ANNALS OF BOTANY

VOL. XIX

Oxford
PRINTED AT THE CLARENDON PRESS
BY HORACE HART, M.A.
PRINTER TO THE UNIVERSITY

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EDITED BY

ISAAC BAYLEY BALFOUR, M.A., M.D., F.R.S.

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

D. H. SCOTT, M.A., PH.D., F.R.S.

HONORARY KEEPER OF THE JODRELL LABORATORY, ROYAL BOTANIC GARDENS, KEW

AND

WILLIAM GILSON FARLOW, M.D.

PROFESSOR OF CRYPTOGAMIC BOTANY IN HARVARD UNIVERSITY, CAMBRIDGE, MASS., U.S.A.

ASSISTED BY OTHER BOTANISTS

VOLUME XIX

With Thirty-three Plates and Seventeen Figures in the Text

London

HENRY FROWDE, M.A., AMEN CORNER, E.C.

OXFORD: CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1905

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Vol. XIX. No. LXXIII. January, 1905. Price 14s.

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Recent Researches on the Parasitism of Fungi[1].

BY

H. MARSHALL WARD, Sc.D. CANTAB.; HON. D.Sc. VICTORIA.

President of the Cambridge Philosophical Society; Fellow of Sidney Sussex College, and Hon. Fellow of Christ's College, Cambridge; and Professor of Botany in the University.

INTRODUCTORY.

THE definition of 'recent' must always be relative, and in the present connexion it refers to very late times indeed: times not only within the period of the lives of all of us, but in the recollection of most of us. For it should not be forgotten that we have not yet reached the jubilee of the germ-theory of disease, or that of modern pathology and mycology.

Even if we take as our starting-point the period of Cohn's classical work (48) on *Empusa Muscae*, 1855, and of A. Braun's monograph (35) on *Chytridium*, 1855; of De Bary's Untersuchungen ü. d. Brandpilze (4), 1853, and of Tulasne's splendid Mémoires sur les Ustilaginées comp. aux Urédinées (184), 1847 and 1853—a period rich in mycological investigations—we are still only just at the jubilee of the foundation of the modern anatomical school of mycology.

It was not until De Bary's supreme classic, Recherches sur le développement de quelques champignons parasites (5), in 1863, followed by his Morphologie u. Physiologie d. Pilze, &c. (7), in 1866, that the foundation of the parasitism of Fungi, as now understood, was established, so that I am well within the limits of historical truth in insisting on the fact that we have not yet arrived at the jubilee of the demonstration of parasitism and infection on a scientific basis.

As regards bacteriology, events are even more recent, for I think I am right in dating the whole of the modern germ-theory of disease from 1876, the date of Cohn's publication of Koch's paper on Anthrax (99), though, as we shall see, the way had been paved here also by the indefatigable labours of earlier botanical investigators.

But these were merely the foundations. No consistent doctrine of the pathology of plants, a branch of science which has now grown to enormous

[1] An address delivered before Section K, British Association, at Cambridge, on August 22, 1904.

[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

dimensions, with libraries and laboratories of its own, was possible until they were securely laid, as the following historical sketch will show.

Mycology.

Prior to the publication, in 1857, of Berkeley's Introduction to Cryptogamic Botany (28), which we may regard as the last important pre-Darwinian work on Fungi, comparatively little had been done with these plants beyond collection and classification—into the service of which figures of extraordinary merit had been pressed—and what system existed was rapidly degenerating into chaos.

But much of the little that had been done was good. Stimulated by the activity in other departments of microscopy, and by the rapid improvements made in optical appliances, as also by the increasing conviction that the history of development alone could save the situation, numerous observations on the mycelia, sclerotia, sporophores, and fructifications of both lower and higher Fungi were being made by workers of the stamp of Corda (50), Henfrey (82), Berkeley (28), Leveille (104), Bonorden (31), and others, including Sachs, in his early paper on *Crucibulum* (148), in 1855.

Then came the immediate and rapid crisis which culminated in the revolution for mycology, as for other branches of cryptogamic botany, in the publication of De Bary's Morphologie und Physiologie der Pilze, Flechten und Myxomyceten (7), in 1866.

Among the pre-Darwinian works it is impossible to overlook Tulasne's papers in the Annales des Sci. nat. on *Elaphomyces* (177), 1841, *Sclerodermа* (179), *Polysaccum* and *Geaster* (181), 1842, on *Onygena* (182), and on *Nidularia* (183), 1844, *Secotium* (178), 1845, various Lichens (180), in 1851–3, and especially the papers on Ergot (176), and on Uredineae, Ustigalineae (184), and Tremellini (174), in 1847, 1853, and 1854; leading to the Fungi Hypogeai (175), in 1851, and culminating, in 1861–5, in the Selecta Fungorum Carpologia (173), works of supreme beauty and undying reputation. With some exceptions, chiefly concerned with the germination of spores, the work of the Tulasnes was still anatomical, however, and it must never be forgotten that the methods of anatomy and comparative morphology alone are not sufficient for the elucidation of the life-history of small organisms. Striking as many of Tulasne's conclusions were, and especially in the later works in which much was done to establish the polymorphism of the Fungi, it can never be overlooked that the mere juxtaposition of two forms of Fungi growing together may readily lead to, and often did lead to error.

The clear recognition of this, and the stricter adherence to the method of continuous observation under the microscope, checked by experimental work, are the characteristics by which the three greatest men in this connexion are to be marked—viz. Cohn, Pringsheim, and especially De Bary.

Indeed Cohn's papers on *Pilobolus* (42), 1852, *Chytridium* (43), 1855–6, *Empusa* (48), 1855, and Über Pilze als Tierkrankheiten (47), in 1854; Pringsheim's on *Achlya* (137), in 1857; and De Bary's on *Achlya* (13), 1852, *Aspergillus* (14), 1854, and Myxomycetes (11), 1858, in the recently founded Botanische Zeitung, and above all, his Untersuchungen ü. d. Brandpilze (4), 1853, laid a foundation for an edifice of philosophical science which has been building ever since, and becoming statelier and statelier as it grows.

Darwin's Origin of Species (54), which appeared in 1859, of course deals in no way especially with Fungi, but we must note that it gave impetus in a marked degree to such work as I have just referred to, because in no branch of investigation had the problems of evolution and of species come more prominently into the foreground; and the fullest acceptance of the developmental method and of the theory of evolution was already evident in De Bary's Morphologie und Physiologie, &c. (10), which came out in 1866, i. e. seven years after the Origin of Species.

In this classical work De Bary not only brought together the results of his discoveries on the germination of spores and the proofs of direct infection by means of germ tubes in Peronosporeae, Uredineae, &c. (first given (5) in the Ann. des Sc. Nat., 1863), but he also generalized on the real significance of parasites on and in plants and insects, &c., and rendered clear the distinction between saprophytes and parasites.

De Bary also brought together the then recently discovered facts concerning sexual reproduction in the Fungi; and although some of his conclusions have not withstood the test of time, no investigator can afford to overlook the importance of his ideas.

Of equal, or even greater, importance to the development of the subject was his chapter on so-called pleomorphy and alternation of generations in Fungi. Tulasne had (176), in 1851, called attention to the fact (anticipated to some extent by Corda (51)) that one and the same species of Fungus may produce a succession of two or more totally different kinds of spores and of spore-bearing apparatus; and had thus wiped out at one blow, as it were, a number of genera of Fungi which were nothing more than descriptions of a Fungus in one particular stage of its development, which same Fungus in another stage (when bearing one of its other kinds of spore) had hitherto been described as belonging to some other genus. But Tulasne's proofs, as was usual with him, depended chiefly on observation of the contiguity of forms. De Bary, however, proved by direct sowing of the Fungus, and observation of its development, that a particular spore produced a mycelium which then bore the second form of spore; and thus showed how to criticize the numerous reckless statements of careless observers which were rampant then and soon afterwards.

But the most striking of all De Bary's numerous striking discoveries was his proof of heteroecism in *Puccinia graminis*, first recorded in his paper

'Neue Untersuchungen über die Uredineen,' &c. (9), in the Ber. d. Berl. Akad., 1865, and referred to in the present book in a modest paragraph on p. 221.

The germ of yet another discovery, destined like the last-named to have extensive consequences, first saw the light on p. 291 of this remarkable book, namely, the view that Lichens are not autonomous plants, but consist of a Fungus and an Alga associated in a peculiar way and living a life in common. It is true that this idea was only generalized, so as to include all Lichens, two years later, in 1868, by Schwendener (160), and did not emerge triumphantly from the storm of controversy which it evoked until considerably later, after Bornet (32), Famintzin and Baranetzki (68), 1867 and 1868, Treub (171), 1873, and Stahl (166), 1877, and others had isolated and cultivated the gonidia, and synthetically reproduced a Lichen by allowing the Alga and Fungus to come together; and after De Bary in 1879 had himself definitely launched the doctrine of symbiosis (17), and strengthened his argument by numerous other cases.

Enough has now been said to show you how firmly established are the claims of De Bary's classical book to fame, and I pass to a short review of some of the chief fruits of his inspiration.

A rapid development of the new mycology, founded on the rigid application of the study of development of Fungi, at once followed. De Bary himself, and his collaborator Woronin, produced a rich harvest of discoveries published in their Beiträge zur Morphologie und Physiologie der Pilze (18), begun in 1864; and in 1884 we had from the hands of the master the Comparative Morphologie und Biologie der Pilze, &c. (67), in which he developed his ideas on the great series of Ascomycetes and their allies, on the one hand, and of the Basidiomycetes and certain smaller groups on the other. These were based on his comprehensive account of *Pythium* and the Peronosporeae in the Beiträge (18) for 1881, and in the Botanische Zeitung (21) of the same year, where he established the doctrine of gradual inception and accomplishment of apogamy—the abandonment of the sexual process—in the group. The researches of Pringsheim on *Achlya* (138), 1882; of Van Tieghem (167), Bainier (1), and Le Monnier, in 1873–5 and 1883; and of Nowakowski (122), and Schröter on Chytridiaceae (159), 1876–8, and others also furnished materials for a comprehensive survey of the Phycomycetes as a whole, and from this date onwards the definite establishment of this alliance of Fungi was assured.

In the Ascomycetes proper, De Bary's celebrated contention for sexuality was argued at length, on data derived from his own work (6) on *Eurotium* and *Erysiphe*, &c., in 1870, and from numerous papers by his pupils and others, among which those of Van Tieghem (169), 1875–7, of Kihlmann on *Pyronema* (89), 1883, and of Janczewski on *Ascobolus* (88), 1871, are important. The extraordinary pleomorphism of the group is also set forth in considerable detail.

The Uredineae, a special subject of De Bary's, come in for exhaustive treatment; his further researches on Witches' Brooms (15), 1867, and *Aecidium abietinum* (12), 1879, as well as investigations by Schröter (154), 1869–75, Wolff on *Aecidium* (*Peridermium*) *Pini* (203), 1876, and others, having yielded several other cases of heteroecism as well as materials for more definite lines of classification.

Then follows an excellent discussion of that difficult and enormous group, the Basidiomycetes, the materials for which were still chiefly anatomical. Although Hesse (83) had succeeded in germinating the spores of *Cyathus* in 1876, and Eidam (59) those of other Nidularieae in the same year, and Woronin (207) had traced the whole life history of *Exobasidium* in 1867, and although a considerable amount of developmental work had been done by Brefeld (36) on *Coprinus* in 1879, and on *Agaricus melleus* (78) by Hartig in 1874, nevertheless the materials for full treatment were scanty; and De Bary's comparison of the teleutospores of the tremelloid Uredineae with the basidiospores of the Tremellineae proper—a comparison which bore abundant fruit later at the hands of Brefeld—as well as his able comparative treatment of the Gastromycetes, are evidence of the keen morphological insight he possessed.

Bacteriology.

Ehrenberg (58) in 1838 described a considerable number of minute motile organisms which he regarded as animalcules and called *Vibrionia*, some of which had been observed as long ago as 1683 by Leeuwenhoek (103), and again by Müller (114), who classified them in 1786 under the names *Vibrio*, *Spirillum*, and *Bacillus*. Further attention was devoted to these apparently unimportant, though interesting living particles by Dujardin (57) in 1841, and by Perty (127) in 1852; and it was about this period that the attention of microscopists was first attracted to the organisms in drinking water as possibly having something to do with an epidemic of cholera; indeed, the suspicion gained ground that the micro-organisms of sewage-contamination, &c., were of vital importance from the sanitary point of view. Hassal (80), in 1850, gave an account of these organisms in London water, and in 1852 and 1866 Cohn (42) made a very thorough and, for the period, wonderfully exhaustive investigation of certain of these organisms, thus starting a subject which soon grew into a special study.

Meanwhile, Pasteur (124) had published the results of his investigations on lactic, butyric, and other fermentations; and in 1866, as we have seen, De Bary's classical Morphologie und Physiologie der Pilze (10) appeared, in which he was able to summarize what was then known of the ferment and putrefactive organisms, as well as his own discoveries on the parasitism of Fungi, in which he had definitely demonstrated the fact of infection

by means of spores. We thus see that the period was ripening for great things, though little did the investigators of those days foresee what startling events were ahead of them.

In 1870–6 Cohn (44) brought forward that splendid series of papers which will always establish his fame as the founder of bacteriology. Not only did he describe with marvellous accuracy, considering the technique of his day, a considerable number of Bacteria; but he made observations on their movements, spore-formation, cell-contents and life-history, which put them at once on a definite footing as organisms to be classified and dealt with as were other plants, conclusions by no means so obvious then as now. Not only did Cohn establish the plant-nature of Bacteria, however, but he gave (45), in 1875, a scheme of classification of the various genera which has had to be reckoned with ever since, although the numbers of species or forms now known vastly outnumber the score or so known to Cohn in 1875; as may be seen by comparing Winter's list (139) of sixty-nine species in 1884 with Migula's estimate (111) of nearly 1,000 in 1896. Moreover, the definitions and characters of genera and species have undergone great changes, as is readily seen on comparing the principal schemes of classification proposed by successive authorities, e.g. Winter (139), 1884, Van Tieghem (168), 1884, Zopf (209) in 1885, Flügge (73), De Bary (19), and Hueppe (86) in 1886, De Toni and Trevisan (170) in 1889, Fischer (70) in 1897, and Migula (111) in 1896, each of whom has attempted to include the general results of the various and rapidly following discoveries concerning the general morphology, pigments, cilia, spore-formation and germination, mode of growth and division, and putrefactive, pathogenic, and other physiological properties of these organisms.

In 1876 Cohn was enabled to introduce to a startled and incredulous world a practical result of the patient and faithful study of development introduced so successfully by De Bary, Pringsheim, and himself, which, taken together with De Bary's proof of the infection of plants by the germ-tubes of parasitic Fungi in 1863, may be looked upon as the foundation of the parasitic or germ-theory of disease. This was Robert Koch's paper on Die Aetiologie der Milzbrandkrankheit (99), in which he gave the principal phases of the life-history of *Bacillus anthracis* inside and outside the animal body, and thus started that important series of researches which, together with Pasteur's work and the labours of numerous pupils, have brought such maladies as anthrax, tetanus, diphtheria, tubercle, and several other infectious or contagious diseases out of the region of mystery into the clear light of science.

It is of course beyond the scope of this address to enter into the numerous collateral branches of successful research to which these results gave the initial impulse; but I may remind you of the important position in any history of the science of the last thirty years which such subjects as

antisepsis, the theory of toxins and vaccination, and other outcomes of these fundamental researches of Cohn and De Bary—to which should be added those of Lister and Pasteur—must always occupy ; and we shall see that these are by no means the only directions in which exact botanical researches have effected far-reaching results on the well-being of mankind.

It is more germane to my immediate subject to refer to another great question which the work of Cohn, De Bary, and Pasteur, brought to a series of tests which finally rid the world of a bugbear.

The idea of spontaneous generation, although it had lost prop after prop, as the conditions of development of the more minute organisms were carefully and conscientiously examined, had for some time apparently secured a stronghold among the Bacteria, from which it seemed impossible to dislodge it.

Cohn's work (49) on the Hay Bacillus, in 1876, during the course of which he observed the formation of the endospores, brought forward very clearly the fact of the resistance of spores to extremes of temperature, and his experiments, together with those of Pasteur on the organisms of the atmosphere (125), 1862, and those of Tyndal (186), 1876, on discontinuous sterilization, laid the spectre of abiogenesis, which had repeatedly arisen at intervals since the time of Van Helmont (81), 1745, and Needham (119), and had recently been revived to great activity by Bastian (25) ; and we should observe that Cohn did not merely devise experiments which showed that a something or other got into a putrescible liquid and fermented it or not according to circumstances, but he showed what that something was—the spore of the Bacillus—and followed its development to complete proof of the relation it bore to the phenomenon.

Yet another question of general importance arose in connexion with Bacteria. Several observers had laid stress on the fact that forms of definite shape, to which generic and specific names had been given by Cohn and others, altered their sizes and shapes during development, and assumed morphological characters which had been used to define other genera and species. Billroth (29), 1874, Nägeli (116), 1887, and Zopf (208), 1882, carried their views so far as to deny, more or less categorically, any constancy of form whatever among these organisms, or even to assert that all the forms were derived from one or a few only. It seems strange to us at this distance of time that so much research should have been necessary to prove that the so-called pleomorphism of the Bacteria was merely due to the species in question having been described and named by different observers who saw them in different stages of development. Exactly similar conditions of affairs would arise if an Oak seedling was named by a man who knew not the tree, and the latter by another who did not know it was developed from the former.

Plant-Pathology.

Our present extensive knowledge of the pathology of plants has been developed chiefly from the study of two branches of botany, viz. the physiology of nutrition and the parasitism of the Fungi, and dates from that active period of research at the zenith of which appeared Kühn's Die Krankheiten der Kulturgewächse (100), 1858, and De Bary's Morphologie u. Physiologie der Pilze, &c. (10), 1866, on the one hand, and Sachs's Experimental Physiology (149), 1865, on the other.

True, general treatises and papers on the diseases of plants had been written before these dates, of which Berkeley's papers on Vegetable Pathology (27), in 1854, and the works of Unger (187), 1833, Wiegmann (200), 1839, Meyen (110), 1841, and Ratzeburg (140), 1839 and 1866, may be especially mentioned ; nor would full justice be done to the subject without reference to numerous publications on agriculture and forestry which appeared before 1866. Most of these earlier efforts suffered from two prominent deficiencies, viz. a lack of understanding of the meaning and significance of parasitism, and of comprehension of the fact that pathology is abnormal physiology. De Bary first rendered clear the former, and Sachs was the pioneer who cleared the way to the latter.

During the period intervening between then and now, a considerable number of important treatises have appeared, of which the following may be deemed to have exerted the greatest influence on what is now a very special branch of knowledge :—

Hartig, R., Wichtige Krankheiten d. Waldbäume (78), 1874 ; Die Zersetzungserscheinungen d. Hölzer (79), 1878 ; Frank, Krankheiten d. Pflanzen (74), 1882 (and 2nd ed. 1894-6) ; Hartig, R., Lehrbuch d. Baumkrankheiten (77), 1882 (2nd ed. 1889) ; Sorauer, Handbuch d. Pflanzenkrankheiten (164) (2nd ed. 1886) ; Kirchner, Die Krankheiten, &c., unserer landwirtschaftlichen Kulturpflanzen (90), 1890; Prillieux, Maladies d. plantes agricoles, &c. (136), 1895 ; and Tubeuf, Pflanzenkrankheiten (172), 1894.

Terminology.

Perhaps the most striking summary of the effects of the half-century of work glanced at above is afforded by the number of new terms, or of terms with new connotations, which have been introduced into Botany. Here I have only time to advert to a few, but the following brief list will serve to remind you of a whole dictionary of names of new ideas. Antiseptic. Biologic forms. Toxins and anti-toxins. Form-species. Heteroecism. Infection-tube. Infection. Separation-cultures. Immunity. Spraying. Germ-tube. Adaptive parasites. Specialized parasitism. Bridging species. Pure cultures. Susceptibility. Area of infection.

Wound-parasites. Sub-cultures. Facultative and obligate parasitism. Saprophyte. Incubation-period. Symbiosis. Stomatal infection. Abiogenesis. Cuticular infection. Fermentation. Predisposition. Enzyme. Capacity of infection. Prototrophic, metatrophic, and autotrophic. Haustoria. Appressorium. Pleomorphism. Inoculation. Chemotaxis. Positive and negative chemotropism. Infection vesicle. Adaptability. Intra- and inter-cellular mycelium. Mycoplasm. Pleophagism. Mycorhiza. Endo- and exo-trophic. Lipoxeny.

Uredineae.

It is now time to pay some regard to the practical limits of my subject, imposed alike by the time at command, and by the patience of my hearers. It would be quite impossible in this address to cover the whole of the ground implied in the parasitism of the Fungi, and I therefore propose to deal more especially with the group in which the most important victories in our struggles for new knowledge of parasitism have been won. This group is the Uredineae, a group which has become especially interesting, not only on account of the great tax its ravages have imposed on our corn and other cereal crops, but because it is the focus of the classic work done by De Bary, referred to above, and of the important discoveries since made, matters which I propose to deal with below.

Heteroecism.

Almost simultaneously with De Bary's classical researches (9) on *Puccinia graminis*, *P.* '*Rubigo-vera*,' and *P.* '*coronata*,' in 1864–5, where he showed that the latter is heteroecious on *Rhamnus*, as is the former on *Berberis*, Oersted (123) proved that the so-called *Roestelia* on Pomaceae is merely the aecidium-form of the *Gymnosporangia* of Junipers.

Thereupon followed a period of busy seeking after alternate hosts, with such success that by 1870 as many as seven, and by 1880 fourteen well-established cases had been accepted, chiefly the results of investigations by Magnus (106), 1874, Schröter (156), 1875, Wolff (205), 1874 and 1877, Rostrup (143), 1874, Winter (202), 1874–5, Nielsen (121), 1877, Reichardt (141), 1877, and De Bary (9): of these the most interesting was probably Wolff's demonstration that *Peridermium Pini* was merely the aecidium of *Coleosporium Senecionis*.

By 1890 the number of cases of heteroecism had amounted to fifty, of which Hartig's (78) work on *Calyptospora* deserves to be especially mentioned: you will remember that the remarkable *Calyptospora Goeppertianum* on *Vaccinium* was here shown to be the puccinia-form of *Aecidium columnare* of the Silver Fir.

The number of demonstrated cases of heteroecism now known is about

150, of which the long and hitherto vain search for the alternate form and host of *Aecidium Elatinum*, the Fungus of the Witches' Broom of the Silver Fir, and which Fischer (69), 1902, has shown to be *Melampsorella Caryophyllacearum*, is surely the most interesting.

But another interesting aspect of this fascinating theme was the discovery that heteroecism is not confined to the Uredineae, for Nawaschin and Woronin (117), 1896, have shown that *Sclerotinia heteroica*, an Ascomycete, alternates between *Ledum* and *Vaccinium*.

Equally interesting is the result that several animal parasites, especially certain Vermes, Arthropoda, and Protozoa, are heteroecious on two or even three hosts. An interesting example is that of the insects producing Galls on the Spruce and Larch; but as Mr. Burdon, who has for some time been working at this subject in our laboratory, will give you an account of these phenomena, I pass them by with this reference.

Cycle of Life-History, and Classification.

Most species of *Uromyces*, *Puccinia*, *Melampsora*, *Pucciniastrum*, &c., form teleutospores which germinate only after resting through the winter, and are heteroecious; but there are many cases—e. g. *Endophyllum*, *Puccinia Asparagi*, *P. malvacearum*, *Uromyces appendiculatus*, &c.—where these spores germinate during the summer or autumn of their development and are autoecious.

Moreover, *Endophyllum* and others only form teleutospores, and have a perennial mycelium. A perennial mycelium also occurs in *Gymnosporangium* and others.

In the cases of *Chrysomyxa Rhododendri* and *C. Ledi*, again, we find that the mycelium remains alive in the leaves throughout the winter, and develops teleutospores in the spring, and these germinate forthwith.

In *Cronartium* and *Coleosporium*, on the other hand, it is the aecidial mycelium which is thus perennial; while in *Melampsorella* both the teleutospore- and the aecidium-mycelia are perennial.

An important step forward, after the discovery of heteroecism, was made by Schröter in 1879 (155), in proposing as a basis of classification within the genera *Puccinia*, *Uromyces*, &c., the sequence of spores and their behaviour. Schröter suggested that the various groups should be put into sections according to the existence of aecidia or not, according as they were heteroecious or autoecious, and according to the behaviour of the teleutospores, &c.

Taking, for example, *Puccinia* as the largest and best worked genus, Schröter denominates the aecidial-form I; the uredo-form II; and the teleutospore-form III; and groups as follows:—

Eupuccinia, I, II, III, all known.
Pucciniopsis, I, III known, but no uredo.
Brachypuccinia, spermogonium, II, III only known, but no aecidium.
Hemipuccinia, II, III only known ; no aecidium or spermogonium.
Micropuccinia, III only, germinating *after* the winter rest.
Leptopuccinia, III only, but germinating at once.

In like manner the genus *Uromyces* falls into *Eu-uromyces*, *Uromycopsis*, *Brachyuromyces*, &c., and so on with the other forms in so far as they form aecidia or not.

Under each we then have subdivisions according as they are autoecious or heteroecious ; and, further, account may be taken of the spring or the autumn germination of the teleutospores, and of perennial mycelia, &c.

The biological groupings are of course provisional, since at any time we may find the aecidium of a supposed hemi-form—as has recently occurred with *Melampsorella Caryophyllacearum* (see p. 10). As another example of like kind I may mention my proof in 1881 of the existence of teleutospores in *Hemileia*, the Fungus of the Coffee leaf-disease.

There can be no doubt that Schröter's ideas, extended by Dietel, Klebahn, and Brefeld, saved the situation, which was rapidly degenerating into chaos.

Sexuality.

It is impossible for me to go into the alleged sexuality of the Uredineae, or into the further details of classification based on spore-formation, &c. But reference ought to be made to Mr. Blackman's (33) summary of the matter, and his own interesting and important histological work, 1904 ; however, as he will himself give you his results, I may here pass over the matter with the mere expression of opinion that Sapin-Trouffy and Dangeard have not established a case sufficiently strong to upset Brefeld's contention that the Uredineae like the Basidiomycetes have no recognized sexual process ; whether Blackman has succeeded in doing so is matter for further investigation, and there can be no question of the importance of his results.

Spore-distribution.

It is astounding how little we actually know of the distribution of the spores of the Uredineae. I refer more particularly to the modes of distribution, and the number of spores conveyed ; and to what distances they may be carried and still remain alive.

I showed clearly enough in 1881 (192) that the uredospores of *Hemileia* are carried by wind, and can be caught on slides smeared with glycerine and exposed in the air ; and the fact that wind is the principal agent of dispersal of such spores cannot be doubted.

That such spores may retain their germinating power for from sixty-

one to ninety-four days at least, has also been shown by Miss Gibson and myself in the case of the Chrysanthemum Rust and of *Puccinia dispersa.*

Klebahn also points out that insects must carry many such spores. The only contribution I can offer here is the fact that flies may have spores attached to their feet, and that a small dipterous larva (*Diplosis*) commonly occurs in large numbers feeding on the uredospores, and itself dusted with them. I found these larvae in abundance on Coffee in Ceylon in 1880–1, yellow with uredospores of *Hemileia*, and have since observed them very commonly on Bromes, and similarly coloured with the uredospores of *Puccinia dispersa.*

Mr. Salmon has shown the same to be true of Erysipheae, the spores of which are eaten with avidity by a *Diplosis* larva, possibly identical with the above, which he has reared and examined in this laboratory.

But there can be little doubt that other insects co-operate in the carrying of spores—e. g. *Aphides.* I commend the subject to any one who will undertake what should be an interesting and definite subject for research.

The Germination of Uredospores.

As is now well known uredospores and aecidiospores usually germinate easily in water ; but it is a common experience that, of those sown, the percentage of spores which germinate freely varies. In some cases, moreover, very few, or even none at all, will be found to have germinated : and Eriksson refers to these phenomena as ' capricious.'

Now there can be little doubt that such a term only implies that some condition or conditions have not been realized in our experiments : at any rate, we have no warrant for supposing that any spore is not amenable to changes of conditions. Indeed we have now gone some way towards proving this.

I often find, as also did Freeman, that thorough drying—such as would result from passage through the air on a high wind—facilitates germination ; and Eriksson showed that chilling to the point of freezing—another way of drying out superfluous water—quickens the germination of such uredospores.

My results also point to the necessity for perfect ripeness of the spores, in order that they may germinate freely : and no doubt water is expelled during maturation.

Klebahn found that spores which germinate badly—as measured by the percentage of germinating spores in those sown, e. g., in a watch glass—may nevertheless infect readily, and Freeman showed something of the same kind.

Uredospores do not germinate freely if plunged under water ; and Evans, working in my laboratory, found that it is often better to have the surface of the leaves merely damp, not thoroughly wet, so that Klebahn

is probably right in saying that germination and infection occur better in dew and mist, alternating with sunshine, than in rain—apart from the fact that spores may be washed off during showers.

But I have evidence to support the generally accepted view that external conditions of other kinds affect the germination of uredospores, as they are known to do that of other spores.

That the temperature is an important factor must be conceded ; but it is surprising how little has been done in this connexion as regards Uredineae. I found (190, p. 269) the optimum for *Puccinia dispersa* to be near 20° C. ; the minimum being below 10°–12° C. ; and the maximum about 27° C. These uredospores failed to germinate at 30° C., and were killed at 65°–70° C. ; though their age and ripeness affect the matter.

As regards age, De Bary found that the uredospores of *P. graminis* lost their capacity for germination in from one to two months.

Klebahn (98, p. 26) was able to infect with spores of *Peridermium Pini* after keeping them five weeks ; and with spores of *P. Strobi* after keeping twenty days.

I found the uredospores of *P. dispersa* germinated after being kept dry for sixty-one days, and the experiments were only closed then for lack of material ; while Miss Gibson, working in my laboratory, kept aecidiospores of *Phragmidium* for fifty-four days, and the uredospores of the Chrysanthemum Rust for ninety-four days, when they still germinated. Again the work had to be stopped, and we do not know how much longer they would have continued to live.

Jacky, also, found the uredospores of the Chrysanthemum Rust alive after keeping them from December 1 to February 5, in gauze bags in the open (87).

I can also give you a few other data, bearing out the same point. Barclay (2, p. 234) gives as the limits of germination-capacity for various species, from two to eight months ; and Bolley (30) says that *P. 'Rubigo-vera'* infected after thirty days' exposure to air and sunshine.

Even in cases where the teleutospores do not germinate until the following spring, it is clear that facts such as the above will have to be reckoned with before any one can deny the possibility of a uredo being carried safely through the winter period.

Further research—and the subject is a good one—will have to decide how far the climate, shelter, lurking in crannies or concealed in dry tufts of herbage, and so forth affect the matter. It is true that perennial mycelia have been sought for in vain in many of the forms here concerned ; nevertheless Magnus states that *P. Caricis* passes the winter in the uredo-stage, and the same is affirmed of others—e.g. by Dietel and Schröter of *Uromyces Junci* and *Puccinia Luzulae*, and by Barclay of *P. coronata*, the uredo of which he found throughout the winter in sheltered places.

Lagerheim (102) found the uredo of *P. Poarum* just after the melting of the snow about it; and Dietel (56) describes two kinds of uredospores in *P. vexans*, the thicker walled of which he regards as resisting the winter.

I myself found germinable uredospores of *P. dispersa* on Bromes during every month throughout the year 1901–2, even in February and March, the least favourable of times.

Plowright in 1882 also found uredospores on *Agropyrum repens* at the end of December and in March, and says that those of *P.* '*Rubigo-vera*' can be got all the year round.

Hitchcock and Carleton (84) found uredo on Wheat all through January, February, and March, and Carleton says that *P.* '*Rubigo-vera*' *Tritici* lives all the year round in the uredo-state as we pass from the Southern to the Northern States; while Bolley confirms this, and asserts, further, that even in the Northern States he finds the germination-capacity preserved through the winter.

Even Eriksson (62, pp. 153–4) admits Nielsen's statement that uredo withstands the winter on green leaves; and other cases given by Kuhn (110), Blomeyer (34), Rostrup (142), McAlpine and Cobb (40), go to swell the evidence that we cannot afford to overlook such facts as the above in discussing the question of the period of infection, or of the limits of germination-capacity of these spores.

Specialized Parasitism.

Before proceeding to consider the details of infection and consequent phenomena, it is necessary to break the thread of our story, as it were, in order to render clear a subject which becomes more and more involved in the cycle of phenomena yet to be described. After the period of research in which investigators had been concerned chiefly with the hunt after new cases of heteroecism, and with the unravelling of the species of Uredineae from the threatening chaos to which their rapidly increasing numbers was leading, the next step of fundamental importance was the discovery of specialized parasitism.

Before going into this question I may remind you that the matter has a history behind it. The older observers, e. g. Persoon, of course named each Fungus on a different host as a distinct species, or even genus, as the words *Aecidium*, *Peridermium*, *Roestelia*; *Puccinia*, *Coleosporium*, *Gymnosporangium*; *Uredo*, *Uromyces*, &c., sufficiently attest.

Then came the controversy regarding polymorphism, and the Tulasnes and De Bary especially did yeoman's service in proving that even heteroecious forms passed through such stages as *Uredo*, *Puccinia*, and *Aecidium*. The next stage was the proof that species like *Puccinia graminis* and *Peridermium Pini*, in the old sense, were really composite forms; and that the aecidia of the different cereal forms were developed on plants other

than the Barberry, and the uredo-stage of the *Peridermium* on hosts other than *Senecio*. Here, again, the investigations passed through several stages of discovery and of controversy, but the upshot was that in the first place *morphologically* different Fungi growing on grasses were extracted from the collective species hitherto named *P. graminis*—e.g. De Candolle's (38) *Uredo Rubigo-vera*, 1815, and Schmidt's (152) *U. glumarum*, 1816, and Corda's (50) *P. coronata*, 1837, began to emerge as definitely morphological species, especially after De Bary (9) in 1866 had shown that *Aecidium Asperifolii* belonged to *P. Rubigo-vera* and *Aecidium Rhamni* to *P. coronata*; and similarly, the various forms of *Gymnosporangium* and *Roestelia* were sifted out, in connexion with various species of *Crataegus*, *Pyrus*, &c. Researches along these lines culminated in the demonstration that, quite apart from the morphologically distinguishable species of rust, there are other forms which, although utterly undistinguishable by the microscope, are nevertheless sharply distinct in respect of their parasitism. Eriksson especially has proved by careful and long-continued experiments that, for instance, the *Puccinia graminis* which grows on Wheat does not attack Rye, Barley, Cocksfoot, *Alopecurus*, *Aira*, *Agrostis*, or *Poa*, if its uredospores are sown on these Grasses. Nevertheless *P. graminis* is common on these hosts. Similarly the uredospores from the *P. graminis* growing on Rye and Barley will not directly infect Wheat or Oats, &c.

Now, since there are no morphological distinctions between these forms of *P. graminis* on different hosts, while there *are* physiological or biological invisible differences, it is clear they must be distinguished. It should be pointed out that De Bary (5), in 1863, had already insisted on the intimate choice of hosts (*choix rigoureux*) of the Rust Fungi for particular cases; and had insisted that the morphological differences between the aecidium-forms of *Chrysomyxa Rhododendri* and *C. Ledi* were so slight that these are 'rather biological than morphological species.'

Schröter, who first called special attention to this matter in 1879 (153, p. 69) in the case of *P. Caricis*, in 1893 (158, p. 31) proposed to term such forms, sister species (*Species sorores*). Rostrup (144, p. 40) in 1894 called them *biologiske Arter*—i.e. biologic species, using practically the same term as Klebahn, who called them *biologische Spezies*, had proposed in 1892 (93, pp. 258 and 332).

Hitchcock and Carleton in 1894 (84) proposed the term *physiological species*.

Eriksson in 1894 (60, p. 292) suggested the term *specialisierte Formen* or *formae speciales* (specialized forms) and has supported his term with much admirable work.

Rostrup in 1896 (145, p. 37) suggested the term biologic races (*biologische Rassen*); while Magnus in 1894 (107, p. 82) proposed *Gewohnheitsrassen* (adapted races) as the preferable term for such forms.

There is one observer who has done excellent service in this connexion, to whom full justice has hardly been done, and the matter appeals to us especially here because he is almost a local man—I refer to Dr. C. B. Plowright of King's Lynn.

Between 1880 and 1900 Dr. Plowright published a large series of papers on the Uredineae, as well as a monograph (129) on the British Uredineae and Ustilagineae, and it is to be noted that this long-sustained and important work (so far as the Uredineae are concerned) was done in the intervals of a busy practice as a medical man.

Not the least of Dr. Plowright's contributions must be reckoned his clear-sighted recognition of a series of forms which are only distinguishable by their biological, and not by their morphological peculiarities. Among others I may mention the *Gymnosporangia*, *Puccinia Phragmitis*, *P. Phalaridis*, and the various forms of *Puccinia* on *Carex* (129–35).

Eriksson's *Formae Speciales*.

It is to Eriksson, however, that we owe the clearest exposition of the idea of specialized parasitism, since his paper in 1894 (60) on the breaking up of the old species *P. graminis* into at least two species and a number of special forms; as well as his work on the unravelling of the chaos of special forms in the species *P. glumarum*, *P. dispersa*, *P. coronata*, and *P. coronifera*.

The application of Eriksson's results at once led to the establishment of specialized forms for the species of *Puccinia* on *Phalaris*, *Carex*, &c., and those of *Melampsora* on Willows.

In his paper in 1901 (61, p. 101) Eriksson summarizes the results of infection experiments which led him to break up the older *Puccinia graminis*, *P. Rubigo-vera*, &c., into finer 'species' and races. These results may be put shortly as follows:—

Species I. *P. graminis*, Pers., the Black Rust of cereals, is heteroecious on Barberry, and has at least six specialized forms:

F. sp. 1. *Secalis*, on Rye, Barley, *Triticum repens*, and some other Grasses; but not on Wheat or Oats.

F. sp. 2. *Avenae*, on Oats, *Dactylis*, *Alopecurus*, and some other Grasses; but not on Wheat.

F. sp. 3. *Tritici*, on Wheat, but not on the other cereals or Grasses.

F. sp. 4. *Airae*, on *Aira caespitosa* only.

F. sp. 5. *Agrostis*, on *Agrostis* only.

F. sp. 6. *Poae*, on *Poa* only.

Species II. *P. Phlei-pratensis* (Er. and Henn) is the Black Rust of *Phleum pratense* and *Festuca elatior*, and was formerly regarded as identical with *P. graminis*; its aecidium is as yet not known. It was hitherto included under *P. graminis*, but owing to its inability to infect Barberry or Wheat it must be separated.

Species III. *P. glumarum* (Er. and Henn), the Yellow Rust of Wheat, was formerly

included with the next nine species in *P. Rubigo-vera*, DC., but it has no aecidium form as yet known. There are at least five specialized forms of it, as follows:

F. sp. 1. *Tritici*, on Wheat; but not on Barley, Oats, or Rye.

F. sp. 2. *Secalis*, on Rye, but not on Wheat, Barley, or Oats.

F. sp. 3. *Hordei*, on Barley, but not on the other cereals.

F. sp. 4. *Elymi*, on *Elymus*.

F. sp. 5. *Agropyri*, on Couch Grass.

Species IV. *P. dispersa*, Er. This is the Brown Rust of Rye, and is heteroecious on *Anchusa*, where it produces the *Aecidium asperifolii* of previous authors.

Species V. *P. triticina*, Er., the Brown Rust of Wheat, has no aecidium as yet known, and is not heteroecious on *Anchusa*.

Species VI. *P. bromina*, Er., is the Brown Rust of the Bromes. Its aecidium is as yet unknown. [Since this was written, F. Müller has shown that the aecidium is formed on *Symphytum* and *Pulmonaria*, &c.]

Species VII. *P. Agropyrina*, Er., is confined to *Agropyrum repens*, and has no known aecidium.

Species VIII. *P. holcina*, Er., is confined to *Holcus*, and no aecidium is known.

Species IX. *P. triseti*, Er., occurs only on *Trisetum flavescens*, and has no known aecidium.

Species X. *P. simplex*, Kcke. Er. and Henn, is a dwarf species, hitherto regarded as a variety of *P. Rubigo-vera*, DC., occurring only on Barley and with no known aecidium.

