differentiation, repeats in its ontogeny its phylogenetic development.' Strasburger, Noll, Schenck, and Schimper: A Textbook of Botany, Second English edition, 1903, p. 49.

the nucleus of each of these contains fourteen chromosomes. The nuclei of the tetrasporic plant contain each fourteen chromosomes; and it therefore seems reasonable to assume that the tetrasporic plants arise from carpospores. In the first division of the nucleus of the tetraspore-mother-cell the number of chromosomes is reduced one-half, so that seven chromosomes enter the nucleus of each tetraspore. It seems probable that on germinating the tetraspore gives rise to an individual whose general morphological relations and vegetative structure are similar to those of the plant producing tetraspores, with two significant exceptions, (1) the nuclei show at mitosis seven chromosomes instead of fourteen, and (2) the individual bears sexual organs instead of asexual spores. In other words, in *Griffithsia* a sexual plant is probably succeeded by an asexual plant of similar morphological relations.

The proof of this hypothesis must rest on actual cultural experiments, and it is much to be desired that such experiments be carried out.

Since Strasburger (78) showed that in the Archegoniates the double number of chromosomes is characteristic of the sporophyte and the single number of the gametophyte, the main facts have been confirmed in so many forms that many botanists have come to consider that the chromosome number alone is a trustworthy guide for the identification of the two generations: that plants showing the diploid condition of the nucleus necessarily belong to the sporophyte, and that where the haploid condition of the nucleus obtains, the gametophyte is necessarily involved. Botanists have come to speak of the sporophyte as the '$2x$-generation' (Lotsy, 51), and of the gametophyte as the 'x-generation'; and undoubtedly within the Archegoniate series such a conception is very useful. However, even in Archegoniates, where the rule is so generally applicable, recent work tends to show that the diploid condition of the nucleus is not necessary for the differentiation of the sporophyte (Yamanouchi, 94), nor is the haploid condition necessary for the differentiation of the gametophyte (Farmer and Digby, 30).

In thallophytes, the evidence at hand indicates great diversity in the point at which the numerical reduction of chromosomes takes place. Even in the single group of Rhodophyceae the point of reduction occurs at different places in the life-history of different species. When one comes, therefore, to regard the chromosome number as the sole test for the delimitation of sporophyte and gametophyte, it seems probable that confusion will result. With this in mind I shall now review briefly the opinions expressed by workers in this field as to the alternation of generations in the red algae, and shall venture to offer some suggestions as to the meaning of the rather complicated nuclear life-histories of members of this group.

Oltmanns (58), after a careful study of the development of the cystocarp in four genera, came to the conclusion that the sporogenous cells

constitute a generation similar to the sporophyte of the Archegoniate series. Later (59, ii), he elaborated this conception and expressed the opinion that the sporophyte is of antithetic origin, i. e. that it became gradually intercalated in the life-history by a series of stages of increasing complexity. In those forms in which the tetraspores are borne on distinct plants, he regards the tetraspore-producing plant as a 'facultative gametophyte', which is a result of a process of differentiation similar to that which produced dioecism in many of the Archegoniates. The tetraspores he considers analogous to the gemmae of certain liverworts. Admitting the possibility that a numerical reduction of the chromosomes may take place in the tetrasporangium, he expresses the opinion that the number of chromosomes is not the final test of alternation of generations. 'Ich vermute, die vergleichende Untersuchung des ganzen Entwickelungsganges führt eher zum Ziel, oder aber die Kombination beider Methoden' (59, ii, p. 273).

The work of Wolfe on *Nemalion multifidum* (90), in which he found a numerical reduction of chromosomes just previous to the production of carpospores, furnished a cytological analogy between the cystocarp of the Rhodophyceae and the sporogonium of the Bryophytes, and strengthened the position of Oltmanns.

Yamanouchi (93), after a very complete cytological study of *Polysiphonia violacea*, reached the following conclusion: 'The sexual plants and the tetrasporic plants present the two distinct phases of an antithetic alternation of generations, with the cystocarp a part of the sporophytic phase' (p. 433). This conclusion is based on the discovery by Yamanouchi that the dividing nuclei of the tetraspore-producing plant throughout its history, as well as those of the sporogenous cells of the cystocarp, show forty chromosomes (the $2x$ number), while the nuclei of the sexual plants show twenty chromosomes (the x number). The number of the chromosomes is reduced in the divisions of the nucleus of the tetraspore-mother-cell; the double number is restored by the union of the nuclei of the gametes. In discussing the origin of the tetraspore, Yamanouchi surmises that in some such form as *Batrachospermum*, in which monospores are borne along with gametes on the sexual plants, reduction may have been suppressed in the formation of the carpospore, 'so that it germinates with the sporophytic number of chromosomes, producing a plant which consequently becomes at once a part of the sporophytic phase. It is quite possible that the first tetraspore-mother-cells corresponded to monospores on the sexual plant, except that they had the double number of chromosomes, since such reproductive cells would very naturally become the seat of the delayed reduction phenomena. The resemblance in general morphology of the tetrasporic plants in the red algae to the sexual plants would be expected because they live under similar environmental conditions' (p. 435).

The views of Oltmanns and Yamanouchi coincide so far as regarding

the sporogenous cells of the cystocarp as belonging to the sporophytic phase of an antithetic alternation of generations. The point of departure lies in the interpretation of the tetraspore-producing plant. Because of its general morphological identity with the gametophyte, Oltmanns regards this as a part of the gametophyte, which has become differentiated for the production of tetraspores. Because of the diploid condition of its nuclei, Yamanouchi regards the tetrasporic plant as a part of the sporophyte, whose resemblance to the gametophyte is stamped on it by 'similar environmental conditions', a view in which Bower (12, p. 81) seems to concur.

For the purposes of the present discussion, I shall assume from the cytological evidence what it will take cultural experiments to prove, namely, that in those red algae in which tetraspores and gametes are regularly formed on separate individuals there is an actual succession of sexual and tetrasporic plants, the reproductive bodies of one kind of plant always producing the other kind of plant.

Yamanouchi's suggestion (93) that the tetrasporic plant may have arisen phylogenetically by the postponement of the phenomena of reduction from the formation of carpospores to the production of asexual spores seems to be rendered probable by what we know of other groups of plants. In the simplest plants which have been investigated from this standpoint, the position of reduction in the life-history seems to be at the first divisions of the fusion nucleus, as is described in *Coleochaete* (Allen, 2), certain desmids (Klebahn, 47), and in *Spirogyra* (Karsten, 45). Beginning with the simple Bryophytes, the familiar Archegoniate series shows a progressive removal of the point of reduction from the point of fusion of the sexual nuclei. These and other examples seem to show that there is a general tendency throughout the plant kingdom to prolong the diploid condition of the nucleus through the greater part of the life-history (see Bower, 12, p. 77).

Nowhere is this more plainly shown than in the Uredineae (Blackman, 8; Blackman and Fraser, 9; Christman, 16, 17). This group is characterized by a succession of phases, or generations, which have been shown by Christman to be morphologically equivalent, though each ends in a distinct form of spore. Now nuclear association, which has been regarded as the equivalent of fertilization in this group, occurs, in all forms in which the aecidial stage is present, in those cells of the mycelium which give rise to the aecidium. The process of numerical reduction of the chromosomes, or, to speak more accurately, of the chromatin, occurs always in the last spore-form preceding the production of aecidia, in the teleutospore when present. The diploid condition, extending from the aecidium to the teleutospore, is lengthened by the intercalation of new phases, which, in some cases, seem to have the power of continuing the diploid generation indefinitely. The haploid generation, from the teleutospore to the binucleate cells at the base of the aecidium, is never lengthened by the inter-

calation of new phases. In other words, in the Uredineae the diploid generation has become prolonged throughout the greater portion of the life-history.

In the red algae, it seems likely that a similar postponement of reduction has taken place. In the Nemalionales, which is considered the most primitive group of the Rhodophyceae, the point of reduction is removed from the point of nuclear fusion only by the few cell-generations in the cystocarp (Wolfe on *Nemalion*, 90). In the higher forms, such as the Rhodomelaceae (Yamanouchi on *Polysiphonia*, 93), and the Ceramiaceae (*Griffithsia*), nuclear reduction is separated from nuclear fusion, not only by the cell-generations of the cystocarp, but also by all the divisions of the vegetative cells of the tetrasporic plant. That is, the diploid phase has come to occupy the greater portion of the life-history.

The biological meaning of this apparently general tendency in the evolution of plant structures is hinted at by the experiments of Gerassimow (32). After studying the growth of vegetative cells of *Spirogyra* in which nuclei had been induced to fuse, Gerassimow came to the conclusion that the growth of a cell which has an unusual amount of nuclear material is more vigorous than that of a cell with the usual nuclear content. The cell-wall, the chromatophores, and apparently the protoplasm, grow more vigorously. Such cells divide only after they have reached a size noticeably larger than normal. (See Bot. Gaz., xxxv, 1903, pp. 224–5.)

If, then, the presence of nuclei with the double chromatin content imparts greater vigour to the cell, we should expect to find some evidence of this greater vigour not only in the size, but in the rate of growth of the tetrasporic plants of *Griffithsia*. A comparison of sexual plants with tetrasporic plants does not reveal any constant difference in the size of the resting nuclei or in the size of the cells of the two kinds of individuals. However, a comparison of those cells of the diploid individual which produce sori of tetraspores with the occasional cells of the haploid plant, which form similar structures, shows a difference in size, the cells with the diploid nuclei being larger (p. 672).

More important from this standpoint is the fact that not only in *Griffithsia*, but in red algae generally where tetrasporic and sexual plants occur side by side, the tetrasporic plants are, as a rule, more abundant (p. 640). In *Griffithsia Bornetiana* the number of tetraspores produced is certainly much greater than the number of carpospores, and we should expect, therefore, if the two kinds of spores were equally vigorous, that the number of sexual plants would greatly exceed the number of tetrasporic plants; whereas the reverse is the case. It seems possible that the carpospores have a greater capacity for development than the tetraspores. Cultural experiments along this line are much to be desired.

If the view is correct that a postponement of reduction has occurred in some Rhodophyceae, it is evident that besides the alternation of the

gametophyte (the sexual plant) with the antithetic sporophyte (the sporogenous cells of the cystocarp), there is a succession of homologous phases, inasmuch as a tetrasporic individual regularly succeeds a sexual individual of identical morphology. This latter condition is not paralleled in the Archegoniate series; and since the terms gametophyte and sporophyte have come to have a special significance in connexion with such conditions as are found in the Archegoniates, neither of these terms should be applied to the tetrasporic plants of *Griffithsia* and *Polysiphonia*. The tetrasporic plant has probably been intercalated in the life-history of the red algae, but there is no evidence for the belief that it has been intercalated by gradual integration and differentiation of a simple product of the germination of the zygote, which product was at first unlike the sexual plant and which represents a new departure in the life-history; and the intercalation, by amplification, of an *unlike* phase seems to be the very pith of the theory of antithetic alternation (see Bower, 11; and 12, p. 47; Yamanouchi, 94, p. 310).