Species XI. *P. coronifera*, Kleb., was previously confused with *P. coronata*, Corda. It is the Crowned Rust of Oats, &c. It is heteroecious on *Rhamnus catharticus*; but not on *R. Frangula*. There are at least six specialized forms, as follows:

F. sp. 1. *Avenae*, on Oats.

F. sp. 2. *Alopecuri*, on *Alopecurus*.

F. sp. 3. *Festucae*, on *Festuca elatior*.

F. sp. 4. *Lolii*, on *Lolium perenne*.

F. sp. 5. *Glyceriae*, on *Poa aquatica*.

F. sp. 6. *Holci*, on *Holcus*.

Species XII. *P. coronata*, Corda, the Crown Rust proper, produces its aecidium on *Rhamnus Frangula*, and has at least five specialized forms, as follows:

F. sp. 1. *Calamagrostis*, on *Calamagrostis arundinacea*, &c.

F. sp. 2. *Phalaridis*, on *Phalaris arundinacea*.

F. sp. 3. *Agrostis*, on *Agrostis stolonifera*.

F. sp. 4. *Agropyri*, on Couch Grass.

F. sp. 5. *Holci*, on *Holcus*.

Species XIII comprises a third species of the *P. coronata* group; with two forms, as follows (neither has any known aecidium, and they were previously included with the two preceding species under *P. coronata*):

F. sp. 1. *Epigeii*, on *Calamagrostis Epigeios*.

F. sp. 2. *Melicae*, on *Melica nutans*.

That we must accept these data as the experimental results of a good investigator is unquestionable; but there is room for differences of opinion

as to the validity of Eriksson's 'species,' and as to the exact value of his 'form-species.' My own opinion is that no species can be accepted as valid until it is capable of definition in morphological terms; and we shall see that the results of cross-infections have shown that bridging species (of host-plants) seriously affect the question of autonomy regarding the form-species.

Much remains to be done, however, before we can settle these questions, and I would point out that here, again, a definite line of research exists in the inquiry after the as yet undiscovered aecidia, and the testing of form-species on different hosts.

Eriksson, meanwhile, appears to insist on the rigid specialization of these forms, each to its own hosts; and in one case at least the aecidium form discovered for *P. dispersa*, f. sp. *bromina*, bears out his contention, though other results shake his conclusions, as will appear in the sequel.

The researches of the last few years have brought out the fact, soon suspected and looked for, that specialized parasitism is not confined to the Uredineae.

Magnus (107, p. 81) in 1894 pointed out that *Peronospora parasitica* and *Ustilago violacea* show a, so to speak, choice of hosts which points to the same phenomenon; and Rostrup in 1896 (146, p. 116) called attention to several Ascomycetes, e.g. *Dasyscypha Willkommii*, *D. calycina*, *D. abietis*; species of *Sclerotinia*, *Epichloë*, &c.; *Sphaerotheca pannosa*; as well as certain Ustilagineae, &c., as indicating by their behaviour a similar specialization.

In 1902 (120, p. 342) Neger showed, by means of cultures, that specialized parasitism is probably quite common in the Erysipheae, at any rate as regards the conidial form; and the phenomena have been very exhaustively followed out by Salmon (150), who has proved the question up to the hilt, and has shown that the course of events is similar in all respects to what occurs in the Uredineae. Much of this work was done in my laboratory, and I can testify to the accuracy and thoroughness of Salmon's work.

Giesenhagen (76, p. 319) in 1895, and Ludi (105, p. 1) in 1901, have also discovered similar phenomena in Exoasceae and Chytridiaceae; and Beijerinck's (26) results with the bacillus of the leguminous root-nodules point to the same conclusion, as do many other investigations in Bacteriology.

Stäger in 1903 (165) showed the same to be true for *Claviceps*; see also Ed. Fischer (71, p. 53).

On the other hand, *Cystopus*, *Botrytis*, and others appear to be pleophagous so far.

Nor is specialized parasitism confined to plants. Species of *Chermes*, according to the researches of Cholodkowsky (39), and of Burdon—the latter working in my laboratory—are specialized more or less to certain trees; and similar results obtain for the Pine-beetle, Willow Galls, and even the Hessian Fly and, according to recent work, the root Nematodes.

The Bromes and their Brown Rust.

In 1902 I published the results (191 and 199) of my first experiments with the Bromes and their Rust-Fungus, *P. dispersa*, f. sp. *bromina*, and have continued these during the intervening years.

My point of view involved some generalizations based on previous experience—for I may be allowed to say that the Uredineae and their parasitism is an old study of mine.

In the first place, it has always appeared to me that we are too apt to overlook the behaviour of the host in its relation to the Fungus, whereas the physiological condition of the host is always a factor of prime importance.

Secondly, the real meaning of infection can never be thoroughly understood merely from culture-experiments in the open: it will be necessary to examine not only the behaviour of the Fungus in detail, and the reactions of the host-plant to the Fungus, but also the effects of the environment on both.

It was an obvious idea that in order to attack a task of this kind properly, care must be taken that both the Fungus employed and the hosts to be infected by it must be definite as regards species, and pure as regards origin. Consequently, my first object was to work with one species of *Puccinia* only, and to confine the infections to one genus of plants. I chose *Puccinia dispersa*, the Brown Rust of Grasses, and the genus *Bromus*, on which one form of this rust is common, viz. the specialized form, f. sp. *bromina*.

There is still some doubt among authorities as to whether this Fungus, growing on the Bromes, and, as we shall see, very closely specialized in that group, should or should not be accorded specific rank. Eriksson has sharply criticized me for continuing to name it *P. dispersa*, and his contention that it should be regarded as a species (*P. bromina*) is perhaps strengthened by F. Müller's discovery that it is heteroecious on *Symphytum*.

But, while according all due respect to Eriksson's opinion, I maintain that a species must be capable of morphological distinction before it can be accepted as good.

Meanwhile this *Puccinia* (formerly included in *P. striaeformis*, Westend.; *P. straminis*, Fuck; *P. Rubigo-vera*, DC.) was first included in *P. dispersa*, Erikss., and then separated as *P. dispersa*, f. sp. *Bromi*, and, later, separated still further as a species, *P. bromina*, Erikss.; while Klebahn, accepting Müller's name, has it as *P. Symphyti-Bromorum*, F. Müll.

Such are the complexities daily being perpetrated in synonomy; and I hold them to be unnecessary until we discover morphological differences in this form sufficient to distinguish it from *P. dispersa*.

Having selected this Fungus for the work, the next thing was to become so familiar with its characters that it could always be distinguished from other morphologically distinct species.

But on extending my collection of Bromes—I have now obtained and grown every species that could be got from all parts of the world—it soon became evident that nothing short of a revision of that genus would suffice to reduce the chaos inflicted on them by well-meaning authorities and amateurs, and I had carefully to work out each species and variety as occasion demanded. Only those who have acquaintance with the forms of *Bromus erectus*, *B. secalinus* and its allies, and such species as the South American *B. unioloides* and the North American *B. ciliatus*, will be able to appreciate the labour here involved; but it was clearly useless to attempt any strict delimitation of the experiments without attempting this.

One of the first general results was the demonstration that uredospores taken from *B. mollis*, of the group Serrafalcus, will not infect *B. sterilis* or *B. erectus*, species of the groups Festucoides and Stenobromus, or do so very rarely; whereas they readily infect *B. secalinus* and its allies of the same group. Again, spores from *B. sterilis* (Stenobromus) refused to infect *B. erectus* (Festucoides) and *B. mollis* (Serrafalcus), but readily infect others of its own group.

In short, the uredo on the species of Brome belonging to any one group are remarkably closely specialized to species of that group; and this was borne out by Freeman, who repeated and extended these experiments.

It was interesting to notice that we both found a few exceptions to the rule, of which more later on.

Immunity and Susceptibility.

Obviously the results above referred to on specialization of parasitism raise in a new form the old question of immunity and susceptibility, and the problem resolves itself into this:—Why is it that, of two closely allied species, or varieties, both equally exposed to infection, one will prove immune and the other susceptible?

The suggestion that the immunity depended on structure, and that immune varieties would be found to have thicker cell-walls, fewer or smaller stomata, more or longer hairs, waxy cuticles, &c., had been made from several sides, but I believe it was Cobb, in Australia, who first put this question to the test in 1890–3 (41), and he thought that a thicker cuticle, more stomata, and absence of waxy bloom, made for immunity.

Eriksson was unable to confirm this (62, pp. 355–63).

My researches, published in 1902 (190, p. 302), for which I claim greater exactness in measurement, counting, &c., than had hitherto been attempted, showed that the curves of infectibility and of numbers, sizes, &c., of stomata, hairs, and so forth, not only do not correspond, but they show no relations whatever; and clearly led to the conclusion that the matter has nothing to do with anatomy, but depends entirely on physiological reactions of the protoplasm of the Fungus and of the cells of the host.

In other words, infection, and resistance to infection, depend on the power of the Fungus-protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins; and, reciprocally, on that of the protoplasm of the cells of the host to form anti-bodies which destroy such enzymes or toxins, or to excrete chemotactic substances which repel or attract the Fungus-protoplasm.

The histological examination of the host naturally led to the investigation of the microscopic characters and behaviour of the Fungus in the tissues of the host.

This was done, and the results published in a paper on the Histology of *P. dispersa* (195) in 1903, in which I demonstrated the whole course of normal infection, the development of the haustoria, the behaviour of the nuclei, and the growth of the Fungus in the leaf of the Brome.

Eriksson's Hypothesis.

The necessity for this thorough examination of the host and parasite, and their mutual behaviour one towards the other, had become the more imperative in view of the extraordinary position which had been taken up by Eriksson, than whom no man had earned a better right to speculate, be it said, with regard to epidemics of rust.

Eriksson, it will be remembered, had been driven by the results of experience in the field to conclude that the enormous epidemics of rust which break out so suddenly, at certain seasons, cannot be explained by the incidence of normal infection by wind-blown uredospores.

He therefore framed the hypothesis that the Fungus is able to so combine some of its living protoplasm with the living protoplasm of the host, that a condition of symbiosis is established, both remaining alive and capable of passing from cell to cell of the host-plant.

This symbiotic union Eriksson termed *Mycoplasma*—a word we translate *Mycoplasm*. This mycoplasm was assumed to be capable of remaining dormant in the leaf, or even in the seed into which it had passed, for many weeks or months; and then, under favourable circumstances of the environment, the fungal element—hitherto unrecognizable by any method known to us—would suddenly sort itself out, assume morphological autonomy, and

grow out of the cell into a mycelial form, which in due time extended and developed uredo-patches on the leaf.

The first assertion of this extraordinary hypothesis by Eriksson appears to have been made (63 and 64) in 1897, and the principal grounds on which he based it were, so far as I can discover, the following:—

(1) Certain forms—i. e. cultivated varieties—of Wheat always show rust in a short interval after sowing, e. g. 4–5 weeks, no matter what time of year the seed is put into the ground. But it should be noted that, in Sweden, the rust appears in summer earlier on winter-sown than on spring-sown crops.

(2) The Barberry, which carries the aecidial form of *P. graminis*; the two species of *Rhamnus* which carry the aecidia of *P. coronata* and *P. coronifera* respectively; and the Borragineae which bear the aecidia of *P. dispersa*, are by no means always present in the neighbourhood of these sudden outbreaks of rust, and cannot possibly be credited with the infection. Similar statements have been made by others—e.g. Barclay (3) in 1892 pointed out that *P. graminis* appeared in India at a distance of 300 miles from any Barberry; Cobb (40) in 1891 had assumed that the uredo-form must be perennial in Australia, and so on.

We may safely accept the fact that outbreaks of rust frequently occur at distances so remote from any plant known to carry the aecidial form, that it is almost impossible to believe that spores blown from such plants are responsible.

(3) Dismissing the probability of wind-blown aecidiospores as explaining the outbreaks, Eriksson proceeds to demolish the idea that lurking uredospores, which have passed the winter in the died-down stocks of grasses, may be responsible for the outbreaks.

Here we may note that De Bary in 1865 (20, p. 23) had sought in vain for a perennial mycelium of *P. graminis*, persisting through the winter, in *Agropyrum repens* and *Poa pratensis*; and Eriksson confirmed this in 1896 (62, p. 40), and also failed to find any such wintering uredo on *Dactylis glomerata* or *Agrostis vulgaris*.

On the other hand, Blomeyer in 1876 (34, p. 405); Rostrup in 1884 (147); Plowright in 1882 (132, p. 234); Kühn in 1875 (101, p. 401); Hitchcock and Carleton in 1893 (85, p. 11); and Bolley in 1898 (30, p. 894), specifically demonstrated that the uredospores can live through the winter on Wheat.

(4) But the principal evidence on which Eriksson relies is, perhaps, that obtained by growing Wheat, &c., in glass cases in his experimental fields.

Herewith I append a summary and analysis of these experiments, compiled from Eriksson's account:—

TOTAL EXPERIMENTS.

	Year.	No. of Plants.	Successes.	Failures.
(a)	1892	15	0	15
(b)	1892–3	126 at least	0	126
(c)	1893	35	0	35
(d)	1893 [1] (*tubes*)	10	0	10
(e)	1893	90 at least	0	90
(f)	1894–9 [2] (*tubes*)	32 at least	7	25
(g)	1894 [3]	14	2	12
(h)	1894	20	0	20
(i)	1895	20	1	19
(j)	1897 [4]	14	9	5
(k)	1898	15	0	15
	Total . .	391	19	372

(a) Oats in cases, summer 1892. To see if they can be cultivated in cases, and what importance may be attached to the presence of spores in soil. Three cases. *One* = 5 seeds Oats covered with *T. repens* + Black Rust. *Two* = 5 seeds in sterile soil. Sowed June 5. No trace of rust to August. Eriksson says all grew too abnormal to develop the mycoplasm germ out. Yet inoculations on Aug. 23 and 24 gave + results in 10–20 days!

i. e. 15 seeds sown and all proved failures.

(b) Autumn Wheat in cases in autumn 1892 and through winter to 1893. Michigan Bronze and Yellow Rust, to which it is susceptible. 14 cases. Questions: (1) Can seed or seedling be contaminated by *Uredo glumarum*? (2) Or by *Pucc. glumarum*? (3) Can disease proceed from diseased seed? (4) Can disinfection (heat dry, or Jensen's hot water) of seed destroy the Fungus?

Case.	Began.	Soil.	Seed.
1	Sept. 12	Not sterilized	9 seeds with *Pucc. glumarum*.
2	,,	Sterilized 5 h.	,, ,,
3	,,	Not sterilized	,, no teleutospores.
4	,,	Sterilized 5 h.	,, ,,
5	,,	,,	,, no teleutospores, and disinfected by Jensen.
6	Sept. 13	,,	,, with teleutospores and disinfection dry, 4 h. at 40–43° C.
7	,,	,,	Whole spike with *Pucc. glumarum*.
8	,,	Not sterilized	9 seeds quite free of rust.
9	Sept. 14	Sterilized 5 h.	,, ,,
10	Sept. 15	,,	,, ,, but *Uredo glumarum* added.
11	,,	,,	,, ,, but added *Puccinia*.
12	Sept. 16	,,	9 *seedlings* devoid of Fungus, but added uredospores.
13	,,	,,	9 seedlings and added teleutospores.
14	Sept. 17	,,	9 seedlings and no addition.

[1] No rust but abundance of *Erysiphe*!

[2] In midst of badly infected rusted Wheat.

[3] Cases appear to be openly ventilated.

[4] Aphides in one of the successful cases.

All gave negative results, although in the fourteenth case further infections were made with *teleutospores* on Sept. 22 and 24. On Mar. 29, 1893 = plants still growing, but died soon after. *No rust.*

i. e. at least 126 sown and 126 failures.

(c) Spring Cereals in cases in summer, 1893. Used 7 cases. All began May 15.

Case.	Soil.	Seed.
1	Not sterilized	5 Barley, showing *Pucc. glumarum.*
2	Sterilized 3 h.	,, ,,
3	Not sterilized	5 Barley, free of Fungus.
4	Sterilized 3 h.	,, ,,
5	Not sterilized	5 Oats, free of Fungus, but added *Puccinia.*
6	Sterilized 3 h.	,, ,, ,,
7	,,	5 Oats, free of Fungus, and nothing added.

Results totally negative to Aug. 9.

i. e. 35 sown and 35 failed.

(d) Autumn Wheat in long tubes in spring of 1893.

Used 10 seedlings, each in a tube, but although they got *Mildew* (*Erysiphe*) they had no rust. N.B. Eriksson points out that there was very little rust anywhere, as it was a poor year for the Fungus!

i. e. 10 seedlings and 10 failures.

(e) Autumn Wheat in ventilated boxes, autumn, 1893. Ten cases used.

Case.	Sown.	Soil.	Seed.
1	Aug. 29	Not sterilized	9 seeds showing *Puccinia.*
2	,,	Sterilized 3 h.	,, ,,
3	,,	Not sterilized	9 seeds, free of Fungus.
4	,,	Sterilized 3 h.	,, ,,
5	,,	,,	Whole spike *rusted.*
6	,,	,,	9 seeds showing *Puccinia* and sterilized 3 h. at 48° dry.
7	,,	,,	9 grains. These were inoculated on Sept. 8 on roots or leaves with *uredospores.*
8	Sept. 4	,,	9 grains, free of Fungus, also inoculated on Sept. 10–11 with *teleutospores.*
9	,,	,,	9 grains, Fungus free.
10	,,	,,	,, ,,

Here, although the cultures in the cases were in every respect excellent, yet none showed rust. In *no case* were positive results obtained. The only remarkable point here is that of case 7, where the after-inoculation with *uredospores* also failed.

i. e. at least 90 sowings and 90 failed.

(f) Autumn Wheat in tubes in 1894 to 1899 and in case in the spring of 1894.

In spring, 1894, put 10 seedlings in tubes:—

In 4 tubes = plants died in 3–4 weeks.
In 2 tubes = cotton carried away by wind.
In 2 tubes = plant died in 3 months.
In 2 tubes = *pustules showed in about* 40 days.

These tubes were in the midst of badly rusted Wheat of the same plot, &c. Another tube, started May 11, showed *pustules* in about 40 days. A large case, covering a tuft, showed *pustules* in 2 months after covering, and soon after rust appeared outside! In 1895 = 5 tubes of Horsford on April 23 and 5 tubes Rye May 20, and in 1896 on May 8 = 5 tubes of the same Wheat.

All gave negative results, and Eriksson says because it was a poor rust year.

But 1897 was a favourable rust year. Here we have 5 tubes on May 5, of which 2 soon died, 3 bore pustules.

Yet Eriksson says these cases prove non-infection from outside, because

(1) Where could infective spores come from?
(2) Uredospores could not survive the winter!
(3) Even if they could they would need but 10, not 40–41 days for incubation!

Yet Eriksson admits that *Erysiphe* appeared in such tubes, and, later, that *Aphides* appeared. Does he intend to argue that *these* were carried in the seed?

He argues that the pustules were (1) either due to *teleutospores*, which is absurd; or (2) to *mycoplasm*!

The results of (f) may therefore be summarized as follows:—

10 + 1 + 15 + 5 = 31 tubes.
1 case = 1
Total 32

i. e. = at least 32 plants were used, of which 2 + 1 + 3 = 6 tubes and 1 case gave *positive results*, while 25 tubes resulted in failure.

(g) Barley in cases during the summer of 1894.

In each case = 7 seeds sown on May 11 and continued to Aug. 7, i. e. 88 days.

Results = 1 case totally free.
1 case had 2 plants infected.

i. e. of 14 plants 2 yielded positive results.

(h) Autumn Wheat in cases in the autumn of 1894.

There were 4 cases, each with 5 grains.

Case.	Sown.	Soil.	Seed.
1	July 7	Sterilized 3 h.	5 seeds, badly attacked with *Puccinia*.
2	,,	,,	5 seeds, free of Fungus.
3	,,	,,	,, ,,
4	Sept. 6–13	,,	5 seedlings, infected with teleutospores.

No results to Nov. 14.

i. e. 20 seeds, &c., and 20 failures.

(i) Barley, Oats, and Twitch in cases in the summer of 1895. Began May 15 in 4 cases.

Case.	Sown.	Soil.	Seed.
1	May 13–June 1	Sterilized 3 h.	5 seeds Barley, attacked with *Puccinia.*
2	May 14	„	5 seeds, free of *Puccinia.*
3	„	„	5 seeds Oat, free of Fungus.
4	May 15	„	5 rhizomes of Twitch, washed and ? free.

On July 4 case 1 showed *one* plant with *pustules.* No others in this or other case.

i. e. 20 seeds and plant with 19 failures and *one success.*

(j) Barley in cases in the summer of 1897.

Three cases used, sterile soil and 5 seeds each.

Case.	Sown.	Results.
1	May 31	1 *pustuled* in 26 days and others followed till *all* 5 were *pustuled.*
2	June 1	No rust at all on any plant.
3	June 3	4 seeds only germinated. *Pustules* visible on July 25, and next day discovered the presence of *Aphis, and aphides rapidly increased till numerous.*

This insect was 'traced' *via* a crack and *pustules increased daily.*

Results = 14 plants, of which 9 *rusted* and 5 *failed.*

(k) Spring Wheat and Barley in cases in the summer of 1898. Began May 26 and used 3 cases, with 5 seeds each. On September 6 = all free of rust.

i. e. 15 seedlings and 15 failures.

(l) Experiments in 1896–9. But resulted in no further success.

(5) It was noteworthy that although Eriksson had called attention to certain 'corpuscules spéciaux' (*eigenthümlichen Körperchen*) in the cells of the host, in the neighbourhood of young rust pustules, and had declared that these peculiar bodies were the incipient mycoplasma growing out into the intercellular spaces as mycelium, we were anxiously awaiting figures of these bodies for some time. Eriksson's first enunciation of the mycoplasm-hypothesis was in 1897 (63 and 64) be it remembered; but no figures appeared until 1901–2 (65), when the author gave a plate of outline drawings of these peculiar bodies.

Those of us most familiar with the haustoria of the Uredineae were at once reminded of these bodies. I had made a very special study of these haustoria in the case of *Hemileia*, the Fungus of the Ceylon coffee-disease, in 1880–2 (192), and was convinced of the identity of the bodies in *Puccinia dispersa*; but in order that there should be no jumping to conclusion I worked out the histology of that Fungus from spore to spore, and in 1903 placed the matter beyond cavil (195).

Klebahn and others upheld the same view, and indeed the former seems to have suggested the resemblance already. However, the details are now of the less importance since Eriksson (66 and 67) has practically admitted the validity of my proof.

(6) Eriksson relies to a certain extent on the analogy alleged to exist between his mycoplasm and certain cases of intra-cellular parasites, such as Woronin's *Plasmodiophora* (206), Viala and Sauvageau's *Pseudocommis* in Vines, &c. (188) and others, and claims resemblances between them to which I shall have to refer later on.

Taking these six points one by one, I would point out:—

(1) The rusting of winter-sown Wheat at an earlier period in spring than spring-sown Wheat may obviously be due to the former having been longer exposed to the few spores which have survived the winter: it takes time for the year's crop to become epidemic, and we know how easy it is to overlook the first pustules of the season.

Moreover, the undoubted differences of susceptibility and immunity of various races complicate this question: nor can we overlook the weight of the evidence summed up in the references on pp. 13 and 14.

(2) The absence of the Barberry and other aecidial hosts is conceded in many cases, and would be a real difficulty if the uredospores were found to be so short-lived as has been assumed; but the mere fact that such spores will keep their capacity for germination for from sixty-one to ninety-four days (see pp. 11 and 12), and the evidence that we do not yet know the limits, should make us cautious here, to say nothing of the fact that germinable spores of *P. dispersa* have been found all the year round. See also p. 14.

(3) Here again the reply is partly the facts just referred to, and partly that we are only at the beginning of knowledge as to the transmissibility of spores from Twitch and other weed Grasses, either direct or by means of 'bridging species.' Moreover, the results given in Blomeyer (34), Rostrup (147), Plowright (132), Kühn (101), Hitchcock and Carleton (85), and Bolley (30) are, so far as they apply, dead against Eriksson's conclusions: they all declare for the persistence of uredospores and their retention of the capacity of germination through the winter.

(4) An analysis of Eriksson's experiments in large glass cases and tubes in the open is not easy; but the tubes (d) of 1893, a poor rust year, showed the presence of *Erysiphe* on the plants. Does Eriksson deny that this *Erysiphe* reached his plants by means of spores; or does he also intend us to assume an internal origin for that Fungus also? In the cases where he does get uredo he asks us to believe that it is due to mycoplasm; but if *Erysiphe* spores reached his specimens, surely the uredospores may have done so.

Again, in the tubes (f) of 1894–9, where, of thirty-five plants, seven were rusted, I find there was abundance of rust around the experiment.

Yet Eriksson asks us, Whence came the infection? He argues that uredospores cannot survive the winter; and that even if they could ten and not forty days are necessary for incubation. But here again, both *Erysiphe* and *Aphides* appear in such tubes. Are the *Aphides* derived also from an internal source, like 'mycoplasm'?

In series (g) again, where two cases were used and seven seeds employed, during the eighty-eight days of the experiment one case remained free from rust, while two plants in the other were rusted.

Further, in series (i), where four cases of Barley, Oats, and Twitch were tried, five seeds or rhizomes in each case, of the twenty plants only one was rusted, and this had *Puccinia* on it at the start.

Surely there is something wrong in the distribution of the parasite here, if Eriksson's idea is correct.

With regard to series (j), where three cases of sterile soil were sown with Barley, five grains each, case No. 2 showed no rust; case 1 showed rust in twenty-six days; and case 3 was rusted in fifty-two days and had *Aphides* in it.

Now, apart from the question, Does Eriksson imagine that the *Aphides* arose from within? is it not suggestive that *Aphides* can carry the spores of Uredineae about on their bodies?

(5) Since Eriksson has admitted (67) that his 'corpuscules spéciaux' figured in 1901–2 (65) are haustoria, in accordance with my explanation of them (195), I need say no more on this subject here. Further details regarding histological points will be dealt with later.

(6) With regard to analogies afforded by other parasites, Eriksson has in my opinion fallen on somewhat unfortunate examples.

Nawaschin (118) has shown that the amoebae of *Plasmodiophora* are perfectly distinct, and remain so, from the cytoplasm of the cells they invade, and modern staining methods make it impossible to believe that where the nuclei of the host-cells and of the parasite stain so distinctly as they do in Uredineae no better traces of them could be obtained than Eriksson supposes.

In support of his hypothesis, Eriksson might have used the then mysterious seed Fungus of *Lolium temulentum*; but Freeman has now shown, in my laboratory (75 *a*), that the Fungus is perfectly traceable in the developing seed. It is interesting to note by the way that Lindau has since found this in Egyptian seeds buried for about 4,000 years (104 *a*).

With the question of *Pseudocommis* I have dealt on p. 34.

Infection.

When the sporidia (*basidiospores*), developed from the promycelium (*basidium*) of the germinating teleutospore of *P. graminis*, are sown on the Barberry, the germ-tube put forth pierces the cuticle; and we now know that

many parasitic spores, only capable of attacking young organs, adopt this mode of cuticular infection.

In the case of uredospores and aecidiospores the process is different, as De Bary first showed; their germ-tubes enter the stomata, and it is with this stomatal infection only that we have here to do.

But further research shows that two events must be sharply distinguished, in addition to the preliminary act of germination of the spore itself

First, we have the entry of the germ-tube *via* the stoma, preceded by a swelling of the tip into the so-called *appressorium* over the external orifice of the stoma. The tip then sends a slender offset through the orifice into the respiratory cavity, and there it swells into the *sub-stomatal vesicle*, and the entry of the Fungus is assured.

Secondly, we find this sub-stomatal vesicle puts forth one hypha (or more) which grows towards the cells bounding the inter-cellular spaces, attacks them by sending in haustoria, and thus the act of *infection* is completed.

It will be evident that we have in the process of *entry* or *inoculation*, a phenomenon quite distinct from and independent of that of *infection*.

We cannot assume that either the stomatal or the mesophyll-cells of the host are passive or inert in this matter. As regards the former it is suggestive that inoculation is best effected by sowing in the evening; it often fails in bright daylight, and apparently always does so if the temperature is high, or the plant in darkness.

In 1882, at Owens College, I observed the following phenomenon. A slice of bean-stem in a hanging drop, to which some zoospores of a *Pythium* were added, was under observation. A zoospore was seen to gyrate rapidly in smaller and smaller circles, and suddenly dart on to the exposed cut surface of the bean section, where it remained stationary, and in half an hour had begun to germinate. In a couple of hours its tip was boring through the cell-wall.

My attention at the time was directed to other matters; and it is to this fact and to my lack of that particular form of insight which is called genius that must be ascribed the stupid failure to see that here was a case of what we now know as chemotaxis.

In 1883 (128, p. 524) Pfeffer published his first paper on the directive actions of chemical stimuli, which he subsequently named chemotaxis, and which he showed to be so potent a factor in attracting the tips of hyphae to enter plants; a theme worked out more in detail in this connexion by his pupil Miyoshi (113), 1894, and (112), 1895.

I have always maintained that in parasitism we must consider not only the power of the parasitic Fungus to attack a given host-plant, but also the reaction of the host on the parasite (198), 1890, and (197), and also (190), and it is characteristic of modern work that this view is spreading.

Massee (109), in an interesting summary of experiments on chemotaxis with certain Fungi, in which he confirms previous results, goes so far as to say that 'infection is due to positive chemotaxis,' and that immunity is due to the fact that the plant,' owing to the absence of the chemotactic substance in its tissues necessary to enable the germ-tubes of the Fungus to penetrate, remains unattacked' (p. 23).

But, apart from the fact that chemotaxis is also a factor in saprophytic life, and that the entrance of a hypha into a stoma is only a preliminary act, not necessarily indicative of parasitism, there are other objections to the title of Massee's paper, 'On the Origin of Parasitism in Fungi.'

Miss Gibson, in my laboratory, has shown that the germ-tubes of almost every Uredine she tried will enter quite the wrong host by the stomata, as the annexed table shows:—

Spores.	*Species of Fungus.*	*from*	*Host.*	*Result.*
Uredospores	*Uredo chrysanthemi*	*Chrysanthemum sinense*	*R. Ficaria*	Enter freely.
Aecidiospores	*Phragmidium rosae-alpinae*	*Rosa*	,,	,, ,,
,,	*Uromyces poae*	*Ranunculus repens*	,,	,, ,,
,,	*Aecidium bunii*	*Bunium*	,,	Negative.
,,	*Puccinia poarum*	*Tussilago*	*Caltha*	Enter freely.
Uredospores	*Uromyces geranii*	*Geranium*	,,	,, ,,
Aecidiospores	*Puccinia Menthae*	*Mentha*	,,	Enter not very freely.
Uredospores	*Puccinia*	*Carduus*	,,	Enter freely.
Aecidiospores	*Phragmidium Sanguisorbae*	*Poterium*	,,	2 or 3 doubtful entries.
Uredospores	*Puccinia glumarum*	*Triticum vulgare*	,,	1 certain entry.
,,	*Puccinia graminis*	*Poa aspera*	,,	Enter freely.
,,	*Uromyces poae*	*Poa pratensis*	,,	,, ,,
,,	*Puccinia taraxaci*	*Taraxacum*	*Tropaeolum*	,, ,,
,,	*Coleosporium Sonchi*	*Tussilago*	,,	,, ,,
,,	,, ,,	,,	*Valeriana*	,, ,,
,,	*Puccinia pulverulenta*	*Epilobium*	,,	1 entry.
,,	,, *Centaureae*	*Centaurea nigra*	,,	Enter freely.
,,	,, *Menthae*	*Mentha*	,,	,, ,,

It cannot be too clearly understood that the entry *via* a stoma is not infection. Over and over again I have found leaves which are immune, penetrated at hundreds of stomata without effect. This is why I have taken to speaking of the *germ-tube* and *appressorium*, formed outside the stomata, as distinct from the *sub-stomatal* or *infection-vesicle* and *-tube* developed later, in successful cases, in the respiratory cavity beneath the stoma.

Infection proper depends on two sets of factors. On the one hand the relations between host and parasite, involving the power of attack by means of enzymes and toxins of the latter, and the power of resistance, by means of anti-bodies, &c., of the host. On the other hand, the effects of the environment on both parasite and host.

External influences undoubtedly exert important effects. I have given evidence (190, p. 291) to show that too high a temperature during incubation may ruin the Fungus. Internal influences may result in the death of both hypha and host-cell, and then comes ruin to the former.

Klebahn (92, p. 262) shows that sporidia of *P. Convallaria-digraphidis* if sown on *Polygonatum*, and that the sporidia of *Gymnosporangium clavariaeforme* if sown on *Pyrus Aucuparia* (91, p. 150), form poor spots, &c. Also Bolley (30, p. 893) got mere specks on cereals with uredospores; and I have given similar examples (191, p. 298).

Here we have several cases possible: (1) It is possible that the environment stops the growth of the Fungus; (2) too many competing infections may be present; (3) the Fungus may be too weak to overcome the host-cells; (4) the host-plant may be too rich in antitoxins; (5) the Fungus may be too strong, and so kills the cells destructively; or (6) the host is too weak and succumbs too rapidly at the spot attacked.

In this connexion it is instructive to find that Mr. Evans and myself have satisfied ourselves (1) that so-called immune plants furnished us by the agriculturists may be really badly inoculated, bearing innumerable minute yellow and brown spots, each of which is an abortive infection area; (2) that experimental inoculation results in just such spots: the germ-tube goes in, but the walls of the cells in contact with it turn brown and die, and the hyphae are starved; and (3) we can stop the infection-tube, even when well in the leaf, by various modes of interference with nutrition, especially by starving the leaf of carbon dioxide: also by floating the leaf on water and starving it of salts, or by heating or cooling the roots.

That chemotactic actions are factors in the complex fight which results from the antagonism between the Fungus—with its attacking weapons such as enzymes and toxins—on the one hand; and the cells of the host—with their defending armoury of anti-toxins and other enzymes—on the other, cannot be denied. But that it is only one of several sets of factors in an extremely complex phenomenon, seems clear when we see that inoculation—the process of entry of the germ-tube and development of the sub-stomatal vesicle—and infection—the attack of the cells by the infecting tube and haustoria—are two distinct events; that external influences such as temperature, and internal factors such as the age and ripeness of the spore, and the condition of nutrition, starvation, &c., of the host-cells, affect the matter; and that a certain species of host A, though perfectly capable of being infected with, say, *Puccinia dispersa*, will refuse to be infected by spores of that Fungus taken from a host B, while it *will* take it from a host C, and will even take it from C when the latter has received it from B.

It seems justifiable, therefore, to speak of predisposition and immunity in the light of the above facts.

Eriksson's Criticisms.

In 1903 Eriksson made reply (66) to my paper in an article entitled 'The Researches of Professor H. Marshall Ward on the Brown Rust of the Bromes and the Mycoplasm Hypothesis,' in which he courteously appreciates the work, but at the same time maintains his previous position, insisting that my cultural and histological results have little or nothing to do with the matter.

Of my pure cultures in test-tubes (193, 1902, p. 451) he says:—'The pure cultures in test-tubes, described in 1902, where the results were negative in the cases when no infective substance was introduced, prove no more against the theory in question than do the numerous experiments with cultures, equally negative in their results, which I myself had carried out in isolated glass houses during the years 1892–8.'

This I concede: in both cases the absence of positive results is, so far as it goes, dead against the existence of 'mycoplasm' in the seedlings or plants.

Eriksson then proceeds to state that he ventures to regard the existence of mycoplasm 'as proved, at least until sufficiently comprehensive proofs to the contrary have been produced from some other quarter.' Surely this is a travesty of the kind of logic demanded by science! We have given proofs over and over again of the course of normal infection; these proofs are in accordance with the experience of others and in other parasitic Fungi. It may be quite true that certain possibilities can be conceived. Eriksson states (66, p. 143) that 'if we try to make clear to ourselves the origin of the outbreak of an uredo-pustule fleck of an heteroecious species of rust (e.g. *Puccinia graminis*) we have to suppose several different possibilities. The fleck can arise (1) from an infection by uredospores (e.g. *Uredo graminis*); or (2) from an infection by aecidiospores (e.g. *Aecidium Berberidis*). Numerous experiments have fully proved that both of these modes of origin occur.... It is possible that an uredo-pustule can also arise: (3) from a direct infection by teleutospores (e.g. *Puccinia graminis*) without the intervention of an aecidium-stage; or (4) from a latent germ of disease inherited from the parent plant....'

Surely it does not need my emphasis of one of the fundamental rules in scientific reasoning—viz. that the mere assertion that such and such an idea or hypothesis is possible in no way supports its probability, and still less does reiteration of a hypothesis prove it! Surely Eriksson must see that the *onus* of proof lies with him: not with us! Until we know all about the infection by means of uredospores and aecidiospores—and recent work shows how much we have yet to learn as to the limits of germinating power, infective capacity, and so forth of the uredospores—we have no right

to call in another hypothesis, totally subversive of all the laboriously won doctrine of parasitism founded by De Bary. We may admit the *possibility* of other hypotheses—I would be the last to deny honour to him who proposes and tests such—but we cannot accept such on negative foundations only.

I venture to assert that Eriksson has omitted a precaution which might have saved him much trouble, viz. he has not compared the course of normal infection with the phenomena he describes.

We had already worked out the chief points in the histology of *Puccinia glumarum* when Eriksson's second paper (67) since my investigation of the histology of *P. dispersa* was published in January, 1904, entitled 'Ueber d. vegetative Leben d. Getreiderostpilze.' This work was conjoint with Georg. Tischler, and deals with *P. glumarum* in Wheat.

After a short and luminous introduction, showing how De Bary's proof that *Aecidium Berberidis* belonged to *P. graminis*, *Ae. Asperifolii* on *Anchusa* belonged to *P. Rubigo vera* (*P. straminis*), and *Ae. Rhamni* on *R. catharticus* and *R. Frangula* belonged to *P. coronata*, Eriksson states that this by no means ended the Wheat Rust controversy.

True, De Bary's results were in the main confirmed, especially as regards *P. graminis*. But all three Wheat Rusts have now yielded several other species, and have been shown to be each a multiple species.

P. graminis is found to include two, *P. Rubigo vera* eight, and *P. coronata* two new forms which Eriksson regards as species. Of these only four species—*P. graminis* proper, *P. dispersa*, *P. coronifera*, and *P. coronata*—are known to be heteroecious, and of these only three form teleutospores which pass the winter before germinating, the teleutospores of *P. dispersa* germinating in the autumn of their ripening. Eriksson then goes on to repeat his opinion that it is impossible to explain the outbreaks of Rust in certain seasons by means of aecidiospores or of uredospores which have persisted through the winter; and especially insists on his culture-experiments in the open, as driving to the conclusion that some internal source of infection exists. In other words, Eriksson holds fast to his mycoplasm hypothesis.

But under what a different guise does the hypothesis now appear! Hitherto we were asked to assume that the protoplasm of the Fungus and of the cell were amalgamated in an intracellular symbiosis, and that at certain periods this could be seen to be separating out the Fungus-mycelium as 'corpuscules spéciaux,' now shown to be the haustoria.

Now we are told (67, Pl. 1, Fig. 2) that the vacuolated and granular contents of certain cells are probably the 'mycoplasm.' Neither the figures nor the description give us any sufficient evidence for this assumption, however, and I see no reason for altering my opinion that Eriksson had here before him simply the intact protoplasmic contents of normal cells

which had taken up the stain, and so showed up these cells, distinguished of course from cells which had been cut into.

The references to the obscure *Pseudocommis* of Viala and Sauvageau (188) and to *Plasmodiophora* (206) appear to me particularly unfortunate, for Prillieux (136, p. 47), writing in 1895, was quite unable to accept the views of Viala and Sauvageau; and Tubeuf, writing in the same year (72, p. 545), also points out that there is no proof forthcoming of the existence of such a parasite. Indeed, the autonomy of *Pseudocommis* has been accepted by no authority.

Moreover, I have myself observed, especially in tropical plants, appearances and alterations in the protoplasm of cells quite like Viala's, but which are due to physiological changes; and I may refer you to Miss Dale's work, in this laboratory, on intumescences (52) for some interesting results suggesting how such phenomena may be artificially induced.

The reference to *Plasmodiophora*, so beautifully worked out by Woronin in 1877–8 (206), is still more unfortunate; for not only did Woronin obtain the spores and germinate them, but Nawashin has shown (118) that it is perfectly easy to distinguish the amoebae in the cells throughout their life.

Eriksson, however, not only accepts all the dubious cases of *Pseudocommis*, &c., described by various authors, but suggests that even where hyphae coexist probably the so-called plasmodia 'nichts anders sind als Mycoplasmastadien verschiedener Hyphenpilze' (66, p. 12).

After making the most of this very questionable evidence, Eriksson then proceeds to a curious shifting of his position.

Hitherto, as we have seen, the mycoplasm was intracellular. He now passes to the consideration of a stage (66, Taf. II, Fig. 10) where it is intercellular, but concludes that this position was accidentally attained owing to manipulation during the preparation.

Eriksson then devotes his attention to certain phenomena observed at the edges of incipient pustules.

The first point to notice is that very young hyphae, which he terms *protomycelium*, are figured as partly creeping filaments, and partly as irregular masses filling up the intercellular spaces.

These are described by the author as follows: 'Keine Scheidewände sind vorhanden, auch keine erkennbare Kerne, nur zerstreute etwas stärker färbbare Körnchen, oft mehrere dicht bei einander. Deutliche Membranen heben sich auch nicht von dem Plasma ab; ob solche in der Tat vorkommen oder nicht, muss fortgesetzten Untersuchungen vorbehalten sein zu entscheiden.'

Owing to their 'almost plasmodium-like nature,' and the complete lack of septa, these structures are alleged to differ essentially from the normal mycelium, and are called 'protomycelia.'

We then read: 'Nach den bis jetzt vorliegenden Auseinandersetzungen

und Untersuchungen unterliegt es für uns keinem Zweifel, dass das intracellulare Mycoplasma und das intercellulare Protomycelium genetisch zusammengehören. Nur sind die Einzelheiten im Übergang von jenem zu diesem Stadium noch nicht so vollständig und genügend aufgeklärt worden, dass wir jetzt auf diese Übergangsfrage näher eingehen können oder wollen.'

Eriksson then describes (66, Taf. II, Fig. 11 *a* and *b*) what he regards as swollen nuclei of the host-cells.

Before proceeding further, I may again remark on the one curious omission in all Eriksson's work. Nowhere, so far as I can discover, does he trace his Fungus from the infection-spot, and in no case has he given a figure of the germ-tube entering the stoma, and compared the phenomena with what he finds elsewhere. True, he denies that the investigation of infection by means of spores is the right way to go to work; but one would have thought that it was at least necessary to compare and contrast what happens in normal infection with what is alleged to take place independently of it. Moreover, as was clearly shown in my paper on the histology of *P. dispersa*, Eriksson would have avoided some important errors had a strict comparative investigation been made; and the application of this to the histology of *P. glumarum* leads us to the same conclusion.

The Histology of *Puccinia Glumarum*.

Assisted by Mr. Evans, I have for some time been occupied with the histology of *P. glumarum* in Wheat, under various conditions of growth, normal as well as abnormal; and I would take this opportunity of saying that Mr. Evans has independently worked out the normal life-history and histology in the most thorough manner, as well as helped my part of the work to such an extent that we may regard it as practically conjoint.

The results may be briefly summarized as follows:—

The uredospore of *P. glumarum* germinates on Wheat, and infects it by means of a germ-tube which enters the stoma and puts forth an infection-tube from a vesicle, exactly as in the case of *P. dispersa* on the Bromes.

The sub-stomatal vesicle and the infecting hypha sent out from it are, however, very different in detail from those of *P. glumarum*, the vesicle having a firmer cell-wall and more numerous nuclei, and the infecting hypha being of far greater diameter and containing very many brightly staining nuclei.

Soon after entering the stoma, and having formed the sub-stomatal vesicle, the infection-tube put out from the latter forms its first haustorium, and the further growth takes place much as in *P. dispersa*.

The most remarkable points in these young hyphae are their great diameter (from 3–4 up to 18 μ and more), the rarity of septa, the abundance

of nuclei (often amounting to scores or even hundreds in a short length), and the shortness and stoutness of the branches.

Klebahn (94, p. 255) has this year called attention to the rarity of septa and the abundance of nucleus-like bodies in the hyphae of this Fungus, and has drawn one of the haustoria.

Now it is a noteworthy point that Eriksson figures and describes his 'protomycelium'—i. e. according to him the youngest stage developed from his alleged mycoplasm—as devoid of distinct nuclei.

We find that the nuclei become more and more indistinct, and for the most part diminish in size, as the mycelium ages, or if we *starve it by cutting off carbohydrate food-supplies;* and, without further information, we should certainly have interpreted Eriksson's (66) Fig. 11, on Pl. II, as either older hyphae or starved ones.

In no case have we been able to confirm Eriksson's statement as to the absence of a membrane to the hyphae. It appears very unlikely that among the thousands of preparations, in all stages, examined by us, the so-called 'protomycelium' has been overlooked. Indeed, as already said, we identify it with some of our stages of older or starved hyphae; but these have always a distinct clothing membrane.

It is impossible to avoid the suspicion that, had Eriksson cut serial sections through the patches at the margins of which he finds his 'protomycelia' and 'mycoplasm,' he would have discovered the sub-stomatal vesicle and entering germ-tube, for it should be mentioned that *in the very Wheat he has recommended as so susceptible*—viz. Michigan Bronze—we have seen excellent cases of entry on the *eighth day*, i.e. two days before spore formation may be expected; and in another susceptible Wheat (Red King) we have found the entering hyphae at the stoma on the ninth day. The fact is, the whole of Eriksson's work demands repetition from this point of view: we want not only the preparations from the margins of the young pustular-patches, but from every part of the infected area to its centre. At that centre the presumption is that he will find some of the cases of entry described, and will then see that he is reading the phenomena backwards, as I have all along contended he is doing.

With regard to the haustoria, it is the less necessary to say much, since Eriksson has now accepted my explanation, and withdrawn his erroneous interpretation of the 'corpuscules spéciaux' (66, p. 17). Klebahn had also noticed the similarity of these 'corpuscules' to haustoria (95, p. 89) in 1900.

We are, however, unable to confirm several of Eriksson's statements respecting the mutual behaviour of the haustorium and cell-nucleus, and are quite unable to explain his identification of the 'swollen nuclei' in his Fig. 11 *a* and *b* (66, Taf. II). The criticism of these and other details may be reserved for the full paper.

Susceptible and Immune Varieties, and their Hybrids.

A question of fundamental importance for our purposes is that of the immunity of certain forms of Wheat, and arising from this is the question, Can immunity be induced or propagated?

Mr. Biffen, working at our Experimental Farm, has done excellent service in recording the behaviour of certain races of Wheat, and in hybridizing and selecting them with great care and with an industry and perseverance not easily appreciated by those unacquainted with the kind of work involved. Much of his work has been directed to the testing of Mendel's law, a question into which I do not however propose to enter here; and this is the less necessary since he will himself bring this matter to your notice.

Some time ago I asked Mr. Biffen to select for me grains of a susceptible Wheat, of an immune Wheat, and of a cross between the two, and Mr. Evans and myself undertook to test them according to our own methods.

The races Mr. Biffen was good enough to supply were A, Rivet Wheat, a form found to be almost immune, even when growing in the midst of Rusted Corn; B, Red King, a very susceptible race, covered with Yellow Rust in the season; and C, a crossed Wheat obtained by pollinating Rivet with Red King.

This crossed form, on sowing and cropping, was found to be highly rusted the first year; but in the second year yielded some plants which showed Rust and others which appeared to be practically immune, in the proportion of practically 3 : 1, whence Mr. Biffen concluded that susceptibility to Rust is a dominant and transferable character. This question does not, however, concern us.

We sowed these several grains in separate pots of similar soil, similarly treated, and infected them in the ordinary way with spores from the same source.

The susceptible form B (Red King) showed signs of pustules on the tenth day, and by the twelfth day was covered with pustules of Yellow Rust.

The 'immune' form A on the tenth day showed what looked like very early stages of pustules, but these as a rule did not pass beyond the stage of pale flecks, and on the twelfth day showed a few spore-bearing pustules only.

On this twelfth day the proportions of pustules on A and B respectively were as follows, counted on three leaves each:—

A. Leaf 1 = 94 pustules.
„ 2 = 7 pustules.
„ 3 = 2 pustules.

B. Leaf 1 = }
„ 2 = } Many hundreds of pustules: far too numerous to count.
„ 3 = }

As regards the crossed Wheat C, one or two incipient pustules only could be detected on the tenth day, and on the twelfth it was as badly infested as the susceptible form, the numbers of spore-bearing pustules being far too many to count.

We then fixed the infected areas of leaves of each at intervals on the third, fourth, fifth to the eleventh days respectively, cut, stained, &c., and examined the serial sections. Several of each were left growing to determine their behaviour as regards producing spores.

We have made a prolonged series of preparations of all three Wheats, and find that inoculation and infection occur very readily in the susceptible form, the sections showing scores of entries of the germ-tubes: the hyphae then pursue the normal course, are typically stout, branched, and contain hundreds of nuclei. They also form numerous haustoria, and the attacked cells show no evident signs of injury to the chlorophyll-corpuscles or nuclei until a late stage of growth. It was particularly evident that the hyphae show no signs of degeneration until the period when spore-formation begins; but a tendency of the smaller nuclei to become massed together may be observed from about the sixth day onwards.

The microscopic examination of the 'immune' form yielded some very curious results.

It was evident, from the number of entries we found, that the uredo-spores germinate and send tubes into the stomata as frequently, or nearly so, as they do into the susceptible Wheat. Moreover, the course of events is at first similar: so much so that I thought at first that we were either mistaken in the information received as to the behaviour of these plants in the open, or that the so-called immunity was a mere delusion.

The behaviour of these hyphae after the fourth to sixth day, however, gave us the clue to the mystery.

In the first place the hyphae do not extend far from the point of infection, and they diminish in diameter; moreover, the contents become granulated or almost fibrous and present a starved appearance, while the nuclei not only diminish in size and number, but especially in distinctness and staining capacity, so that it often appears as if they were absent.

In short, these hyphae show evident signs of degeneration in all respects, and we conclude, from comparison with experimentally starved hyphae, that they are undergoing death-changes owing to one of two events, viz. they are either starving for want of food-supplies, or they are being poisoned.

Even more striking are the changes observed in the cells of the host in the immediate neighbourhood of these degenerating hyphae.

In the susceptible Wheat, even where the hyphae are particularly large and abundant, and full of nuclei and deep-staining protoplasm, the adjacent cells, including those into which haustoria have been sent, are still turgid

with well-staining nuclei and chlorophyll-corpuscles, and the cell-walls do not take up the fuchsin.

Here, on the contrary, the cells in the immediate neighbourhood of the hyphae are often collapsed, their nuclei and chlorophyll-corpuscles disintegrated and flowing together into shapeless masses, and deeply stained red, as are also the collapsed cell-walls.

That this is a purely local phenomenon, confined to the immediate neighbourhood of the hyphae—which rarely send haustoria into the cells—is seen by the normal appearance and staining of cells further away.

It is therefore impossible to accept the view that this collapse and abnormal behaviour of these nests of cells are due to the effects of preparation.

The only conclusion we can come to is that the hyphae attack the cells too vigorously at the outset. Instead of capturing them, as it were, by putting in haustoria which delicately tap the cell-contents and make them serve as sources of food-supplies, the Fungus clumsily kills these cells, and is in consequence subjected to all the exigencies of starvation, or worse.

Starvation Phenomena.

Our reasons for this conclusion are not based only on the histological evidence afforded by these so-called 'immune' Wheats.

We have induced starvation phenomena of substantially the same kind, even in susceptible Wheats, in which infection and the growth of the Fungus proceed normally under normal conditions; and this in several ways.

If infected leaves are cut off on the third day or so after infection, and floated on water, the Fungus may continue to grow, but it soon shows signs of starvation; for although the leaves are exposed to light and go on forming carbohydrates, the mineral and soluble carbohydrate supplies soon run short, from being washed out, and other changes occur which bring the normal functions to a stop.

The effects of mere mineral starvation have already been demonstrated by me (194, 1902), but it is clear from our experiments that although they do not kill off the Fungus forthwith, they have their effect on the structure of the mycelium.

Another way by means of which we have induced starvation phenomena is to keep the roots of the infected plants too hot or too cold for normal growth. This was effected by placing three pots of infected plants in reservoirs, plunged in water, in tin boxes. One, the control, was kept at the ordinary temperature; the other was kept heated to temperatures about 30°–35° C. day and night by means of a lamp below; and the third was kept chilled, by ice round the pot day and night, to near 5° C. and below. This treatment refers to the roots only: the foliage was freely exposed to

the light and air of the Eastern greenhouse. Not only did we trace starvation effects on the hyphae, but in the heated pots we found corroded nests of cells as in the immune plants. Since our work is not yet finished, however—we have yet to examine thousands of sections of these leaves—I defer discussion of the details for further communications.

There is one other series of experiments to which I must refer, however.

We have been much occupied with infections on leaves deprived of carbohydrates by keeping them in the dark, or in light from which the red-orange end is filtered off, &c. But especially important are the attempts to grow the Fungus in leaves from which all atmospheric carbon dioxide is cut off by potash, but which are normally supplied with water and light, and in some of the experiments with minerals in normal culture solutions.

I select one series for illustration.

Experiment 15.

On May 18 three pots were prepared, and in each were sown fourteen grains of Michigan Bronze, a variety selected because it was known to be very susceptible to *Puccinia glumarum*, and was used by Eriksson.

On June 15 the plants were strong and vigorous, each showing the fourth leaf. On June 15, 5 p.m., uredospores obtained from Michigan Bronze were sown on eleven of the first leaves, all over the upper surface, and the inoculated plants were placed under damp bell-jars. The whole growth, and subsequent operations, took place in the Eastern greenhouse.

The inoculated plants were then left for twenty-four hours, to initiate infection; and at 5 p.m. on June 16 were treated as follows:—

Six of the inoculated leaves were cut off close to the base, and each placed in a separate tower-tube, the tubes labelled A, B, to F. The base of this tube contained distilled water, over which was a light plug of wet cotton-wool, on which the cut surface of the leaf rested. Into this basal water plunges the exit end of a tube which carries the air to be drawn through the tower, which air, after passing over the leaf, escapes at the top of the tube.

The tower-tube described above is linked up to a second tower, arranged in exactly the same manner, except that it contains no leaf on a cotton-wool plug, but merely acts as a receptacle of water to wash the air coming through the tube.

This latter is linked to a triad of bulbs charged with a strong solution of potassium hydrate, the proximal end of which is open to the air. In some cases Pettenkofer tubes were employed.

Of the six leaves selected, four were placed as above in tubes thus

linked up to potash-bulbs. The other two were arranged in an exactly similar manner, except that their tower-tubes were not connected with potash-bulbs, and the air passing over their contained leaves passed through distilled water only. Clamps at various points enabled me to regulate the rate of ingress of air.

All six tower-tubes containing leaves were now joined up to a central large flask, by means of tubes linked together, and the flask communicated with a vacuum-pump. On joining up the system, and starting the pump, a little regulation of the clamps easily enabled me to set up a current of air through the towers at uniform rate, and at a low pressure, viz. 20–50 mm. of mercury—and this was done so that about one bubble per second escaped from the afferent tubes and passed over the leaf above.

The histological examination of these infected leaves showed that as soon as the leaf feels the deprivation of carbon supplies the Fungus-hyphae begin to degenerate and starve; and in some of the cases we find corrosions of the tissues beneath the spores on the epidermis. We have made out numerous interesting details regarding the degeneration of the nuclei, breaking up of the chlorophyll-corpuscles, thinning of the cell-walls, and so forth; but since there is still much to be done before all the facts can be linked together I do not propose to go further into the matter here.

The case of the plants with roots kept at high temperatures also promises to be interesting in several directions.

I pointed out some time ago (190, p. 291) that infections are difficult to carry out in hot weather, and gave evidence which goes to show that the mycelium suffers when the temperatures inside the leaf pass beyond certain limits. I also gave some experimental data showing that the temperatures in grass leaves exposed to a summer sun may rise far higher than is usually supposed.

It is interesting to note that Dr. Blackman, working in our laboratory at assimilation and respiration, and using thermo-electric measurements and methods of greater accuracy than were at my disposal, has confirmed and extended these data; as he will himself bring the matter before you in detail, I need say no more now.

These failures of infection in hot weather are so common—we have experienced them several times this summer—and the phenomena are so similar to what occurs in our heated plants, that I am disposed to urge that both cases stand in the same category.

But that is not all. The phenomena—starvation of hyphae in a nest of dead cells, or the corrosion of cells beneath the spores sown on the leaf—*are similar in all these cases to what occurs on the so-called immune plants we have dealt with.*

Bridging Species and their Significance.

In 1903 (191, p. 145) I summarized the results of a long account of experimental work, as follows:—

'The table gives the results of nearly five thousand experimental infections made with the uredospores from eleven species of *Bromus* belonging to the three sections *Stenobromus* (*B. sterilis*, *B. diandrus*, and *B. crinitus*), *Libertia* (*B. Arduennensis*), and *Serrafalcus* (*B. arvensis*, *B. secalinus*, *B. mollis*, *B. intermedius*, *B. japonicus*, *B. patulus*, and *B. brizaeformis*), on sixty-four species and varieties representing all the five sections into which the genus *Bromus* is sub-divided.' I then gave the tabular results showing that in the vast majority of cases the uredospores from a given species of *Bromus* only infect the same species or, usually in diminishing proportions, species closely allied to it and in the same group.

In the same paper (191, p. 139) I referred to some exceptional cases, which had occurred during the three years over which the experiments had extended, and on which I was at first inclined to lay no stress, regarding them as possibly errors; but since they were found to recur, in certain species, so frequently, I was forced to conclude they had an important significance.

Bromus erectus of the group *Festucoides* was once in thirty-seven trials found to take the infection from spores derived from *B. mollis* of the group *Serrafalcus*; *B. sterilis* (*Stenobromus*) four times out of ninety trials by spores from *B. mollis* (*Serrafalcus*); *B. Madritensis* (*Stenobromus*) once out of thirteen trials by spores from *B. secalinus* (*Serrafalcus*); and *B. maximus* (*Stenobromus*) once out of seventy-four trials with spores from *B. mollis* (*Serrafalcus*); and I raised the question, 'Is this a case of species raised on *B. mollis* adapting themselves to *B. sterilis* and *B. erectus*, &c., or of the latter proving individually less resistant than their species generally do to the infection?'

I then showed that the phenomenon turned out to be commoner than was suspected.

Freeman (75), working in my laboratory, confirmed my previous results, and showed that in the case of five species infections occurred with spores both from *B. sterilis* and *B. mollis* as follows:—

B. Gussonii (*Stenobromus*) was infected successfully thirty-seven times out of sixty with spores from *B. sterilis*, and six times out of fifty-three with spores from *B. mollis*; *B. Krausei* (*Serrafalcus*) succeeded fourteen times out of twenty-nine with spores from *B. sterilis*, and twenty-seven times out of twenty-seven with spores from *B. mollis*; *B. pendulinus* (*Serrafalcus*) twelve times out of fifty-three with spores from *B. sterilis*, and twice out of twenty-six with those from *B. mollis*.

I then showed (191, p. 150) that *B. Arduennensis* of the group *Libertia* is not only particularly susceptible to spores from its own species, but

also to those from *B. mollis* and *B. patulus* (*Serrafalcus*), and, in the case of its variety *villosus* at any rate, also to spores from *B. sterilis* (*Stenobromus*) as well.

So that *B. Arduennensis* is a 'bridging species' by means of which *P. dispersa* can pass into three of the five groups of Bromes.

I therefore concluded (191, p. 150):—'It seems to me that we have in these cases of "bridging species" the clue to an explanation of a phenomenon which must be assumed to occur in nature, whatever hypothesis we accept regarding the origin and signification of adaptive parasitism, viz. *the passage of the Fungus from species of one circle of alliance to those of another, in spite of the fact that it is usually closely adapted to species of one section of the genus only*. . . . We may suppose a uredospore from *B. sterilis* to infect *B. Arduennensis* var. *villosus*, and the crop of spores produced on this to further infect *B. Arduennensis*: thence the Fungus could pass to *B. secalinus*, and, further, to *B. brizaeformis*. According to Table III it would appear possible that an odd spore from the latter could infect *B. carinatus*, and if so, this would have for result the passage of the Fungus to four out of the five sections of the genus.'

Further experience only confirms the above, and convinces me that in these 'bridging' forms we have the clue to the phenomenon of the ever-widening cycle of adaptation.

In nine hundred and ninety-nine times out of the thousand the spores educated by, or adapted to, a given small circle of host-plants cannot successfully break through the defences of another circle; but in the thousandth case a single spore just manages to infect the alien host, and, once established, its progeny can go on infecting the new host. Or, it may be, that in nine hundred and ninety-nine times out of the thousand the spores from all kinds of alien sources fall on a given host, and it successfully resists any infection even though the germ-tubes enter the stomata; but the thousandth individual, weak in resisting power—whether machinery, or substance, or physiological activity—lets the enemy in, and it is then established.

The evidence compels us to believe that the host reacts upon and affects the physiological powers of the Fungus: these effects are invisible, and have no distinguishable morphological impress on the spores.

But is this always the case? De Bary's results with *Aecidium abietinum* (12) point the other way: they suggest that very slight morphological results follow according as the Fungus has passed its alternate phase (*Chrysomyxa*) on *Rhododendron* or on *Ledum*, and many similar cases can be quoted.

If this is once established, then we have the clue to the graduations of morphological differences sufficiently distinct for the determination of species.

Phylogeny.

It now remains to consider the bearing of some of the above phenomena on the phylogeny of the Uredineae.

In 1881 (192, p. 28) I recorded the fact that when uredospores of *Hemileia vastatrix* from *Coffea* are sown on *Canthium campanulatum*, another rubiaceous plant of Ceylon, the germ-tubes 'blocked up the stomata, sent their branches into the leaf, and commenced to form a normal mycelium in the intercellular passages of the leaf, as in coffee.' I also put forward the view that the origin of leaf-disease on Coffee was due to the spores of *Hemileia* having passed from the native plant, *Canthium*, to the introduced one, Coffee.

Although the words 'specialized parasitism,' 'adaptive races,' or 'biologic species' were not used, it will, I think, be conceded that here was expressed the idea that a native jungle Fungus-parasite had adapted itself successfully to an alien host.

But the idea of specialization of the parasite to the host was also present in De Bary's mind, when he wrote (7, p. 358, Engl. ed.): 'We encounter on the other hand in these Fungi [parasites] a very long and varied series of phenomena of one-sided or reciprocal adaptation between the parasite and the living organism on which it feeds. . . . Every parasite species lives on certain host-species, and the limits within which it can choose its host are different in different species.'

And again (7, p. 359): 'These facts and gradations would lead us to expect that there must also be differences in the aggressive behaviour of a parasite to the different varieties and individuals of a host; or, to express the matter in the converse way, in the *predisposition* of the individuals for the attacks of the parasite. In this direction also there are all possible gradations.'

Again (7, p. 386), De Bary wrote: 'The Fungi which are parasitic on plants naturally exhibit, within the limits of the chief phenomena of parasitic vegetation and its effects, . . . a variety of special adaptations in respect of their choice of a host and their spreading in, upon, or along with it.'

But probably the clearest example illustrating De Bary's attitude is the following:—

In 1879 De Bary (12) worked out the heteroecism of *Aecidium abietinum*, and proved that *Chrysomyxa Rhododendri* is its alternate form; but he found that at lower altitudes, although no Rhododendrons were present, the aecidium-form occurs, and showed that here the uredo-stage was *Chrysomyxa Ledi*. Although certain extremely fine distinctions exist between the two forms, which led De Bary to maintain them as species

provisionally, he asks 'ob beide Pilze durch Aenderung der Wirthpflanzen in einander überführbare Formen *einer Species* zu nennen sind oder *zwei* distincte, wenn auch sehr nahe verwandte Species.' It is almost certain that De Bary would, in the light of our present knowledge, have agreed with Klebahn (98, p. 131) that they are 'mehr biologische als morphologische Arten.'

Caeoma Laricis is the aecidium-form of an Uredine on the Larch, the connexion of which with an uredo-form has only lately been demonstrated; it is now known to be the aecidium of a *Melampsora* found on willows (98, p. 149). Klebahn's investigations have shown that spores from some of the aecidia (*Caeoma*) on the Larch readily infect *Salix viminalis, S. cinerea, S. aurita*, &c.; less readily *S. Caprea, S. fragilis, S. daphnoides*, &c., *S. acutifolia*. Other spores from aecidia, quite indistinguishable morphologically, readily infect *S. daphnoides*, and especially *S. acutifolia*, but have so far obstinately refused to infect *S. aurita* or *S. Caprea*; while *S. viminalis* and *S. cinerea* seem to be immune or very nearly so.

Klebahn concludes that we have here two Fungi on the Larch which, though biologically distinct, have not yet become sharply differentiated one from the other (98, p. 150). In other words, the Larch *Caeoma* is in process of splitting up into two as yet incipient species.

Puccinia Pringsheimiana, Kleb., and *P. Ribis nigrae-acutae*, Kleb. (I am not concerned in defending the bad nomenclature), both grow on *Carex acuta*, L., and form their aecidia on species of Ribes: this aecidium was long known as *Ae. Grossulariae*. Klebahn (98, p. 150) found that although *P. Pringsheimiana* and *P. Ribis nigrae-acutae* are practically indistinguishable by any morphological characters, the former develops its aecidia with ease on the Gooseberry and some other species of Ribes, except on the Black Currant: occasionally the latter host may be very feebly infected, but out of all comparison with the virulent infection of the Gooseberry. *P. Ribis nigrae-acutae*, on the other hand, infects *R. nigrum* easily and virulently: it also infects some other species of Ribes, but on *R. Grossularia* the infection is poor and uncertain, and rarely proceeds further than the development of *spermogonia*.

Here we have, according to Klebahn, a case similar to the last, but the separation has proceeded further, and the biologically incipient species are more differentiated.

The Fungus previously known as *Puccinia Bistortae*, Strauss, may be cited as a third case.

Infection experiments appear to show that at least two forms of *Puccinia* are comprised under this name on *Polygonum Bistorta*. One of these forms its aecidium-stage on *Conopodium*, as Soppitt showed (161, 1893, p. 4, and 162, 1895, p. 773), and was the first instance known

of a heteroecious *Puccinia* which develops its teleutospores on a Dicotyledon. It is now known as *P. Conopodii-Bistortae*, Kleb.

Another, known as *P. Angelicae-Bistortae*, Kleb., was found by Klebahn to develop the aecidium-form not on *Conopodium*—which does not grow in that part of Germany—but on *Carum*, and also, and still better, on *Angelica*. Ed. Fischer in 1902 (72, p. 12) confirmed the infection of *Carum*. But it appears that yet a third *Puccinia*, forming its teleutospores on *Polygonum viviparum*, is here comprised. It is known as *P. Polygoni-vivipari*, Karsten, and it also infects *Angelica* and forms aecidia on that plant, which again infect *P. viviparum*, but can also, though feebly, infect *P. Bistorta*, and there can be little doubt that there are other cases.

Whether Klebahn's conclusion that *P. Conopodii-Bistortae* and *P. Angelicae* are representative (or geographical) species is correct or no, there seems little room for disagreeing with his view that we have in the case of *P. Angelicae-Bistortae* and *P. Polygoni-vivipari* two forms adapted in different degrees to their mutual hosts.

A series of puccinias on *Digraphis*, formerly comprised as *Puccinia sessilis*, Schneid., offer a still more complex case.

Aecidia on *Allium ursinum*, *Arum maculatum* and certain Orchids, &c., have been proved to belong to this set. Soppitt showed that one of them—now called *P. Convallariae-Digraphidis*, Sopp.—develops its aecidium only on *Convallaria* (163, p. 213); on *Polygonatum* it gets no further than the formation of brown flecks—i. e. it appears to infect this plant, but the infecting tubes cannot grow to maturity, but die in the tissues. Klebahn showed and Soppitt confirmed (161, 1896, pp. 257 and 324, and 1897, p. 8) that *Majanthemum* remains immune.

Another was shown by Plowright (131, 1893, p. 43) to form aecidia on *Paris* but not on *Convallaria*.

Again, Magnus, Wagner, and Klebahn have found the *Puccinia* from *Digraphis* successfully infecting *Convallaria*, *Polygonatum*, *Majanthemum*, and *Paris*, and this form is now described as *P. Smilacearum Digraphidis*, Kleb.

But Klebahn has since found that, whereas *Polygonatum* is richly and easily infected, and *Convallaria* nearly as well; *Majanthemum* and *Paris* are but feebly attacked, and although more work is required, it certainly does look as if we had a *Puccinia* on *Digraphis* here accommodating itself in various degrees to a number of other hosts on which to form its aecidia. Nor is this all. Klebahn (98, p. 151) mentions a form, practically indistinguishable from *P. Convallariae-Digraphidis*, but distinguished by its feeble capacity for infecting *Paris* and perhaps *Majanthemum*.

Moreover, infection experiments have now separated *P. Ari-Phalaridis*, Plowr., forming aecidia on *Arum* but not on *Allium* (135, 1888, p. 88),

and Dietel (55, p. 43) confirmed Plowright's results; though Klebahn found it infect both *Arum* and *Allium*, but not *Convallaria*, *Polygonatum*, *Majanthemum*, *Orchis*, or *Listera*. However, teleutospores *obtained from Arum* the following year refused to infect any but *Arum*. Now the question before us is, Are the forms now known as *P. Allii-Phalaridis*, Kleb., *P. Ari-Phalaridis*, Plowr., *P. Convallariae-Phalaridis*, Sopp., *P. Smilacearum-Phalaridis*, Kleb., *P. Paridi-Digraphidis*, Plowr., *P. Orchidearum-Phalaridis*, Kleb., &c., all with their puccinia-form on *Digraphis* and their aecidium-form on the host referred to, to be accepted as species?

To me it is impossible. Until morphological characters can be found which separate it satisfactorily into specific forms, the *Puccinia* on *Digraphis* must be regarded as one and the same morphological species. But it does appear as if this species was adapting itself more and more closely to different alternate hosts, on which to develop its aecidia. Whether this adaptation will ultimately result in the breaking up of the various races into species is another question.

There are several possibilities, suggested by experiment but by no means as yet decided, to be kept in view in future investigations.

It is, for instance, quite possible that these adaptations are local, in the geographical sense; that a *Puccinia* which in one geographical area is in the habit of infecting one alternate host, in another, where that host is rare or absent, has to adapt itself to another. Klebahn's results with *P. Conopodii-Bistortae* and *P. Angelicae-Bistortae* (*P. Cari-Bistortae*) may well be quoted in support of such a view.

Another possibility, in support of which Klebahn's results with *P. Convallariae-Digraphidis* and *P. Smilacearum-Digraphidis* might be quoted, is that the age of the plant, or the complex of events we term weather, may decide which of a number of potential host-plants shall be chiefly infected during a particular season.

Yet again, we may find that different conditions of culture of the teleutospores may impress on them differences in infective capacity for different alternate hosts, as Klebahn (98, p. 152) has suggested for certain cases. Whichever view comes to be most accepted in the future, however, it seems impossible to escape from the conclusion that specific susceptibility on the part of the host will also have to be taken into account. So far our speculations have concerned the adaptation of the parasite in the alternating phases of its existence—e. g. of the *Puccinia* to a second host on which it can form its aecidium.

But the peculiarity of specialized parasitism, in the more modern sense of the phrase, and due chiefly to Eriksson's work, is that each phase of the Fungus—e. g. the uredo-form—also adapts itself to various species, races, or varieties, and I now propose to inquire into the meaning of this. Eriksson's experiments resulted in not only an extension of the above

results to the heteroecism of the Rusts of Wheat, but also in the proof that the uredo-stage is by itself capable of specialization on particular species and races of Wheat, &c. My experiments with the Bromes showed the same thing for various well-recognized species and varieties within this genus with the uredo of a particular form-species of *Puccinia dispersa.*

Returning to the bearing of these matters on the relatively immune wheat described on p. 37 (A. Rivet Wheat), we see that it is in no degree immune in the sense of being able to prevent the germ-tubes from entering the stomata, or even to keep the infection-tubes from attacking the mesophyll-cells. What seems to occur is that the cells attacked, usually—but not always—succumb at once to some influence, poisonous or otherwise, exerted by the hyphae, and the latter find themselves involved in the ruined débris of these cells. This is an inimical environment for such hyphae, and they die off. Sometimes, indeed, the poisonous action of the Fungus seems to be exerted at the very surface of the leaf.

Unfortunately, although we are justified in assuming the existence of poisons or enzymes in the Fungus, no one has as yet established the fact of their presence in these Uredineae.

De Bary (24) and I myself showed long ago (196) very convincingly that such poisons and enzymes occur in other parasites—e.g. *Botrytis*—and numerous observers have proved the existence of about a dozen different kinds of enzymes in various other Fungi, but I failed to extract any such body from *P. dispersa* (190), and Dr. Green, who at my suggestion has undertaken the investigation of spores and mycelia of *Puccinia glumarum*, has also as yet failed to obtain results beyond the probability that diastase occurs, and possibly cytase also. However, since Dr. Green is continuing this work, we may hope that he will eventually be able to throw more light on the matter.

Now it is interesting to observe in this connexion that we frequently, and indeed usually, find 'immune' plants flecked with small yellow or orange spots. Miss Gibson has recently observed the same kind of thing on leaves of an 'immune' form of Chrysanthemum she was trying to infect with Chrysanthemum Rust during the recent hot weather.

That these flecks are due to the dead tissues shining through appears certain from our examination of a number of cases, and I find that such flecks of corroded tissue in which infection has failed have been observed in the open by other observers. My remarks on p. 298 of the paper 'On the Relations between Host and Parasite in the Bromes and their Brown Rust' (190) refers to the same phenomena.

Moreover, Klebahn states (98, p. 36): 'Keimschläuche und Nährzellen sterben ab, und in Folge der Braun- oder Rotfärbung des Inhalts der Nährzellen erscheinen braune oder rote Flecken an den Impfstellen. So

beobachtete ich es an *Polygonatum*-Pflanzen, die mit den Sporidien von *Puccinia Convallariae-Digraphidis* besät worden waren.'

I have facts which go to show that infection-hyphae thus arrested in development—whether owing to the high temperature prevailing at the time or to reactions of the host-cells and independent of temperature—are not killed, but may lie dormant, and *may later on resume their growth.* If we can show that such inhibition and arrest of growth can be prolonged for more than a few days, the persistence of such dormant mycelia might explain a good deal. But it would not go to strengthen the mycoplasm hypothesis. But there is another point.

I have already stated (p. 13) that several observers agree that uredospores can live and remain capable of germination for long periods. I found those of *P. dispersa* retained their germinating power for sixty-one days (191, 1903, p. 138), and only brought the experiments to a close from lack of material. Miss Gibson has found uredospores of Chrysanthemum Rust germinate after keeping for ninety-four days, and has not yet reached the limit. Magnus (108, p. 18) found the uredo of *P. Caricis* persisted through the winter on the plant; Schröter found (156, p. 3) the same thing with a *Puccinia* on *Luzula*; and Barclay (2, p. 227) states that the uredo of *P. coronata* was found in winter; while Lagerheim found that of *P. Poarum* on *Poa* after the melting of the snow (102, p. 124). I myself found germinable uredospores of *P. dispersa* during every month of the year 1901–2, even in February and March (191, p. 132), and other cases are cited on pp. 13 and 14.

Conclusion.

Seeing that uredospores can be found nearly or quite all the year round; that they can be developed on odd tufts of grass here and there during the winter; and that they will retain their germinating power for two to three months—perhaps longer; that, further, specialized forms are not absolutely adapted to their hosts, but can occasionally infect races of Wheat, &c., which normally prove immune, or can pass from one hitherto excluded variety to another by means of 'bridging' species, where is the necessity of the mycoplasm hypothesis?

Moreover, it has been clearly shown—and is now conceded—that the so-called 'special corpuscules' supposed to be the 'mycoplasm' were merely haustoria; and I have traced all the phases of infection by means of sections at all stages shown in the field, and by means of similar sections at intervals of one, two, three to eight, or ten days after inoculation.

It has been shown that pure cultures of the Uredine give no evidence which lends the slightest support to the mycoplasm hypothesis; and all the evidence obtained from the study of starvation phenomena is equally non-supporting.

In fine, no trace of the alleged mycoplasm can be discovered, and all the facts point to the necessity of a thorough revision of the hypothesis in the light of comparison with the normal course of infection, and with what we now know of the behaviour of the spores.

Eriksson claims that the mycoplasm hypothesis is 'proved, at least until sufficiently comprehensive proofs to the contrary have been produced from some other quarter.' But this kind of argument cannot be accepted: the *onus* of proof is with him. I think that if the infecting spores are sought for, by means of serial sections through the flecks which yield the 'protomycelium' and 'mycoplasm' at their edges, they may be found, because we often find the entry even on the eighth or ninth day after inoculation, and are generally able to detect *the place of entry of the germ-tube in pustules on Wheat in the open.* Until this is done, I can only maintain that the mycoplasm hypothesis depends on the reading backwards of the course of events, on the part of an able and eminent observer, for whose work in other departments, and especially in the field, we must all have the greatest respect.

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On the vegetative life of some Uredineae[1].

BY

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I. Introductory Remarks.

IN the middle of 1860 Anton De Bary succeeded in proving by experiments the power of some corn-rust Fungi to live in different forms on two or more distinct hosts, that is to say, their so-called Heteroecism.

At first, and for some time, there was no doubt that the question of the corn-rust had been solved through this discovery. The aecidium-forms on *Berberis*, *Rhamnus*, and *Anchusa* were considered to be indispensable generations of *Puccinia graminis*, *P. coronata*, and *P. rubigo-vera*. Then, this being the case, one must get rid of the corn-rust by rooting out from the neighbourhood of the cornfields as radically as possible the plants attacked by the aecidia. In several countries of Europe and America they have begun a war of eradication against the plants mentioned above, especially against the barberry bush.

It is true that many researches during the following decennia have confirmed the results of De Bary in essential points. Repeatedly it has been shown that aecidiospores of barberry, &c., if sown on the young leaves of corn, infect them and produce the uredo and then the puccinia on them. Conversely, the sporidia of the puccinia, if sown on the young leaves of barberry, &c., infect them and produce the spermogonia, and then the aecidia on them.

But at the same time it has become more and more manifest that the process of development of the corn-rust diseases cannot be quite as simple as it was considered at first.

[1] Read before the Botanical Section of the British Association, Cambridge, August 22, 1904.—Conf. J. Eriksson, *Sur l'appareil végétatif de la rouille jaune des céréales*, Compt. Rend., 1903, p. 578. *Über das vegetative Leben der Getreiderostpilze*, I, Kgl. Sv. Vet.-Ak. Handl., 1904, Bd. xxxvii, Nr. 6. *Nouvelles recherches sur l'appareil végétatif de certaines urédinées*, Compt. Rend., 1904, p. 85. *Über das vegetative Leben der Getreiderostpilze*, II–III, Kgl. Sv. Vet.-Ak. Handl., 1904, Bd. xxxviii, Nr. 3.

[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

Now we know that we have to count with twelve instead of three species of corn-rust Fungi. *P. graminis* is divided into two species, *P. coronata* into two, and *P. rubigo-vera* into eight. Among these twelve species we find only four, *P. graminis* sens. strict., *P. coronifera*, *P. coronata*, and *P. dispersa*, which are heteroecious, and among these four species only the three first-mentioned have their teleutospores germinating in the spring after they are developed. The teleutospores of *P. dispersa* already germinate in the same autumn as they are developed.

Consequently we can explain the reappearing of the corn-rust in the following summer, in accordance with the principles of De Bary, only so far as concerns the three species with the teleutospores lasting the winter. In the case of *P. dispersa* that is impossible, because the aecidia on the *Anchusa*, like the *Anchusa* itself, end their lives before winter sets in.

Further, we must notice that *Berberis*, *Rhamnus*, and *Anchusa* do not at all occur so commonly as to explain the ubiquity of the corn-rust. In consequence it has become more difficult now than formerly to explain a rust-epidemic on our cornfields in a satisfactory manner.

Finally, I wish to observe that very numerous detailed studies executed in the open air during the last decennium, as well as many isolated cultures during the same time, have indicated the great probability of there existing an internal source of disease in the corn-plant itself, these internal germs being inherited from the parent plant.

In several works[1] I have submitted the hypothesis that the rust-fungus in the corn-varieties, extremely susceptible to rust, must live for a long time a latent symbiotic life in the cells of the plant itself—this symbiosis being called *mycoplasm*—and that only a short time before the eruption of the rust-pustules, when external conditions are favourable, it enters upon a visible state, assuming the form of a *mycelium*.

The mycoplasm hypothesis has been contested by many authors in several countries. However, through the criticisms presented I am not convinced of the error of the hypothesis. On the contrary I will now demonstrate some new investigations which appear to afford a good support to the theory already suggested.

Since the summer of 1902 I have been working at a cytological research on the vegetative life of the corn-rust Fungi. In this work I was effectively supported in the summer of 1902 and 1903 by Dr. George Tischler from Heidelberg.