According to this view, the tetrasporic plant probably arose, when first produced, with the complete differentiation characteristic of this species. The best evidence for this conclusion is based on the morphological identity of the tetrasporic with the sexual plant. Similar environmental conditions would hardly suffice to produce identity of form in two individuals unless the individuals were from the beginning identical. The tetrasporic plant of a red alga may be said, then, to be homologous with the sexual plant.

That the two phases are homologous is evidenced, not only by their similarity of structure, but by the fact that either seems capable of producing the morphological equivalent of the reproductive structures of the other. It has been known since Bornet first called attention to the fact (10) that in many species of red algae structures resembling tetraspores are occasionally found on the sexual individuals. This phenomenon has been carefully investigated in *Polysiphonia violacea* and *Griffithsia Bornetiana*. In *Polysiphonia*, Yamanouchi (93) found that the development of these tetraspore-like structures ceases at the mother-cell stage; cleavage of the cytoplasm may begin, but normal nuclear division is absent. In *Griffithsia*, the phenomena observed are similar to those noted in *Polysiphonia*, except that by the time the abortive cleavage begins, the nucleus has divided to form several. The cleavage planes have never been observed to reach the centre of the cell, and it is quite evident that tetraspores are not formed, since the whole cell becomes withered and wrinkled, resembling the degenerated tetrasporangia described on p. 668. In one instance, however, a mother-cell was observed in which no trace of cleavage of the cytoplasm was apparent, and in which the number of nuclei had increased to sixteen, the whole structure resembling very much the early stages of germination of a normal spore. It seems quite possible that the tetraspore-mother-cells

borne on the sexual plants sometimes germinate as monospores, though this can be ascertained only by cultivation.

On the other hand, tetrasporic plants may at times produce structures

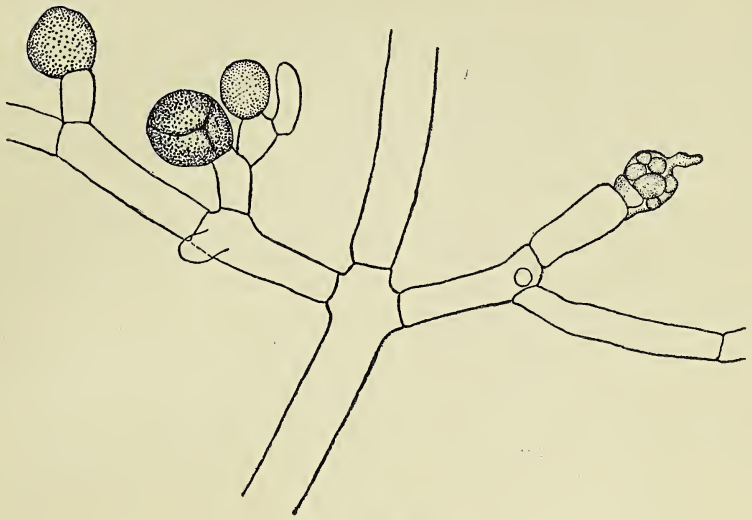

Fig. 1.

morphologically identical with procarps. Text-fig. 1 is taken from a tetrasporic plant of *Spermothamnion Turneri* collected at Wood's Hole in August, 1907, and shows tetraspores and procarp on opposite branches of the same filament. Text-fig. 2 shows a section of the tetrasporangium in which the definitive tetraspores are formed, though not yet separated. Antheridia have not been reported as occurring on *Spermothamnion Turneri* at Wood's Hole, and cystocarps are very rarely produced. Whether functional gametes are ever produced on an individual which bears normal tetraspores is not known.

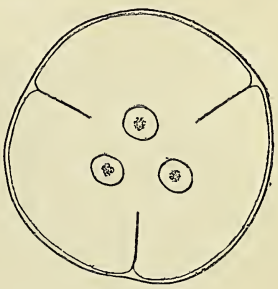

Fig. 2.

In *Spermothamnion Turneri*[1] from Helgoland, Pringsheim states (63, p. 26) that 'Kapselfrüchte, Vierlingsfrüchte und Antheridien normal zusammen auf denselben Exemplaren auftreten'. This case is obviously of so great theoretical interest that I took occasion, in the summer of 1908, to collect this plant at Helgoland. Hundreds of plants were collected, the great majority of which bore only tetraspores. In a few individuals

[1] The species called *Spermothamnion roseolum* by Pringsheim is stated by Professor Kuckuck to be identical with *S. Turneri*. In this connexion I wish to offer my thanks to Professor Kuckuck for the privilege of working at the Biologische Anstalt at Helgoland.

tetraspores were found together with antheridia or procarps, but in no case were tetraspores and cystocarps found on the same plant. Cytological investigation of *Spermothamnion* is much to be desired.

The theory of homologous alternation in the red algae outlined above is almost identical with the view of Pringsheim as to the relations within this group (64). He states (p. 897), 'die Annahme ist nächstliegend, dass bei Florideen und Dictyoteen zwischen Exemplaren mit Kapselfrüchten und Exemplaren mit Vierlingsfrüchten eine Abwechselung besteht.' Pringsheim's view was based, however, on a very different kind of evidence from that brought forward in the present paper.

The evidence at hand seems to justify the following conclusions:

1. There is in *Griffithsia* an antithetic alternation of generations, the gametophyte being represented by the sexual plants, the sporophyte by the sporogenous cells of the cystocarp.

2. In addition to this, there is a regular succession of tetrasporic individuals and sexual individuals. The tetrasporic individuals resemble the sporophyte in number of chromosomes; they resemble the gametophyte in morphological differentiation. They are to be considered as a phase of an homologous alternation of generations, not the equivalent, wholly or in part, of the sporophyte of Archegoniates.

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EXPLANATION OF PLATES XLIX–LIII.

Illustrating Dr. Lewis's paper on *Griffithsia Bornetiana*.

Lettering of figures: *a.n.*, auxiliary nucleus; *aux.*, auxiliary cell; *b.c.*, basal cell; *carp.*, carpogonium or carpogenic branch; *c.c.*, central cell; *f.n.*, fusion nucleus; *p.c.*, peripheral cell; *pl.*, placenta; *pl.c.*, placental cell; *sp.c.*, sporogenous cell.

PLATE XLIX (Figs. 1–26).

Fig. 1. Small specimen, female plant. × 2.

Fig. 2. Portion of male plant, showing caps of antheridia over the tips of the terminal cells, and hairs. × 30.

Fig. 3. Portion of female plant with cystocarps. × 30.

Fig. 4. Portion of tetrasporic plant with tetraspores. × 30.

Fig. 5. Optical section of the wall of a single cell, on the left from near the apex, on the right from the base, showing characteristic lamination. × 1800.

Fig. 6. Longitudinal section through an intercellular connexion. × 700.

Fig. 7. Section showing accumulation of nuclei and cytoplasm on the wall. × 1200.

Fig. 8. Transverse section through an intercellular connexion, showing the pore surrounded by nuclei; among the nuclei are deeply staining balls of proteid. × 700.

Fig. 9. Surface view of an apical cell, in which all the nuclei are undergoing division. × 500.

Fig. 10. Resting nucleus from a tetrasporic plant; around it are chromatophores. × 1800.

Figs. 11–26. Stages in mitosis of nuclei from tetrasporic plant. All × 3600.

Figs. 11–18, prophases; 19, metaphase showing spindle, chromosomes, and kinoplasmic caps; 20, polar view of equatorial plate; 21–23, anaphases; 24–26, telophases.

PLATE L (Figs. 27–54).

Figs. 27–31. Stages in mitosis of nuclei from male plant. × 3600.

Figs. 27, 28, prophases; 29, 30, polar view of equatorial plate at metaphase; 31, metaphase.

Fig. 32. Surface view of chromatophores, showing arrangement; one nucleus in the field. × 1200.

Fig. 33. Dividing chromatophores. × 3600.

Figs. 34–41. Stages in cell-division, longitudinal sections. × 330.

Fig. 34, accumulation of protoplasm in tip of the cell; 35, formation of dome-shaped membrane; 36, beginning of vacuole; 37, slightly older stage; 38, small 2-celled lateral branch, with neighbouring hair; 39, beginning of formation of ring-shaped constriction; 40, 41, stages in ingrowth of ring-shaped partition.

Fig. 42. Hair-like organs of different ages side by side. × 330.

Fig. 43. Same after older hair has elongated. × 125.

Fig. 44. Three rhizoids on single cell, longest one toward base of cell. × 30.

Fig. 45. Tendril-like rhizoid twining around support. × 45.

Fig. 46. Rhizoids connecting neighbouring cells. × 15.

Figs. 47–50. Stages in regeneration, showing how continuity is restored in filament upon the death of a cell. × 30.

Fig. 51. Young attaching disk, attaching cells shaded. × 30.

Fig. 52. Older stage of same. × 15.

Fig. 53. Surface view of young antheridial branch, with hair. Antheridial papillae cover tip of apical cell. × 130.

Fig. 54. Longitudinal section of apical cell bearing antheridial papillae. × 350.

PLATE LI (Figs. 55–89).

Fig. 55. Antheridial papillae cut off from mother-cell. × 1200.

Figs. 56, 57. Division of antheridial papillae. × 1800.

Fig. 58. Antheridial papillae lying in swollen wall of mother-cell. × 1800.

Fig. 59. Same, after division of the papillae. × 350.

Figs. 60, 61. Stages in formation of antheridial filaments. × 1800.

Figs. 62, 63. Antheridial filaments with mature spermatia. × 1200.

Figs. 64, 65. Formation of new antheridial branches after terminal cell has ceased to produce antheridia. × 20.

Fig. 66. Surface view, to show 2-celled procarp; the small apical cell has been pushed to one side. × 100.

Fig. 67. Longitudinal section of similar stage. × 500.

Fig. 68. Longitudinal section of 3-celled procarpic branch. × 500.

Fig. 69. Surface view, showing first peripheral cell. × 500.

Fig. 70. Surface view, showing second peripheral cell. × 500.

Fig. 71. Surface view of later stage; 3-celled carpogenic branch shaded. × 500.

Fig. 72. Procarpic branch metamorphosed into vegetative shoot; on either side are ordinary vegetative branches. × 150.

Fig. 73. Almost mature procarp; carpogenic branch shaded. × 500.

Fig. 74. Section of very young carpogonium. × 1800.

Fig. 75. Mature procarp; spermatium attached to tip of trichogyne. × 500.

Fig. 76. Longitudinal section of procarp of same age as in Fig. 71. × 500.

Fig. 77. Neighbouring longitudinal sections of mature procarp; two spermatia attached to trichogyne; two male nuclei in trichogyne. × 500.

Fig. 78. *a*. Longitudinal section through trichogyne, carpogonium, and auxiliary cell. × 500. *b*. Tip of same trichogyne. × 1800.