Material of leaves, straws and ears of several corn-varieties, collected in several seasons and in varying stages of development, was fixed for the

[1] J. Eriksson, *Vie latente et plasmatique de certaines urédinées*, Compt. Rend., 1897, p. 475. *Der heutige Stand der Getreiderostfrage*, Ber. d. Deutsch. Bot. Ges., Bd. xv, 1897. *Sur l'origine et la propagation de la rouille des céréales par la semence*, Ann. d. Sc. Nat., Bot., sér. 8, t. 14–15, 1901–2. *The Researches of Professor H. Marshall Ward on the Brown Rust on the Bromus and the Mycoplasm Hypothesis*, Ark. f. Botanik, Stockholm, 1903, s. 139.

most part in Flemming's Fluid, but sometimes in Hermann's Fluid, in Absolute Alcohol, in Corney's Fluid or in Merkel's. The washing, the hardening and the paraffin-embedding were done in the usual way. The staining took place mostly with Flemming's Saffranin-gentian-violet-orange, but now and then with Heidenhain's Iron-alun-haematoxylin or with Fuchsin-methyl-green.

II. Perennial Mycelium. Does it exist in the wintering Corn-plant?

The first pressing question which had to be solved was whether there exists in the wintering corn-plant (Winter Rye, Winter Wheat) a mycelium surviving from the late autumn of the one year until summer of the following year, at which time the new rust-pustules are breaking out.

To explain this I fixed and embedded species of the leaf of three Wheat-varieties (Horsford's Pearl, Michigan Bronze, Squarehead), and one Rye-variety (Pirna Rye) in the year 1902 on October 6, 14, and 27, and in the year 1903 from the same plots on April 28, May 28, June 5, 11, and 18, and lastly on July 4. From October 6, 1902 until June 18, 1903 there was no trace of rust-pustules to be discovered on the plots. On July 4 we saw solitary spots of yellow rust-pustules (*Uredo glumarum*), breaking out on the wheat-leaves (Horsford's Pearl), but the small pieces for embedding were taken as far away from these pustules as possible, on those parts of the leaves which looked wholly sound. The Rye leaves were clean also on July 4.

From the embedded pieces of leaves very numerous series of sections were cut and examined. On this examination we have seen in none of the sections—and the research involved the examination of thousands of such—the least trace of mycelium either in the wheat or in the rye-leaves, not even in the wheat-sections (Horsford's Pearl) of July 4, which were taken far from the pustules.

From this examination we can conclude, I think, with a great degree of certainty, that the outbreak of *Uredo glumarum* on the wheat-leaves in the first days of July, and of *Uredo dispersa* one week later, could not be explained from *a perennial mycelium in the corn-plant itself.* Such a mycelium *was not at all to be found there.*

I ought to observe that the period of the biennial corn-plant's life, in which no mycelium is to be discovered, must be very varying in different years. For the season 1902–3 this period lasted from the sprouting in the beginning of October until the beginning of the following July, that is to say for nine months. In other seasons, when external conditions are favourable for the development of the Fungus, we can already see proleptic outbreaks of rust-pustules at the end of October or in November, and the

principal outbreak in the new year can take place in the beginning of June or even earlier, in the course of May. Thus in the past season 1903 I have seen on November 2 pustules of *Uredo glumarum* abundantly in several wheat-plots on the experimental-field, and new pustules were already breaking out in great abundance in the first days of May. In such a season the period lacking mycelium in the wheat-plant's life must be short, two or three weeks in October after the sprouting, and in the new year, two or three months, March–May, in the early spring.

III. The Intracellular Mycoplasm-Life of the Fungus.

The results of the researches carried out have, however, by no means been only negative. In all embeddings of varieties especially susceptible to the disease we find *some cells more or less filled up with a particular dense plasmatic substance*, at first commonly fine-grained, later more reti-form and vacuolar. In the younger stages—*the resting-stage of the myco-plasm*—the cell-nuclei seem to be of normal size, but later they swell to twice their normal size and lose to a certain degree their normal fibrillar structure. Gradually they also lose their normal form and appear as if eroded on the surface. At last they usually disappear as organized bodies, vacuoles frequently appearing as well as some small bodies, which take the saffranin stain. Now the mycoplasm enters into its *ripening-stage*.

From this time the Fungus must be considered as a true parasite, beginning to dissolve the cell-nucleus. Simultaneously one sees in the mycoplasmic reticulum small spherical bodies, which take the saffranin stain. These bodies are surrounded by hyaline areas and are believed to be the nucleoli of *the nuclei of the mycoplasm*, the hyaline area sur-rounding each nucleolus being the body of the nucleus. The spherical bodies are very variable in size.

IV. The Intercellular Mycelium-Life of the Fungus.

Now the plasmodium is ready to force itself out of the host-cell, in order to develop an intercellular mycelium. On its forcing itself out no dissolution of the cell-wall, either total or partial, takes place. It seems to be the case that the plasmodium effuses through the subtile pores that must be supposed to exist in the cell-wall, that is to say in the same way as the plasmodesms between the cells.

At this stage of development we often find places in the preparations, where wholly *corresponding portions of plasma are to be found inside and outside the cell-wall.* The connexion between the two plasma-portions is only broken off in consequence of the plasmolysis of the cell-contents.

The inner and outer plasma-portions otherwise show quite the same structure, and react in the same way towards staining agents.

When expelled from the cell the nucleoli of the plasmodium are dissolved and their contents are transported into the outer plasma-portions. One often finds distinct threads growing from the larger nucleoli towards the cell-wall. In this case we have before us a body very like a young haustorium, as that is generally described and represented. But there is a great difference, in that we have before us here a growth that must be considered as coming from within. I will name it an *Endohaustorium*. The contents of the former nucleolus gradually get exhausted, and then only the outer light halo remains as a wide vesicle still connected with the outer mycelium and transporting nourishment to this from the cell-residues.

In company with these round-headed bodies we find now and then some elongated ones, otherwise of the same structure. Probably they also must be considered as endohaustoria.

It sometimes happens that the production of mycoplasm-nucleoli is very reduced or quite absent, and nevertheless the plasmodium is forcing itself out. This is probably due to an accidental weakness of the mycoplasm.

At first one observes no distinct nucleoli in the intercellular *Protomycelium*, only various small grains of plasma, which take the violet colour more deeply than the other parts of the plasma. These grains are to be considered as a preliminary stage of nucleoli.

Very quickly, however, a new stage begins, in which are formed large distinct nucleoli, surrounded by a light halo.

Hitherto there were no partition-walls in the intercellular fungus-body, but later on such walls are formed, simultaneously with the dissolving of the nucleoli.

After this we get a true *mycelium*, and finally a tissue of threads winding round the remaining leaf-cells, that is to say a *pseudoparenchyma*. Gradually the tissue of the host-plant is consumed by the Fungus. The chlorophyll-corpuscles are agglomerated into an irregular lump, and at last all the remaining parts of the cells are devoured by the Fungus.

Now the Fungus is ready to produce spore-pustules. This seems to be initiated by a new appearance of large nucleoli in the pseudoparenchyma. The spore-forming threads grow outwards and the spores are produced.

With the production of spores a new period, the fructificative period, of the fungus-life is introduced, the vegetative period being ended.

So far the investigation has as yet been followed up only to the stage of development of the corn-plant, where its tender germ is sprouting out of the earth. The question, where the plasmodia in the leaves of the corn-plants have come from, must be left for further investigation.

The Relation of Root to Stem in Calamites.

BY

ARTHUR J. MASLEN, F.L.S.

With Plates I and II, and a Figure in the Text.

Introduction.

THE identity of Williamson's genus *Astromyelon* with the roots of *Calamites* has been shown by Renault [1] and later by Williamson and Scott [2].

Casts and impressions of Calamitean stems showing attached adventitious roots have often been figured and described. Lindley and Hutton figure in their 'Fossil Flora of Great Britain [3]' specimens with roots inserted just above the nodes of the stem, and one of Grand' Eury's figures, which is reproduced by Seward in his textbook [4], shows three nodes each with adventitious roots inserted either at or immediately above them. Weiss also gives good figures of Calamitean stems with attached roots, and some of these show clearly the continuity of the central cylinder of the roots with the vascular bundles of the stem at the node [5].

Renault also figures roots inserted on the nodes of the stem [6].

In most cases the roots arise in whorls, but according to Weiss they are sometimes grouped in tufts which arise close to the insertion of the lateral branches [7].

So much has long been known, but, nevertheless, much difference of opinion has been expressed with reference to the exact mode of attachment of the roots to the stems.

In *Equisetum*, with which it is natural to compare *Calamites*, the adventitious roots arise from the base of the lateral branches, one

[1] Nouvelles recherches sur le genre *Astromyelon*, Mém. de la Soc. des Sci. Nat. de Saône-et-Loire, 1885.

[2] Further Observations on the Organization of the Fossil Plants of the Coal-Measures, Part II The Roots of Calamites, Phil. Trans., vol. 186, B. 1895.

[3] Vol. I, Plates LXXVIII and LXXIX. [4] Fossil Plants, vol. i, Fig. 77, p. 316.

[5] Steinkohlen-Calamarien, Abhandlungen zur geologischen Specialkarte von Preussen, 1884, Part II, Pl. 2.

[6] Renault and Zeiller, Flore houillère de Commentry, Part II, Pl. LVII, Fig. 1.

[7] Loc. cit., Part II, Plates VIII and IX.

[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

(sometimes more) on each branch. Each bud usually produces a single root below the first leaf-sheath and from the lower side of the branch. In the aerial shoots the roots usually abort, but from the buds formed on the rhizome the roots develop whether the buds themselves grow farther or not[1]. In *Calamites* we can discover no trace of these 'rhizophoric buds': the roots pass right through the wood of the main stem and are directly connected with its primary xylem.

Many observers have tried to show that the roots of *Calamites* were in some way connected with the infra-nodal organs of Williamson. Thus Renault states that: 'Les racines adventives, quand elles se développaient, étaient en rapport avec ces organes que nous considérons comme des organes particuliers expectants, que nous distinguerons sous le nom d'*organes rhizifères*[2].' Again, in describing a specimen of his *Arthropitus lineata* exhibiting some large roots, he says: 'Les racines avortées ne sont représentées que par les organes rhizifères dont nous avons déjà plusieurs fois parlé; dans l'échantillon qui nous occupe ces organes sont bien conservés; ils sont dirigés du centre à la périphérie en s'abaissant un peu dans leur course; leur section transversale est elliptique; ils sont nettement formés d'une partie centrale composée de cellules polyédriques à minces parois, et d'une gaine de cellules prismatiques allongées dans le sens de l'organe et dont les parois portent de nombreuses ponctuations; là où les racines se sont développées les organes rhizifères n'existent plus[3].'

The 'organes rhizifères' of Renault are certainly the same as Williamson's infra-nodal organs, as Renault himself agrees[4]. Jeffrey, following Renault, and comparing the Calamites with their modern representatives, goes so far as to state that the more conspicuous series of nodules on casts of Calamites are not impressions of infra-nodal canals, but of the short cylindrical medullary cavities of modified rhizophorous branches, homologous with those of Equiseta[5], i. e. with the little developed buds from which many of the roots of the rhizome arise, and described by Jeffrey as 'rhizophoric buds.'

Grand' Eury[6] has recently described the distribution of the infra-nodal tubercles on the stems of *Calamites*, and he states that they are absent on the horizontal rhizomes and occur only on the ascending portions of the subterranean stems. If this account of their distribution is correct, their absence from the rhizomes is difficult to account for on the assumption that they are rhizophorous.

[1] Campbell, Mosses and Ferns, p. 447.

[2] Flore fossile du bassin houiller et permien d'Autun et d'Épinac, Part II (text), 1896, p. 89. Published in Études des Gîtes Minéraux de la France.

[3] Loc. cit., p. 106.

[4] Loc. cit., Flore fossile d'Autun, Part II, p. 89.

[5] Mem. Bost. Soc. Nat. Hist., vol. v, No. 5, 1899, p. 188.

[6] Forêt fossile de Calamites Suckowii, Comptes Rendus, 1897.

More recently, and after an examination of some of the sections described later in this paper, and of Renault's specimens in Paris, Jeffrey contradicted his previous statements and declared that: 'It is apparent from these recent observations that there is no necessary relation between the presence of roots and the occurrence of infra-nodal tubercles,' and that the roots were not attached to them although they were present in abundance in the same specimens [1]. He thus comes to a conclusion which was long ago arrived at by Williamson. Detailed examination of the large series of slides on which the present paper is based, many of them showing infra-nodal organs and roots in the same slide, confirms Williamson's conclusion that there is no connexion between these two sets of organs. The infra-nodal organs are always distinctly below the nodes and pass right through to the pith of the stem: the adventitious roots are inserted about on a level with the nodes (i. e. the level of the leaf-traces), and can also be traced right through to their connexion with the primary xylem of the stem. At no point in their course are the two sets of organs connected with one another. Indeed, the functions and homology of the infra-nodal organs remain as great a mystery as ever.

The principal object of the present paper is to describe and illustrate some recently obtained specimens and sections of the basal part of the stem of *Calamites* in which the connexion with the roots is clearly shown. Renault has already figured specimens with Astromyelon structures on Calamitean stems, and has shown that similar appendages were borne on the stems of the sub-genera *Bornia* (*Archaeocalamites*) and *Calamodendron* [2], as well as on those of *Arthropitys* (Göppert), the form to which most of the petrified Calamitean stems in the British Coal-Measures may be referred. Specimens showing the connexion between stem and root, and also exhibiting structure, are rare in our British collections of fossil plants. Williamson and Scott in their memoir on 'The Roots of Calamites [3]' only instance one example in the Williamson Collection, and Seward in his textbook [4] figures one other example from a section in the Cambridge Botanical Laboratory Collection.

The reason for the paucity of such sections is doubtless to be found in the fact that all the largest roots, as far as is at present known, were adventitious and only occurred at the base of the main aerial stems and on the underground rhizomes, while most of the fragments of Calamitean stems which are found represent portions of the axis above the root region.

All the sections have been skilfully prepared by Mr. James Lomax of Bolton, and all were in the possession of Dr. D. H. Scott, F.R.S.,

[1] Annals of Botany, vol. xv, 1901, pp. 139–140.
[2] Loc. cit., Flore fossile d'Autun, Part II (Atlas), Plates XLIII and LIX.
[3] Loc. cit., p. 685.
[4] Fossil Plants, vol. i, Fig. 92, p. 347.

by whom they were given to me for examination. Some of the slides have now been incorporated in Dr. Scott's collection and are indicated by the letter S in the following description; the others, marked M, are now in my own collection.

Description of the Specimens.

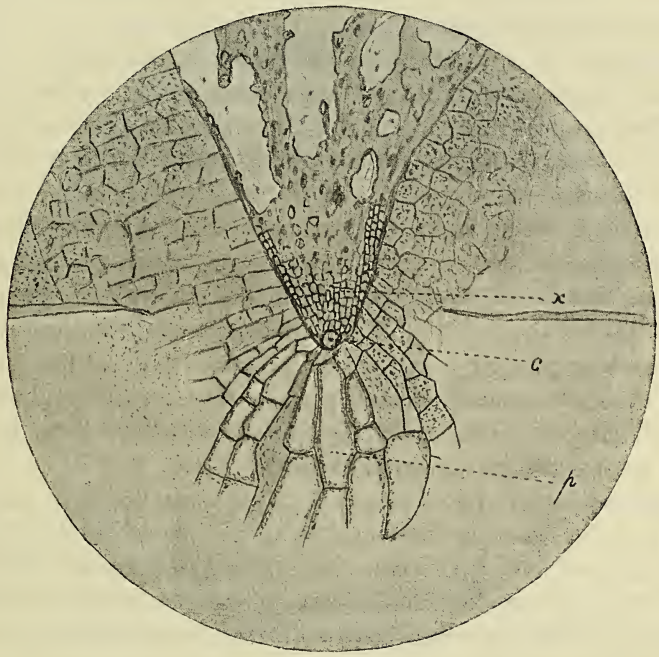
The specimen shown in Plate I, Fig. 1, was contained in the collection of the Chadwick Museum, Bolton, and for the photograph we are indebted to the curator, Mr. W. W. Midgley, F.R.Met.S. A series of twelve sections has been made from the specimen by Mr. Lomax (slides 1092–1103 S).

It measures just over seven inches in length, and tapers at its lower end almost to a point, and so comes to resemble in form the familiar medullary casts of the branches of Calamitean stems. It is not, however, a mere medullary cast, as the sections which have been made from the specimen clearly show the internal structure of the wood. Passing right through the xylem from its inner border outwards are seen in the sections a considerable number of adventitious roots arranged in whorls, from four to six from a node. The arrows in the photograph indicate the position of these whorls of roots, each of which is visible on the outside of the specimen as a distinct elevation. The lower tapering part of the stem is about three inches long, and on this portion the whorls of adventitious roots are crowded together. In the upper four inches there is only one whorl of roots. Some of the roots show an evidently fractured surface (see the middle node of the tapering part of Fig. 1), indicating that they have been broken off subsequently to fossilization.

It would appear that we here have the lowest part of a main aerial stem of *Calamites*, obviously in a young condition from its comparatively small size. The way in which the root tapers below to a blunt point would indicate the absence of a persistent primary root and that all its roots were adventitious. Probably the lower tapering part and some of the stem above were embedded in the swampy soil in which the plants grew, and it was propped up by its whorls of adventitious roots in very much the same way as in some Monocotyledons at the present day. As it is known that *Calamites* was provided with an underground rhizome from which the erect stems grew, it is highly probable that our specimen was attached at its narrow end to such a rhizome. If so the attachment must have remained a small one. This is of course quite different from the familiar cases of the attachment of pith-casts of branches of stems or rhizomes by little more than points. Williamson long ago explained the latter by showing that, although the pith became very narrow at the junction of branch and stem or of one branch with another, the enclosing secondary wood became much thicker, so that the real attachment was

not narrow but broad. In our specimen the wood also is preserved, and the real area of attachment to the rhizome must have been a small one. After leaving the rhizome the pith and wood rapidly dilated.

Passing to the sections which have been cut from this specimen it is unfortunate that the preservation of the tissues is peculiar and not good. The hollow pith-cavity has been filled with sand and the inner part of the wood has been to a certain extent destroyed. Some of the xylem wedges which project into the pith-cavity are however complete, and they show their carinal canals quite clearly and sufficient to demonstrate the



TEXT-FIG. 1. Part of a transverse section of a stem of Calamites (slide 1095 S) showing one of the xylem wedges. *c*, carinal canal; *x*, xylem; *p*, peculiar cells.

fact that the main axis is really a stem of *Calamites*. The points of the xylem wedges are surrounded by peculiar large cells, which, if in their natural condition, as they certainly appear to be, are different from those of any other Calamite stem with which we are acquainted (Text-Fig. 1). In vertical sections these cells are often nearly square in outline. Similar cells can be seen forming the pith of the lateral roots [1].

The next series of slides (548–557 S) consists of nine longitudinal sections and one transverse one made from a flattened stem found by Mr. Lomax in the Halifax Hard Bed. Slide 1783 S appears to have been cut from the same block.

[1] E. g. in 1093 S.

The longitudinal sections are cut parallel to the longer diameter of the transverse section of the flattened stem, and the series passes completely through the xylem of one side, then across the pith and the wood on the other side, and finally into the matrix in which the stem is embedded. The sections pass through one node, and at least five roots can be seen springing from this node. One of the roots branches when it is free from the wood of the main axis.

There is very considerable difficulty in distinguishing the stem-branches from the root-branches in sections which are taken close to the insertion of the lateral members on the primary wood of the parent axis, though it is usually easy enough when they are cut farther out.

Good figures of stem-branches are given in Williamson and Scott's Memoir[1], and they show, while still embedded in the xylem of the main axis, a relatively wide pith surrounded by a ring of vascular bundles each provided with a small canal (carinal canal) marking the positions of the protoxylem groups. The presence of the canals renders the identification of such sections as stems and not roots easy. The difficulty increases with the nearness of the branches or roots to their seat of origin, especially since, as Williamson and Scott have shown, the characteristic inter-nodal or carinal canals, which mark the position of the disorganized protoxylem groups of the Calamitean stem, are not present at the actual base of the branch[2].

Given then an isolated section passing transversely or longitudinally through the base of a stem-branch or an adventitious root, it is usually practically impossible to determine which of the two it is. A number of serial sections cut from the same specimen may, however, enable one to follow the stem or root outwards and so to determine its character. With regard to the position of the stem-branches, Williamson and Scott have shown that they are placed immediately above the node (i.e. the level of the leaf-traces), and usually between two of the bundles coming in from the leaves.

Plate I, Fig. 2 (slide 551 S) is from a tangential longitudinal section of the stem showing two roots in approximately transverse section. The main axis is evidently a stem as it shows clearly the usual bifurcation of the bundles at the node, leaf-traces (*l. t.*) passing through the nodal wood, and Williamson's 'infra-nodal organs' (*i. n. o.*). As the latter always occur below the node[3] they enable one to orientate the sections correctly, i. e. to distinguish the upper and lower ends. The section is evidently cut near to the pith of the stem, and passes through the inner portion of the wood:

[1] Further Observations, &c., Part I, Calamites, Calamostachys, and Sphenophyllum, Phil. Trans., vol. clxxxv, B. 1895, Pl. LXXII, Figs. 5 and 6.

[2] Loc. cit., p. 891.

[3] Small enlargements at the *lower* ends of the medullary rays (i. e. above the node) often occur, but these are usually easily distinguished from the infra-nodal organs.

this is clear from the distinctness of the vascular bundles (*x*) and wide medullary rays (*m. r.*). This photograph shows only one of the two roots which can be seen in the section (the other one is shown in Fig. 3), but the centre of the one figured (*r.*) appears to be exactly in a line with the leaf-trace bundles (*l. t.*) of the stem. This is therefore a first point of difference from the stem-branches, which always arise distinctly above the level of the leaf-traces, i.e. above the node.

Fig. 3 is made from the same slide as Fig. 2, but it shows the other root which is coming from the same node, and is more highly magnified than the latter. This photograph shows clearly two of the stem-bundles (*x. x.*) with a wide medullary ray (*m. r.*) between them and a root (*r.*) at the node above. The section becomes more tangential in the upper part, and the root shows the connexion with the secondary xylem of the stem above and with the nodal wood below. The position of the root with reference to the bundles of the stem is quite clear. It lies midway between the two bundles which are coming up from below, and indeed, in this respect, occupies precisely the same position as the stem-branches. This will be clearly seen by comparing our Fig. 3 with similar sections of stem-branches given by Williamson and Scott. The similarity of the two is very striking[1]. As the vascular bundles in the stem of *Calamites* usually alternate in position in successive internodes, it follows that both branches and roots will usually be opposite to a bundle in the internode above the node from which the lateral member springs, but opposite to a medullary ray of the internode below. This difference may be made use of in determining whether a transverse or oblique section of a stem which also shows one or more roots is cut above or below the node from which the roots arise. It is clear, for example, that the sections from which Figs. 9, 10 and 11 (Plate II) have been made were cut below the node from which the root shown was developed, as the centre of the root is in a straight line with a medullary ray of the stem and not with one of its vascular bundles. That the stem-bundles of successive internodes do not *always* regularly alternate is well known, and indeed an example of it is shown near the centre of Fig. 2, but it is sufficiently constant to make this method of some value.

Fig. 3 does not clearly show leaf-trace bundles quite close to the root, i. e. as close to the root as they are shown to the stem-branches in some of Williamson and Scott's figures. Could they be seen, they would probably appear at the sides of the root instead of distinctly below it, as is the case with the stem-branches. The evidence for this statement is as follows. Although no leaf-traces can be made out quite close to the root shown in Fig. 3, they are clearly seen in other parts of the section, and on the same node as that from which this root arises: the centre of the latter is exactly

[1] Compare, for example, our Fig. 3 and Williamson's figure showing a stem-branch reproduced in Dr. Scott's Studies in Fossil Botany, Fig. 9, p. 30.

in a line with the outgoing leaf-traces, thus confirming the evidence of the root shown in Fig. 2. Moreover, if a comparison is made of similar sections showing lateral branches and roots (e.g. Fig. 3 and Williamson's figure [1]), it will readily be seen that whereas the branches are well above the node and consequently a considerable distance above the level of the infra-nodal organs, the roots are at the node and within a much shorter distance of the infra-nodal organ of the medullary ray immediately below.

Fig. 4 is from a slide (552 S) cut from the same specimen as that from which Figs. 2 and 3 were made. The section is tangential of the main stem as before, but is cut farther out in the secondary xylem, as is shown by the continuous character of the wood (*x.s.*), which is only broken by the infra-nodal organs shown at *i.n.o.* The slide shows the same two roots as before (as well as traces of others from the same node), and one of these is seen in the photograph. The roots are now farther out in the wood; they show the usual 'Astromyelon' structure, and there is no doubt as to their root nature. Comparing this figure with Figs. 2 and 3 it will be seen that the root is somewhat lower, for whereas in the latter sections the roots are distinctly above the level of the infra-nodal organs (*i.n.o.*), in Fig. 4 the centre of the root is about in a line with them. The root, therefore, probably sank a little in its passage through the secondary wood of the stem.

Fig. 5 (slide 553 S) is also from the same specimen, and the slide shows the same two roots. The latter are now still farther out, and the one figured is nearly free from the wood of the parent axis. Its root structure is now very clear.

There can be no doubt then that Figs. 2, 3, 4, and 5 represent roots arising on a stem and apparently exactly at a node of the latter. The differences between the stem and root branches will be clearly seen by comparing our figures with those in Williamson and Scott's Memoir on the stem [2]. The latter figures show that the stem-branches arise considerably *above* the outgoing leaf-trace bundles, and that they soon develop the characteristic protoxylem canals which are quite absent from the roots.

The next figures are from sections cut from another specimen. This was collected by Mr. Knott at Fieldhouse Colliery, Huddersfield, and is a decorticated stem from one and a half to two inches in diameter, and about five inches long. It is clearly the basal portion of a stem, as it shows several verticils of adventitious roots. The block has been cut into no less than forty-six longitudinal and transverse sections by Mr. Lomax. Fig. 6 is from one of the longitudinal sections of this series (slide 11 M). The stem (*s.*) is cut radially, and medullary rays (*m.r.*) are clearly seen passing through the secondary wood. The section also passes radially through a large root (*r.*), which sinks gradually in its passage outwards. The connexion of the pith of the root with that of the stem can be seen. William-

[1] Loc. cit. [2] Loc. cit. Further Observations, &c., Part I, Pl. LXXII, Figs. 5 and 6.

son and Scott have shown that in the stem-branches of *Calamites* the pith terminates inwards in a narrow neck, by which it is continuous with the pith of the stem[1], and Williamson long ago accounted for the insertion of the medullary casts of large branches on their stems by means of a narrow neck, by the narrowing of the pith[2]. Our Fig. 6 shows that the narrowing of the pith inwards also takes place in the roots. Judging from this section, however, and comparing our Fig. 6 with the figures of stem-branches given by Williamson and Scott, it appears that in the roots the narrowing is more gradual than in the stems. Comparison of stem and root branches which are cut approximately at the same distance from the centre of the main axis on which they are borne, but so as to avoid the narrow neck connecting them, shows that the stem-branches have a relatively larger medulla. Compare our Figs. 2, 3, and 4, and Williamson and Scott's Figs. 5 and 6.

It is well known, however, that specimens of *Astromyelon* vary greatly in the relative size of the pith, which may consist of very few cells indeed, so that they can hardly be identified, or it may be relatively large. This extreme variation in size of the medulla is not found in the roots which arise directly on the stems and with which we are here specially concerned, but only in the smaller specimens. Of these there are many scattered through our slides, and they doubtless result from the branching of the larger roots, all of which agree in the possession of a well-developed pith.

Fig. 6 also shows the connexion of the primary and secondary xylem of the main axis with those of the root.

Figs. 7 and 8, Plate II (slides 5 and 7 M) are made from sections cut from the same specimen as Fig. 6, but they do not pass through the same root.

The section from which Fig. 7 is taken is cut longitudinally, and it passes into the pith of the stem. The figure shows a medullary ray (*m. r.*), and one of the infra-nodal organs (*i. n. o.*). Just above the level of the latter, part of one of the nodal diaphragms is preserved (*n. d.*), and in a line with this a leaf-trace bundle can be seen in the section. The leaf-trace can hardly be made out in the photograph, although easily seen in the section; its position is indicated at *l. t.* A root (*r.*) is shown, and its centre is about on a level with the leaf-trace bundle and the nodal diaphragm.

Fig. 8 shows the same root as the last, but it is cut nearer its origin, just above an infra-nodal organ (*i. n. o.*). In both of these cases, as well as in Fig. 6, the central part of the pith has disappeared. There is some evidence in these cases that the disappearance is due to fungal action.

The usual persistence of the pith in *Astromyelon* was one of the

[1] Loc. cit., Pl. LXXX, Fig. 22.

[2] See Organization of the Fossil Plants of the Coal Measures, Part IX, 1878, Phil. Trans., Pl. XXI, Fig. 30 and description of the same.

characters which led Williamson to separate it from *Calamites* (i.e. the root from the stem), and the smaller specimens usually agree in the possession of a solid medulla either relatively small or large. In the larger examples, however, those which arise directly on the stems, the disappearance of the central part of the pith is usual, and there is often a definite line separating the central cavity from the peripheral persistent portion. The latter always forms a wide band of tissue, and the pith never disappears right up to the xylem as is usually the case in the larger stems.

Some slides that we have serve to throw some light on this subject.

Fig. 9 (slide 1685 S) is from an approximately transverse section of a stem which is evidently cut near to (just below) a node, as some infranodal organs (*i. n. o.*) are passed through. A root is shown passing out obliquely through the secondary wood (*s. x.*) of the stem. This root must have passed out much more obliquely than those shown in Figs. 2, 3, 4, 5, and 6, since although the stem is cut nearly transversely the root is also cut more nearly transversely than longitudinally. The connexion of the wood of the root with the secondary xylem of the stem is clearly seen, as well as the contortion of the tracheides (*c. t.*) on the inner side of the root. The pith of the root is here quite entire, but it shows a well-marked differentiation into a small central portion (*c. p.*) of thin-walled cells and a wide peripheral zone (*p. p.*) in which the cells appear to have had thicker walls.

Fig. 10 shows a portion of Fig. 9 more highly magnified. The differentiation of the pith and the contortion of the tracheides are more clearly shown.

Fig. 11 (slide 1748 S) is from a slide cut from the same specimen as Figs. 9 and 10. The root is now farther out, and the central portion of the pith is represented by a space, owing to the destruction of the thinner-walled tissue.

Fig. 12 (slide 22 M) shows a root cut quite close to its origin. The connexion of the primary and secondary wood of the stem with those of the root can be seen, and also clear differentiation of the pith into an outer thicker-walled zone (*p. p.*) and a thinner-walled central portion (*c. p.*). In other sections showing the same root cut farther out from the axis the central part of the pith is hollow.

The differentiation of the pith is visible in a large number of the sections at our disposal, and in nearly all cases the central portion becomes hollow while the root is still embedded in the parent stem. In some cases there is no evidence to show that the destruction of the central cells was other than a natural process, but in other cases fungal action appears to have caused or helped in the disintegration. It may be that the walls of the inner cells were composed of cellulose, while those of the outer part were more or less lignified and so were able to resist fungal action.

On the whole, we incline to the opinion that the larger adventitious roots of *Calamites*, i. e. those which spring directly from the stems, were probably fistular, and the presence of thinner-walled central cells would indicate that this may have been their natural condition. A few of our specimens form an exception to this rule. The sections from which Figs. 2, 3, 4, 5 are taken show roots originating directly from a stem, but the pith of the former is neither differentiated nor hollow. This may be a specific distinction.

This paper is not specially concerned with the smaller roots of *Calamites*, of which there are many scattered through our sections, and which are mainly branches of roots and not borne directly on stems. These are the specimens usually figured by Williamson, and by Williamson and Scott. The medulla varies enormously in relative size, and is sometimes absent altogether. As usually developed, the pith-cells show a gradual increase in size towards the centre, but no differentiation into distinct outer and inner portions such as is commonly seen in the larger roots, and there is no evidence that the pith became hollow. The presence or absence of a pith in these small specimens of *Astromyelon* appears to be an individual variation, or it may vary in the same individual. A curious case is illustrated in Fig. 13 (slide 48 M), which shows a small branching specimen in which the branch has a conspicuous pith while the parent axis has none.

Summary and Conclusions.

The roots of *Calamites* were mainly adventitious, and they usually arose in whorls from the nodes of the lower portion of the aerial stems as well as from the underground rhizomes.

The lowest portion of the ascending stems rapidly tapered to their insertion, probably, on the underground rhizome. The actual connexion was probably a small one. The roots arise in direct connexion with the protoxylem of the main axis, and are not seated on the bases of the branches as in *Equisetum*. Detailed examination of this large series of sections affords no evidence of any connexion between the roots and the infra-nodal organs of Williamson. Roots and stem-branches are difficult to distinguish from one another in sections which are cut quite near to their insertion on the protoxylem of the main axis. The roots resemble the stem-branches in their position relative to the stem-bundles and the outgoing leaf-traces, as seen in tangential sections through the main stem, i. e. in both cases the lateral member is usually placed so that its centre lies vertically above a medullary ray of the internode below and between two leaf-traces.

The roots arising directly on the stems appear to differ from stem-branches in the following particulars:—The roots arise on a level with

the leaf-trace bundles, i. e. at the node, and not above them as in stem-branches; the roots pursue a somewhat downwardly directed course in passing through the wood of the main axis (the actual angle of divergence seems to vary greatly in different cases); the internodal (carinal) canals, representing the disorganized protoxylems, which are characteristic of the stem-branches even when embedded in the wood of the main axis, are not present in the roots; the narrowing of the pith of the root before it joins with that of the stem appears to be more gradual than in the stem-branches, otherwise they appear to be very similar to one another, and there is usually a differentiation of the pith into distinct inner and outer regions. The pith of the large roots usually became hollow by the destruction of the thinner-walled central portion. In nearly all cases there is a wide band of persistent pith, i. e. the whole of the pith does not usually disappear as in the larger stems.

In conclusion, I wish to express my obligation to Dr. D. H. Scott, M.A., F.R.S., who suggested the investigation and supplied the slides on which the work is based. He has also allowed the work to be done in the Jodrell Laboratory at Kew, and has kindly afforded much help during its progress. My thanks are also due to Mr. L. A. Boodle, F.L.S., for great assistance in connexion with the photographs which accompany this paper, and to Mr. W. W. Midgley, F.R.Met.S., who kindly supplied the photograph from which Fig. 1 (Pl. I) is made.

EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Mr. Maslen's paper on 'The Relation of Root to Stem in *Calamites*.'

PLATE I.

Fig. 1. The basal part of an upright stem of *Calamites*, showing nodes with whorls of adventitious roots coming from the stem. The arrows indicate the position of the nodes.

Fig. 2. Part of a tangential longitudinal section of a stem of *Calamites* (551 S), showing a root (*r.*) in approximately transverse section. *l. t.*, leaf-traces; *i. n. o.*, infra-nodal organs; *x.*, vascular bundles of stem; *m. r.*, medullary rays.

Fig. 3. Another portion of the same section as that from which Fig. 2 is made, showing another root from the same node. *x. x.*, two stem-bundles; *m. r.*, medullary ray; *i. n. o.*, infra-nodal organ; *r.*, root.

Fig. 4. Another tangential section from the same block as Figs. 2 and 3. *r.*, root; *i. n. o.*, infra-nodal organs. Slide 552 S.

Fig. 5. Another section showing the same root. *x. s.*, xylem of stem; *x. r.*, xylem of root; *p.*, pith of root. Slide 553 S.

Fig. 6. Radial section of a stem of *Calamites* also passing radially through a root. *s.*, stem; *m. r.*, medullary rays of stem; *s. p.*, pith of stem; *r.*, root; *r. p.*, pith of root. Slide 11 M.

PLATE II.

Fig. 7. Longitudinal section cut from the same block as Fig. 6. *m. r.*, medullary ray of stem; *i. n. o.*, infra-nodal organ; *r.*, root; *l. t.*, position of leaf-trace bundle; *n. d.* nodal diaphragm. Slide 5 M.

Fig. 8. Another longitudinal section from the same block as Figs. 6 and 7, and showing the same root as Fig. 7. *r.*, root, *i. n. o.*, infra-nodal canal. Slide 7 M.

Fig. 9. Approximately transverse section of a stem of *Calamites* with an included root. *i. n. o.*, infra-nodal organ; *s. x.*, secondary xylem of the stem; *c. t.*, contorted tracheides on the inner side of the root; *c. p.*, central part of the pith of the root; *p. p.*, peripheral pith of the root. Slide 1685 S.

Fig. 10. A portion of Fig. 9 more highly magnified. Letters as before.

Fig. 11. Another approximately transverse section from the same block as Fig. 9. The root is now farther out. *c. p.*, central pith of root; *p. p.*, peripheral pith of root. Slide 1748 S.

Fig. 12. Oblique section of a stem of *Calamites*, showing a root cut quite close to its origin. *c. p.*, central pith of root; *p. p.*, peripheral pith of root. Slide 22 M.

Fig. 13. A small branching root of *Calamites*, showing a pith-less specimen bearing a branch with a distinct pith. *x. m.*, xylem of main axis; *x. b.*, xylem of branch; *p. b.*, pith of branch. Slide 48 M.

Fig. 1.

Fig. 2.

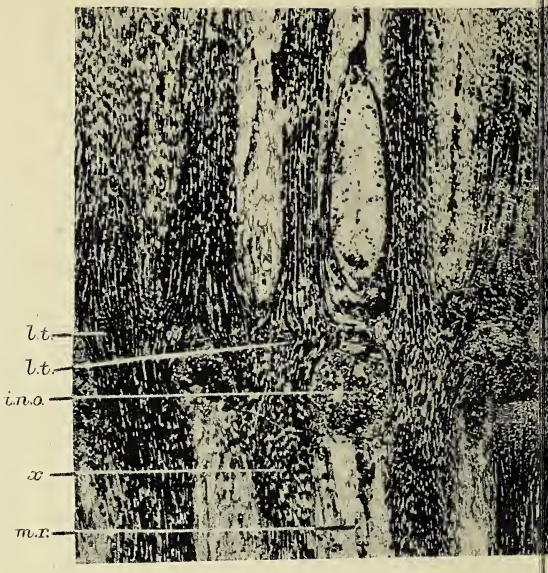
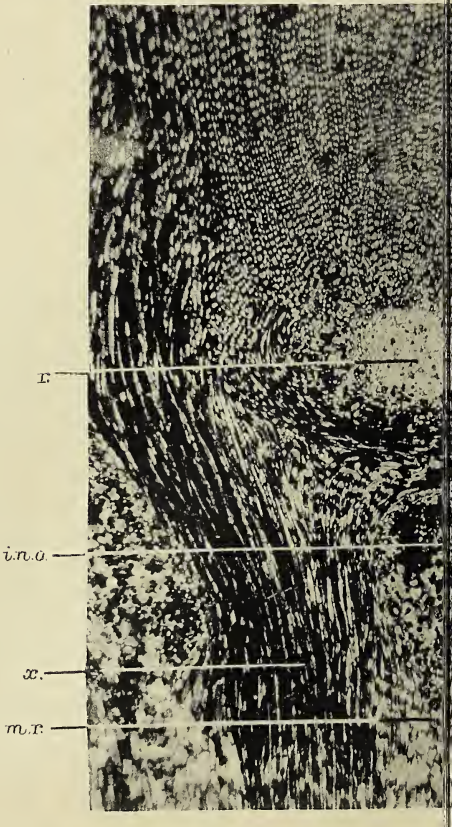
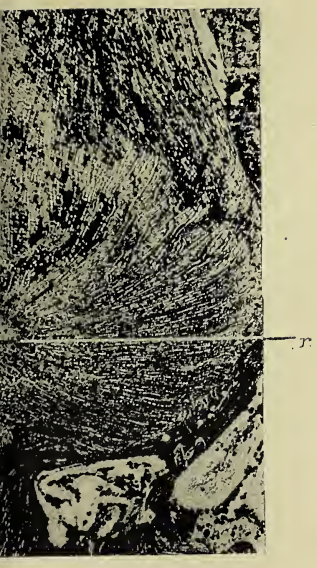


Fig. 3.



A. J. Maslen, photo.

Fig. 4.



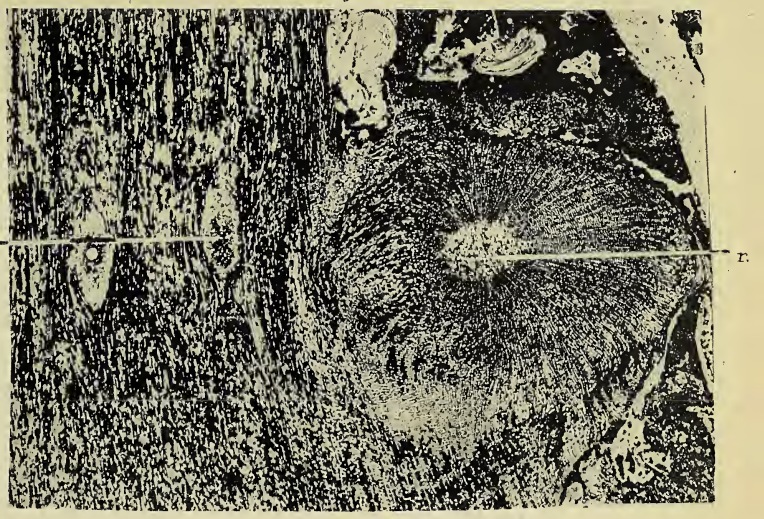
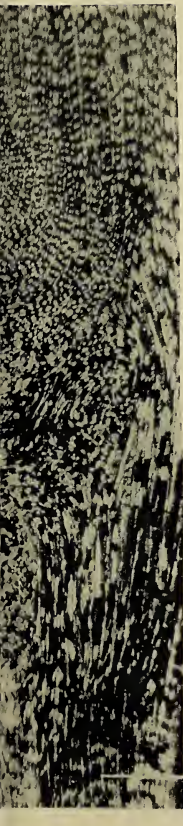


Fig. 5.



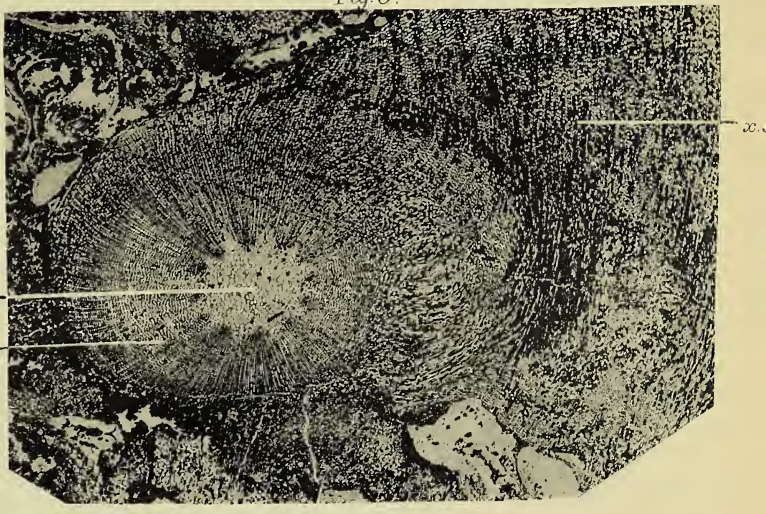


Fig. 6.

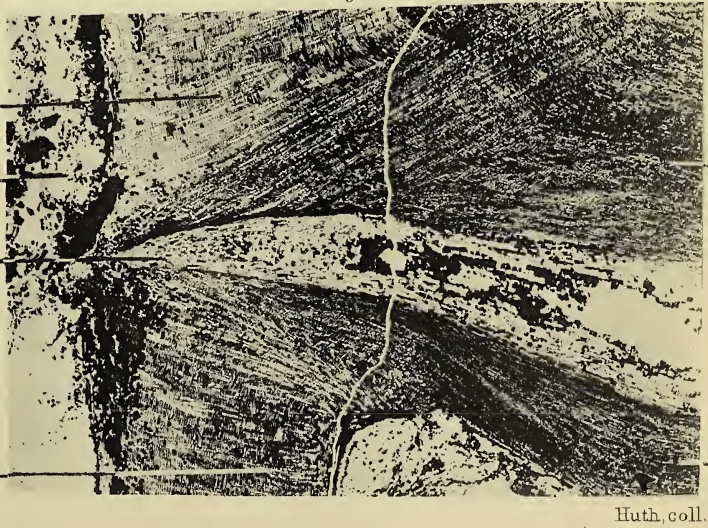


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Fig. 1.

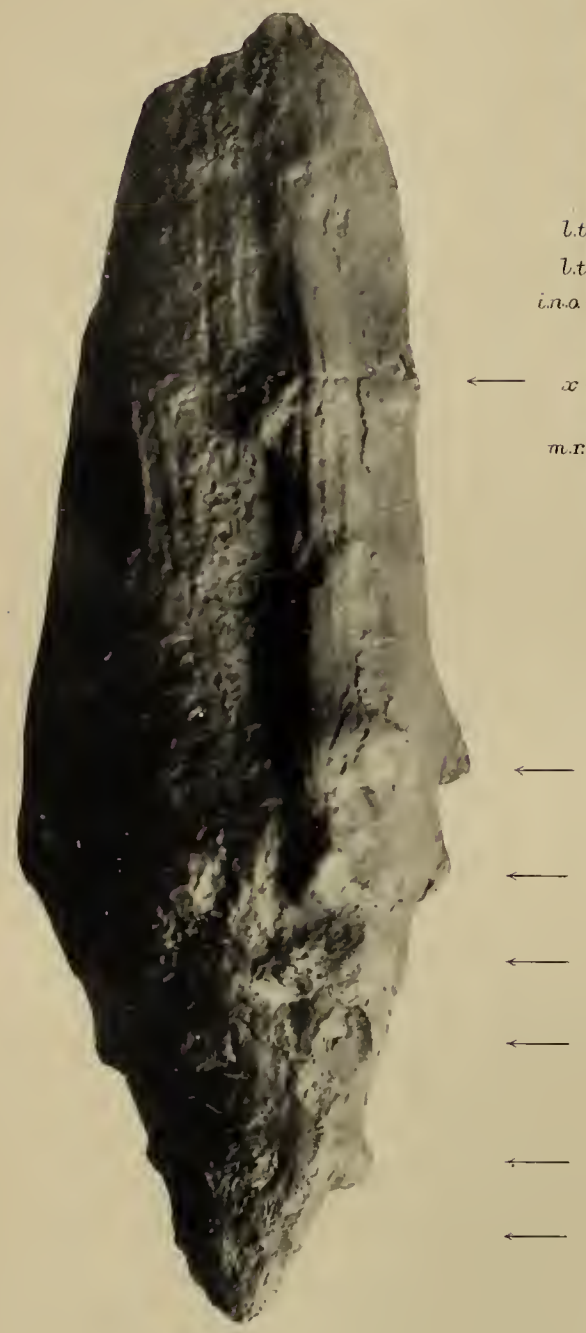


Fig. 2.

Fig. 3.

Fig. 4.

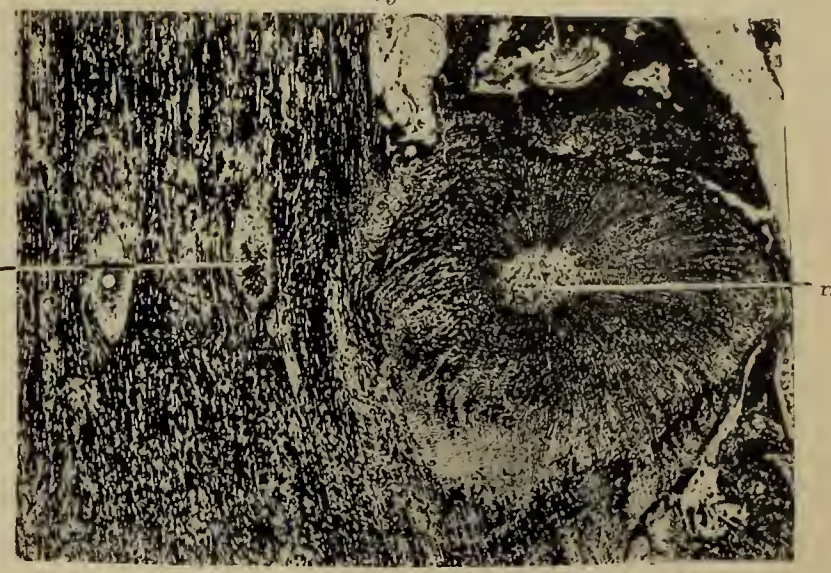


Fig 5.

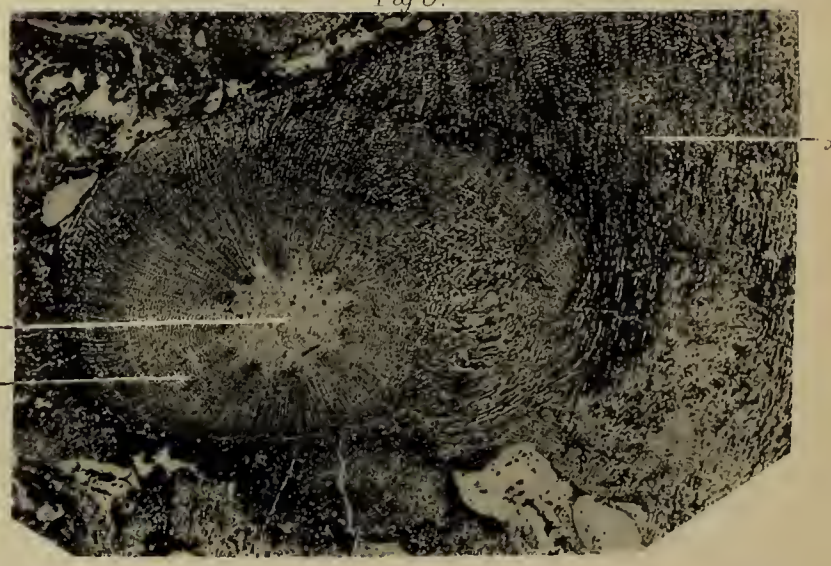


Fig. 6.

A. J. Maslen, photo

Huth, coll

MASLEN. — ON ROOTS OF CALAMITES.

Fig. 7.

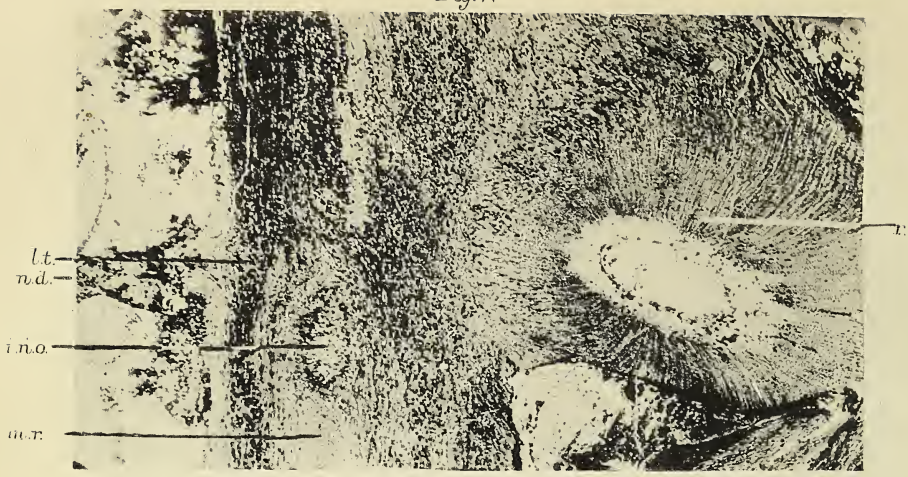


Fig. 11.

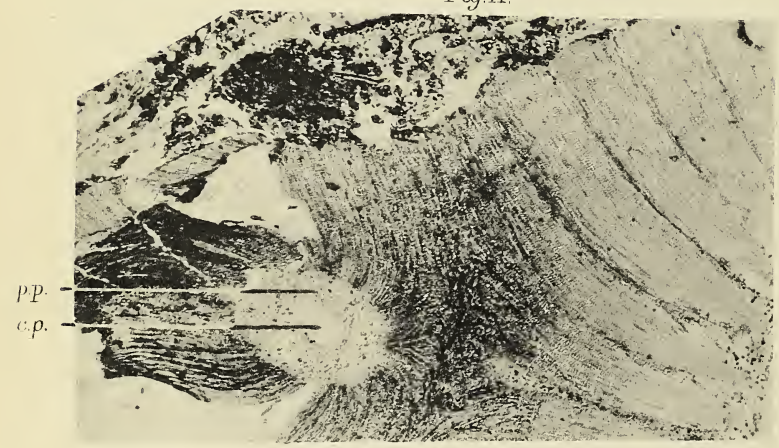
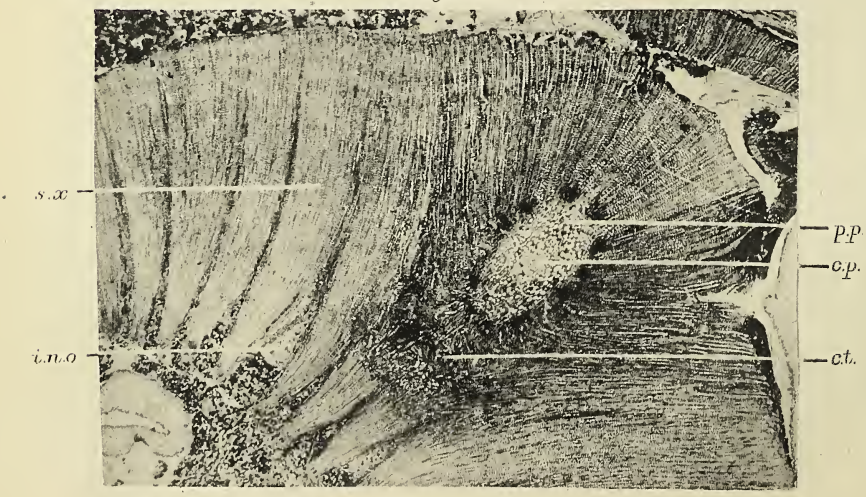


Fig. 9.



A.J.Maslen, photo.

Fig. 13.

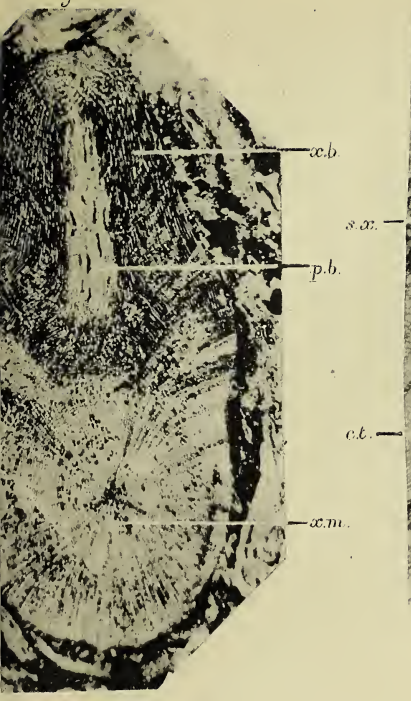


Fig. 10.

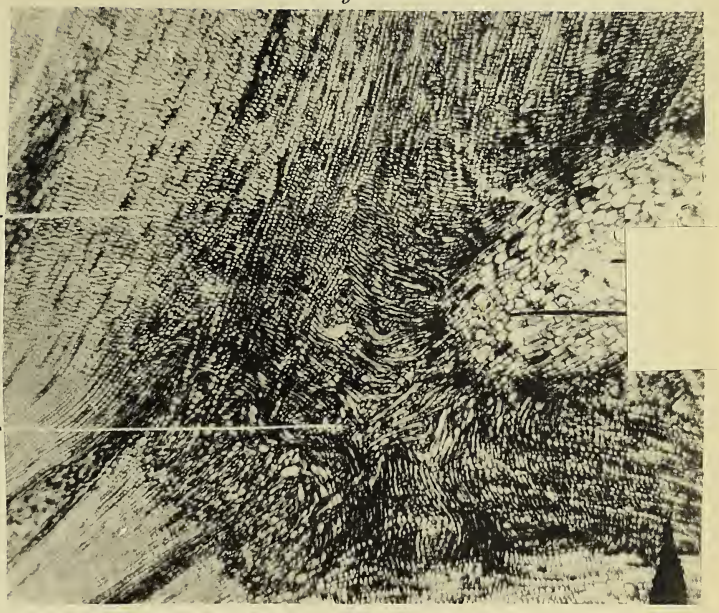


Fig. 8.

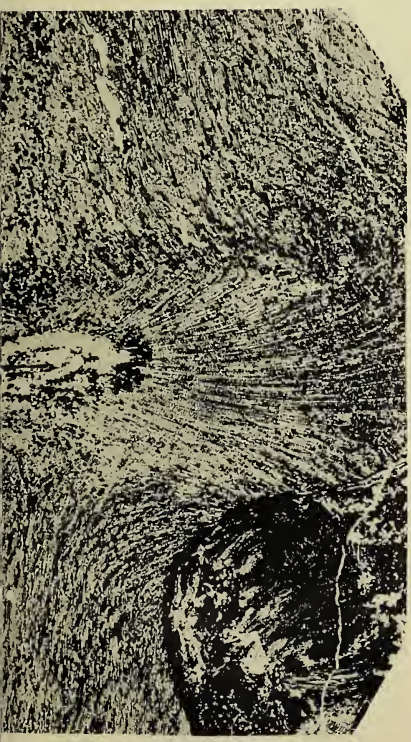


Fig. 12.

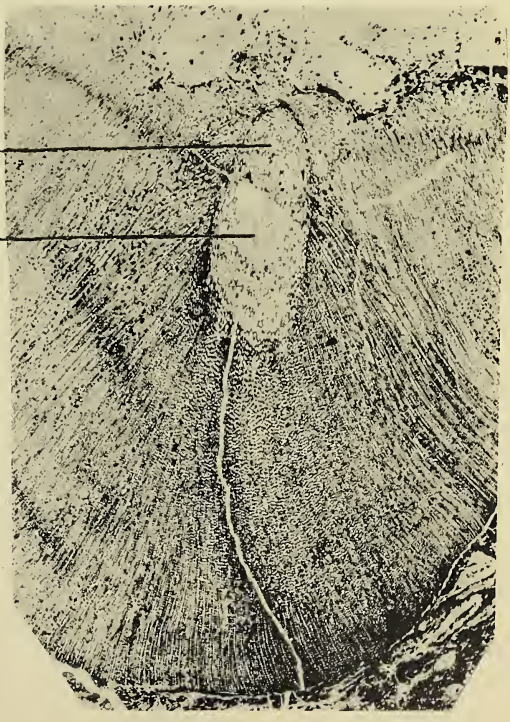


Fig. 7.

Fig. 13.

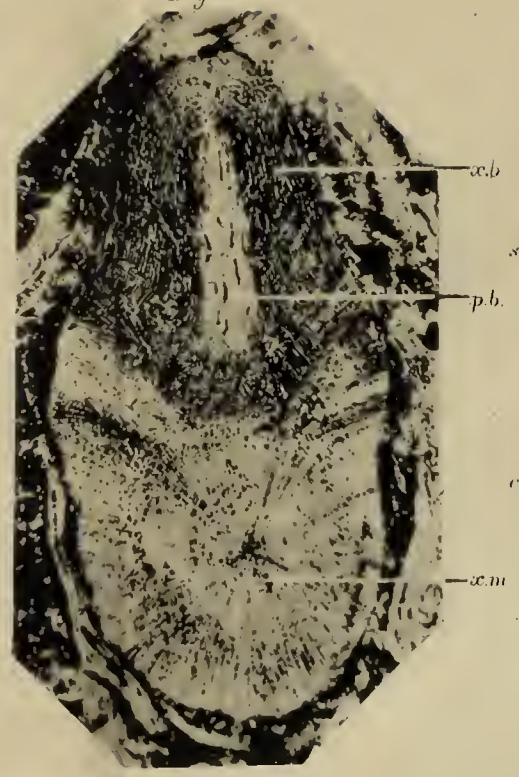


Fig. 10.

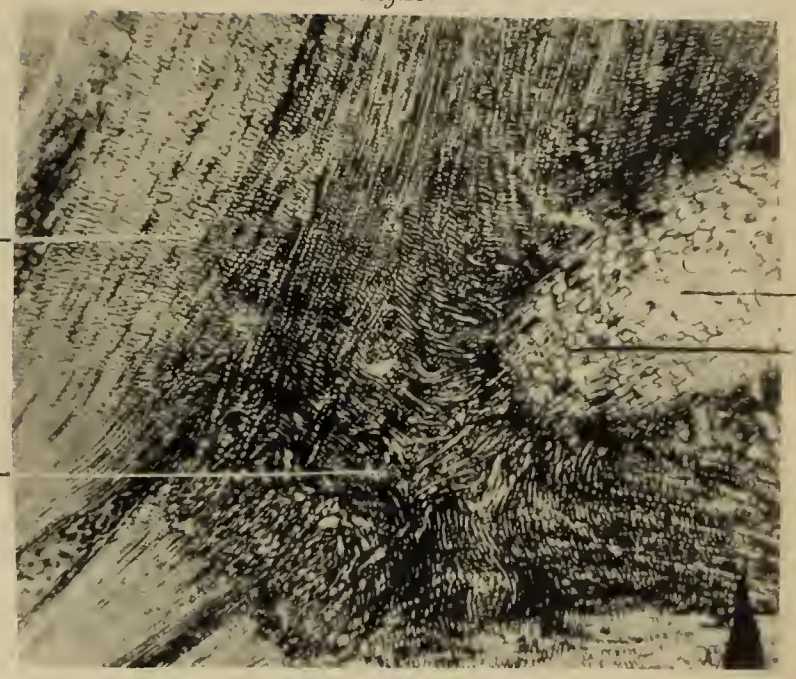


Fig. 11.

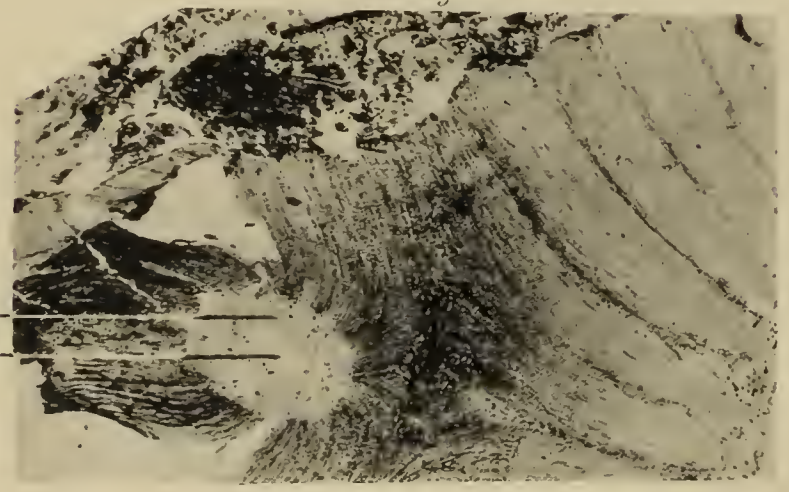


Fig. 8.

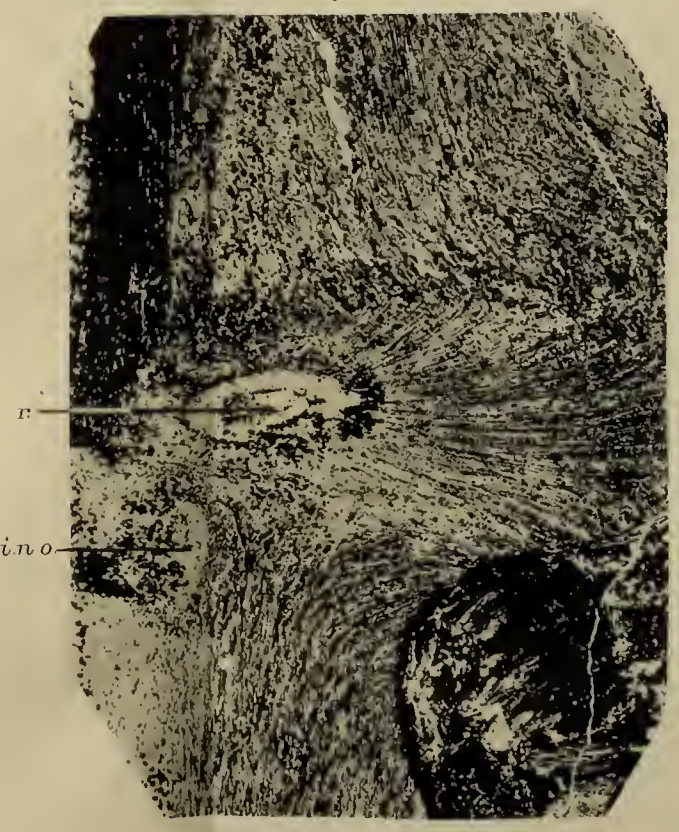


Fig. 12.

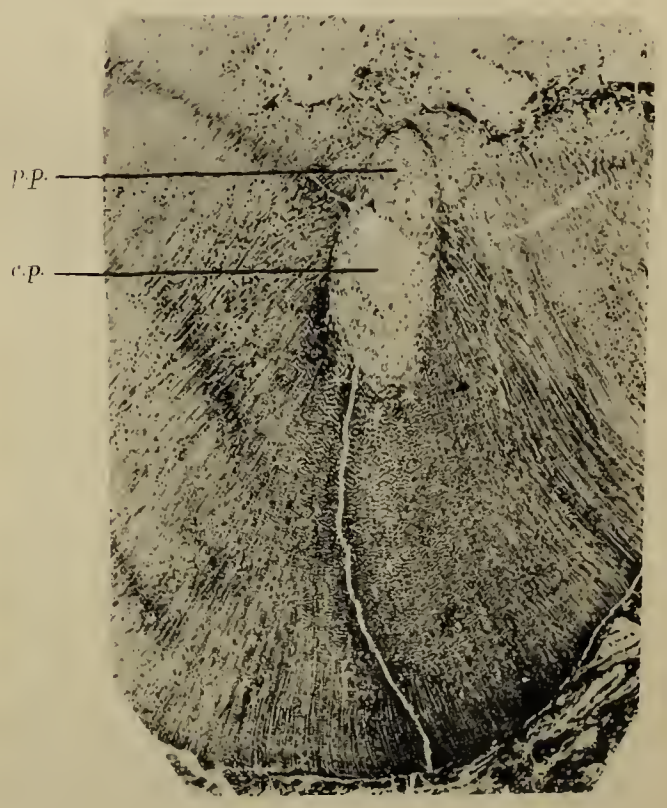
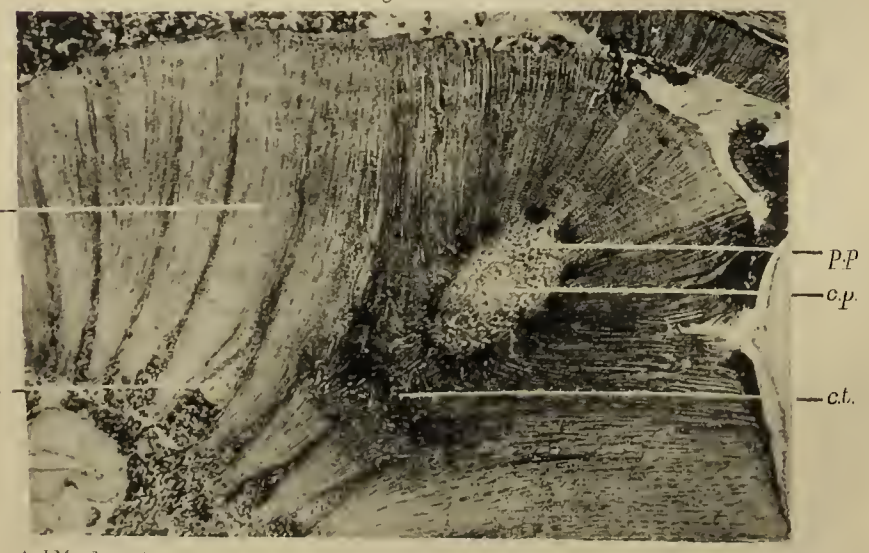


Fig. 9.



A.J.Maslen, photo. Huth, coll.

MASLEN. — ON ROOTS OF CALAMITES.

The Anti-ferment Reaction in Tropistic Movements of Plants[1]

BY

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I.

TO determine whether or no an external stimulus has been perceived by a plant we have generally only one method: namely, to observe whether or not a movement follows the stimulus. The positive observation of a distinct movement is a proof that the stimulus has been perceived. When no distinct movement is to be found, it is possible, either that the stimulus has been perceived, but that a movement for some reason could not be carried out, or that the external stimulus was not perceived.

Therefore in such experiments negative results cannot be utilized. Positive results, moreover, depend exclusively upon observations made on the motor zone. Where perception of stimulus and reaction take place in separate zones of the organ, as in the geotropic perception of the root-tip, discovered by the Darwins, father and son, and in the geotropic curvature in the motor zone of the root, no processes can be detected in the sensory zone by the general method of investigation above mentioned.

My wish to gain some knowledge of what goes on in the sensitive root-tip was realized in 1897, after much fruitless endeavour (1).

A number of root-tips of *Vicia Faba major*, half of which had been geotropically stimulated, while the remainder were unstimulated, were treated in the following manner. Thick longitudinal sections were prepared and boiled in an ammoniacal solution of silver nitrate: they all gave a strong reduction. But when the specimens, carefully squeezed on the slide with the cover-glass, were held towards the light, it was clear that the stimulated tips were always darker than the unstimulated ones. This difference was already distinct long before the first beginning of the geotropic curvature. Another result was found in the fact that unstimulated root-tips placed in a water-emulsion of alcoholic guaiacum solution were coloured blue before the stimulated root-tips; in the same manner the colour tests with alkalin solution of α-naphthol + p-phenylen diamin, or with reduced indigo, were retarded to a remarkable degree. I succeeded therefore at this time in demonstrating the action of an oxydase in the three tests mentioned. But I could not decide whether the decrease in oxydasic effects (after a geotropic stimulation) is caused by a quantitative

[1] Read before the Botanical Section of the British Association, Cambridge, August, 1904.

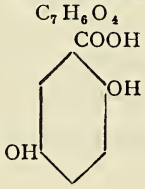
[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

alteration in the amount of the enzyme present, or by some retardation of the oxydasic effects. The substance reducing nitrate of silver, which increases in amount in stimulated roots, was recognized to be a phenol acid, but could not be more closely identified. Analogous changes were also found in the tip of the cotyledon of *Avena* after geotropic stimulation. These results, though of obvious significance, may for the moment be passed over.

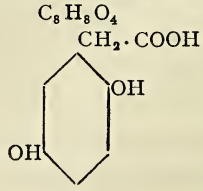
To place my results on an unassailable basis a quantitative method was necessary, and in my search for such a method I was for some time unsuccessful. In the year 1900 it was observed in my laboratory that roots of *Lupinus albus*, being in an asphyxiated state or in chloroform narcosis, showed numerous spherical crystals which could be recognized as those of tyrosin (2). I immediately saw that this precipitate of tyrosin did not occur in the root-tip or in the youngest parts of the growing region, just in the parts where I had observed the strongest reduction of silver. It seemed possible that the reducing substance and the tyrosin crystals were genetically connected. To test this Lupine roots were treated with chloroform water until they were rich in tyrosin crystals, when they were further digested at 28° Cels. in a chloroform atmosphere. At first the cells containing tyrosin did not show any silver reduction; but after a few days the crystals of tyrosin disappeared and the cell-content reduced ammoniacal solution of silver very strongly. Roots of *Lupinus* were then finely ground and treated with chloroform water at 28° until the Millon reaction could not be observed. I then added a little pure tyrosin from Merck, Darmstadt. This also disappeared, as could be seen by the Millon test becoming gradually weaker and finally imperceptible. But the mixture was getting an exceedingly strong power of reducing silver; so that the suggestion that a silver-reducing substance is produced from tyrosin was certainly confirmed. It was also shown that this decomposition of tyrosin is connected with enzyme action.

Now since the year 1891 a substance has been known arising from tyrosin in human and animal metabolism, and possessing an active power of reducing silver. This substance was prepared by Wolkow and Baumann (3), and must be regarded according to these authors as the next higher homologous acid to gentisinic acid. This substance $C_8H_8O_4$ received in the nomenclature of Tiemann the name of 'homogentisinic acid':

Gentisinic acid:



Homogentisinic acid:



Enzymes decomposing tyrosin with formation of homogentisinic acid have been frequently prepared in recent times from animal and vegetable tissues. Such a tyrosinase Bertrand (4) discovered in Hymenomycetes (*Russula*), in the tubers of *Dahlia,* in the root of beet. Gonnermann (5) showed lately, in the case of the tyrosinase of the beet-root, that the substance produced in the fermentative alteration of tyrosin is identical with homogentisinic acid.

The effect of tyrosinase on tyrosin is oxydation accompanied by the formation of ammonia and carbon dioxide. The formation of homogentisinic acid from tyrosin is expressed by the equation :

$$\underset{\text{Tyrosin}}{C_9H_{11}NO_3} + O_3 = \underset{\text{Homogentisinic acid}}{C_8H_8O_4} + NH_3 + CO_2$$

There remains the question, whether the enzyme called tyrosinase is a distinct undivisible substance or a mixture of an oxydizing and desamidizing enzyme.

An important fact established in my laboratory is the occurrence of an oxydizing enzyme in root-tips, which changes homogentisinic acid into a substance not able to reduce silver nitrate. The homogentisinic acid, therefore, cannot be regarded as a final product of metabolism from the tyrosin.

The result of our experiments shows, therefore, that a short time after the beginning of the geotropic induction, there appears a retardation of the normal destruction of tyrosin, to be recognized by an accumulation of homogentisinic acid. We have in the following report to confirm it more exactly and to describe the methods of investigation.

II.

The substance reducing $AgNO_3$ can easily be extracted from the ground root-tips by treating them with alcohol of 96 per cent. After evaporating the alcoholic solution and dissolving the remainder in water a brown solution is obtained, weakly acid to litmus, nearly free from sugar and reducing ammoniacal solution of $AgNO_3$ very strongly. The solution, even when weakly acid or neutral, assumes a dark colour on being allowed to remain in contact with air. Crystals I could never obtain from such extracts. The reactions of those solutions were nearly the same as those described by Baumann for solutions of pure homogentisinic acid. Treated with alkali the solution changes to a reddish brown or a dark brown according to the concentration ; ammoniacal solution of $AgNO_3$ is immediately reduced on warming ; cold Fehling's solution is not reduced, but is feebly reduced on boiling. Iron chloride gives a green colour, iron vitriol a blue-violet. Millon's reagent gives a yellow colour ; acetate of lead gives a precipitate. To identify homogentisinic acid (which is difficult

to crystallize) Baumann recommends the preparation of the easily crystallizable ethylester of the acid, which gives most of the tests in the same manner as does the free acid. For this purpose gaseous hydrochloric acid was passed in large excess in an alcoholic solution of the root-tip substance well cooled with snow. After twenty-four hours water was added, the fluid made alkaline with Na_2CO_3, and shaken with ether. The ether having been evaporated, there remained a syrupy mass containing embedded crystals. These crystals could finally be obtained quite pure by recrystallizing from hot water and washing with alcohol. The solubility and shape of crystals as well as the melting-point (120°) agreed exactly with the properties of the ethylester of homogentisinic acid. The reducing substance of the root-tips is therefore undoubtedly to be regarded as homogentisinic acid. Homogentisinic acid gives a reddish colour with hydrogen-peroxide, and I suppose that the red colour produced in the tissue of *Faba* roots by hydrogen peroxide, as described by Pfeffer, is connected with the presence of homogentisinic acid. Wolkow and Baumann elaborated an excellent method for the quantitative estimation of homogentisinic acid, particularly in urine. This method can be made use of for our purposes without any important modifications. Baumann titrated with decinormal solution of silver nitrate, and determined with weighed quantities of absolutely pure homogentisinic acid how much ammoniacal silver solution is needed to oxidize 1 g. homogentisinic acid. According to this estimation 1 g. of homogentisinic acid corresponds fairly closely to 2·60–2·65 g. silver; 1 cc. decinormal solution of $AgNO_3$ therefore corresponds to 4·1 to 4·2 mg. of pure anhydrous homogentisinic acid.

In determining homogentisinic acid in root-tips I proceeded as follows. From 100 radicles the apical 2 mm. are quickly cut off and immediately ground as finely as possible in a small mortar with 5 cc. distilled water and 2 g. of the purest glass-powder. The contents of the mortar are washed with a small quantity of water into a measuring-flask containing 25 cc. The flask is then filled up to the mark and the contents shaken and filtered. The entire homogentisinic acid of the root-tips is now in the filtrate. Ten cc. of the filtrate are transferred by means of a pipette to a flask. Then 10 cc. of dilute ammonia[1] are added, and immediately afterwards a small quantity of $\frac{n}{10}$ silver solution from the Curette. Generally 1 cc. $\frac{n}{10}$ silver nitrate can be added at once without risk of excess. The mixture is now boiled. To facilitate the filtration of the silver precipitate the flask is allowed to cool for five minutes, then five drops of moderately concentrated calcium chloride solution are added, and ten drops of ammonia carbonate. The contents of the flask are then shaken and filtered.

[1] Commercial concentrated ammonia of sp. gr. 0·900 diluted in the proportion 1·10.

The filter-paper should be as small as possible, and the fluid should drain off thoroughly. Now ammonia and 0·3 cc. of silver solution are again added to the filtrate. If any reduction occurs, $CaCl_2$ and $(NH_4)_2CO_3$ are again added and the solution filtered, and this process may be repeated three or four times. If at last no distinct precipitation of silver occurs, it is necessary to see whether a precipitate of silver chloride appears on adding HCl so as to give a distinct acid reaction. If a white cloud appears there must have been a slight excess of silver solution. In this case 10 cc. of the original filtrate are measured off and treated as before, except that the silver solution is diminished by 0·1 to 0·2 cc. If no excess of $AgNO_3$ can now be detected, the right value must lie between the two results of titration. Since the limits of error cannot be more in this method than $\pm$0·2 cc., the homogentisinic acid can be estimated within $\pm$1 mg. The colour and the quantity of the precipitate in the reduction tests of the extracts prepared from root-tips can be exactly distinguished even if the tests differ in concentration so little that the difference corresponds to 0·3 cc. $\frac{n}{10}$ silver solution; usually the limit of error is found to be about $\pm$0·2 cc. $\frac{n}{10}$ silver nitrate.

In such experiments 5·0 cc. of $\frac{n}{10}$ silver nitrate are usually required for 100 root-tips. Of course by far the greater part of the silver solution consumed is not reduced by the homogentisinic acid, but by precipitation of proteids and other substances. Therefore the coefficient of conversion calculated by Baumann (4·1 mg. homogent. acid = 1 cc. $\frac{n}{10}$ $AgNO_3$) cannot be applied here. It is, therefore, necessary to determine by weighing the share in the entire silver consumption taken by homogentisinic acid in the before mentioned experiments. First of all it had to be made out whether the whole of the reducing substance could be obtained in solution by alcoholic extraction of the dried root-tips. A hundred root-tips were dried in Hofmeister's weighing-glasses and weighed, then powdered with the glasses in a mortar, extracted with absolute alcohol, then the remainder after drying and weighing was extracted with distilled water. The alcoholic and the watery extract were titrated with $\frac{n}{10}$ $AgNO_3$. Two such tests, together with a control experiment without root-tips, gave the following results :—

	I.	II.	Control.
The weight of the glass	1·5634 g.	1·6240 g.	1·0315 g.
The bowls with 100 root-tips	1·5843 g.	1·6421 g.	1·0315 g.
The weight of the root-tips	0·0209 g.	0·0181 g.	—

	I.	II.	Control.
Weight of alcoholic extract	0·0189 g.	0·0188 g.	0·0154 g.
Thereof soluble in water	0·0148 g.	0·0146 g.	0·0102 g.
Titration with $AgNO_3$	0·75 cc.	0·75 cc.	no reduction.
Residue after extraction with alcohol .	2·0327 g.	2·0725 g.	1·4802 g.
The part soluble in water titrated with $\frac{n}{10}$ $AgNO_3$	0·50 cc.	0·50 cc.	no reduction.
Insoluble in water	2·0031 g.	2·0442 g.	1·4564 g.
Insoluble in alcohol and soluble in water	0·0296 g.	0·0283 g.	0·238 g.

In this experiment only 2·25 cc. of silver solution were consumed for the substances soluble in water and alcohol. By far the larger quantity of the reducing substances was soluble in absolute alcohol, as shown by the high titration value of the alcohol extract, a smaller quantity of them was insoluble in alcohol and only soluble in water. This last portion, reducing Fehling's solution and giving the well-known test with phenyl-hydrazine, must contain principally sugar. The fitness of the Baumann method for investigating the processes of metabolism in geotropically stimulated roots was still to be tested by investigating the increased values obtained by silver titration in the case of alcoholic and watery extracts of geotropically stimulated roots in comparison with unstimulated specimens.