Fig. 79. Auxiliary cell and carpogonium in longitudinal section; male nucleus entering carpogonium. × 3600.

Fig. 80. Surface view from above of young cystocarp in which trichogyne has begun to wither. × 500.

Fig. 81. Longitudinal section; fusion nucleus present in auxiliary cell. × 500.

Fig. 82. Surface view of young cystocarp showing withered carpogenic branch falling off as a whole. × 500.

Fig. 83. Longitudinal section showing two fusion nuclei in auxiliary cell. × 500.

Fig. 84. Longitudinal section showing auxiliary cell divided into two, the upper cell the placental cell. × 500.

Fig. 85. Longitudinal section of cystocarp in which sporogenous lobes have been formed from placental cell. × 500.

Fig. 86. Formation of sporogenous lobes from auxiliary as well as placental cell. × 500.

Fig. 87. Fusion of cells in centre of cystocarp; central cell, placental cell, auxiliary, and first peripheral cell are shown taking part in the fusion. × 500.

Fig. 88. Degenerating nuclei from the lower part of the placenta. × 3600.

Fig. 89. Division of sporogenous lobe; crescent-shaped cell is cut off on outside. × 500.

PLATE LII (Figs. 90–128).

Figs. 90–93. Sections of sporogenous lobes, showing mode of division. × 500.

Fig. 94. Mitosis in sporogenous lobe, and division by constriction of cytoplasm. × 1200.

Fig. 95. Surface view of cystocarp producing mature spores; sporogenous lobes of various ages are shown. × 150.

Fig. 96. Section of mature carpospore. × 700.

Fig. 97. Spindle and polar view of equatorial plate from mitoses in sporogenous lobes. × 1800.

Figs. 98, 99. Tetrasporic papillae pushing out through wall of vegetative mother-cell. × 850.

Figs. 100, 101. Tetrasporic papillae cut off from mother-cell. × 850.

Figs. 102–4. Division of primary tetrasporic cell. × 850.

Fig. 105. Basal cell and tetraspore-mother-cell. × 850.

Fig. 106. Second division of basal cell. × 850.

Fig. 107. Basal cell with two tetraspore-mother-cells; in one three definitive spore nuclei are visible. × 850.

Fig. 108. Basal cell with three tetraspore-mother-cells. × 500.

Fig. 109. Connexion of basal cell with tetraspore-mother-cell. × 3600.

Fig. 110. Division of mother-cell into spores; basal cell shows tubular process. × 500.

Fig. 111. Mother-cell; nucleus shows 14 granules. × 850.

Fig. 112. Chromatin granules fewer in number and irregular in outline; nuclear (chromidial) material is shown passing out into cytoplasm. × 850.

Fig. 113. About same stage; chromatin granules show indications of tetrad grouping. × 850.

Fig. 114. Nucleus of tetraspore-mother-cell in prophase; chromosomes scattered through nuclear cavity. × 1800.

Fig. 115. Same stage. × 1800.

Fig. 116. Longitudinal section of tetraspore-mother-cell; nucleus in metaphase. × 1800.

Fig. 117. Metaphase, first mitosis. × 1800.

Fig. 118. Prophase, first mitosis; chromosomes gathering in centre of nuclear cavity. × 1800.

Fig. 119. Prophase; chromosomes seem to be grouped in pairs. × 1800.

Fig. 120. Polar view of equatorial plate showing fourteen chromosomes. × 1800.

Fig. 121. Anaphase, showing separation of two groups of chromosomes (from neighbouring sections). × 1800.

Fig. 122. Telophase; nuclear cavity dividing into two. × 1200.

Fig. 123. Two daughter-nuclei in mother-cell. × 1800.

Fig. 124. Prophase, second mitosis. × 3600.

Fig. 125. Longitudinal section, tetraspore-mother-cell, upper nucleus showing spindle at metaphase, lower showing polar view of equatorial plate with seven chromosomes. × 1800.

Fig. 126. Telophase, second mitosis. × 1200.

Fig. 127. Three definitive spore nuclei visible in mother-cell; into lowest food material is passing from basal cell. × 850.

Fig. 128. Portion of wall of tetrasporangium showing beginning of cleavage. × 3600.

PLATE LIII (Figs. 129–58).

Fig. 129. Partitions about one-third of the way to centre of tetrasporangium. × 850.

Fig. 130. Later stage, partitions double, chromatophores visible around periphery. × 850.

Fig. 131. Partitions almost reach to centre of tetrasporangium; spores nearly separated. × 850.

Fig. 132. Spores completely separated, still enclosed by gelatinized wall of tetrasporangium. × 330.

Fig. 133. Diagrammatic longitudinal section showing mode of occurrence of tetrasporic structures. × 30.

Figs. 134–8. Tetraspore-like structures from male plant. × 850.

Fig. 134. Basal cell with two tetraspore-mother-cells (cf. Fig. 108).

Fig. 135. Later stage, only one tetraspore-mother-cell present.

Fig. 136. Basal cell with tubular outgrowth (cf. Fig. 110).

Fig. 137. Tetraspore-mother-cell showing eight nuclei; cleavage of cytoplasm has begun.

Fig. 138. Sixteen nuclei present in mother-cell, no trace of cleavage.

Fig. 139. Vegetative cell accidentally isolated has produced a rhizoid from basal end. × 100.

Fig. 140. Later stage showing young plant developed from similar isolated cell. × 15.

Fig. 141. Binucleate sporeling. × 850.

Fig. 142. Eight-nucleate sporeling, optical section. × 500.

Fig. 143. Section of sixteen-nucleate sporeling. × 850.

Fig. 144. Sporeling has begun to elongate without division. × 500.

Fig. 145. Two-celled sporeling. × 850.

Fig. 146. Three-celled sporeling. × 850.

Fig. 147. Same, surface view. × 30.

Fig. 148. Three-celled sporeling after elongation, attached to filament of *Lomentaria*. × 100.

Fig. 149. Similar stage, showing difference in relative size of the cells. × 100.

Fig. 150. Sporeling of same age growing on glass; basal cell very long. × 100.

Fig. 151. Similar sporeling after basal cell has reached a suitable substratum. × 100.

Fig. 152. Section showing basal cell of sporeling applied to surface of *Champia*. × 100.

Fig. 153. Section showing basal cell penetrating tissues of *Champia*. × 100.

Figs. 154–6. Development of attaching disk from basal cell. × 30.

Fig. 157. Four-celled sporeling; basal cell branched. × 30.

Fig. 158. Sporeling beginning to branch. × 30.

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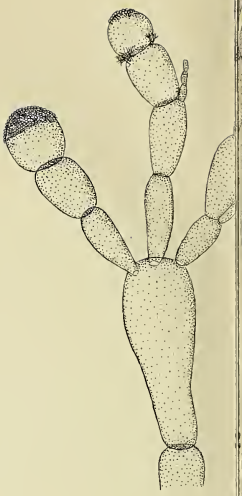

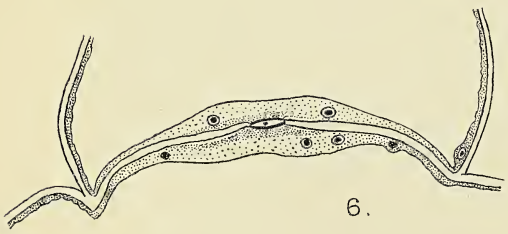

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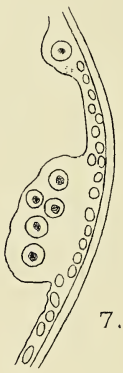

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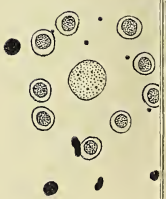

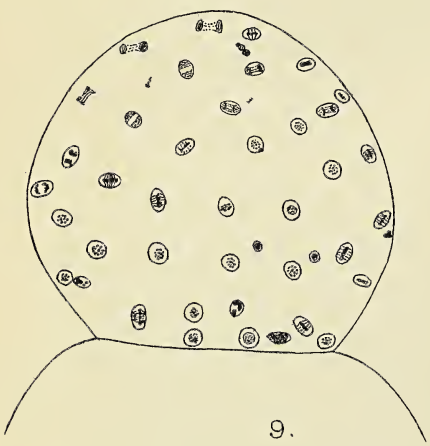

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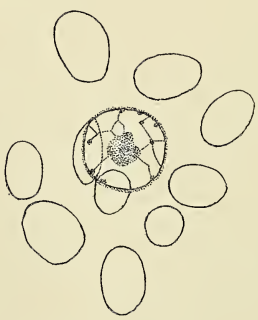

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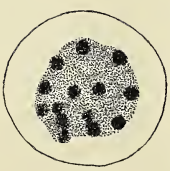

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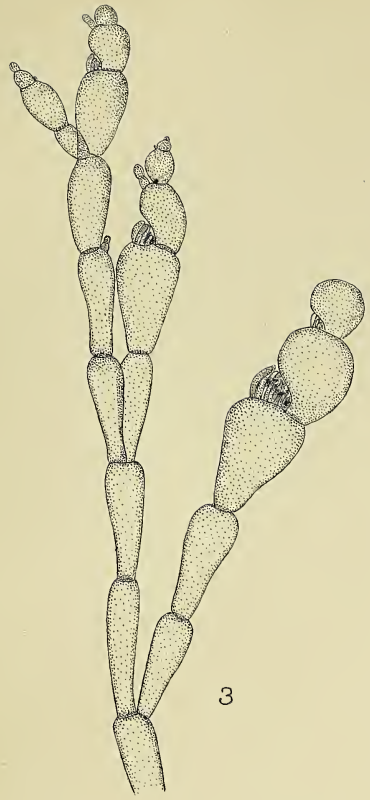

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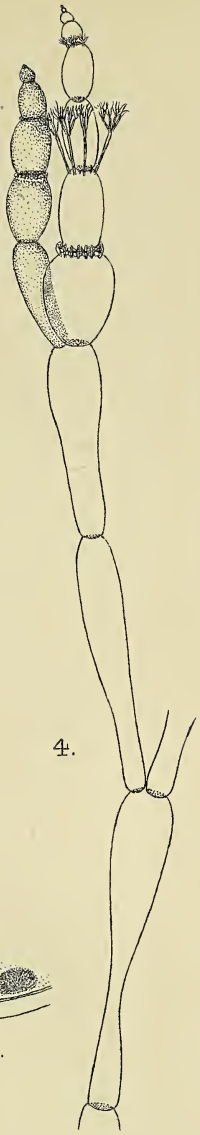

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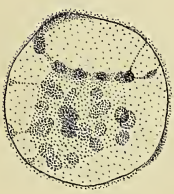

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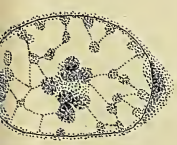

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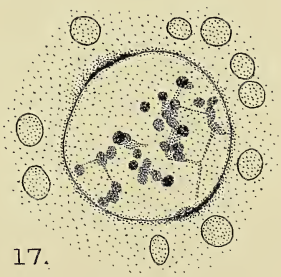