Two sets of unstimulated roots and two sets of roots stimulated by thirty minutes in a horizontal position were used for analysis. A control experiment was included, and the arrangement was otherwise as in the above described instance:—

	Not stimulated.		Stimulated.		Control.
I. The glasses	1·0468 g.	1·4197 g.	1·3377 g.	1·0858 g.	1·3925 g.
II. Glasses with root-tips .	1·0678 g.	1·4461 g.	1·3603 g.	1·1113 g.	1·3925 g.
III. The tips alone . . .	0·0210 g.	0·0264 g.	0·0226 g.	0·0255 g.	„
IV. Alcoholic extract soluble in water	0·0082 g.	0·0093 g.	0·0087 g.	0·0108 g.	0·0053 g.
V. Alcoholic residue soluble in water	0·0182 g.	0·0214 g.	0·0172 g.	0·0190 g.	0·0131 g.
Titration of IV.	0·75 cc.	0·75 cc.	1·00 cc.	1·00 cc.	$\frac{n}{10}$ $AgNO_3$
Titration of V	0·50 cc.	0·50 cc.	0·50 cc.	0·50 cc.	„

The same test was carried out by rubbing up the fresh root-tips directly with glass-powder and absolute alcohol; the subsequent proceedings were the same.

	Not stimulated.		Stimulated.		Control.
I. Alcoholic extract soluble in water	0·0081 g.	0·0084 g.	0·0093 g.	0·0087 g.	0·0048 g.
II. Alcoholic residue soluble in water	0·0211 g.	0·221 g.	0·0223 g.	0·240 g.	0·0170 g.

	Not stimulated.	Stimulated.	Control.
Titration of I	0·75 cc. 0·75 cc.	1·00 cc. 1·00 cc.	$\frac{n}{10}$ $AgNO_3$
Titration of II	0·75 cc. 0·75 cc.	0·75 cc. 0·75 cc.	„

The increasing of the amount of $AgNO_3$ in the titrations after geotropic stimulation therefore depends only on the portion soluble in alcohol, and the substances in the root-tips which are not soluble in alcohol do not participate in augmenting the reducing power.

Therefore the view is a very probable one, that silver-reducing substances other than homogentisinic acid take no share in the alterations of metabolism caused by geotropism. The amorphous yellow residue of the alcohol extract certainly cannot be regarded as pure homogentisinic acid, and may probably contain other distinct silver-reducing substances. But so far as reliance can be placed on calculations from the small amounts available, the reducing power of the residue from the alcoholic extract does not differ much from the results obtained by Baumann for pure specimens of homogentisinic acid.

If the amounts obtained from the control experiments are subtracted from the average amounts for the portion of alcoholic extract soluble in water, the average weight of this portion can be calculated as 3·87 mg. for 100 root-tips; the average value of titration with $\frac{n}{10}$ $AgNO_3$ as 0·875 cc. Therefore 1 cc. silver solution can be taken as equivalent to 4·478 mg. 'homogentisinic acid.' Baumann gives 4·1 to 4·2 mg. for pure homogentisinic acid.

Homogentisinic acid was also prepared from the upper parts of seedling roots by converting the tyrosin contained in them by means of the root enzyme (tyrosinase) by autolysis in chloroform water, and by extracting the product with alcohol. Ten cc. of the alcoholic extract, being a solution of crude homogentisinic acid, reduced 6·3 cc. of $\frac{n}{10}$ $AgNO_3$ and gave 26·4 mg. dried residue; 20 cc. of the same solution reduced 12·4 cc. silver nitrate and gave 51·1 mg. residue. It follows from this result that 1 cc. of the silver solution is equal to 4·1 mg. dried residue, i. e. crude homogentisinic acid.

The absolute amount of homogentisinic acid contained in root-tips can also be calculated from the results before mentioned. Since the average weight of dry substance of 100 root-tips of *Lupinus albus* according to ten estimations can be taken as 22·7 mg., the 3·87 mg. homogentisinic acid contained in the dry substance equal 17 percentage of the dry substance. After a geotropic stimulation this amount is increased by one-quarter and rises above 20 percentage of the dry substance.

By means of the method described the increase of silver-reducing

power after geotropic stimulation can easily be shown in all root-tips, hypocotyls, epicotyls, and, speaking generally, in all organs of plants hitherto investigated by me (7). The increase in homogentisinic acid always appears much earlier than the first traces of geotropic curvature, and the maximum is observed after about thirty minutes in roots kept at 18° to 20° C. The same phenomenon is also generally to be found in phototropic and hydrotropic movements (8); it has not in fact been absent in any tropistic movement investigated up to the present time. When the roots have finished their geotropic curvature, the difference in the amount of homogentisinic acid in comparison with vertical roots is nearly imperceptible. The course of the process may be illustrated by the following figures, giving the result of the titration of 10 cc. of filtrate (entire filtrate 25 cc.) obtained from 100 ground-up roots of *Lupinus albus* which had been previously stimulated:

Duration of geotropic stimulation.	$\frac{n}{10}$ $AgNO_3$ used for 10 cc. of filtrate		
Minutes.	in 100 stimulated roots.	in 100 control roots.	Difference.
5	2·1 cc.	2·0 cc.	0·1 cc.
10	2·0 „	2·0 „	0·0 „
15	2·0 „	1·9 „	0·1 „
20	2·2 „	2·1 „	0·1 „
25	2·2 „	1·9 „	0·3 „
30	2·4 „	2·1 „	0·3 „
45	2·3 „	2·0 „	0·3 „
60	2·15 „ Curvature distinct.	1·9 „	0·25 „
90	2·15 „	2·0 „	0·15 „
120	2·25 „ Curvature finished.	2·15 „	0·1 „
180	1·90 „	1·90 „	0·0 „

The observed differences are certainly above the limits of error of the method. But they are, however, so small, that they can only be regarded as just certain differences, since the quantities are very small and the error of the method rises to 10 per cent. of the values obtained. It became, therefore, a matter of importance to obtain control of a method giving a much closer insight into the metabolism of stimulation than could be got from the direct estimation of homogentisinic acid.

III.

The inhibition of oxidase action which I found accompanying an accumulation of homogentisinic acid must be assumed (in accordance with my present experience) to be in causal connexion with the increased values obtained by silver-titration.

It is easily demonstrated that the ground-up root-tips kept for some

time in chloroform-water lose the power of reducing silver nitrate; even small amounts of homogentisinic acid intentionally added to the preparation cannot be detected after some time. This is an enzyme action: preventable by boiling the mixture, and to be observed even in filtrates from a Chamberland filter. The alcohol precipitate of this filtrate has the power of oxydizing homogentisinic acid in the same degree. We have here an enzyme (oxidase) capable of acting on homogentisinic acid, p-phenyl diamin + α-naphthol, guaiacum, &c. It is clear that either the action of oxidase is somehow inhibited during geotropic stimulation, so that the homogentisinic acid, which is otherwise further acted on, disappears more slowly than under ordinary circumstances, and accumulates to a certain degree: or there must be temporarily a diminution in production of oxidase by the root-tip.

If the latter were the case, it might be supposed that a mixture of an equal number of stimulated and non-stimulated root-tips would show the homogentisinic acid disappearing at a rate halfway between preparations derived entirely from stimulated and unstimulated roots. But the experiments I made show distinctly that such a mixed preparation does not differ in its behaviour from tests prepared entirely from stimulated roots. Therefore the existence of a specific retarding substance seems to be more probable.

I demonstrated (9) that a mixture of eighty unstimulated root-tips and twenty stimulated ones gives the same rate of disappearance of reducing power as a preparation of stimulated root-tips. Further, it was shown that even four stimulated root-tips added to ninety-six normal root-tips gave a distinct retardation of the disappearance of the reducing power. The hindering effect therefore is an exceedingly strong one. Finally, it was discovered that the inhibitory substance can be destroyed by boiling, and can be isolated by filtration through a Chamberland filter, and precipitation by alcohol. The substance therefore has the properties of an enzyme, and may be characterized as an anti-oxydase, on account of its effect being contrary to that of the oxydase of the root-tips. This anti-oxydase has the property of other anti-enzymes, namely, that it is a strictly specific effect. In this way the anti-enzyme of geotropically stimulated hypocotyls of *Lupinus* has an effect on the oxydizing enzyme of a *Lupinus* root-tip, but not on the root-tip enzyme of *Zea Mays* or *Cucurbita*. Between the oxydase and anti-oxydase of nearly related plants, therefore, a mutual action occurs, but not between enzyme and anti-enzyme of plants widely separated in regard to affinities (9). From these experiments on anti-enzymes it may be shown that the anti-enzyme produced in phototropic stimulation does not differ in any way from the geotropic anti-enzyme. It was established that the anti-oxydase is destroyed at 62° C., while the oxydase of the root-tip loses its power at 63 to 64° C. In a similar manner the anti-toxines lose their power at a lower temperature than the toxines,

and it is possible to prepare by means of a certain temperature an effective specimen of toxin, from a mixture of toxin and anti-toxin, by destroying the anti-toxin. In the same way, heating for one hour to 62° entirely annuls the retardation in the disappearance of homogentisinic acid in a preparation of stimulated root-tips; so that the reaction becomes identical with that observed in unstimulated root-tips.

This result is interesting in still another point. The experiments show that preparations of unstimulated root-tips lose their oxydasic effects at a certain temperature without the occurrence of any acceleration of the oxydation of homogentisinic acid. Therefore it may be supposed that the anti-oxydase does not exist in unstimulated roots, but it is formed only by tropistic stimulation.

Thus the interpretation of the process of metabolism in geotropic stimulation discovered by me is more exactly defined. It is essentially a retardation in the metabolism, in other words, in the decomposition of tyrosin. The tyrosin is, by tyrosinase, converted into homogentisinic acid, as occurs normally, but the further oxydation of the homogentisinic acid by the oxydase is inhibited by the production of an anti-oxydase, rendering the oxydase partially inefficient and causing by this means an accumulation of homogentisinic acid.

This process may, for convenience sake, be called the 'Anti-ferment Reaction.'

The exact investigation of the retardation of oxydation by the tropistic anti-enzyme is precisely suited to the application of chemical methods to the movement of plants, because the differences between stimulated and unstimulated organs can be magnified at will by the addition of homogentisinic acid, and can thus be completely removed from the doubts and uncertainties which interfere with the results of the *direct* titration method described in the earlier part of this paper. We thus obtain the very delicate and trustworthy method of chemically investigating the phenomena of irritability, which I proceed to describe.

The root-tips to be analysed are ground as quickly as possible with glass-powder and water (10 cc.), and are thus converted to a thin homogeneous emulsion or mash. The mash is washed without loss into an Erlenmeyer flask of $\frac{1}{8}$ litre capacity, and 50 cc. of standard aqueous solution of homogentisinic acid are then added. The titration of the acid used in my experiments gave 10 cc. as about equivalent to 2·3 cc. $\frac{n}{10}$ $AgNO_3$. Finally, 5 cc. chloroform are added. The mixture is allowed to settle for ten to fifteen minutes, then 5 cc. are taken to determine the initial reducing power. The specimens now remain uncovered in an incubator (28 to 30°), and are shaken several times every day. At intervals of five days 5 cc. are taken to determine the reducing power. In such a way the decrease

in reducing power may be observed during a sufficiently long time. Or more homogentisinic acid may be introduced at first to extend the time of observation still more.

When so much homogentisinic acid is added, the slight error caused by the simultaneous presence of other reducing substances in the mixture, such as sugar, can be still more neglected. Since, after some time, no reduction at all can be observed in the specimens, even these substances must be destroyed by enzymes.

IV.

We must now pass on to a criticism of the results of our experiments. Even if between geotropically stimulated and unstimulated roots there exist constant and certain differences revealed by the anti-ferment test, we must meet the objection that the anti-ferment reaction may not be confined to tropistic stimulation, but may accompany a variety of departures from the normal condition of plant organs. Therefore it must be shown that only tropisms are able to produce the anti-ferment reaction. This can be really shown, and the following results demonstrate that neither chloroform-narcosis nor poisoning by antipyrin, acids, alkalies, nor mechanical hindering of growth by means of gypsum, nor traumatic stimulus, are by themselves able to produce anti-ferment reaction.

When chloroform or any other poison was used, the proper concentration was empirically determined in each case, namely, the strength necessary to prevent growth and curvature without permanent injury, during the period experimentally known to be sufficient for geotropism to occur. In order that the roots may be certainly influenced by the poison, they must stand in the solution during a sufficient time (one hour at 16° C.) in the vertical position, before they are geotropically stimulated by placing them horizontally.

Before beginning the chloroform experiments I convinced myself that the anti-ferment reaction occurs in the same degree in roots kept in damp air as in roots entirely submerged in water. The reducing power of 5 cc. of the filtrate tested in equal intervals of time was found to be—

Unstimulated:		Stimulated by placing them horizontally for thirty minutes:	
in damp air.	in water.	in damp air.	in water.
3·0	3·0	3·0	3·0 cc. $\frac{n}{10}$ $AgNO_3$
2·5	2·5	2·8	2·7 „ „
2·1	2·1	2·6	2·4 „ „
1·7	1·6	2·3	2·1 „ „

The reducing power was, as usual, exactly equalized at the beginning of the experiments. Even between roots growing in damp sawdust and growing in water no difference was noticed. The water (from the water supply) had therefore no influence on the degree of anti-ferment reaction.

The actual curvature, however, began in roots growing in sawdust after one and a half hours, while the curvature of roots growing submerged in water, on account of hindered respiration and growth, did not appear before three hours. I convinced myself definitely that the anti-ferment reaction is not retarded by submerging the roots in water. With roots growing in damp air or sawdust, placing them horizontally for six minutes is sufficient to produce a distinct anti-ferment reaction, as described in the following section. In submerged roots also the anti-ferment reaction was distinctly seen after six minutes' geotropic stimulation. Therefore the roots submerged in tap-water could be considered as being normal controls for comparison with chloroformed roots. From numerous experiments leading to the same result the following may be quoted. For the experiments large glass vessels were used which could be easily turned through 90°, and thus the whole of the roots could be at once changed from the vertical to the horizontal position. In each vessel the roots were fastened with a suitable apparatus. One of these vessels was filled with chloroform-water (one part of saturated solution chloroform-water to seven parts of water), the other vessel was filled with tap-water. All the roots remained vertical for one hour, the temperature of the fluids being 16°. Then the apparatus were rotated so that both lots of 100 roots were placed horizontally for six minutes. All the root-tips were then cut off and prepared for digestion. The experiments gave the following numbers, being the reducing power of 5 cc. filtrate at intervals of five days.

Chloroform solution :		Pure water :	
roots stimulated.	unstimulated.	stimulated.	unstimulated.
3·0	3·0	3·0	3·0 cc. $\frac{n}{10}$ $AgNO_3$
2·7	2·5	2·7	2·5 „ „
2·4	2·0	2·4	2·1 „ „
2·1	1·6	2·1	1·6 „ „
1·8	1·2	1·8	1·1 „ „
1·5	0·8	1·5	0·7 „ „

The anti-ferment reaction is therefore connected only with geotropic induction.

Experiments arranged in the same manner with antipyrin solution 1·1000 gave analogous results. The stimulation lasted thirty minutes.

Antipyrin :		Pure water :	
stimulated.	unstimulated.	stimulated.	unstimulated roots.
3·0	3·0	3·0	3·0 cc. $\frac{n}{10}$ $AgNO_3$
2·7	2·5	2·7	2·6 „ „
2·5	2·0	2·4	2·2 „ „
2·2	1·5	2·1	1·7 „ „
1·9	1·0	1·8	1·3 „ „
1·6	0·5	1·5	0·9 „ „

Dilute acids or alkalies in suitable concentrations gave the same effect. But I could never obtain the anti-ferment reaction without applying tropistic stimulation.

Experiments with roots mechanically hindered in growth by gypsum also tally with the before-mentioned experiments. To work with roots fixed with gypsum, the seedlings (*Faba major*) were pinned into long and narrow wooden troughs filled with soft gypsum. They stayed in the solid gypsum for twenty-four hours in a vertical position, in damp air. And then, in those cases where geotropic stimulation was to be applied, they were placed horizontally for one hour. After being geotropically stimulated, the roots were freed from gypsum, their tips were cut off and the tests arranged. For such experiments the roots of *Vicia Faba major* were used, not being so easily injured as the roots of *Lupinus*: twenty-five *Faba* roots served for a series. The result of such an experiment is given; the numbers have the same significance as above.

Roots growing in sawdust:		Roots in gypsum:	
unstimulated.	stimulated.	unstimulated.	stimulated.
2·7	2·7	2·7	2·7 cc. $\frac{n}{10}$ $AgNO_3$
2·2	2·4	2·3	2·4 „ „
1·7	2·1	1·9	2·1 „ „
1·2	1·8	1·4	1·8 „ „
0·7	1·5	0·9	1·5 „ „

The anti-ferment reaction is therefore caused only by geotropic stimulus, even in this case.

The following experiments show that a wound cannot by itself produce the anti-ferment reaction. The roots were wounded by the amputation of 1 mm. from the tip.

Normal.	Decapitated and placed in vertical position.	Decapitated and placed for thirty minutes in horizontal position.
2·0	2·0	2·0 cc. $\frac{n}{10}$ $AgNO_3$
1·6	1·6	1·8 „ „
1·2	1·2	1·6 „ „
0·7	0·7	1·3 „ „
0·0	0·0	0·7 „ „

Neither can extreme degrees of temperature or light produce the anti-ferment reaction without a tropistic stimulus; and the sum of my experiences is to prove that tropistic stimulation exclusively is able to cause the alterations of metabolism described by me in root-tips, stems, cotyledons, &c.

I must therefore still differ from the view expressed by Noll (10) in the Botanische Zeitung, that the quantitative alterations of metabolism

discovered by me are an expression of general disturbance of the normal state of organs under anomalous conditions. But we must admit that the connexion with the other processes of geotropic stimulus is, at present, entirely hidden from us. What can be made out is given in the following chapters. Speaking generally, it may be said that it is a question of changes demonstrable at a very early stage of the process, and having probably an indirect relation to the phenomena of perception. At present it is hardly to be decided whether important processes or collateral phenomena of the physiological act are to be seen in the anti-ferment reaction. At any rate, the results of my experiments show that the anti-ferment reaction is a very important method for investigating tropisms, and one capable of throwing valuable light on a variety of points.

V.

Hitherto the geotropic curvature of roots was principally used for the investigation of the anti-ferment reaction. And I shall accordingly in this paper deal especially with the phenomena of geotropism.

First of all, it is of interest to know how long a period of stimulation in a horizontal position is needed for the appearance of the anti-ferment effect. The roots were kept horizontally for accurately measured periods of time (by means of a suitable apparatus), when they were placed vertically and underwent the anti-ferment test. Titrations were made, and the result is given in the following table (cubic centimetres of $\frac{n}{10}AgNO_3$ per 5 cc. filtrate), at intervals of five days.

Time of induction, minutes:	0′	3′	4′	5′	6′	
	3·0	3·0	3·0	3·0	3·0 cc.	$\frac{n}{10}AgNO_3$
	2·5	2·6	2·5	—	2·7 ,,	,,
	2·1	2·1	2·1	2·1	2·4 ,,	,,
	1·3	1·3	1·2	1·7	2·2 ,,	,,
	0·9	0·9	0·7	—	2·0 ,,	,,

The shortest period giving a distinct anti-ferment reaction by placing the roots horizontally at 17° C. can therefore be considered as six minutes. The limit is found at five minutes, nor was the limit changed by raising the temperature to 30°. But it is possible that the anti-ferment is not formed as an immediate result of stimulation, but rather by after-effect. Therefore I resolved to place the roots, after being geotropically stimulated, in the vertical position for one-half, one, two and more hours, and then to test for the anti-ferment reaction. But I could not detect even in this way any anti-ferment reaction after less than six minutes of geotropic introduction. On the whole it seems that, under any circumstances, at least five minutes'

stimulation is necessary for anti-ferment formation. This is the shortest period in which geotropic stimulation has hitherto been shown to occur. Formerly I showed (11) that geotropic after-effect on the klinostat can only be detected in roots of *Lupinus* or other sensitive objects after more than fifteen minutes of geotropic stimulation. This 'presentation-time' for geotropic curvature is therefore much longer than the 'presentation-time' for the anti-ferment reaction. If the roots, having been horizontal during six minutes, are allowed to grow vertically, it is possible to find out how long the anti-ferment reaction continues. Under these circumstances the reaction is found slightly diminished after one and a half hours, very much diminished after two and a half hours, and it finally disappears after four hours at 17° C. Thus those parts of the geotropic process which are not externally perceptible come to nothing, precisely as is the case with the visible part of the phenomena. For a root left horizontal for fifteen minutes and then placed vertically shows no visible curvature. For stimulation of six minutes' duration the klinostat fails us as a method of observation, and we have only the anti-ferment reaction to rely on.

When the roots are placed horizontally for longer than six minutes the anti-ferment reaction is observed in about the same intensity as after an induction of six minutes, during at least three hours after the close of the stimulation. The maximum of the reaction is reached very quickly: thus between stimulation-periods of six and fifty minutes no quantitative difference can be observed in anti-ferment reaction; it may be added that at a temperature of 15–20° curvature is usually beginning after fifty minutes. The anti-ferment reaction therefore differs from after-effect test, which occurs much more strongly after thirty to forty-five minutes than after fifteen minutes of geotropic induction. The duration of the anti-ferment reaction after the cessation of stimulation was also determined. When the stimulus lasted ten minutes the anti-ferment test could no longer be observed eight hours after stimulation. After inductions of twenty minutes the anti-ferment reaction seems to come to its end in eight hours. But when the roots were stimulated for thirty minutes the anti-ferment reaction did not disappear earlier than twenty hours after the close of the induction. With forty and fifty minutes' stimulation twenty-four and thirty hours are needed for the anti-ferment reaction to run its course. The effect is therefore more persistent the longer the geotropic reaction continues. As is known, the after-effect on the klinostat increases in a similar manner. The curvature due to after-effect disappears under the straightening influence of autotropism more easily in proportion as the stimulation is of short duration. In the same way the curvature appears later after a short stimulus than after a longer one. Thus the curvature of roots stimulated for twenty minutes is observed after twelve to twenty-four hours, of roots stimulated for thirty minutes after eight hours, of roots stimulated for forty minutes after

three hours, but of roots stimulated for fifty minutes after two hours. This phenomenon may be explained by assuming a superposition of two processes. In the anti-ferment reaction, however, there seems to be no opposing process, so that the curve shows no striking maximum.

The results of continued observation during the process of geotropic curvature are in harmony with the early occurrence of the maximal anti-ferment reaction and its prolonged persistence. The intensity of the anti-ferment reaction remains the same during the first beginning of the downward curvature, and it does not decrease before five hours have elapsed (at 16–17°), when the curvature is already finished. Since the root stays in the horizontal position about one hour, the anti-ferment reaction could not be expected to disappear in less than twenty-four hours, a supposition actually confirmed. Perhaps the result, that direct titration during the progress of the geotropic curvature shows after three hours no augmentation of the normal amount of homogentisinic acid, may seem to be a surprising one. But it is possible, on account of the very small quantity of substances to be estimated, that the method soon becomes useless. Perhaps also after the oxidation of the homogentisinic acid has been checked a regulative diminution in the production of homogentisinic acid takes place, so that even in the presence of the hindering antioxidase the amount of homogentisinic acid decreases a little.

Further investigations on the significance of the anti-ferment reaction were made in reference to the geotropic induction at different angles to the vertical. When the roots are stimulated for thirty minutes no anti-ferment reaction can be observed at deviations of from 1° to 6° from the vertical. The reaction becomes certain at an angle of 7° from the normal position. This therefore is the smallest stimulus able to cause the anti-ferment reaction. At a deviation of 10° and a stimulation of thirty minutes a nearly maximal anti-ferment reaction can be produced, and the progress of the reaction is now the same for all angles from 10° to 170°. Neither the horizontal nor the obliquely upward position have a stronger effect. The effect decreases at 176°, and at 179° the anti-ferment reaction is very little. The inverse vertical position never causes any anti-ferment reaction ; nor is any after-effect to be obtained in roots *fixed* in the inverse vertical position. I have shown elsewhere (12) that the maximal after-curvature is not to be obtained by stimulation in the horizontal position, but at positions of about 135° obliquely upwards, i. e. 45° above the horizontal. Therefore the inquiry whether the anti-ferment reaction does not after all show a maximum effect at some angle of deviation was an interesting one. The method employed was the weakening of the effect by the shortening of the period of induction. If the induction is diminished to six minutes, at a deviation of 10°, no distinct anti-ferment reaction can be observed, but a very distinct one at 170°. These positions had not differed from each

other when the roots were stimulated for thirty minutes. In this way it can also be established that the anti-ferment reaction is much weaker at 45° than at 135°, i. e. at the same distance from the horizontal position in the upper quadrant. But between 60° and 120° no difference could be detected, nor any distinct difference between 90° and 135°. The stimulus can be still more diminished if the roots after six minutes' stimulation stand vertically as long as possible. They may be even left vertical for three to four hours, since, as above shown, the anti-ferment reaction is not destroyed in such a period. Experiments carried out in this manner demonstrate that the anti-ferment reaction at 60° is distinctly weaker than at either 90° or 120°, and some experiments established a stronger anti-ferment reaction at 135–150° than in the horizontal position. The result of the anti-ferment test, therefore, can be taken as supporting my own view and that of Darwin (13), D. Pertz (14), and Nemec (15), that the positions obliquely upwards give a stronger geotropic stimulus to roots than the horizontal position. The detailed experimental proof of the results here only briefly communicated is reserved for an extensive paper to be published in German. Here I am restricting the discussion to the chief points of importance.

VI.

The behaviour of roots on the klinostat was found to be of great interest in reference to the anti-ferment test. I may state at once that the anti-ferment reaction is to be observed in roots rotating on the klinostat without undergoing any curvature. This is the only case where the anti-ferment reaction occurs (according with the general view) without geotropic or analogous induction. But since in other cases we find the anti-ferment reaction strictly connected with tropistic induction we may be right, supposing that geotropic stimulus occurs even in plants rotating on the klinostat; since no curvature takes place, it was hitherto impossible to demonstrate this effect directly. But the anti-ferment test is able to show it easily and certainly, while the phenomena of grass haulms (Elfving (16), Noll (17)) and pegs or heels of *Cucurbita* seedlings (Francis Darwin (18), Noll) have established similar conclusions in isolated cases. The facts and views recently published by Francis Darwin and Miss Dorothea Pertz (19), which are thoroughly confirmed by the chemical method, are of the highest interest in reference to the results mentioned above.

Several lots of seventy-five seedlings each (*Lupinus albus*) were placed in a box made of zinc-plate filled with sawdust, and fastened to the axis of the klinostat (Pfeffer–Albrecht model). The lot B was kept in rotation for fourteen minutes, the period of revolution being twenty-seven minutes, it made therefore half a revolution ; lot C rotated for fourteen minutes with a period of revolution of fourteen minutes, i. e. C made a whole revolution.

Lot D rotated for the same time, but with a period of seven minutes, and made therefore two revolutions. Lot A consisted of control roots placed vertically. The temperature was 20° C. Lots B, C, D were, of course, treated one after the other on the same klinostat. The reducing power of the preparations made from these roots decreased at the following rate (measured at intervals of five days):—

A	B	C	D	
2·0	2·0	2·0	2·0 cc.	$\frac{n}{10}$ $AgNO_3$
1·6	1·7	1·7	1·8 „	„
1·2	1·4	1·4	1·5 „	„
0·8	1·2	1·2	1·3 „	„

In another similar experiment the klinostat made one turn in twenty-five minutes, and the lots of seedlings were allowed to rotate for the following times:—B, for fifteen minutes; C, thirty minutes; D, sixty minutes; A was again a control lot (temp. 20·3 C.).

A	B	C	D	
2·0	2·0	2·0	2·0 cc.	$\frac{n}{10}$ $AgNO_3$
1·5	1·7	1·7	1·8 „	„
1·1	1·5	1·5	1·6 „	„

A third experiment with the same time of revolution (25′) was designed to investigate the effects of more prolonged rotation. Lot A consisted of control roots; B was rotated for three hours, C for six hours, D for twenty-four hours. Care was taken that the roots of the different lots were of the same age at the end of the experiment.

A	B	C	D
2·0	2·0	2·0	2·0
1·6	1·8	1·8	1·7
1·2	1·5	1·6	1·5
0·8	1·2	1·4	1·2

Retardation in the oxidation of homogentisinic acid was therefore to be observed in every case. I may here briefly mention that the accumulation of homogentisinic acid can be demonstrated by direct titration with silver nitrate in roots rotated on the klinostat. We must conclude from these experiences that roots in rotation on the klinostat perceive the geotropical stimulus in the same time as roots standing vertically, and even that this stimulus lasts during the whole time of rotation. Now that it has been shown that the anti-ferment reaction occurs in roots placed horizontally after five to six minutes, the view I formerly held must be given up, viz. that the effect of the klinostat may be caused by the plant remaining in each position for too short a time to allow of perception. Why curvature does

not occur on the klinostat is a question for further investigations. I should suppose that probably extremely small curvatures do take place, which are easily neutralized by the ortho-autotropism of roots, i. e. the tendency to grow in a straight line ; moreover, there must exist interferences between the curvatures.

VII.

My researches on various other applications of the anti-ferment test to geotropical questions, e. g. to the problem of the geotropism of secondary roots, will be reserved for another paper. Here I shall only enter upon the use of the method in a single problem—the question of the geotropic sensitiveness of the root-tip, which has received so much attention since the famous experiments by Charles and Francis Darwin. This problem has gained a new interest from the views established by Nemec (21), Haberlandt (22), Francis Darwin (23), the so-called 'statolith hypothesis.'

As is known, Nemec supposes that the displacement of starch-grains in the young cells of the root-cap supplies the mechanism of perception, and that only the root-tip (more exactly the part containing the young root-cap cells) can perceive the geotropical stimulus. To this view, which has been supported recently by experiments published by Francis Darwin, which harmonize with other physiological experiences, I have especially objected as follows: that in my experiments with glass tubes much more than the root-cap must be bent; at least 1·5 mm. of the root-tip must be bent laterally before the perception-zone is entirely separated from the zone of growth and curvature. I further succeeded in demonstrating, in one of my later papers, that the accumulation of homogentisinic acid in decapitated roots could only be hindered by cutting off 1·5 mm. of the root-tip.

There remains to complete these experiments by applying the better and stricter anti-ferment test.

One hundred roots of *Lupinus albus*, from which 0·5 mm. of the tip had been removed, were placed in a vertical position in sawdust; another lot of one hundred roots treated in the same manner were placed horizontally for thirty minutes. Then all the tips were ground and prepared for the test. The reducing power decreased in the following ratio:—

Unstimulated roots	2·0	1·6	1·2	0·7 cc. $\frac{n}{10}$	$AgNO_3$
Stimulated roots .	2·0	1·7	1·4	1·2 „	„

The same experiment, cutting off 1·0 mm. of root-tip:—

Unstimulated roots	2·0	1·6	1·2	0·7	0·0 cc. $\frac{n}{10}$	$AgNO_3$
Stimulated roots. .	2·0	1·8	1·6	1·3	0·7 „	„

The same, cutting off 1·5 mm.:—

Unstimulated . .	2·0	1·6	1·2	—	0·4 cc. $\frac{n}{10}$ $AgNO_3$
Stimulated . . .	2·0	1·7	1·4	—	0·6 „ „

The same, amputating 2 mm.:—

Unstimulated . .	2·0	1·6	1·1	—	0·4 cc. $\frac{n}{10}$ $AgNO_3$
Stimulated . . .	2·0	1·6	1·2	—	0·5 „ „

It was shown by a preliminary experiment that decapitation is not able to produce the anti-ferment reaction to any degree without geotropic stimulation.

Neither wounded nor stimulated	2·0	1·6	—	0·6	0·2 cc. $\frac{n}{10}$ $AgNO_3$
Decapitated, not stimulated . .	2·0	1·6	—	0·6	0·3 „ „
Decapitated and stimulated . .	2·0	1·7	—	0·9	0·6 „ „

The parts cut off were immediately ground and added to the other parts of the root-tips used for the test.

I may mention that the anti-ferment reaction can be investigated in isolated root-tips of 5 mm. length placed horizontally.

These experiments show that the anti-ferment reaction is to be obtained in spite of removing the tissues of root-tip up to the motor zone, and that at least 1·5 mm. of the root-tip must be removed to hinder the anti-ferment reaction. This is in complete agreement with my previous experiments, and contradicts the statolith hypothesis in a manner which demands an explanation. Nemec (24) recently expressed the view that possibly cells containing statolith starch could still exist in a length of nearly 1·0 mm. of the root-tips. But according to my experience, in roots of *Lupinus* decapitated to 1·0 mm. there are never any statolith cells, nor can there be any regeneration of such cells in the period of stimulation, viz. half an hour.

Therefore other possibilities must be examined. Thus it is not impossible that the starch-cells of the root-cap are to be considered as being the chief organs for perception of the geotropic stimulus, but that the displacement of bodies contained in other cells may be available for the statolith function, though in a less effective manner. There is even the possibility that such displacements produce anti-ferment reaction, but no complete or normal curvature. That there exist processes connected with the anti-ferment reaction, but never producing any visible curvature, can be shown by the phenomena of roots laterally illuminated, as mentioned in the sequel. Unfortunately, in the case of geotropism this is difficult to decide, because the stimulus of wounding has itself an influence, and the failure of curvature may just as well be due to the effect of wounding as to the non-perception of the geotropic stimulus.

Finally, I cannot exclude the possibility that the anti-ferment reaction in decapitated roots is stronger in the part of the root-tip remaining after amputation than is normally the case in this upper part of the root-tip. Here also the decision is a difficult one.

On the whole the application of the anti-ferment reaction to the question of the localization of the sensory zone demonstrates that all stimulation effects are not absent when all cells containing statolith starch are as carefully as possible removed. Therefore it may still be doubted whether geotropic perception is caused only by means of such cells. But the principle of the statolith hypothesis is not yet refuted by the anti-ferment experiments, and according to the present state of my experience and deliberations I cannot consider the appearance of anti-ferment reaction in decapitated roots as refuting directly the hypothesis of Nemec. I say so because it is, generally speaking—and not merely in this case—dangerous to draw conclusions as to normal processes from the result of operations. The statolith hypothesis, however, must explain many other difficulties before it can rank as a permanent acquisition to our knowledge. At any rate, we gain from it a valuable impulse to new experimental studies. What position may now be ascribed to the anti-ferment reaction in the chain of processes constituting geotropic action in roots? As has been shown, the anti-ferment reaction takes place long before the curvature, and occurs in the root-tip before any alterations can be discovered in the motor zone. Further, the anti-ferment reaction is not influenced by the shock of decapitation in the same degree as those phenomena of irritability which are seriously interfered with when heliotropic and geotropic curvatures are for a time checked by injuries to grass seedlings and roots.

Let us imagine these processes schematically expressed as following each other in a chain of consecutive changes[1]. We might, for example, have:

1. Statolith effect.
2. Anti-ferment reaction.
3. The processes which are hindered by shock.
4. Stimulus transmitted to the motor zone.
5. Curvature.

According to this scheme no curvature can exist without anti-ferment reaction (or some unknown process in causal connexion with the anti-ferment reaction). But statolith action can take place without anti-ferment reaction, which is shown by roots placed inversely undergoing movement of the statoliths, but showing no anti-ferment reaction. It is at present unexplained why the displacement of statoliths produces no geotropic stimulation in roots inversely placed, in contrast with roots in any other positions. In contemplating these remarkable phenomena we are reminded of the macula lutea of the retina.

[1] These actions might in reality go on in part simultaneously, and not one after the other.

VIII.

I had already shortly mentioned in a previous paper (25) that the anti-ferment reaction occurs in roots laterally illuminated, which nevertheless show no trace of heliotropic curvature, even on the klinostat. As a source of light incandescent gas was used. It was found that roots of *Lupinus albus* do not respond to illumination by curvatures under any conditions. But they nevertheless show a distinct anti-ferment reaction when laterally illuminated.

In a large glass trough were placed 200 roots of *Lupinus*; 100 of them were in direct contact with the glass wall, so that they could be illuminated laterally; 100 other roots were completely surrounded by earth and kept in darkness. At a distance of one meter was placed the gas lamp, and the trough was laterally illuminated during two hours. The temperature near the roots in the earth was at first 20° C., at the end of the experiment 21° C. The roots were then taken out and prepared. The reducing power was decreased in the following manner:—

Illuminated	2·0	1·8	1·5	1·2	0·9 cc. $\frac{n}{10}$ $AgNO_3$
Darkened .	2·0	1·5	1·1	0·8	0·4 „ „

The same results were obtained with *Sinapis alba* (cultivated in water), *Phaseolus*, *Faba*, *Zea*. Of these only the roots of *Sinapis* showed distinct heliotropic curvature. In all other species of roots I could not obtain distinct curvatures even by means of the klinostat.

The anti-ferment reaction was, however, very distinct and certain in all cases, and this behaviour proves that the investigated roots were all sensitive to lateral illumination, even if they were mostly not able to show curvature.

The effect of coloured light was investigated by means of ruby glass and of gelatine paper of several colours. Behind the ruby glass seedlings of *Avena*, which are very sensitive to the heliotropic stimulus, showed no curvature. Behind red, yellow, or blue gelatine paper, heliotropic reaction was observed. The anti-ferment test (with *Lupinus* roots) gave results strictly analogous to the direct observation of curvature in *Avena*:—

Red gelatine paper .	2·0	1·8	—	1·1 cc. $\frac{n}{10}$ $AgNO_3$
Control	2·0	1·6	—	0·6 „ „
Yellow gelatine paper	2·0	—	1·2	0·9 „ „
Control	2·0	—	0·8	0·4 „ „
Ruby glass	2·0	—	0·9	0·4 „ „
Control	2·0	—	0·8	0·4 „ „
Blue gelatine paper .	2·0	—	1·4	— „ „
Control	2·0	—	0·6	— „ „

Roots of *Lupinus* or of other seedlings equally illuminated on all sides never gave any anti-ferment reaction. This is proved by the following experiment with roots of *Lupinus*. Two lots of 100 roots were placed vertically in damp air in glass troughs; their cotyledons were wrapped in wet cotton wool. One of the troughs was covered with an opaque box, the other was illuminated from both sides by equidistant incandescent lights during two hours. The reducing power in this experiment decreased in the following rate:—

Darkened .	2·0	1·6	1·1	0·7 cc. $\frac{n}{10}$ $AgNO_3$
Illuminated	2·0	1·7	1·2	0·8 „ „

There was, therefore, no distinct difference; in other words, only lateral illumination gives an effect. In the above experiments the heating effect of the lamps has not been considered. To test this possibility, large glass dishes 20 cm. wide were placed before the troughs containing the roots, and through the dishes a stream of cold water was allowed to flow. During the two hours that the experiment lasted the temperature was taken at three places, and it was clearly ascertained that the temperature on the illuminated side was not higher than on the dark side. The roots were then prepared and tested in the usual way. The decrease in reducing power was:—

Control .	2·0	1·6	1·1	0·7 cc. $\frac{n}{10}$ $AgNO_3$
Illuminated	2·0	1·8	1·5	1·2 „ „

In spite of excluding any local effects of higher temperature the anti-ferment reaction was found to be distinct.

Another experiment, analogous to this, but made with roots growing in damp air, gave the following results:—

Control .	2·0	1·6	1·2	0·8 cc. $\frac{n}{10}$ $AgNO_3$
Illuminated	2·0	1·8	1·5	1·2 „ „

I consider it, therefore, an undoubted fact that lateral illumination alone (under the conditions generally necessary for heliotropic curvature) is able to produce anti-ferment reaction in all seedling roots. We have certainly a right to consider this a rudimentary tropistic reaction, and these experiments are the only ones up to the present time that can demonstrate the general occurrence of heliotropism in roots. We may hope that the anti-ferment reaction will prove to be an available method for demonstrating sensibility to tropistic stimuli where no curvature or only uncertain reactions are observable. I have here in mind chemotropism, osmotropism, thermotropism in roots and in other plant organs. Investigations of these questions will be carried out in my laboratory.

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The Dissemination and Germination of Arceuthobium occidentale, Eng.[1]

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With Plates III and IV.

INTRODUCTION.

'SO much has already been written on this genus of the Loranthaceae that many will no doubt be surprised that there should be anything new to be said on the subject.' From this modest introduction Johnson (1888) proceeds to a description of *Arceuthobium Oxycedri*, which is the more remarkable for its excellence when one realizes the meagreness of the material at his disposal. If it were not that I have had the opportunity to study another species alive and out of doors, as well as in the laboratory, to see it disseminating its 'seeds,' and to observe their germination, I should have no excuse for attempting to add anything to Johnson's paper. As it is, I hope to be able to clear up certain matters which have hitherto been obscure.

The material of *Arceuthobium occidentale*, Eng., which I have studied was on trees, young and old, of *Pinus radiata*, D. Don., the Monterey Pine[2], which grows wild mainly along the shore of Monterey Bay on the coast of California. It is also abundantly planted about San Francisco Bay, and elsewhere. The affected trees which I studied were either in the Arboretum of Stanford University, where *Arceuthobium* has of late suffered greatly from the general improvement of the Arboretum, or in the forest on Point Piños, one of the heads bounding Monterey Bay. This forest protects the town of Pacific Grove from the inward blowing sand which, instead of piling up as at present in fine dunes, would otherwise blow over and bury the town. The preservation of this forest is therefore important, a matter made very serious from the extraordinary number of untoward influences, natural and artificial, now operating there. A careful study of one of the enemies of this pine, therefore, may lead to practical advantages as well as to facts of some botanical interest.