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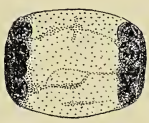

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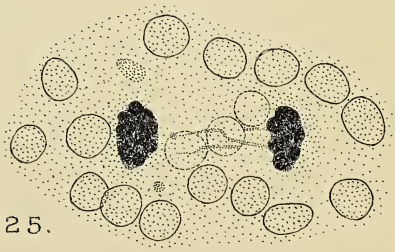

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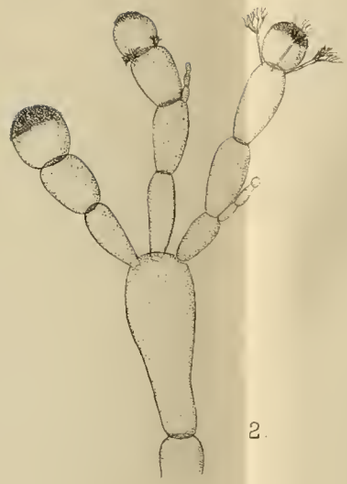

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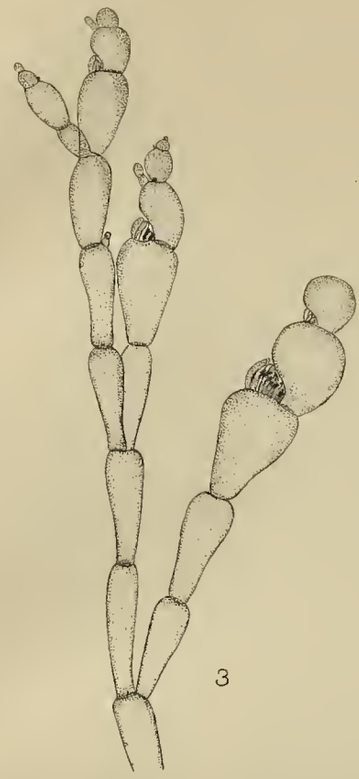

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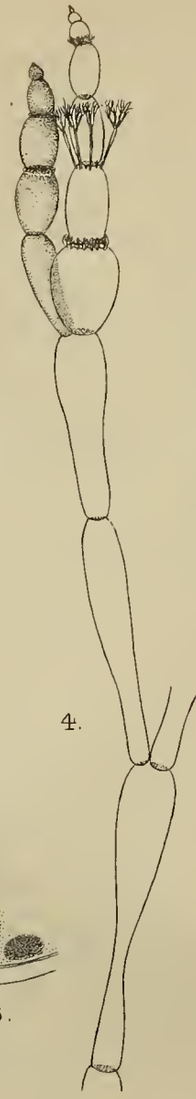

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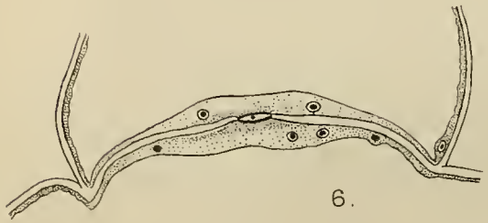

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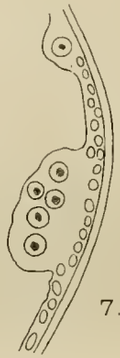

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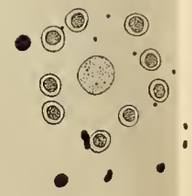

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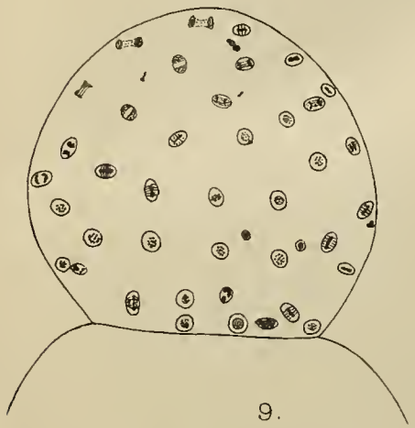

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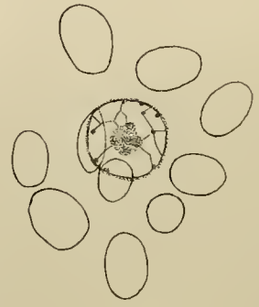

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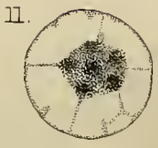

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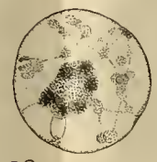

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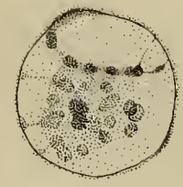

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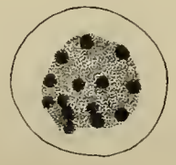

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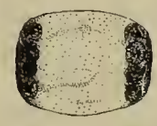

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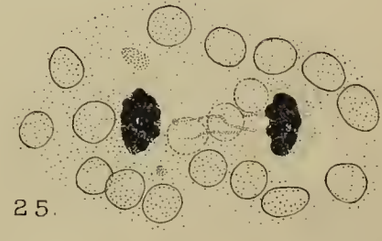

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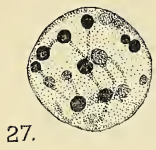
I.F.L. del

Huth, lith et imp.

LEWIS — GRIFFITHSIA BORNETIANA.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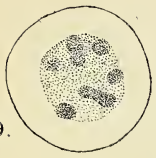
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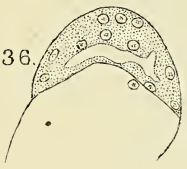
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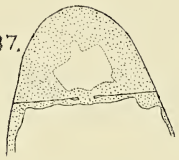
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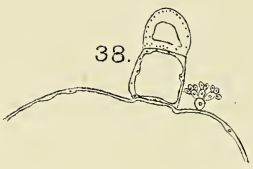
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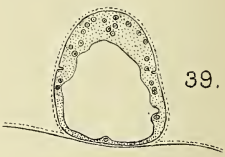
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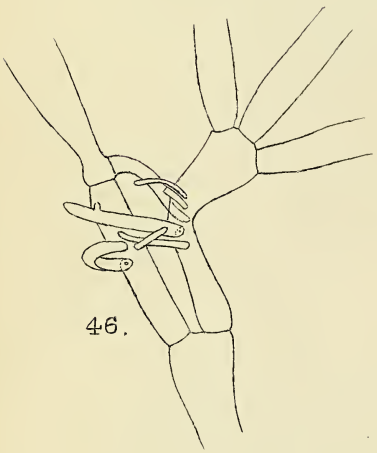
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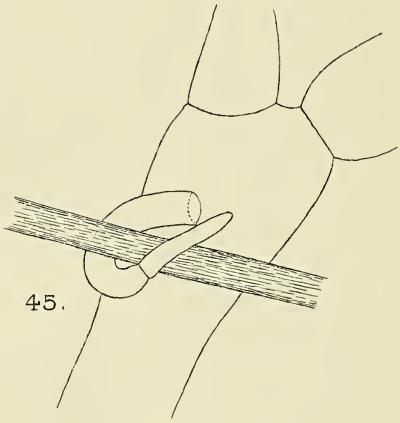
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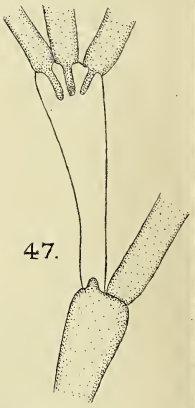
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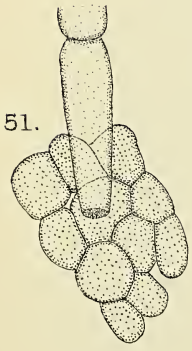
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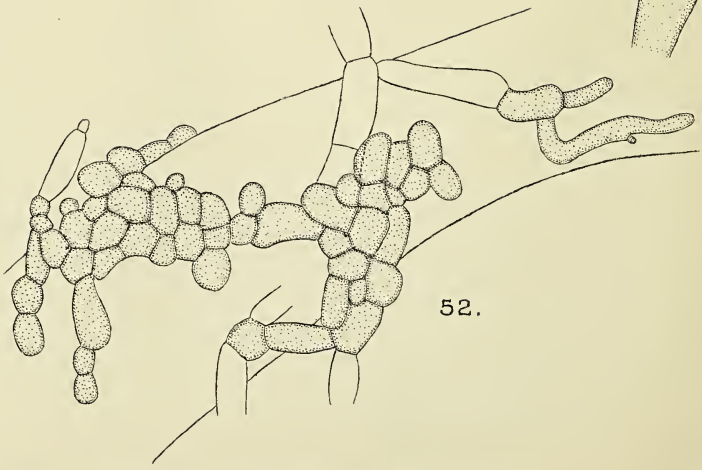
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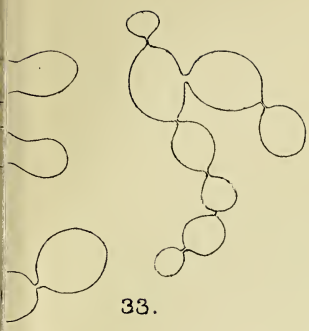
LEWIS—GRIFFITHSIA BORNETIANA.



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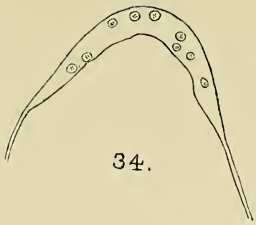

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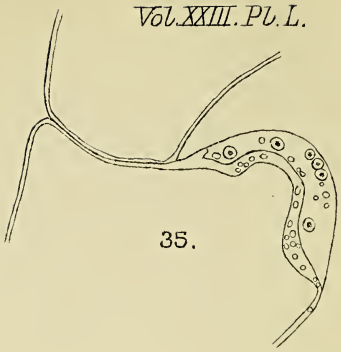

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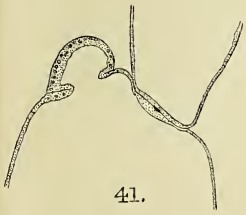

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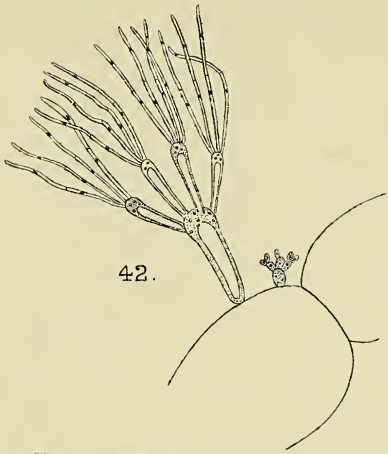

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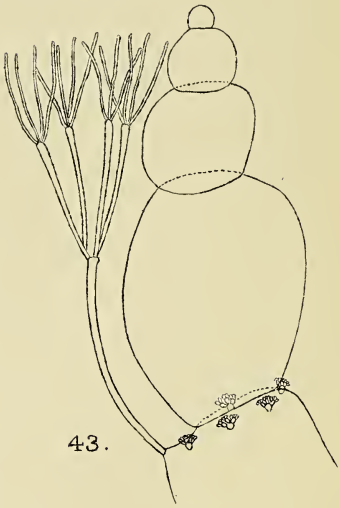

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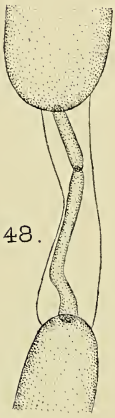

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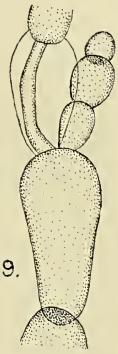

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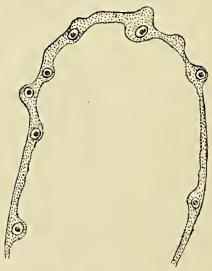

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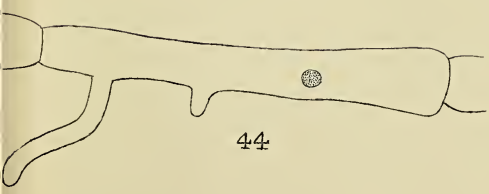

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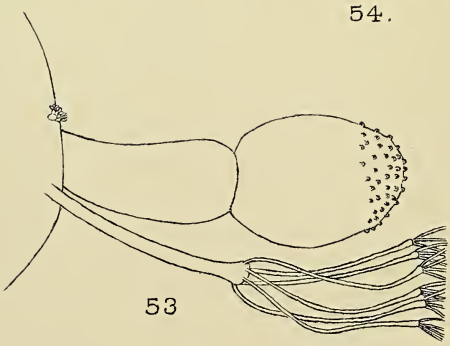

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Huth, lith et imp.


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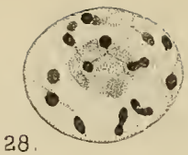
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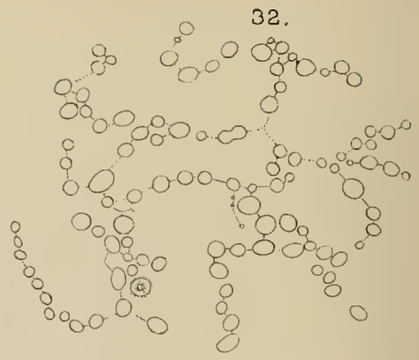
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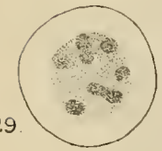
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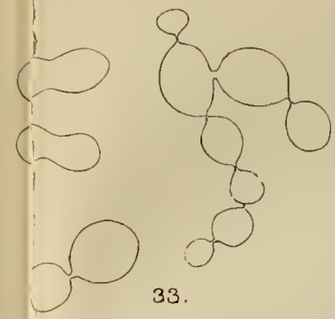
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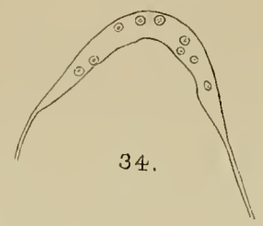
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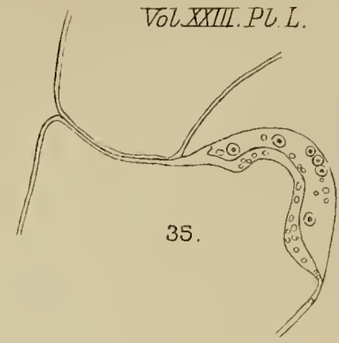
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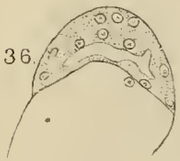
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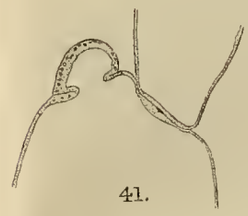
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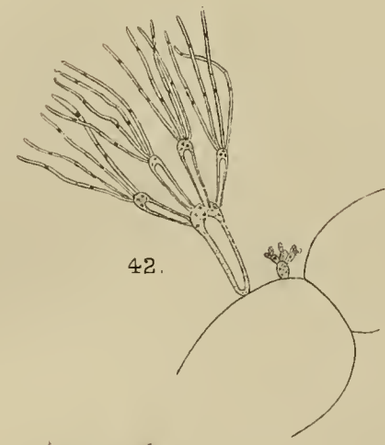
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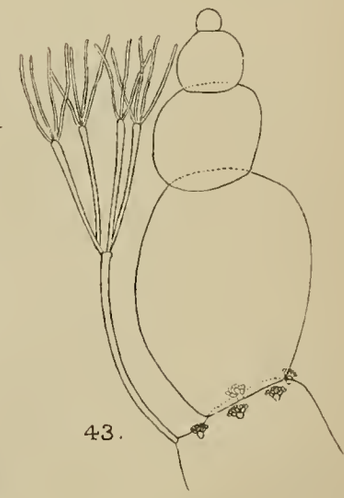
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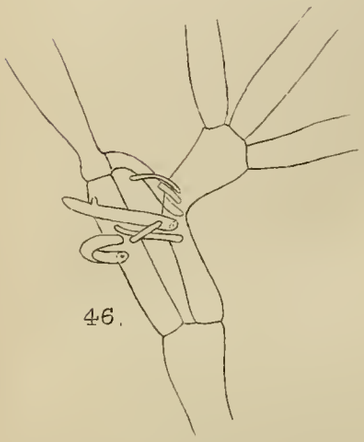
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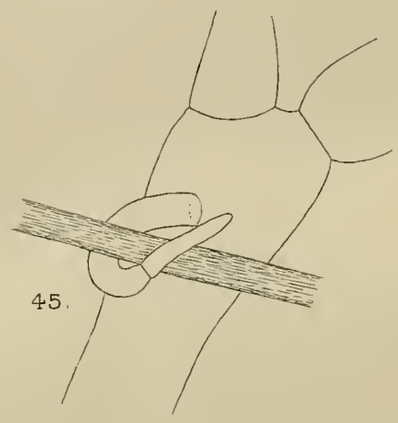
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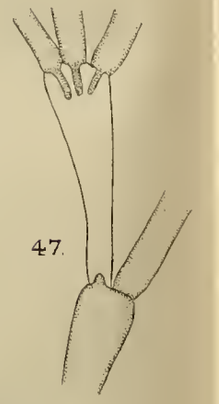
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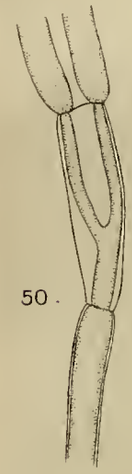
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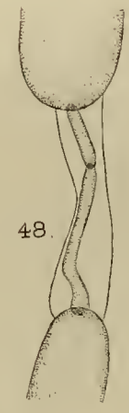
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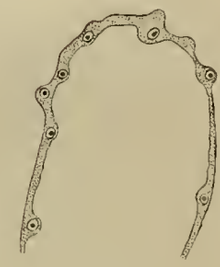
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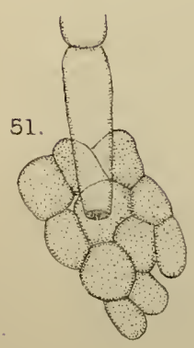
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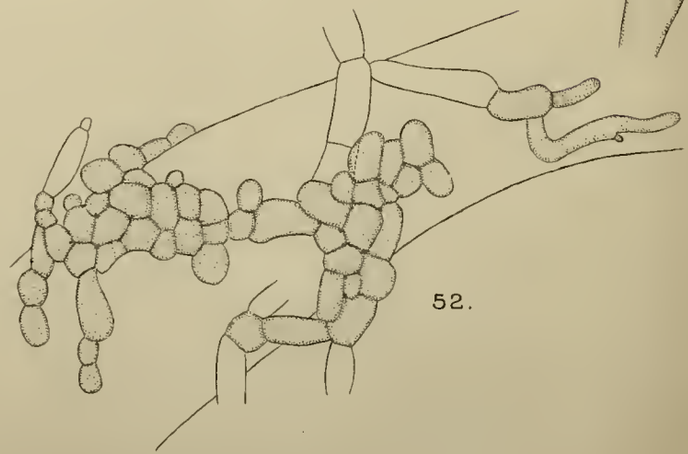
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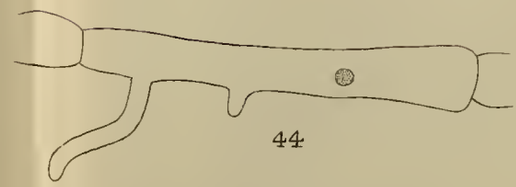
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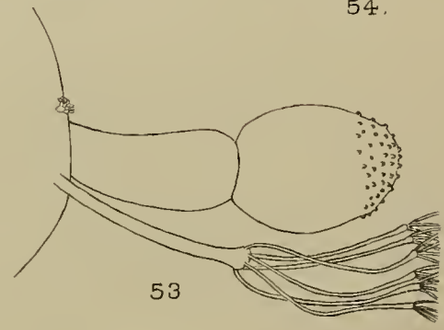
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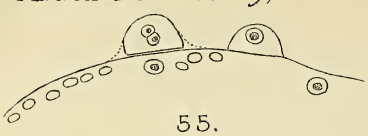

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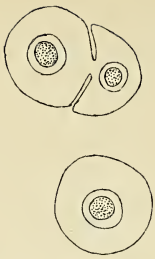

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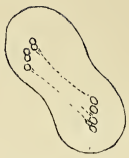

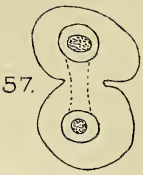

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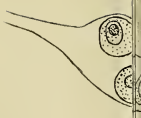

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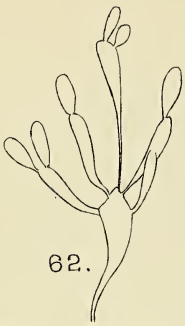

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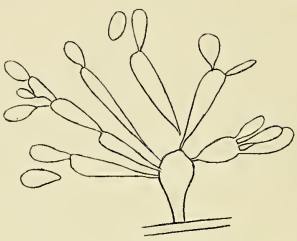

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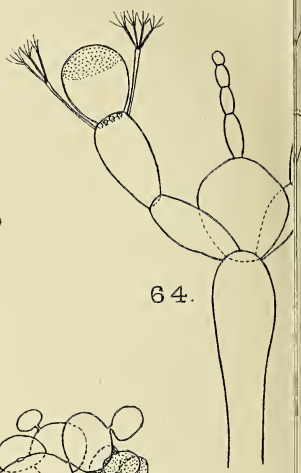

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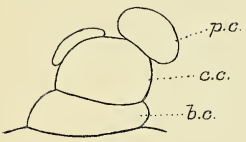

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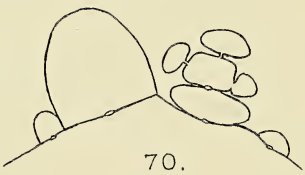