[1] Read before the Botanical Section of the British Association, Cambridge, August, 1904.

[2] For a study of the effects of another parasite which attacks this pine see Peirce, G. J., Notes on the Monterey Pine, Botanical Gazette, vol. xxxvii, 1904.

[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

Some of the material used was alcoholic, but always controlled by the examination of fresh. The fresh material was studied out of doors in the Arboretum and in the forest, and in the Hopkins' Seaside Laboratory at Pacific Grove, as well as at Stanford University. I had fresh material sent to me repeatedly from Pacific Grove at times when I was unable to go there. The study of the dissemination and germination of this parasite must include the observation of the climatic conditions under which the plant lives, especially the humidity of the air. At Stanford University, which is three miles from San Francisco Bay, and cut off from the ocean winds by mountains ranging from 1,000 to nearly 3,000 feet in height, the air is much drier and the winter temperature lower than at Pacific Grove, which is on the Bay of Monterey, and is only a mile from the open sea. Though in both places the rainy season is only from October to April, the rainfall is heavier at Pacific Grove than at Stanford University. The winter temperature at Stanford University is considerably lower than on the coast, where in most places frost occurs scarcely oftener than once in a decade. There was no frost this last winter. On the other hand, the summer temperature of Stanford University is decidedly higher and the air dry, while the coast is cooler and likely to be buried in fog. Fog prevails on the coast during much of the time when the fruits are ripening, while further inland it occurs only at night. When the fruits are ripe the comparatively humid air of the coast keeps them from drying. When rain comes in addition their water content is still greater. But further discussion of the relations of climate to this plant may be deferred for the moment.

Dissemination.

Arceuthobium occidentale flowers from September to January: in a year from that time the fruits are ripe. If heavy rain and high wind come at this time, all or nearly all of the fruits are shaken off; the plants may even be broken to pieces, for they are brittle, and hence the whole crop of two seasons' growth may be destroyed. In some years I have been unable to get fruits after mid-November. This current year, however, I found the fruit very abundant in the woods at Pacific Grove in the Christmas holidays (1903–4).

It is well known that the fruits of *Arceuthobium* are explosive. Before passing to a description of the anatomy and mechanics of the explosive fruit, I should like to call attention to the conditions under which the fruits were disseminating the 'seeds' at Christmas time, 1903.

The weather was showery, but whether the sky was overcast, with or without rain falling, or whether the sun was shining, the air was damp and the temperature mild. In the woods at Pacific Grove the air was still

damper than elsewhere, and dampest of all in the thickets where the pines were small and close together. It is precisely in these thickets and close stands of young pines that *Arceuthobium* is most abundant. On some of these young trees, fifteen to twenty feet high, I counted over twenty separate bunches of *Arceuthobium*. On trees farther apart, or isolated, *Arceuthobium* occurs in far less numbers, if it is to be found at all. Some young trees in the thickets were dead, from no other apparent cause than the great number of *Arceuthobium* plants which they had borne. In many cases where a branch bore a particularly large and vigorous bunch of *Arceuthobium* the branch was dead beyond the parasite, as Cannon (1901) observed on oaks attacked by *Phoradendron*.

In the damp air of the thickets little water is lost by evaporation from the fruits; if rain falls, still less. The fruits seemed to me plumper and more translucent than the few which I had found in the Stanford Arboretum. Without any apparent disturbance the fruits would explode; I could hear them, I might even be struck by the flying 'seeds'; but if the wind gently shook a bunch of fruiting *Arceuthobium*, or the raindrops fell upon the fruits, or I lightly struck a branch of pine on which the fruiting parasite grew, there would be a momentary fusilade, the 'seeds' flying in all directions, and sticking to whatever they struck, provided always the speed at the time they struck were low enough to be offset by the adhesion of their gelatinous outer-coats to the object struck. That the speed with which the 'seeds' leave the parent plant is high any one knows who has been struck by the hard, pointed little bodies. The speed must be great to carry the 'seeds' far, for they are heavy. It is impossible to determine the distance to which a 'seed' may be thrown. I can only estimate this from experiments. The longest distance to which a 'seed' was thrown in the laboratory was fifteen feet. The air of the laboratory was dry, but I had kept the material, sent from Pacific Grove, in an air-tight preserve-jar in which there was a little water. In other words, the air surrounding the material was as moist as that at Pacific Grove, and the fruits were as plump and tense. I have no doubt, from the relative positions of my work-table and of the book-shelf on which the 'seed' stuck, that it could have gone at least ten feet further.

Let us turn now to the structure and mechanics of the fruit. In Fig. 1, Plate III, we see a small fruiting branch, natural size, which was growing a few years ago in the Stanford Arboretum. At Pacific Grove the fruits may be as many again on a branch. The fruits are complex. Morphologically they include the receptacle adherent to the ovary (Engler and Prantl). Skrobischewsky (1890)—only reviews of whose paper I have been able to see—describes the fruit of *A. Oxycedri* as consisting of (1) a one or two-layered epidermis, (2) underlying this four or five layers of collenchyma, (3) parenchyma with vascular bundles embedded in it, (4) the gela-

tinous seed-coat, (5) then three or four layers of tannin cells, (6) the endosperm, (7) and finally the embryo. Thus we have seven concentric layers of differentiated tissues. As *A. occidentale* is slightly different I may go into some detail as to the structure of the fruit. Figure 3 is a diagram of a longitudinal section of a fruit with the 'seed' still in it, and with a part of the stalk still attached. The diagram falls into three parts from top to bottom. The uppermost part, cut off from the rest by the oblique line *a–b*, is covered by heavily cutinized epidermis (*c. ep.*), one-layered, with the outer cell-walls much thickened, and many stomata, the guard-cells of which are depressed (see Fig. 5). Underlying this are several layers of parenchymatous cells (*c.p.*) containing chlorophyll grains in abundance, and with cellulose walls. Under this are two or three layers of somewhat elongated cells (*l.*) with lignified walls more or less spirally thickened. This conical layer is continuous nearly or quite to the top. Within this, again, are parenchymatous cells in which part of the gelatinous 'seed' is embedded. Below the line *a–b* the structure is surprisingly different. The epidermis (*ep.*) is scarcely cutinized, and has no stomata. Underlying this is a very gelatinous collenchyma (*g. c.*), the thin places in the cell-walls of which are still cellulose. This gelatinous collenchyma is several layers thick, and abuts within upon the gelatinous 'seed' coat (*g.f. c.*). This coat extends nearly around the 'seed,' is absent on the end which is to be forward when it is thrown out, and is thickest at the top, which will be the back end in flight. The 'seed' is covered all around with a sclerotic coat (*s.f. c.*) from one to three cell-layers thick. Enclosed within this is the endosperm (*end.*), in one end of which the embryo (*emb.*) lies. At the line *c–d* is the so-called abscission layer, a single row of very thin-walled cells (Fig. 6) lying between masses, above and below, of thick gelatinous-walled cells.

A cross-section of a fruit from which the seed has been expelled is shown in Fig. 4.

Passing now to the mechanics of discharge one sees at once that the top of the fruit is so made as to be able to resist considerable pressure from within the fruit, that the middle and lower parts will develop pressure whenever they can absorb enough water, and that the line of mechanical weakness is the so-called abscission layer, where the fruit is attached to the stalk. Given, then, the conditions such as I have described above—abundance of water in the soil and in the host, from which the parasite absorbs water and the gelatinous parts of the fruit take it up, and air so moist that little water is lost from the fruit by evaporation—it is inevitable that a very considerable pressure must develop in the fruit. MacDougal (1899), describing another species, says: 'During the ripening period the contents of the expulsory layer undergo such chemical changes as to give the contents a very high isotonic coefficient. The consequent osmotic

attraction of water into this layer sets up a turgescence which could not be measured, but which probably amounted to many atmospheres.' In *A. occidentale* we see that it is the imbibition of water by the material of the cell-walls rather than the osmotic activity of the cell-contents which brings in water; that the water is taken up and held by the swelling gelatine of the walls. What the resulting pressure amounts to is unknown, and may for the present remain unguessed.

Finally, the pressure within the fruit becomes too great, under the conditions which I have described, and, with or without some jar to set it off, the fruit breaks at the base. The conical shape of the 'seed,' with the larger end at the back, gives it a cumulative impulse from the top and sides of the fruit, the sides compressing and indirectly propelling it, the top propelling it directly since, before the fruit breaks, much of the pressure has been against the tough epidermis and the lignified layer at the top, stretching these upward.

Passing now to the 'seed.' Morphologically this is a seed enclosed in the inner part of the ovary (Engler and Prantl). The greater part of it is covered with a gelatinous layer sticky enough to attach it to smooth objects as well as rough. This layer will take up still more water from damp air, swell, and so come into contact with a still more extended surface than it at first touched. Furthermore, this gelatinous material dries only very slowly even in dry air. But damp air, only moderately cool, is necessary for germination.

The structure of the 'seed' is essentially as described and figured by Johnson (1888), but as *A. occidentale* appears to differ somewhat I may give some details. The gelatinous outer layer (see Fig. 7, a 'seed' fixed in dilute Flemming's solution, and Fig. 12, a longitudinal section of an ungerminated 'seed') consists of much-elongated cells, their much-thickened walls consisting of two layers (Fig. 8), an outer of gelatinous material, an inner of cellulose spirally thickened. The cell cavity is very narrow. These long cells are attached obliquely or at right angles to cells of the sclerotic layer of the 'seed.' On the outside (as Fig. 8 shows) 'dirt' of all kinds may adhere. Figures 9 and 10 show the extent to which these gelatinized cells may expand. Figure 9 shows a cross-section of these cells near the tip, tangential to the seed, which had lain for some weeks in 95 % alcohol. Figure 10 is the outer part of the same section after it had lain for a quarter of an hour in water on the slide.

With the slow loss of water which takes place as the air dries, these long cells, attached at their tips to whatever the 'seed' has struck, and at their bases to the firm sclerotic coat, contract, shorten. Owing to the spiral thickening of the inner cellulose wall, the shortening of these cells pulls the 'seed' closer and closer, and attaches it more and more firmly, to whatever it has struck. This attachment may hold for many months, for I have seen 'seeds'

a year old still attached to the leaves on branches which they struck and on which they had failed to germinate. The attachment of the 'seed' is then very firm, and may be increasingly firm as time goes on.

So far as my observation goes, the majority of the 'seeds' strike the leaves of the pine, either of the tree on which they grew or of one near by. Owing to the various positions of the fruits, the directions in which the seeds are shot are very various. Some must go to the ground at once, others up into the air, but the majority certainly nearly horizontally, for in the close clumps of trees where *Arceuthobium* is most abundant the parasites are, most of them, on branches from four to ten feet from the ground. I have never seen the plant high up on old Monterey Pines, though it is reported on the high branches of other pines elsewhere. In the Stanford Arboretum it is not on the branches at all, but forms a more or less complete ring around the trunk of the tree two or three feet from the ground. Comparatively few of the enormous number of seeds produced where *Arceuthobium* flourishes reach places favourable for germination and for penetration into a host. The explosive fruit, therefore, is a very powerful but very wasteful means of disseminating the plant, much less efficient than the less astonishing fleshy fruits of *Viscum*, *Phoradendron*, and other Loranthaceae.

Germination.

The 'seeds' of *Arceuthobium occidentale* will germinate on anything, if they will germinate at all. I have seen germinating seeds on pine-needles, dead as well as living, on dead branches, on trees and shrubs of other sorts, on a fence board, anywhere where the air was moist and warm enough. But I have not been able to grow the plants at Stanford University. The reason for this, I am sure, is the cold of winter and the drier air. Until the parasite has penetrated into the host it can get water only from the air. It is therefore very dependent upon such a degree of moisture in the air as will not rob the gelatinous coat of the seed of more water than the embryo requires for its growth and penetration into the host. The gelatinous coat is the water-reservoir of the embryo as well as the means of attaching the seed to the host—two very important functions, requiring the differentiated cell-walls above described. Germination will not be successful, however, unless the seed fall on a branch, the rough surface or the angles of leaves or branches of which oppose the gliding of the growing root over the surface. On leaves the root will continue to grow, gliding over the surface, to an astonishing length, three centimetres or even more, until no longer enough food can be taken out of the endosperm.

The embryo is a simple cylindrical structure lying in the endosperm at the upper end, that is, toward the tip of the fruit. The cotyledons are represented only by a slight notch at the lower end. The epidermis is the

only tissue differentiated. This lies at first in close contact with the endosperm, but as germination progresses it is no longer in contact. The embryo must therefore absorb dissolved food diffusing out of the endosperm into a layer of water which surrounds the embryo. Whether the embryo forms enzymes which, diffusing into the endosperm, dissolve the solid foods stored therein, or whether the endosperm cells themselves dissolve these solid foods, which thereupon diffuse outward toward the embryo, there is at present no means of telling. As all attempts at experimental germinations have hitherto failed, there is no hope for an immediate answer to the question, though an answer might throw an interesting light on the physiological chemistry of germination in exalbuminous plants. The embryos are bright green, and contain much starch, at least in the earliest stages of germination. The radicle has no cap at any time, and the tip for some distance back, even in the later stages of germination, consists of merismatic cells, with only the dermatogen at all differentiated.

It may be of some systematic value to record that, though quite by chance, I found two embryos in one 'seed,' as shown by Fig. 14. These two embryos are of unequal size, and only one could possibly have developed into a new plant. Johnson (1888) records having always found only one embryo, but remarks (p. 158) that 'the general result of the investigation tends to show that in the possibility of the formation of two embryos and in habit the affinity of *Arceuthobium* to *Viscum album* is closer than was generally supposed.' I cannot consider the habit of this species of *Arceuthobium* at all like *Viscum* or the allied genus *Phoradendron*, as will appear later on.

The root does not appear to be geotropic, but as in other Loranthaceae (Pfeffer II. 575, 1900) it is negatively phototropic in marked degree, growing always away from the light, down a needle or along a branch toward the central and darker part of the tree (see Figures 15, 16, 17). I have never seen a root-tip pointing in any other direction than away from the light, and often it was necessary for the root to make a turn of over 90° to do this (Fig. 16). I have seen roots turned 180°, but in these the curve was always a spiral, downward as well as backward. We may have here the influence of gravity supplementing that of light, but as the light reaction is so marked and the geotropic reaction so slight, we may doubt, until experiment furnishes evidence, whether these roots are geotropic at all. In this respect they are like *Viscum*, the roots of which are not geotropic.

The roots do not appear to be very sensitive to contact. They grow generally in fairly close contact with the surface of pine-needles or branches, but they do not stop growing and form holdfasts on the needles, though the contact with the surface of the needle may be long continued. The root may even grow for some distance over the comparatively rough surface

of the bark of the Monterey Pine. Until its further growth is opposed by some obstacle—a slight elevation of the surface or the base of a bunch of leaves or of a branch (Figs. 17, 18, 19, 21)—the root grows on, always toward the trunk of the tree. In colour it is usually more or less claret-red.

When the further growth in length is blocked, the root forms a thick holdfast. This is shown in Figs. 13, 17, 18, 19, 21. The holdfast consists at first of an undifferentiated mass of dividing and growing parenchymatous cells covered by a single layer of dermatogen cells which above differentiate into ordinary epidermis cells, and below, where the cells are in contact with the bark, elongate somewhat and firmly attach the holdfast to the bark, much as is the case in *Cuscuta* (Peirce, 1893) and other haustoria-forming parasites. Into this growing foot the material in the upper (cotyledonary) end of the embryo or seedling is transferred. In consequence this upper end shrinks as the lower grows, the 'seed' coats become loosened and may blow away, and the seedling becomes mainly a foot with a small and shrivelled prolongation (see Figs. 18, 19). In this foot (shown in section in Fig. 13) differentiation begins, vascular tissues form, and then the central part of the foot grows out into the bark. That both pressure and chemical action co-operate in the penetration of the haustorium into the host is highly probable, to judge from other cases (Peirce, 1893, 1894), but the effects of pressure are far from evident. Instead, appearances all point to the much more important action of substances excreted by the cells of the haustorium, enzymes which dissolve the walls of opposing cells and hence permit the easy and rapid penetration of the haustorium through the dead outer bark into the living cortical parenchyma of the host. The haustorium has no cap, the cells at the tip elongate and spread out as in *Cuscuta* (Peirce, 1894), forming strands of infecting-cells which grow in various directions toward the medullary rays of the host, grow through these to and through the cambium, dissolving the opposing cells as they grow. Finally, the haustorial cells effect a direct attachment with the young cells of the wood of the pine branch. These last haustorial cells and those above them differentiate into tracheids, some of the cross-walls may disappear, and a direct connexion by conducting tissue is established between the wood of the host and the mass of *Arceuthobium* cells in the cortex of the host.

It is to be noted that, while the cells at the tip of the haustorium are growing out and forming infection strands which penetrate the medullary rays of the host, the main part of the haustorium is increasing in size, forming a mass of parasitic cells in the cortex of the host (Fig. 22). Morphologically this mass is a part of the haustorium, itself a special outgrowth of the tip of the radicle of the seedling. This mass presently differentiates into conducting and parenchymatous tissues, buds form which develop into branches which grow out through the bark into the air (Figs.

20, 21). These branches are pale green, later they become much greener. They at first vegetate and later flower. We have here an instance of regeneration without wounding, amputation, or other pathological stimulus. The small part of the seedling which penetrates the host forms and develops stem and leaves, a small part of one organ—the root—develops into a complete plant by forming the missing members.

Goebel (1900) says, 'Als Organe "sui generis" dürfen wir auch betrachten die Haustorien der Parasiten.' He supports this assertion *first* by saying that in *Cuscuta*, where the haustoria most closely resemble lateral roots, there is no positive proof of their being roots though they arise endogenously, and in the *second* place by the haustoria of the Rhinanthaceae, Orobanchaceae, and Balanophoreae, which are certainly not root-like in appearance. In *Arceuthobium* the haustorium almost immediately ceases to be root-like in appearance, yet it is plainly a modified root-tip. The same is the case in *Pharadendron* and *Viscum*. The single root of the seedling grows into the host, and in the tissues of the host the tip becomes a mass of cells, at first parenchymatous, later including prosenchymatous cells, and sends out strands which penetrate the medullary rays and form a direct connexion with the tracheids of the host. The primary haustorium of *Arceuthobium* is evidently the primary root of the seedling; the succeeding haustoria are branches of this primary root. In *Cuscuta*, on the other hand, the haustoria arise in the stem of the parasite from that layer of cells which in other and similar stems gives rise to lateral roots. It is not merely the endogenous origin of the haustoria of *Cuscuta*, but their origin from that cell-layer which ordinarily gives rise to lateral roots, which causes one to believe them to be lateral roots (Peirce, 1893). Similarly the lateral roots and the root-tubercles of leguminous plants originate endogenously, but it is only when one sees that the root-tubercles originate from the same layer exactly as lateral roots, and in their earliest stages are indistinguishable from lateral roots except by the presence of the infecting strands of bacteria in the cells, that one can believe them to be morphologically the same (Peirce, 1902). When one realizes the marvellous plasticity, adaptability, of root, stem, and leaf, the idea of organs *sui generis* becomes unnecessary.

After the parasite has once formed a foot or holdfast on a pine branch the succeeding stages above described are passed through very rapidly. The vegetative branches emerge much sooner than one would naturally expect. There is very little evidence of pressure being exerted by these branches in emerging from the bark, probably because they arise as buds on the outer part of the mass of parasitic cells in the cortex and are covered mainly by the dead and weathering tissues of the outer bark of the host. Later, however, as these branches increase in diameter they plainly push back the host cells by which they are surrounded.

When one compares Figs. 1 and 2 with figures of *Viscum* and

Phoradendron one sees at once that the habit of *A. occidentale* at least is entirely different from that of members of the other two genera. In *A. occidentale* the stem is composed of very evident segments, rectangular or square at the base, and cylindrical at the top of each segment. The colour is much lighter, the abundant chlorophyll being masked by a brownish pigment in the walls of the epidermal cells. The brittle stem breaks into pieces much more readily near the nodes than near the middle of the internodes, in this respect resembling the grasses and other 'jointed' plants. The stem branches profusely, the stems of *Viscum* and *Phoradendron* comparatively sparingly. The leaves are reduced to scales subtending the chlorophyll-containing branches.

In the reduction of the leaf surface, the thickening of the outer walls of the epidermis, the sinking of the guard-cells of the stomata below the surface (described by Cannon in *Phoradendron* also), the screening of the chlorophyll by another pigment or pigments (see also Cannon), we have very decided differences from the *Viscum album* of northern and moister Europe, and very evident protective adaptations to conditions which, at certain seasons of the year at least, approach much more nearly those of the desert than those of Europe. Certain species of *Arceuthobium* are found in the Sierra Nevada Mountains, where evaporation into the dry air is very rapid in summer, and in parts of Arizona and New Mexico, which are really semi-desert. While *A. occidentale* is actively vegetating, in the spring, the air may be comparatively moist, and from its host it can absorb water in plenty as the soil under the pines is full of moisture, at least below the surface. But in the summer, when no rain falls and the air is very dry, except when fogs sweep in from the ocean, the parasite must retain as much water as possible lest it perish. There is a very decided contrast, therefore, between summer and winter conditions. While the conditions for germination are those of a mild and humid climate, those of vegetation and fruiting are those of a dry and warm climate. To these two sets of conditions the parasite has adapted itself. In this respect it is like *Phoradendron villosum*, but it has carried its adaptation to the summer conditions further than has *Phoradendron*. The conditions for germination are probably the same in both plants, for only once have I succeeded in germinating seeds of *Phoradendron villosum*, and then the young plants soon perished, though I have tried at least five years.

Something more should now be said of the anatomical relations of parasite and host, and of the effects of the parasite upon the host. Figure 2 shows the direct connexion of a branch of *Arceuthobium* with the wood of the host. In Fig. 22 we have a diagram showing two masses (parts of one) of *Arceuthobium* cells in part of a cross-section of a pine branch. The tissues of the parasite are shaded, those of the host white. In this figure we see that strands of cells of the parasite run from the main mass in the

cortex into the medullary rays of the host, traversing the cambium and passing into the wood. In Fig. 23, a diagram of part of a cross-section of an infected pine branch, we see that from a vascular bundle of the parasite (shaded) in the cortex of the host (unshaded) a vascular bundle occupies the middle of the branch of the parasite which has penetrated into the wood of the host through a medullary ray. Figure 24 represents a tangential section of an infected pine branch through the wood, and shows the direct contact of a tracheid of the parasite (shaded) with a tracheid of the host. Figure 25 is also a tangential section of a branch of pine, but through younger wood, and shows, in the centre, the strand of tracheids and vessels connecting with the older wood, and at the sides the direct connexion of younger *Arceuthobium* cells with the young tracheids of the host. At *a* in this figure we see how thin is the membrane through which the young *Arceuthobium* cell absorbs water from the pine tracheid. By preserving these thin places in the walls, the transfer of water from the cells of the host to the adjacent cells of the parasite is made as easy probably as the transfer of water from tracheid to tracheid through the wood of the host. I have seen no cases in which no membrane at all intervened between the tracheids of host and parasite, though there may be such places.

In this perfect connexion of the xylem-tissues of parasite and host we have nothing uncommon. A more interesting question is as to the relations of the phloem-tissues of the two. In tangential and other sections through the bark I have sought vainly for anything more than contact between the cells of *Arceuthobium* and the sieve-tubes of pine. Fig. 26 at *a* and *b* shows *Arceuthobium* cells (shaded) in direct contact with sieve-tubes of pine. This tangential section was so thin that under an oil-immersion objective I could plainly see the sieve-plates in actual section, but I could never find any place where the pine formed a sieve-plate on the side of the tube toward an adjacent *Arceuthobium* cell. There may be such places, but I have searched long and carefully enough to doubt it. In this respect, therefore, *Arceuthobium* resembles other chlorophyll-containing parasites, e. g. *Viscum*, *Phoradendron* (Peirce, 1893). But from the anatomical fact of the absence of sieve-plates in the walls between the sieve-tubes of the host and the living cells of the parasite the inference is not justified that the parasite absorbs only food-materials, not elaborated foods, from the host (Pfeffer i. 355; Peirce, 1903). In fact Fig. 26 shows plainly that osmotic transfer must take place through the thin walls separating the sieve-tubes of the host from the cells of the parasite whenever the physical conditions make osmotic movement through the thin walls in either direction necessary. The presence in either host sieve-tube or parasitic cell of a greater proportion of dissolved substance, food or other, will inevitably entail the movement of that substance to the cell that contains less.

The life-history of this perennial parasite supports this view. The branches at first vegetate in the air, and later flower and fruit. After their crop of 'seeds' has been discharged they die and fall away. At this time no part of the parasite may be visible outside the body of the host. The persisting part of the parasite, embedded and concealed in the host, contains no chlorophyll. It corresponds with *Rafflesia*, *Balanophora*, &c., and at this stage is altogether different from *Viscum* and *Phoradendron*. When *Arceuthobium* becomes active again, as it does in the spring, forming buds and developing these into branches, it must do this work either at the cost of foods elaborated by itself and stored in the mass of parenchymatous cells of its own in the cortex and medullary rays of the host, or at the cost of foods drawn from the host, or both. As I saw no evidence of any great accumulation of foods in the parasitic tissues embedded in the host, I am inclined to believe that, though its aerial parts contain chlorophyll, *Arceuthobium* is a much more complete parasite than *Viscum* and *Phoradendron*, and, in spite of having no sieve-plate connexions with the sieve-tubes of its host, that it absorbs and uses foods elaborated by the pine. The perennial part of *Arceuthobium* is probably completely parasitic so long as it has no aerial branches. During the many months when it has aerial branches it may not be a complete parasite, and may be merely a 'water-parasite.'

Arceuthobium, therefore, shows a distinct advance over *Viscum* and *Phoradendron* toward complete parasitism, and is an interesting link in the chain connecting independent plants and completely parasitic forms.

Owing to the presence in the host of a perennial part of *Arceuthobium*, it is out of the question to exterminate the parasite by merely removing the aerial branches. The spread of *Arceuthobium* in a forest can, however, be prevented by removing the fruiting branches before the fruits are ripe. If this were done every two years through a series of years, and the infected trees gradually thinned out, the parasite could be exterminated. Thinning the forest, thus exposing the parasite to greater dryness during the summer and greater cold during the winter, would tend to keep it in check. In a natural forest of thick growth combatting the parasite is not likely to be successful. In a planted forest, carefully watched, there should be no danger of any great damage from this source. When we learn in America to take as intelligent and skilful care of our forests as we now do of our gardens the phanerogamic parasites will do little damage to the trees.

As a result of the presence of *Arceuthobium* in the pine, the trunk and branches, especially the latter, exhibit considerable distortions. At a point where there is a bunch of *Arceuthobium* of considerable size, the diameter of the branch may be three or even four times greater than just below. The infecting strands of the parasite do not grow for any distance upward and downward through the cortex of the host. Instead they penetrate the

medullary rays and grow in length only as the diameter of the branch increases from year to year, exactly as do the 'sinkers' of *Viscum* and *Phoradendron*. On the other hand, the infecting strands increase greatly in width, becoming sometimes many times as broad as the medullary rays which they once penetrated and absorbed. Thus in Fig. 27 we see a tangential section of a pine branch one centimetre below a bunch of *Arceuthobium*. Here the medullary rays are only one cell broad and a few cells deep. In Fig. 28 we have a tangential section of the same branch, but in the part infected by *Arceuthobium*. The infected medullary rays have been completely displaced by absorption, and the tracheids of the host have been pressed apart, upward and downward but mainly laterally, by the strand of parasitic tissue. It is by this means that the distortions arise. When such distortions occur in the trunks of pine trees the timber is useless as lumber, and has only an inferior value as fuel. However, as pointed out above, such distortions rarely occur in the trunks of Monterey Pines, and these pines have only local use for any other purpose than fuel. The Monterey Pine is not a good timber tree.

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EXPLANATION OF FIGURES IN PLATES III AND IV.

Illustrating Dr. Peirce's paper on *Arceuthobium*.

The figures were drawn either free-hand or with a Zeiss drawing-prism.

PLATE III.

Fig. 1. Fruiting branch of *Arceuthobium occidentale*. Nat. size.

Fig. 2. Section of branch of *Pinus radiata*, showing part of mass of *Arceuthobium* tissue in the bark, the connexion of this with the wood of the pine and the aerial branch of the parasite. Nat. size.

Fig. 3. Diagrammatic sketch of a longitudinal section of a fruit of *Arceuthobium*. *a–b*, the line above which the epidermis is heavily cutinized. *c–d*, the abscission layer. *c. ep.*, cutinized epidermis with many stomata. *c. p.* parenchyma cells with cellulose walls and chlorophyll grains. *l.*, the layer of cells with lignified walls. *e. p.*, epidermis scarcely if at all cutinized, with no stomata. *g. c.*, gelatinous collenchyma. *g. f. c.*, gelatinous fruit coat. *s. f. c.*, sclerotic fruit coat. *end.*, endosperm. *emb.*, embryo, with radicle up. × 10.

Fig. 4. Cross-section near middle of fruit from which the 'seed' has been discharged. Large-celled thin-walled parenchyma with small but many chlorophyll grains and some yellow oil-drops. *v. b.*, the vascular bundles. × 29.

Fig. 5. Epidermis from upper part of fruit, showing heavily cutinized and thick outer walls, and depressed guard-cells. × 300.

Fig. 6. Part of a longitudinal section through a fruit, at the base, showing very thin-walled abscission layer between thick-walled tissues. × 300.

Fig. 7. Wet 'seed' much swollen, showing markings on the gelatinous surface and the small hard ungelatinized end of the 'seed' whence the radicle will emerge. × 24.

Fig 8. Section through gelatinous outer coat of the 'seed,' showing long cells with narrow cavities, outer part of walls gelatinized, inner cellulose (spirally thickened). Long cells attached to thick sclerotic cells forming continuous coat around the 'seed.' × 300.

Fig. 9. Tangential section of gelatinous coat of a 'seed.' In 95 % alcohol. × 334.

Fig. 10. Part of the same section after lying in water fifteen minutes on the slide. The swelling of the gelatinous outer portion of the cell is very marked. × 334.

Fig. 11. 'Seed' germinating on a pine-needle. × 3–4.

Fig. 12. Longitudinal section of 'seed,' showing gelatinous outer coat, the sclerotic inner coat, the endosperm with the undifferentiated embryo at one end. The radicle is up, the cotyledons down and indicated only by the slight notch. × 20.

Fig. 13. 'Foot' or holdfast formed by the tip of the root when it catches on rough bark. Longitudinal section. From the centre the infecting haustorium will penetrate the host. × 29.

PLATE IV.

Fig. 14. Longitudinal section of 'seed' showing two embryos of very unequal size. × 20.

Fig. 15. Germinating 'seed' on branch of pine, showing root already bent away from the light (negative phototropism) toward centre of the tree. × 8.

Fig. 16. Somewhat older germinating seed with root growing away from the light. × 8.

Fig. 17. Seedling still older, with root forming foot attaching it to the bark. × 6.

Fig. 18. Showing the growth of the foot at the expense of food in the upper part of the seedling from which the coats have already fallen away. × 6.

Fig. 19. Still older and larger foot; the upper part of the seedling much shrivelled and nearly empty. × 6.

Fig. 20. Aerial branches already emerging after recent infection of the branch. × 6.

Fig. 21. Young bunch of *Arceuthobium* branches. Note that the 'seed' has not yet fallen away and that the pine-branch is already enlarged. × 2.

Fig. 22. Cross-section of pine branch showing mass of *Arceuthobium* cells (shaded) in the

cortex (unshaded) and the branches from this mass growing where medullary ray cells were before infection took place. *m. r.*, unabsorbed group of medullary ray cells surrounded by the parasite (shaded). *ph.*, phloem part of a vascular bundle of the pine. *xyl.*, xylem part. *c.*, cambium ring traversed by branches of the parasite growing into the wood. × 50.

Fig. 23. Diagrammatic cross-section of a part of infected pine branch, showing continuous strand of tracheids (and vessels) from the wood of the host (unshaded) to a part of the parasite (shaded) in the cortex. *c.p.*, cortex. Other letters as above. × 300.

Fig. 24. Tangential section of wood of pine showing direct contact of *Arceuthobium* tracheids (shaded) with those of pine (unshaded). × 416.

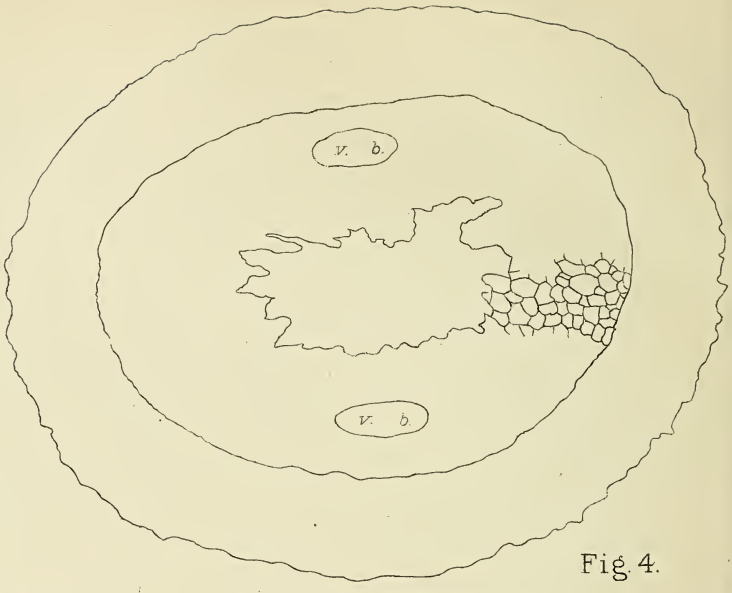
Fig. 25. Similar tangential section, but in younger wood, showing (*a*) that young and forming *Arceuthobium* tracheids thicken their walls at places that correspond with the thicker parts of the walls of the pine-tracheids. × 450.

Fig. 26. Cross-section of pine, showing sieve-plates in section in the walls of the sieve-tubes of pine, but no sieve-plates in the walls of the *Arceuthobium* cells directly in contact. × 900.

Fig. 27. Tangential section through wood (*xyl.*), cambium (*c.*), and phloem (*ph.*) of pine branch 1 cm. below a branch of *Arceuthobium*. Note the comparatively small size of the medullary rays. × 84.

Fig. 28. Tangential section in same branch in the bunch of *Arceuthobium*. Note the great increase in size of the infected medullary rays the cells of which have been entirely displaced and absorbed by *Arceuthobium* cells. × 84.

Fig 1.



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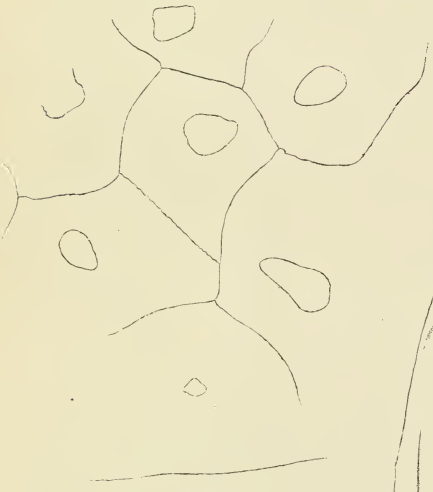

Fig. 10.

Fig. 7.

Fig 9.

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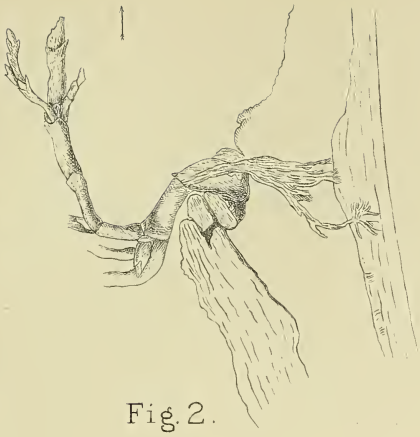
PEIRCE—ARCEUTHOBIUM.

Fig. 8.



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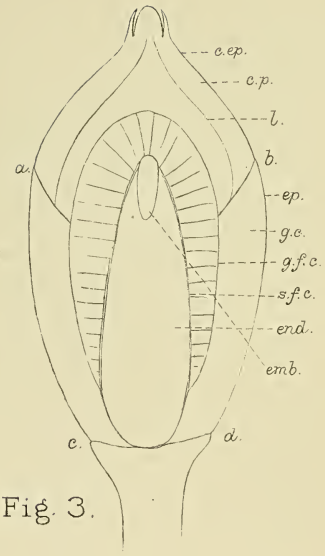

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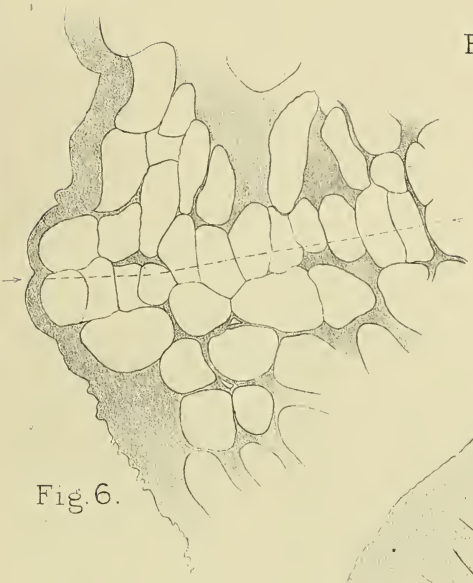

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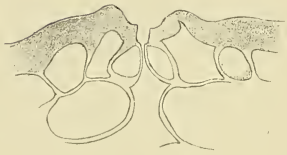

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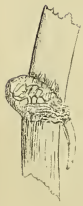

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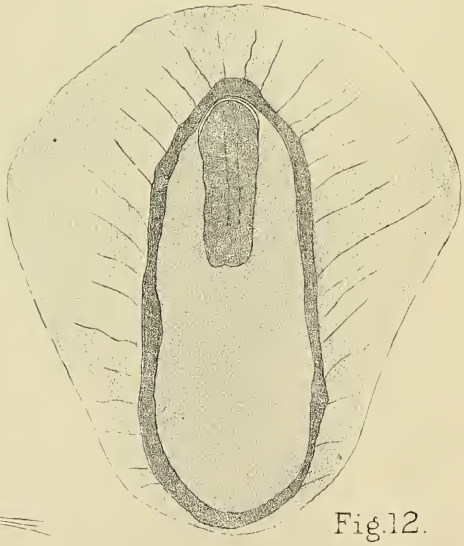

Fig. 12.

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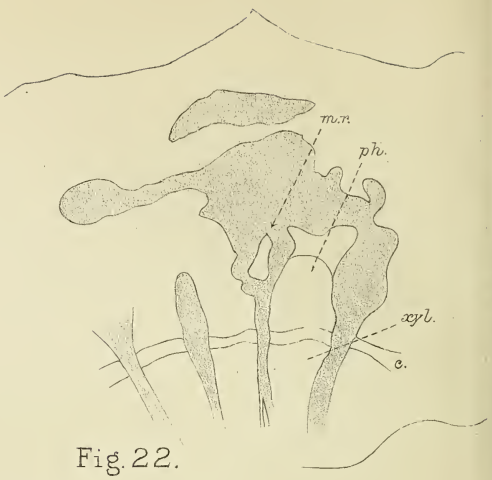
Fig. 14.



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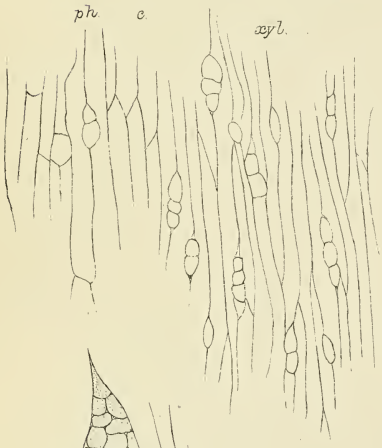

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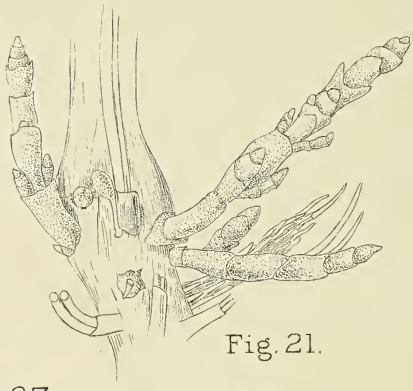

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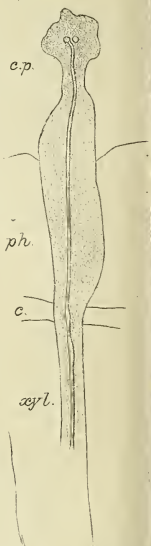

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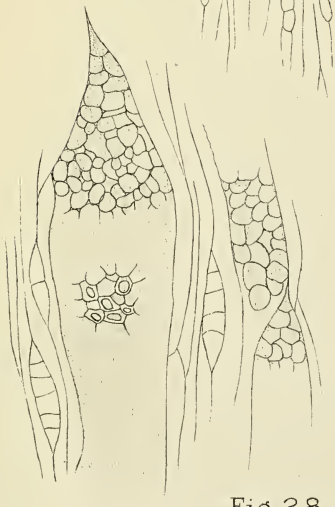

Fig. 28.