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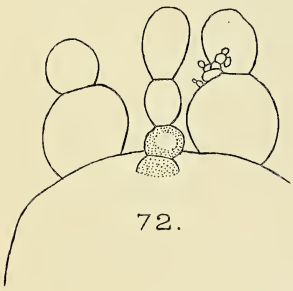

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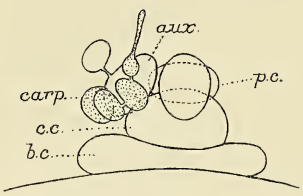

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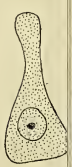

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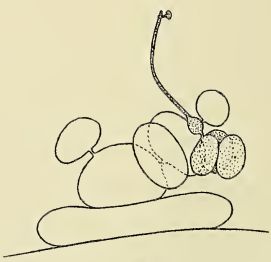

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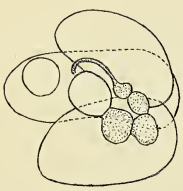

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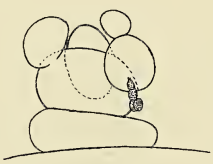

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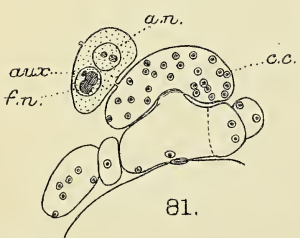

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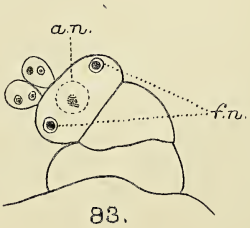

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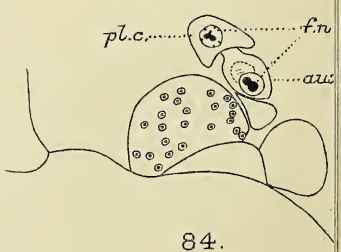

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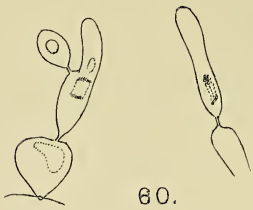

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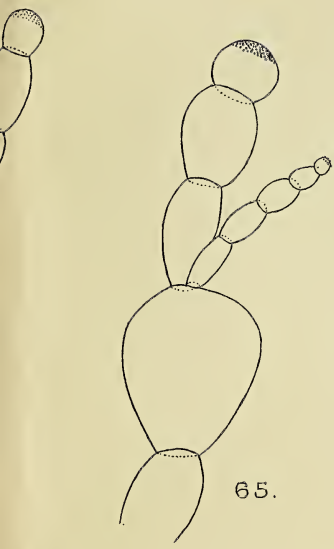

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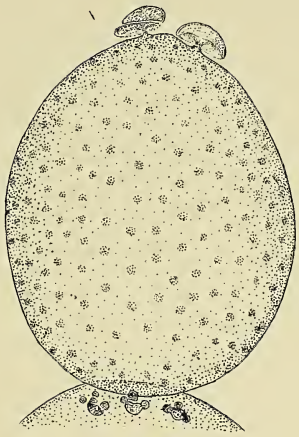

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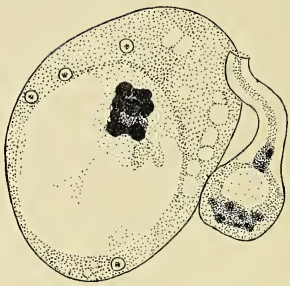

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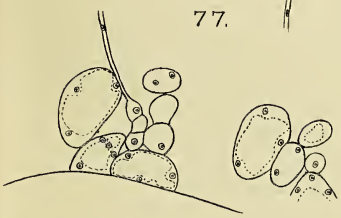

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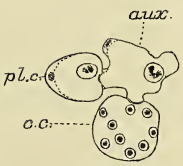

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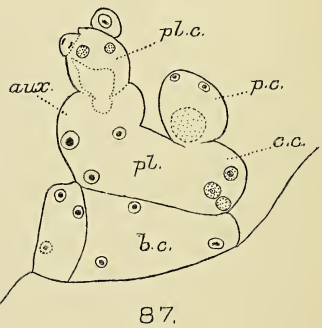

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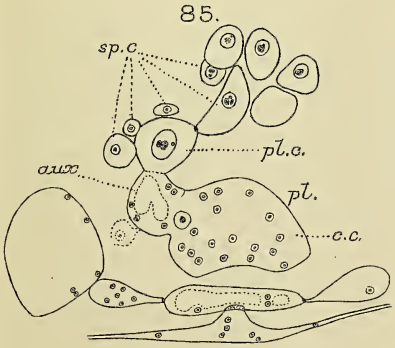

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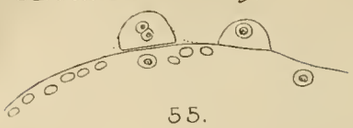
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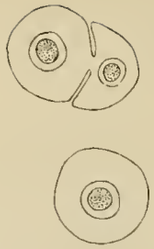
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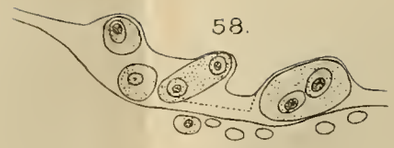
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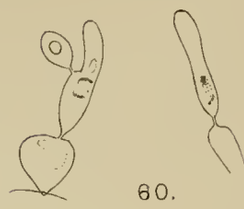
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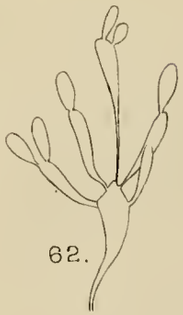
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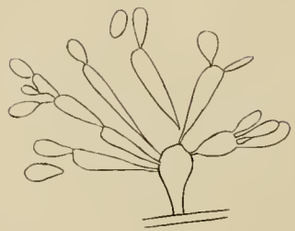
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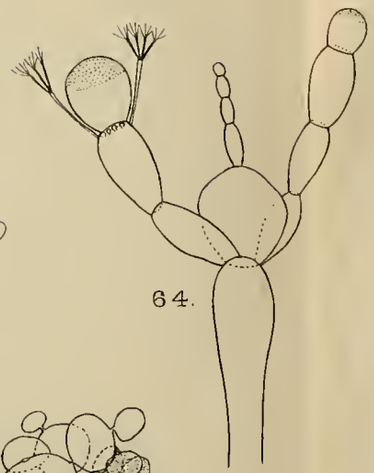
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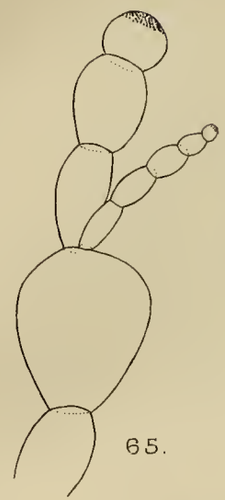
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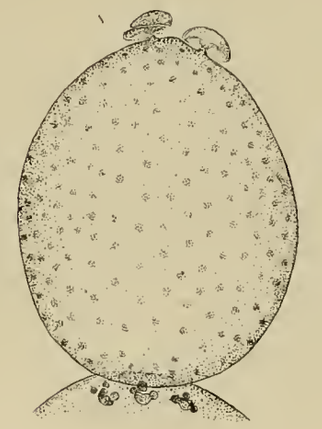
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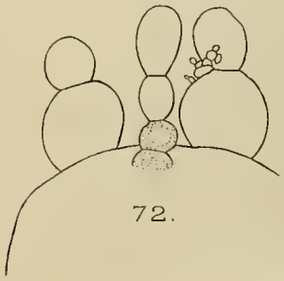
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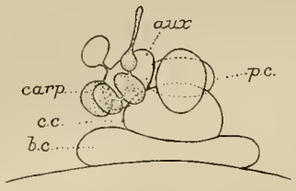

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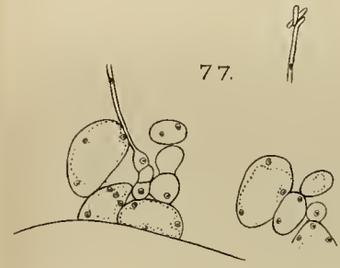
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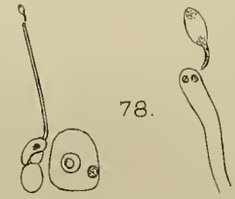
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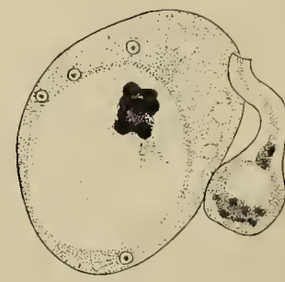
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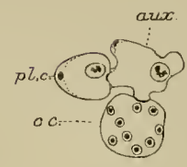

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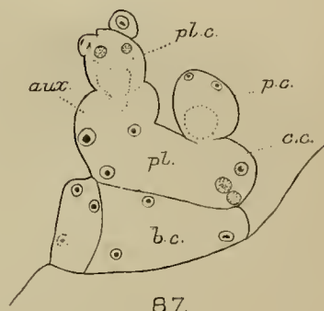

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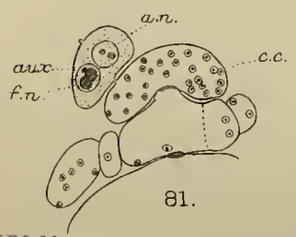

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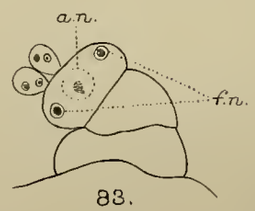

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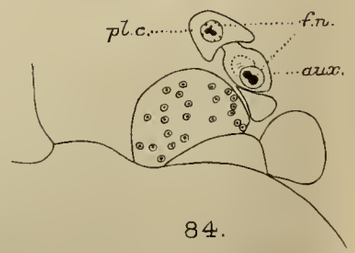

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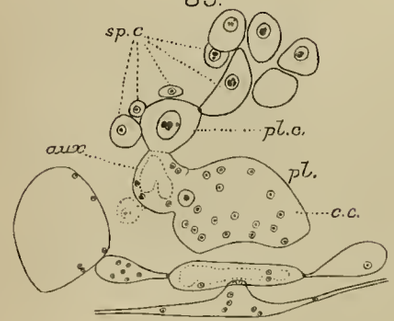

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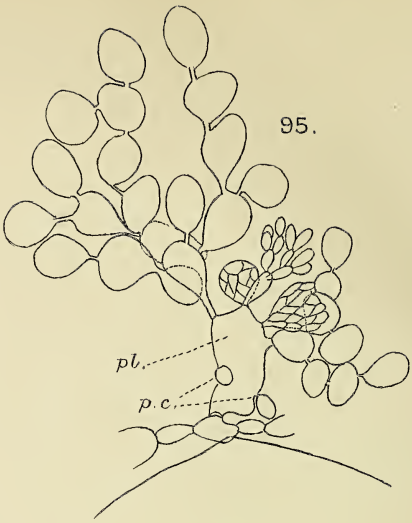

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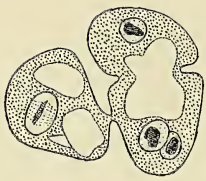

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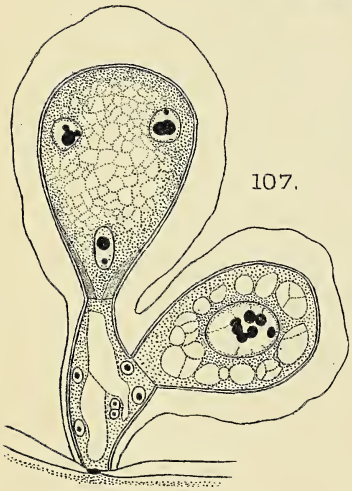

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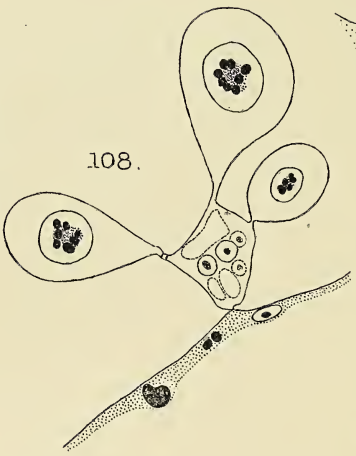

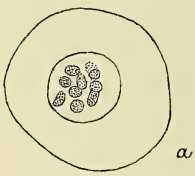

a

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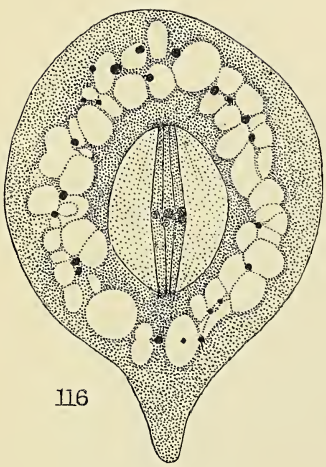

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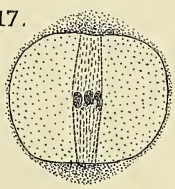

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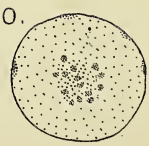

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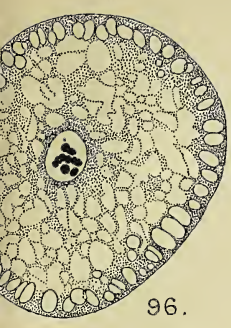
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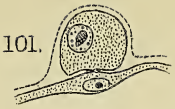
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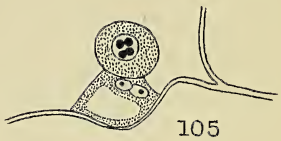
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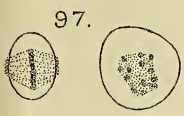
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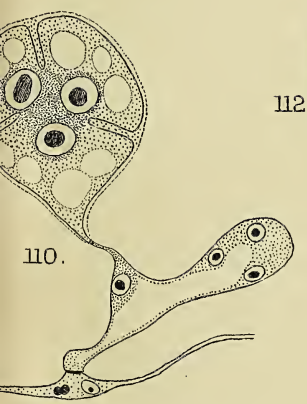
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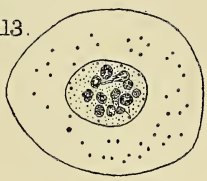
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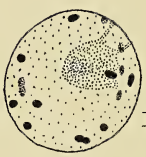
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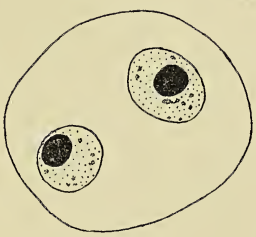
114 a


b


a b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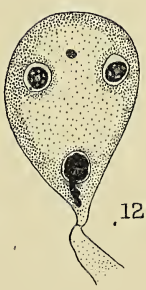
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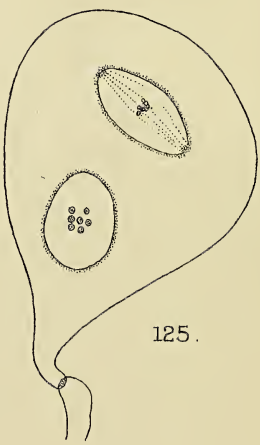
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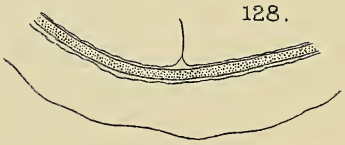
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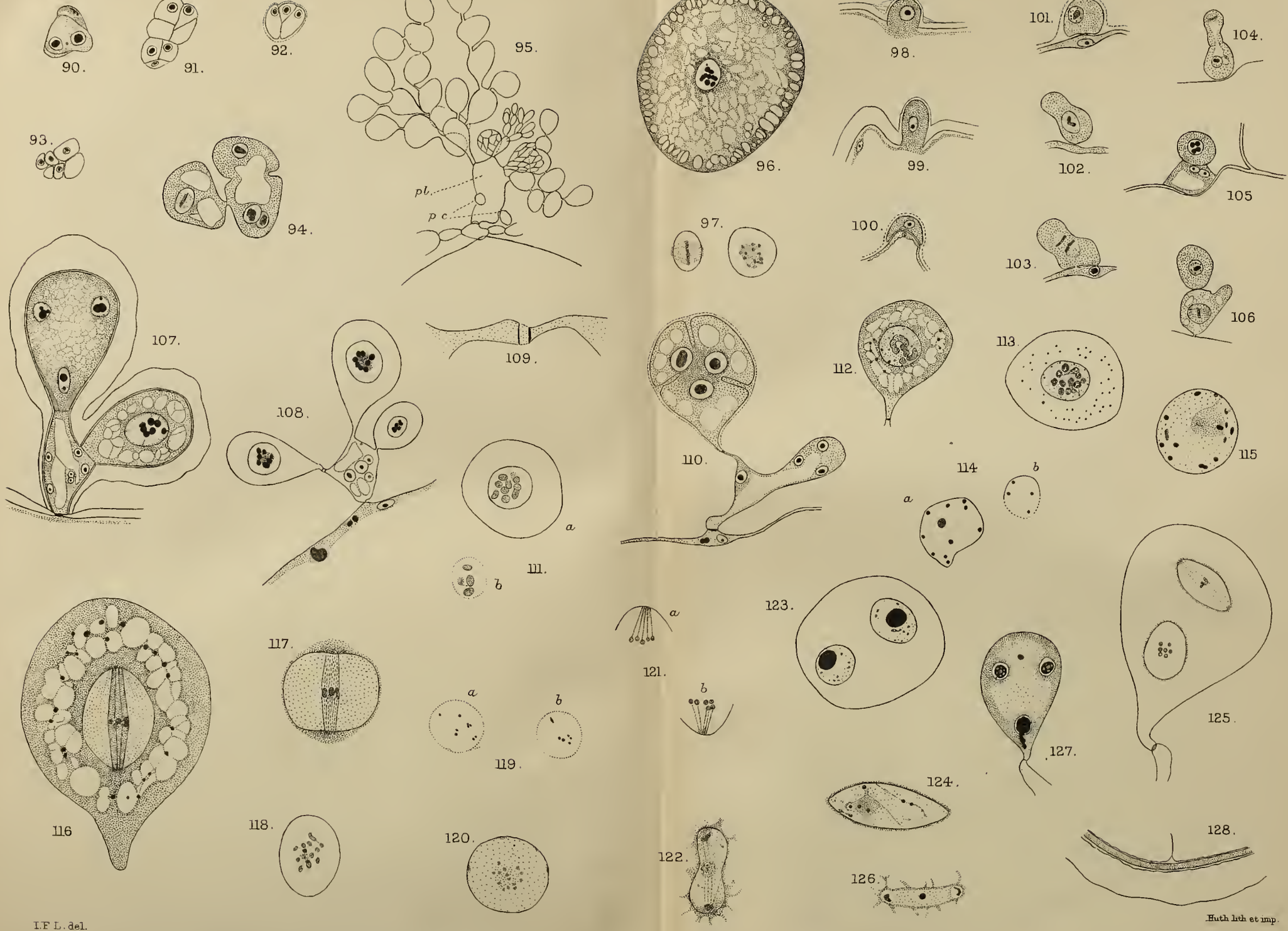

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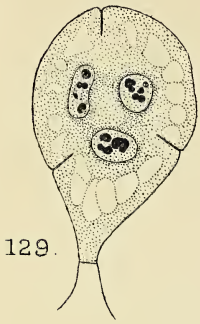
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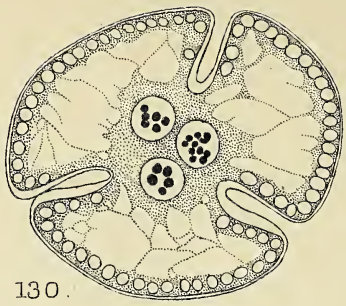
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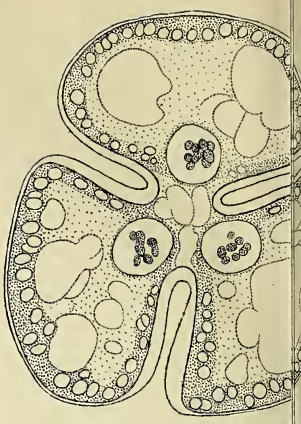
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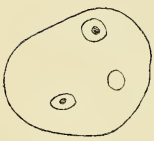
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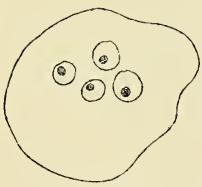

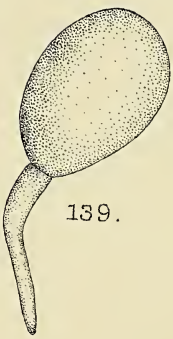
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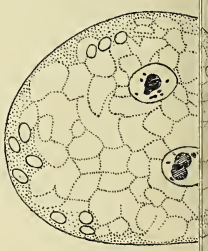
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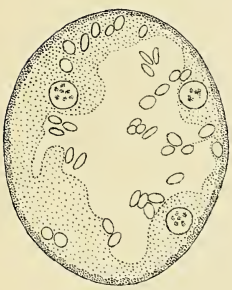
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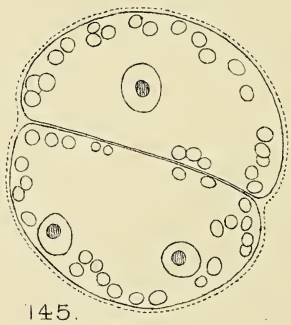
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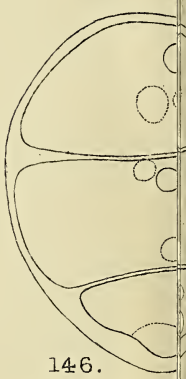
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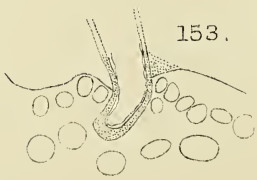
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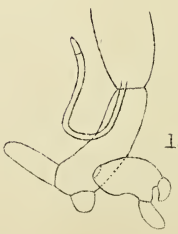
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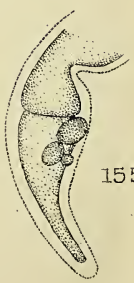
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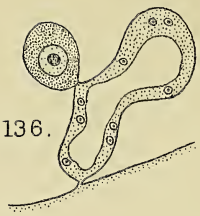
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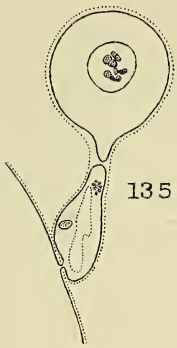
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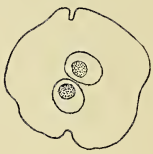

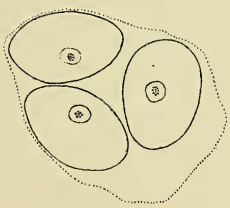
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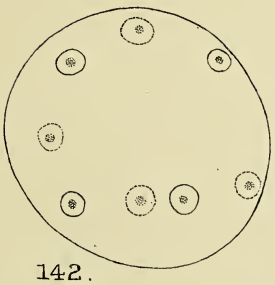
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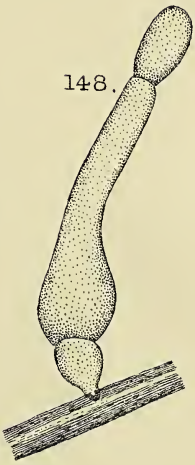
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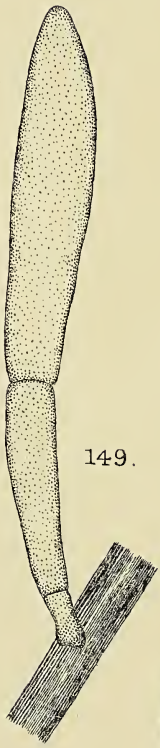
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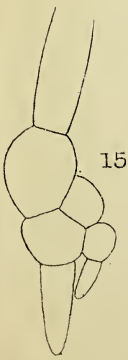
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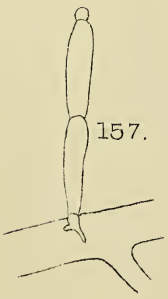
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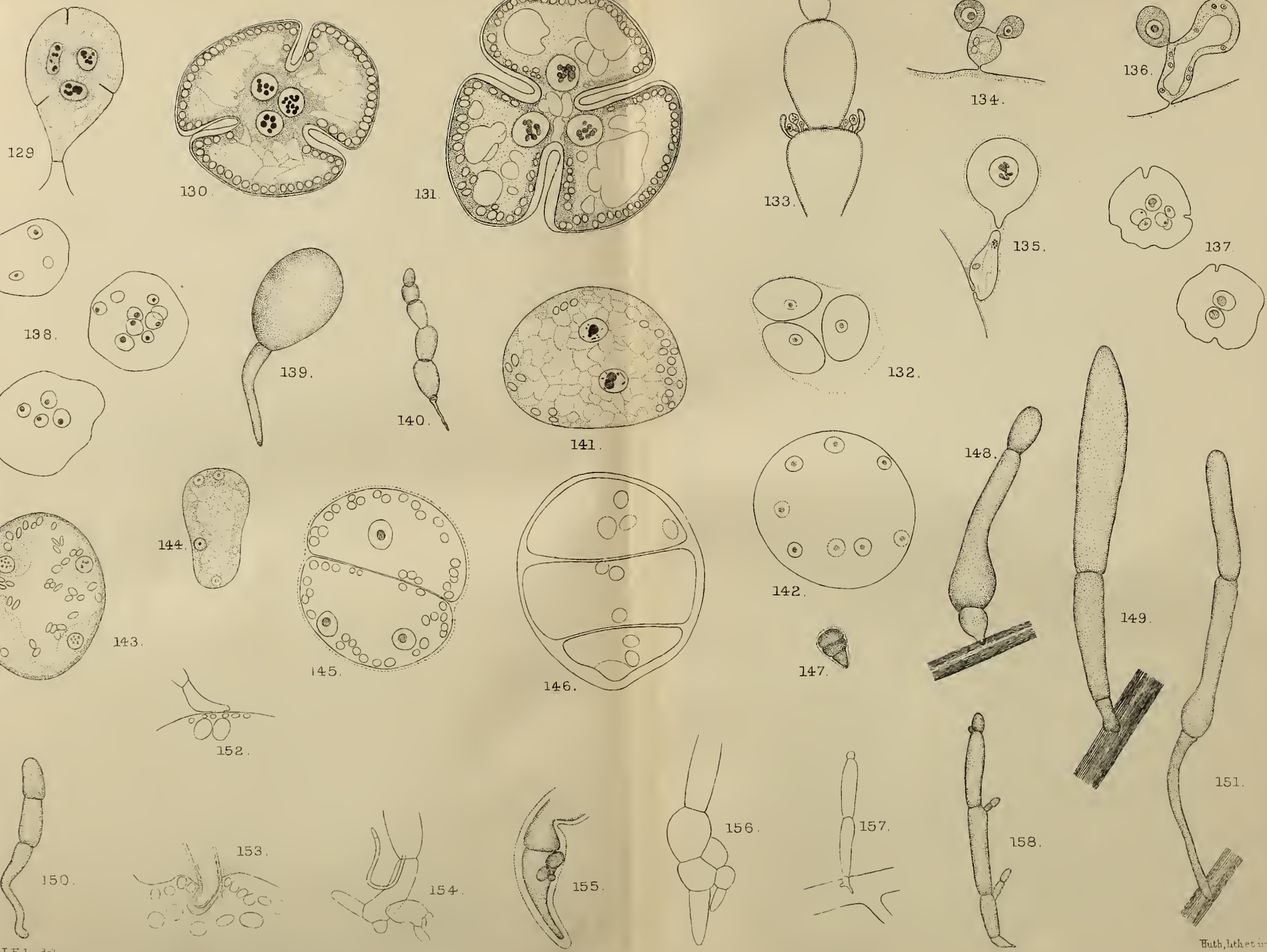

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Huth, lith et imp

LEWIS – GRIFFITHSIA BORNETIANA.

NOTES.

THE PROTHALLIUM AND EMBRYO OF DANAEA.—PRELIMINARY NOTE.—In July, 1908, a fine series of prothallia and young plants of *Danaea* was secured in Jamaica. The greater number of specimens belonged to *D. Jenmanii*, Underw., but there was a good series of *D. elliptica*, Sm., and a smaller number of specimens of *D. jamaicensis*, Underw. The latter were collected at Morce's Gap, the others in the vicinity of Vinegar Hill, both stations being a few miles distant from the Cinchona Botanical Garden.

The prothallia differed from those of *D. simplicifolia*, Rudge, described by Brebner (On the Prothallus and Embryo of *Danaea simplicifolia*, Rudge, Ann. of Bot., x, 1896, p. 109), in their much larger size and elongated form. Most of the larger specimens, which reached a length of twenty-five mm., were several times longer than wide, and often the posterior end was much attenuated and quite thin, the archegonial cushion not extending into it. Forked prothallia were also found, and one specimen of *D. Jenmanii* had four archegonial cushions.

The margin of the prothallium is often deeply lobed like that of *Osmunda* or *Gleichenia*. The rhizoids, as described by Brebner for *D. simplicifolia*, are multicellular.

Archegonia and antheridia resemble in form those of the other Marattiaceae, but the former are remarkable for the imperfect development of the ventral canal-cell, which in many cases could not be demonstrated at all.

The embryo becomes elongated in the dirction of the archegonium-axis before division, and at this time resembles that of *Botrychium obliquum*. The first division-wall is transverse, as in other Marattiaceae, but the hypobasal cell either does not divide at all or divides only once and forms a short suspensor, all the organs of the embryo arising from the inner or epibasal cell. The latter undergoes a somewhat irregular quadrant division, the two lower quadrants forming the foot, the upper two giving rise to the stem-apex, the leaf, and later the root.

A single large apical cell can usually be demonstrated in the stem-apex at a very early period. No single initial is present in the young leaf, which does not appear to be always formed at the same point.

No trace of the root can be made out until the embryo has reached a considerable size. The root is strictly endogenous in origin and its single initial cell arises nearly in the centre of the embryo, probably from the stem-quadrant. With the elongation of the root downward, it carries with it the foot, which covers the growing point of the root like a root-cap.

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June, 1909.

FURTHER OBSERVATIONS ON THE STRUCTURE OF THE OVULES OF MYRICACEAE AND ALLIED GROUPS.—In the last number of this JOURNAL I described the structure and development of the ovule of *Myrica Gale*, drawing special attention to two characters in the structure of the ovule—namely, the free character of the nucellus and the vascular supply to the integument. These were suggested to be possibly ancestral characters retained, and a comparison was made with similar characters in fossil seeds of the *Trigonocarpus* type.

Since then I have had opportunity of examining ovules of another species of *Myrica—Myrica Faya*, and also find the two characters well developed there. An almost identical case is found in the ovules of the allied order Juglandaceae. The ovule is orthotropous as in the Myricaceae, the nucellus stands freely within the single integument, and there is a well-developed integumentary vascular system, which becomes quite obvious to the naked eye in the ripe seed.

Extending this investigation to ovules of the Amentiferae, I find that these characters prevail in many of the genera. The ovules of *Fagus sylvatica* possess a free nucellus and a fairly well-developed integumentary vascular system. The same characters are found in *Carpinus Betulus*. The ovules of *Quercus Robur*, *Corylus Avellana*, *Castanea vesca* and *Alnus glutinosa* also possess an integumentary vascular system. This is figured and described by Lubbock[1] for *Quercus* and *Corylus*, but at that time there could be no suggestion of the possible phylogenetic value of the character. I cannot state with certainty how far the nucellus is free from the integument in these three genera, until more careful observations have been made on their ovules in various stages of growth. The occurrence of these 'ancestral' characters, together with the chalazogamy and other features observed by Miss Benson[2] in these same genera and regarded by her as primitive, seems to emphasize the primitive nature of this group. The exact value of the characters in determining the position of the Amentiferae in the whole group of Angiosperms, can scarcely be estimated until more comparative work on the ovules of allied groups is completed.

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[1] Lubbock, Seedlings, vol. i, p. 71, 1892.

[2] Benson, Contributions to the Embryology of the Amentiferae. Trans. Linnean Soc., London, vol. iii, part 10, 1894.

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