Fig. 15.

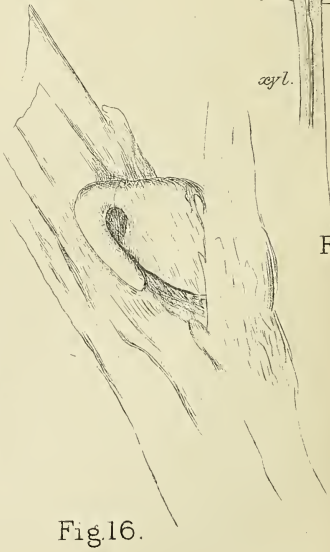

Fig. 16.

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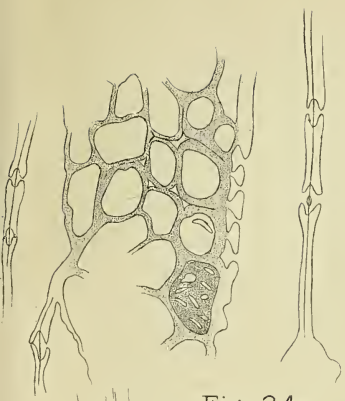
PEIRCE—ARCEUTHOBIUM.



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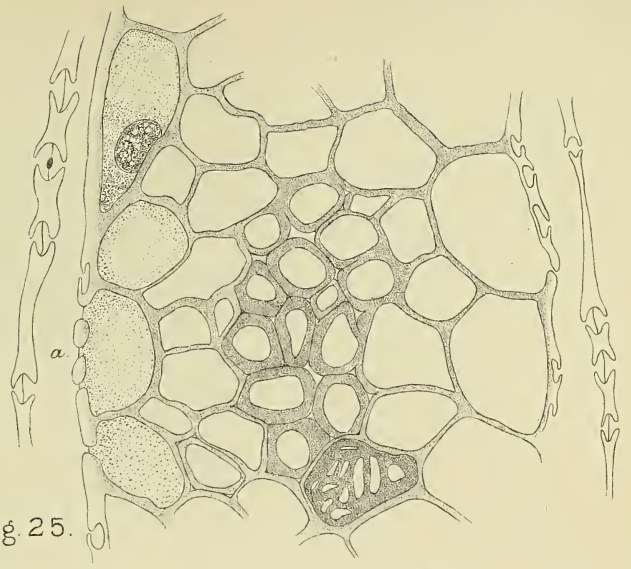

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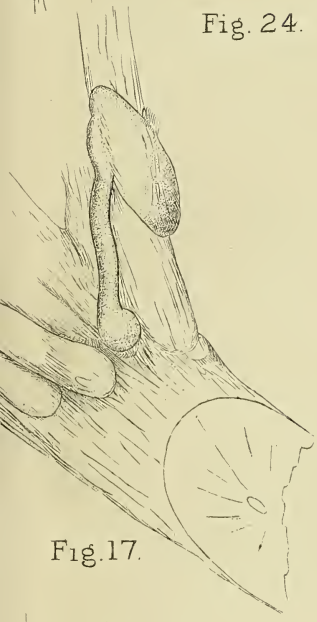

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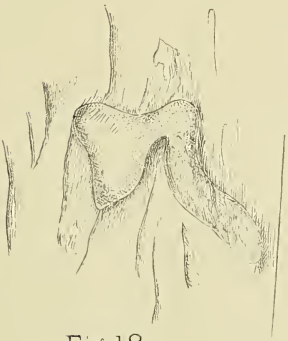

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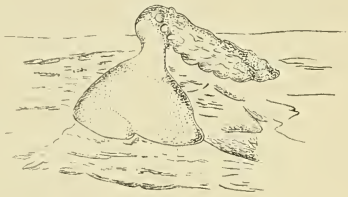

Fig. 19.

Fig. 20.

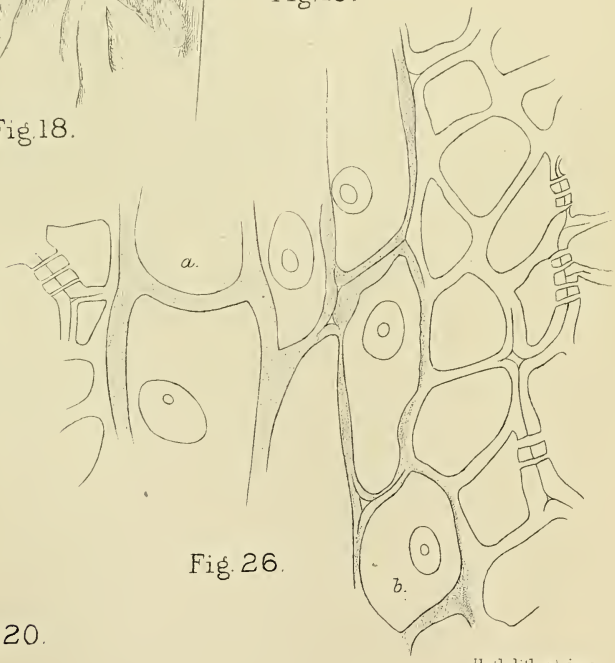

Fig. 26.

Huth lith. et imp.

Fig. 14.

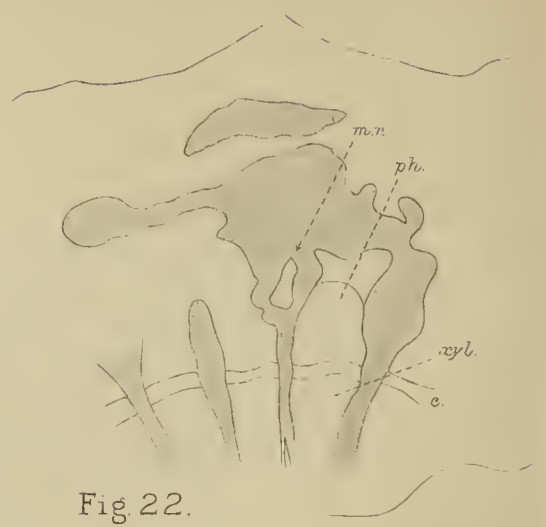

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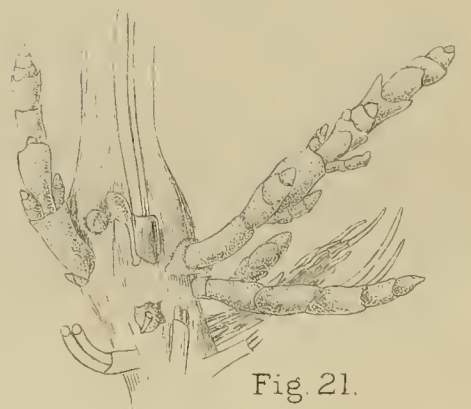
Fig. 27.



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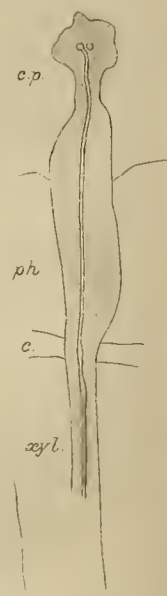

Fig. 23.

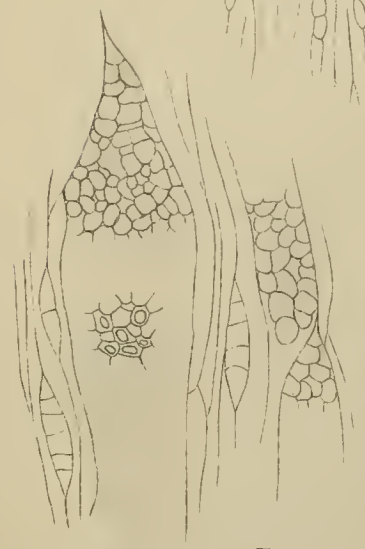

Fig. 28.

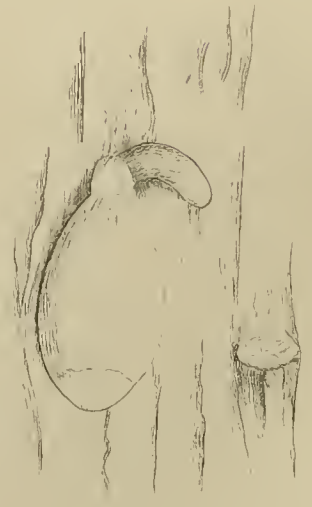

Fig. 15.

Fig. 16.

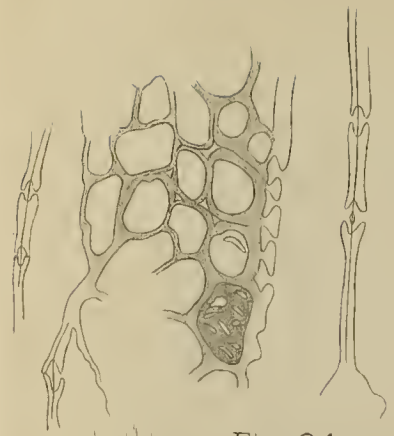

Fig. 24.

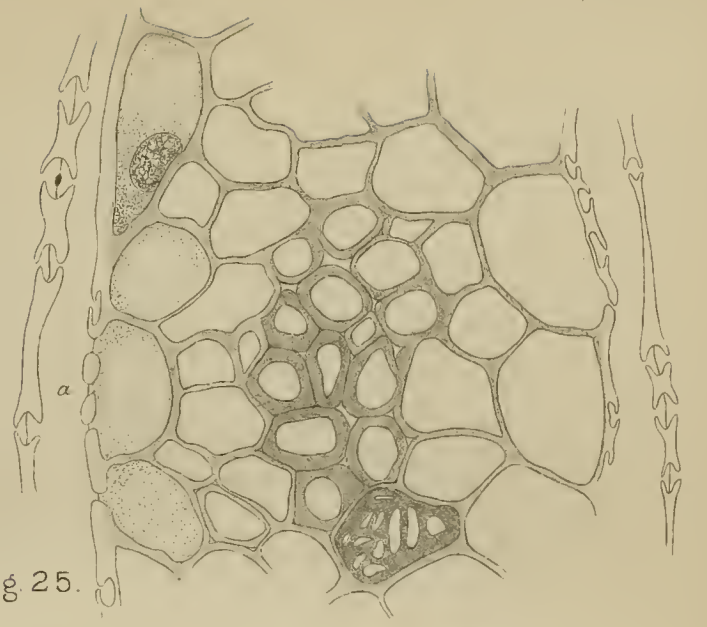

Fig. 25.

Fig. 17.

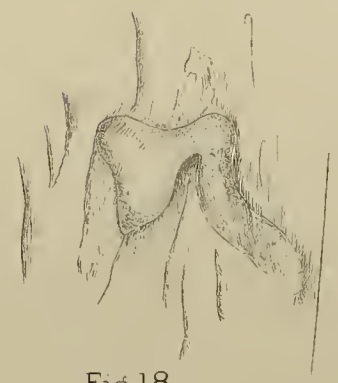

Fig. 18.

Fig. 19.

Fig. 20.

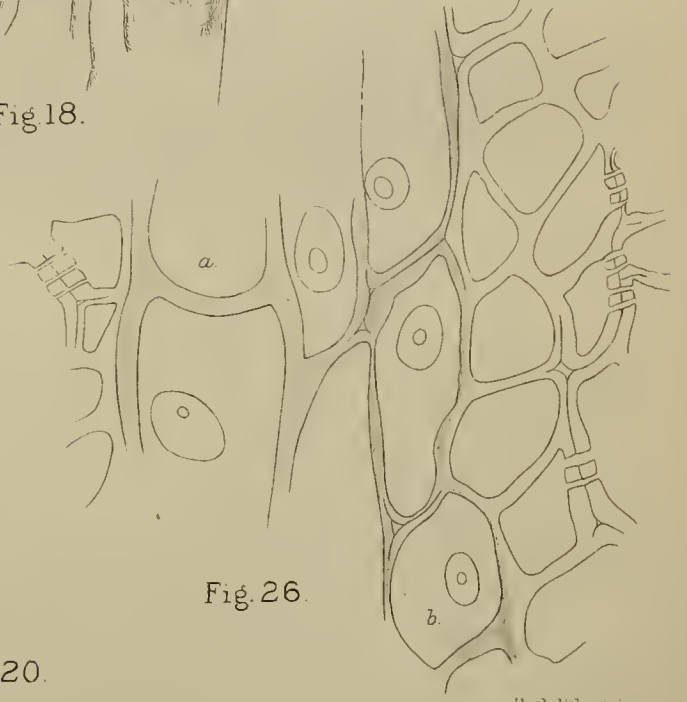

Fig. 26.

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PEIRCE—ARCEUTHOBIUM.

The Anatomy of the Scutellum in Zea Maïs.

BY

ETHEL SARGANT AND AGNES ROBERTSON, B.Sc.

With Plate V.

BOTANISTS have long been familiar with the external structure of the Grass-embryo, which is sufficiently different from that of the embryo in other Monocotyledons to leave some doubt as to the homology of their parts. We began the examination of several Grass seedlings in the autumn of 1902, hoping that their anatomy at a period soon after germination might throw light on this vexed question. We did not propose to monograph the family from this point of view, but, having chosen a few genera from very different parts of it, to compare their seedlings anatomically with each other, and with those of the Monocotyledons already worked out by one of us.

Even this skeleton scheme has not yet been completed, for we have not succeeded in getting all the material desirable. Side issues of some importance have been raised, however, and in particular the anatomical structure of the Maize scutellum offers some features of interest which we believe to be still undescribed[1]. They are clearly bound up with its function as a sucking organ, and the subject is thus a digression from the main morphological line of research, and is more conveniently treated in a separate form.

External Morphology of Embryo.

Within the ripe fruit the main axis of the embryo is triply protected. Embryo and endosperm alike are enclosed in the dry covering of the grain. When a part of this is removed, the whole embryo is seen lying against one face of the endosperm (Fig. 3, Pl. V, cf. also Figs. 1 and 6). The second covering is formed of the scutellum (*sc.* in Figs. 1, 5, 7), a cushion-like structure which is wrapped round the embryonic axis and still conceals the greater part of it in the first days of germination (Fig. 3).

[1] Ethel Sargant and Agnes Robertson, 'On some Anatomical Features of the Scutellum in Zea Maïs.' Report Brit. Assn., Southport, 1903, p. 860.

[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

Finally, each growing-point has its own sheath: the coleoptile encloses the plumule (*cl.* in Fig. 1), and the coleorhiza the radicle (*cr.* in Figs. 1–6). The insertion of these sheaths on the axis, and of the axis on the scutellum, is shown in Fig. 1.

The coleoptile is sharply pointed and stiffened on either side by a vascular bundle. It protects the young stem until the whole shoot is clear of the soil, and is sometimes over an inch long. The rolled-up leaves of the stem-bud finally break through the coleoptile, near the apex but a little to one side of it.

In Fig. 2 the root-sheath or coleorhiza is seen emerging from the covering of the grain. It never attains the length of the stem-sheath, and is soon penetrated by the primary root. Though less differentiated than the coleoptile it is a true sheath, and not a mere mass of tissue within which the radicle is formed endogenously.

The primary root (R, Figs. 1 and 6, Pl. V) grows vigorously when once started, and continues for many weeks to be the chief root-organ of the seedling. Two cauline roots appear very early between the scutellum and the young stem (*r' r'* in Figs. 6, 7, 8, 9). They are symmetrically placed on either side of a median section through the whole embryo, such as that shown in Fig. 1 on Plate V, and accordingly their insertion only (*r'*) is shown in that figure. They grow upwards, appearing over the top of the scutellum a few days after germination (Fig. 9). These twin roots break through the tissues of the young stem just at the base of the cleft dividing it from the upper part of the scutellum (Fig. 6). They press against the inner face of the scutellum in growing upwards, and the first effect of their growth is to widen this cleft by pressing the stem-bud outwards (Figs. 5 and 6). This mechanism no doubt aids the ascending axis to get clear of the seed, and it is possible that the cleft itself—which in the damp soil is sure to be filled with water—may be useful as a reservoir, tapped for a few days by the twin roots as they lengthen.

The twin roots curve sharply downwards so soon as they are clear of the seed and enter the soil, where, like other cauline roots, they serve as auxiliaries to the main root. When growing Maize in pots in a greenhouse, we often observed the seed with the lengthening stem-bud attached to be raised above the surface of the soil on a tripod formed of the primary root and the twin cauline roots. This recalls the stilt-roots of *Pandanus*[1]. But we do not know whether the habit is characteristic of Maize germinating under natural conditions.

The shape and position of the scutellum are shown in Figs. 1, 3, 4, 5 and 6 on Pl. V (cf. also Figs. 7–10). It is in contact with the endosperm over almost the whole of its dorsal surface. It supplies food to other

[1] Kerner and Oliver, 'The Natural History of Plants,' vol. i, p. 756.

parts of the embryo from the stores laid up in its own tissues, and from those of the endosperm. The contents of the endosperm are by degrees dissolved and absorbed by the scutellum, which transmits them in solution to the growing parts of the embryo.

Epithelium and Glands.

The epidermis of the embryo is of normal structure except on the dorsal face of the scutellum. Wherever this face is in contact with the endosperm, that is over the whole of it with the exception of the extreme base and a small area corresponding to the insertion of the pedicel, it is covered with a well-marked epithelium. This tissue has often been described, and is shown in our Figs. 16, 17, 18.

The scutellum is always more or less wrinkled on its dorsal face. The outline of this face is marked in section by a nearly continuous dark line of varying thickness (Fig. 17), and the wrinkles are seen as depressions, sometimes filled with a dark mass. This appearance is no doubt due to a viscous secretion from the epithelium cells, which covers their free surface with a thin sticky layer and sometimes collects in the wrinkles like water in a furrow. We have seen it most clearly in preparations cut from an embryo which had been dissected from the ungerminated seed and fixed in methylated spirit (Fig. 13). In this specimen the secretion, whatever its nature, has been exposed to the solvent action of alcohol only, whereas all the other preparations figured are cut from germinating seeds, in which the tissues were of course thoroughly penetrated with water before they were plunged in alcohol. The depressions in the dorsal outline of the scutellum are also deeper and more numerous in sections from the ungerminated embryo than in those cut from seedlings in which germination has begun.

The epithelium is of course folded in on itself more or less sharply in each wrinkle, and the effect in section is that of a pit lined with epithelium cells and containing a dark mass of secretion (Fig. 13). But leading out of such furrows we sometimes find narrow clefts, also lined with epithelium and penetrating deeply into the tissues of the scutellum (Figs. 11 and 13).

In the ungerminated seed these clefts are already fully formed. They always contain a layer of secretion, but they are often so narrow that it appears in section as a dark line of no great thickness separating one surface of epithelium from the other.

The corresponding glands found in the scutellum of germinating embryos have commonly a very thin layer of secretion (Figs. 11 and 12), or it may be completely absent (Fig. 14). The two surfaces of epithelium then seem to be in contact. In such cases the secretion has no doubt been

washed out by the water in which the tissues of the young seedling are soaked, and perhaps the internal tissues which lie round the gland have swollen, closing its narrow cleft and expelling the secretion at its mouth. Such swelling of internal tissues may also account for the comparative absence of wrinkles in the dorsal surface of the scutellum belonging to a growing embryo. In such embryos many glands open out on a smooth layer of epithelium, whereas in the dry seed we cut they nearly always started from the base of a depression.

The glands vary greatly in size. Some of the smallest are funnel-shaped pits, others shallow slits, and it is very difficult to draw the line between structures which deserve the name of glands and mere depressions or wrinkles. The largest we measured were clefts of considerable size. The opening of one was ·66 mm. long, and its maximum depth ·1 mm. Another reached a depth of ·28 mm.

The number of glands found in a single scutellum is also very variable. Three sets of our serial sections include the whole of the scutellum; and we have counted the number of glands in each series. The numbers thus obtained have no great absolute value. Two difficulties stand in the way of exact determination. The line between well-marked depressions and small glands is not easily drawn, and glands which run nearly parallel to the plane of section are readily passed over. The numbers recorded are therefore in all probability too small.

In seedling A_9 (Fig. 9, Pl. V), six days old, the scutellum was cut transversely. Thirty-eight glands were counted.

In seedling *a*, two days old, the scutellum was cut transversely. Only seven glands were found, all small. This is probably an exceptional case.

In seedling *b*, two days old, in which the scutellum was cut longitudinally, twenty-nine glands were counted.

Sections were also cut transversely through an imperfect scutellum, dissected out of an ungerminated seed. The extreme tip and extreme base were absent, but thirty-eight glands were counted in the remainder.

The majority of glands counted in those seedlings which were cut transversely run parallel with the longer axis of the scutellum, while in seedling *b*, which was cut longitudinally, the majority are more or less perpendicular to the longer axis. In other words, more glands were always counted in the direction perpendicular to the plane of section, and this result is clearly due to the difficulty of recognizing a gland which lies parallel to the section. But we believe that longitudinal glands are really rather more common than transverse ones.

The glands are scattered over the whole surface of the epithelium except where it covers the top of the scutellum. In seedlings A_9, *a*, and *b*, no glands are found above an imaginary line drawn across the scutellum at a distance from the apex of one-third its whole length. The apex of

the scutellum is wanting in the fourth series of sections—that cut through the embryo of a dry seed—but glands are found near the beginning of this series, and they probably stand above the corresponding line. In all four series glands are more numerous in the middle zone than below it, but they are occasionally found even at the extreme base. They are also more frequent on the wings of the scutellum than on or very near the median spine (Figs. 6–8).

We have not followed the development of these glands, for they are fully formed in the ripe seed, which is the youngest stage we have examined. Their appearance in section suggests that they are infoldings of the epithelium.

Vascular System of the Scutellum.

The bundles of the scutellum are inserted on the stele of the axis at the great vascular junction on which the root-insertion r' is marked in Fig. 1, Pl. V. The bundle shown in that figure as entering the lower part of the scutellum is one of several large vascular branches which ramify there and supply its needs. There is no main bundle in this part of the scutellum, and the xylem of the various branches never becomes completely lignified.

A single massive bundle runs from the vascular junction into the upper part of the scutellum, and ends just below the extreme apex. This bundle, with its slender but numerous branches, constitutes the entire vascular system of the upper scutellum. A few days after germination its xylem is completely lignified, standing out sharply in stained sections.

Just above its insertion on the axis the main bundle is circular or oval in transverse section, and is surrounded by an unthickened endodermis. It lies near the ventral surface of the scutellum, towards which its small xylem group is directed. The phloem group is very large (Fig. 15). In the seedling figured there is hardly any indication of a division into two masses, but in others the two groups of phloem are quite clearly marked, and are partially divided from each other by a group of parenchymatous elements with wide lumen and slightly thickened walls which occupies the position of the sclerenchyma in the bundle of the mature stem. Such elements are present in the section drawn in Fig. 15 (*scl.*), but are not gathered up into a compact group.

The phloem proper or 'soft bast' does not show the regular geometrical pattern characteristic of the transverse section through a mature bundle.

Sections which cut the axis of the seedling transversely pass longitudinally through the main scutellum bundle as it enters the axis. Spiral and annular tracheids are the first xylem elements to be lignified. In a six days' seedling (A_9, Fig. 9) many larger tracheids are also present,

polygonal in transverse section (*x*, Fig. 15), and thickened in a rather irregular scalariform way.

Longitudinal sections through the phloem show the elements marked *scl.* in Fig. 15 to be rather long cells with scanty contents and pitted walls not yet lignified. They clearly correspond to the sclerenchyma of the mature bundle.

The structure of the soft bast is shown in Fig. 19. The section drawn is one of a series cut longitudinally through a seedling only a few days after germination. Within the endodermis (*end.*) lie rows of long elements with much elongated nuclei (*s*, *s*, Fig. 19), which alternate—not very regularly—with rows of shorter elements having round or oval nuclei. The cell contents are very dense in both. To bring out the structure of these elements more clearly some microtome sections, cut transversely through the scutellum of an eight-days' seedling and passing through its main bundle almost longitudinally, were treated with Schultze's solution. No sieve-plates could be found in the elements marked *s*, *s*, but we have little doubt that they represent young sieve-tubes. As the conducting system of the scutellum must be most active early in the life of the seedling, that is during the period when it depends on the endosperm for the whole of its food-supply, perhaps the differentiation of the sieve-tubes may never proceed further. The shorter elements may represent companion-cells.

Ramifications of various size are given off from the main bundle throughout its course, except for a short distance above the junction with the plumular bundles. In following the bundle-system upwards we find that branches are given off much more freely as we approach the apex. Even the largest are slender as compared with the trunk. The position of the latter shifts somewhat as it nears the apex. It moves from the ventral to the dorsal side of the scutellum, but never approaches the dorsal surface very closely (Fig. 16).

The longer branches are commonly inserted on the lateral faces of the bundle-trunk and extend into the wings of the scutellum. They give off short branchlets towards its dorsal surface.

The shorter branches are inserted on the dorsal face of the bundle-trunk and spread out towards the dorsal epithelium, showing in transverse section like the sticks of a fan (Figs. 16, 17). As the scutellum narrows towards the apex these short branches become more numerous, and a fairly thick radial section through the scutellum shows the main bundle feathered on its dorsal face by a close crop of vascular branchlets, all bending outwards (cf. Fig. 1). The bundle-trunk terminates in a tuft of such branchlets, which reach almost to the very tip of the scutellum.

Branches and branchlets alike terminate beneath the epithelium of the dorsal surface, generally ending two or three cell-rows below it (Figs. 17, 18). Their insertion on the dorsal and lateral faces of the bundle-trunk modifies

the structure of the latter. Xylem elements creep round its circumference in order to reach the point from which a branch is given off. In the upper part of its course, where branches are frequent, this leads to the formation of a complete girdle of xylem round the phloem of the bundle-trunk (Figs. 16–18). Its structure in this region is that which Professors Strasburger and Jeffrey call amphivasal, though near the base it is, as we have seen, collateral with a compact group of xylem on the ventral side (Fig. 15).

The larger branches are also amphivasal as a rule (Figs. 16–18), but the girdle of xylem is commonly incomplete in the branchlets. The minute structure of two branchlets is shown in Figs. 20 and 21, both drawn from the same section. The xylem consists of completely lignified tracheids (*x*, Fig. 21) ; the phloem of rather long cells with dense contents and large nuclei, and a few much elongated elements, narrow and almost empty (*c.c.* in Figs. 20 and 21). These form a core to the branchlet. We have called them central cells.

The structure of the branchlets recalls the transfusion tissue described in the rootlets of *Stigmaria* by Professor F. E. Weiss. We have adopted the term albuminoid cell for the thin-walled phloem elements with thick contents (*alb.*, Fig. 20). They are seen in the larger branches to be the direct continuation of the phloem in the bundle-trunk (Figs. 16–18).

Conclusion.

The scutellum of *Zea Maïs* is distinguished by the presence of glands on its dorsal face, and by the transfusion tissue connected with its vascular system. Both these features are undoubtedly connected with its prolonged function as a sucking organ.

We have found neither glands nor transfusion tissue in the scutellum of *Triticum*, *Hordeum*, or *Avena*, but in these grasses the endosperm is floury and less copious than in *Zea*, and it becomes semifluid so soon as germination begins. Its reserve of food is exhausted in a few days, and the whole fruit is then cast off by the seedling which in future shifts for itself. Thus the scutellum acts as a sucking organ for a short time only, and it has to deal with a floury endosperm which is already half dissolved by the action of the water absorbed in germination.

The endosperm of *Zea*, on the contrary, is mainly horny, and is absorbed and dissolved by degrees. Sachs's well-known figure, which we have reproduced with some modification in Fig. 1, shows that part of the endosperm is floury, and in this part digestion probably begins.

It is a significant fact that the floury endosperm as shown in such a section is in contact with that region of the scutellum-surface in which glands are most frequent. Possibly their presence there indicates the

activity of the digestive process during the early stages of germination [1]. For on the one hand the convoluted surface of epithelium in the median zone of the scutellum must secrete a larger quantity of enzyme per unit of endosperm surface than the almost plane face of the scutellum near its apex, and on the other this extra supply of enzyme attacks the more digestible portion of the endosperm and rapidly exhausts it. This is well illustrated in the section of a seed which has begun to germinate: the region of empty cells has at one stage much the same outline as the floury endosperm of the dry seed, though a narrow band of exhausted tissue accompanies the scutellum to its very apex.

If this explanation be true, the floury endosperm is digested very soon after germination by the activity of the highly developed epithelium which borders on it. In this way an immediate supply of food is obtained for the growing embryo while it is producing assimilating organs. The later supply is less liberal, since it is procured by the gradual action of enzyme on the horny endosperm, which is more difficult of digestion and also lies further from the secreting epithelium.

Another member of the Maydeae, *Coix Lachryma-Jobi*, which has a fruit similar in structure to that of the Maize, likewise possesses glands in the epithelium of the scutellum, but they are less well-developed.

The position of the transfusion tissue in the upper part of the scutellum is difficult to understand. Nor is the function of the numerous xylem elements within that tissue clear. The albuminoid cells no doubt serve to convey the proteids from the dorsal tissues of the scutellum to other parts of the embryo. Possibly the tracheids which are lignified so early in the branchlets may convey starch in a soluble form; or they may carry water in the opposite direction, and irrigate the secreting epithelium.

Quarry Hill, Reigate.
July 29, 1904.

[1] See Reed, H. S., 'A Study of the Enzyme-secreting Cells in the Seedlings of *Zea Maïs* and *Phoenix dactylifera*.' Ann. of Bot. vol. xviii, 1904, p. 267.

EXPLANATION OF FIGURES IN PLATE V.

Drawn by Miss Robertson to illustrate the paper on the Scutellum of *Zea Maïs* by herself and Miss E. Sargant.

[Figs. 9, 19, 20, 21 are by Miss E. Sargant.]

The following lettering is used throughout :—*h.e.*, horny part of endosperm. *f.e.*, floury part of endosperm. *sc.*, scutellum. *ep.*, epithelium. *v s.*, main vascular trunk of the upper scutellum. *v.s'.*, one of the vascular strands which ramify in the lower scutellum. *cl.*, coleoptile. *cr.*, coleorhiza. *R.*, radicle. *pl.*, plumule. *r'.*, *r'.*, 'wedging' roots. *end.*, endodermis.

PLATE V.

Fig. 1. Diagram of the grain in radial longitudinal section, founded on the figure in Sachs's Textbook.

Fig. 2. Sketch of a seedling one day old. × 5.

Fig. 3. Same seedling with fruit-coat and seed-coat removed. × 5.

Fig. 4. Sketch of a seedling five days old. × 5.

Fig. 5. Side view of the same seedling. × 5.

Fig. 6. Radial longitudinal section of the same seedling. × 5.

Fig. 7. Diagrammatic transverse section through seedling two days old, showing the position of the rudimentary 'wedging roots.'

Fig. 8. Diagrammatic transverse section through the same seedling, 1·3 mm. above the level of the last figure.

Fig. 9. Sketch of seedling A_9, six days old. Life size.

Fig. 10. Part of seedling A_9. Endosperm removed, root and shoot cut short. The dotted lines give level of sections drawn in Figs. 15 and 16. × 1.

Fig. 11. From seedling A_9. Trans. sec. of scutellum showing epithelium and a vertical gland, g_1. × 120.

Fig. 12. From seedling A_9. Trans. sec. of scutellum showing gland g_2 cut obliquely. × 120.

Fig. 13. From embryo dissected from a dry seed and fixed in meth. sp. Trans. sec. of scutellum showing deep pit filled with secretion and prolonged into gland. × 137.

Fig. 14. From seedling A_4. Trans. sec. of scutellum showing epithelium and two deep vertical glands (g',g''). × 120.

Fig. 15. From seedling A_9. Main bundle of scutellum. × 137.

Fig. 16. From seedling A_9. Complete trans. sec. of scutellum. The main bundle is cut at a higher level than that drawn in Fig. 15 (see Fig. 10); its structure has become amphivasal, and it is giving off ramifications resembling transfusion tissue. × 33 *circa*.

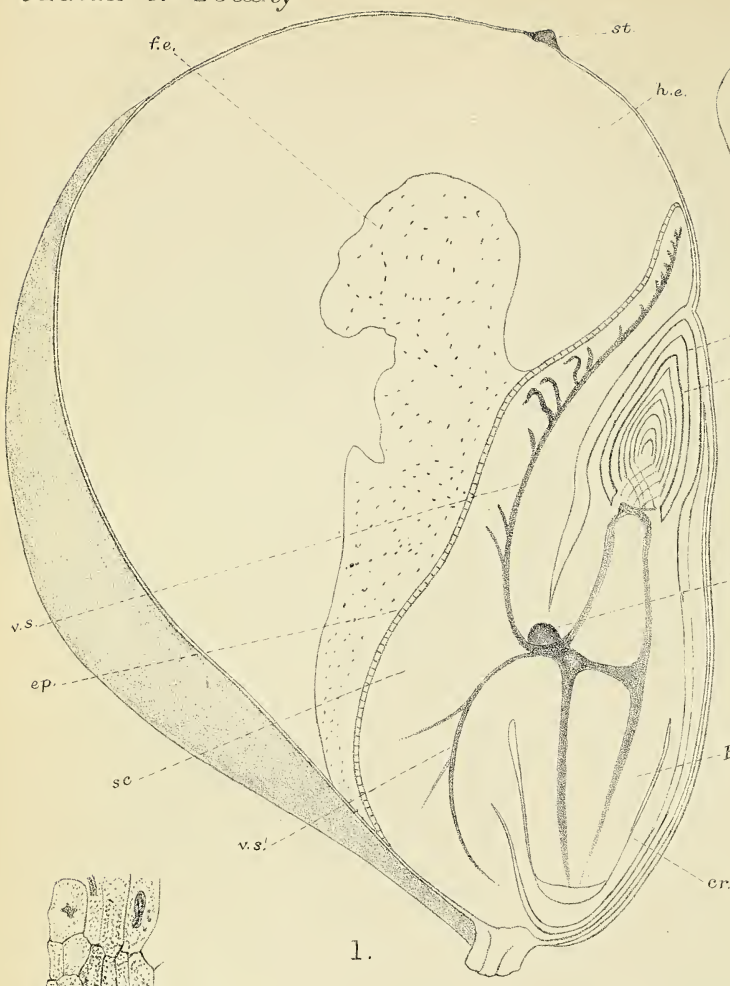
Fig. 17. From seedling A_9. Part of section drawn in Fig. 16, enlarged to show the main bundle and its immediate ramifications. × 125.

Fig. 18. From seedling A_4. Amphivasal structure of main bundle and single ramification with its termination. × 125.

Fig. 19. From seedling A'_1 (just germinated). Part of phloem from radial long. sec. × 240. *s.s.*, probably young sieve-tubes.

Fig. 20. From seedling A_9 (six days old). Phloem from branchlet cut longitudinally. *alb.* albuminoid cells. *c.c.*, central cells.

Fig. 21. From seedling A_9; same section as above. Branchlet cut transversely: *x.*, xylem: *alb.*, albuminoid cells: *c.c.*, central cells.



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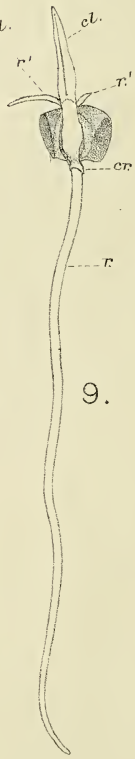

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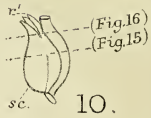

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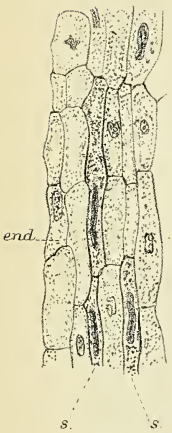

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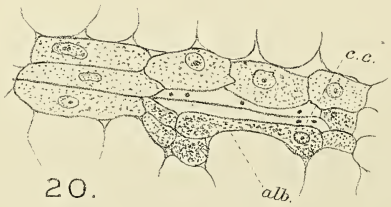

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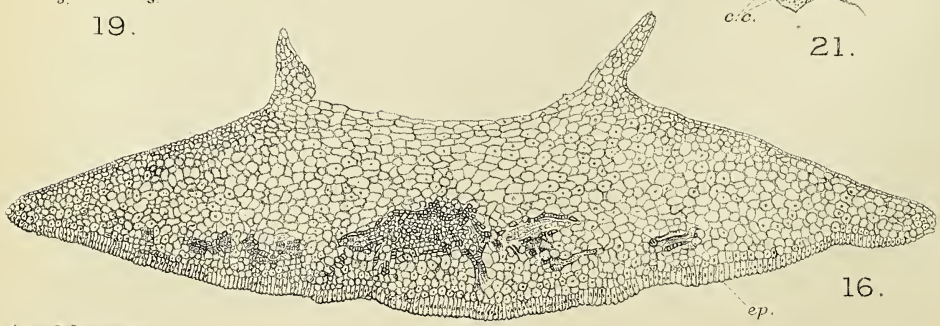

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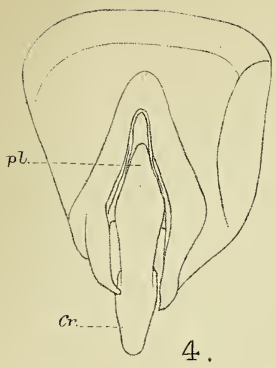

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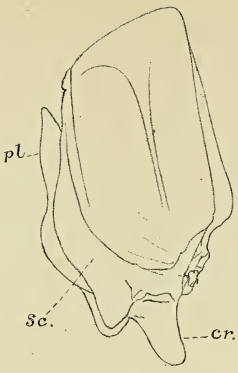

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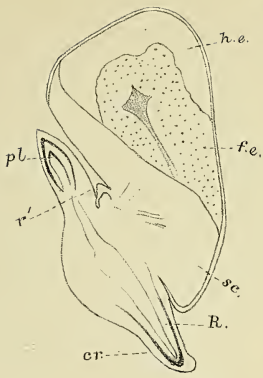

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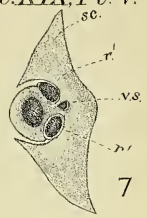

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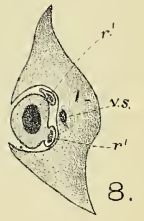

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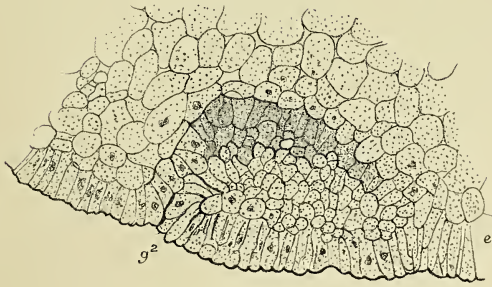

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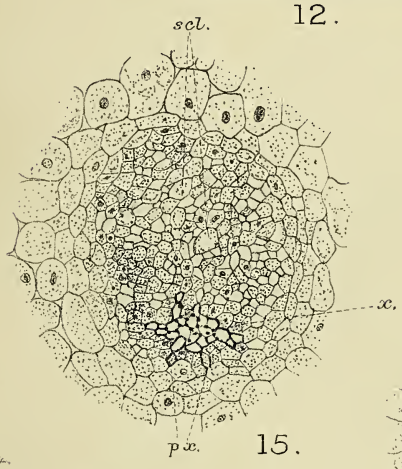

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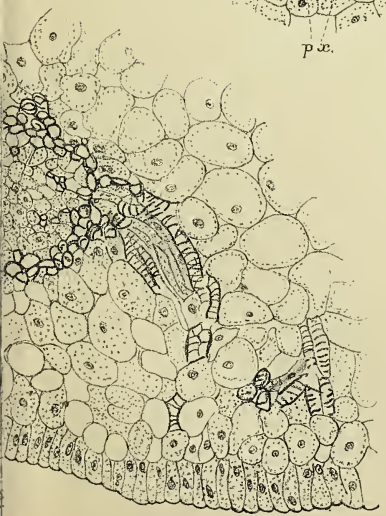

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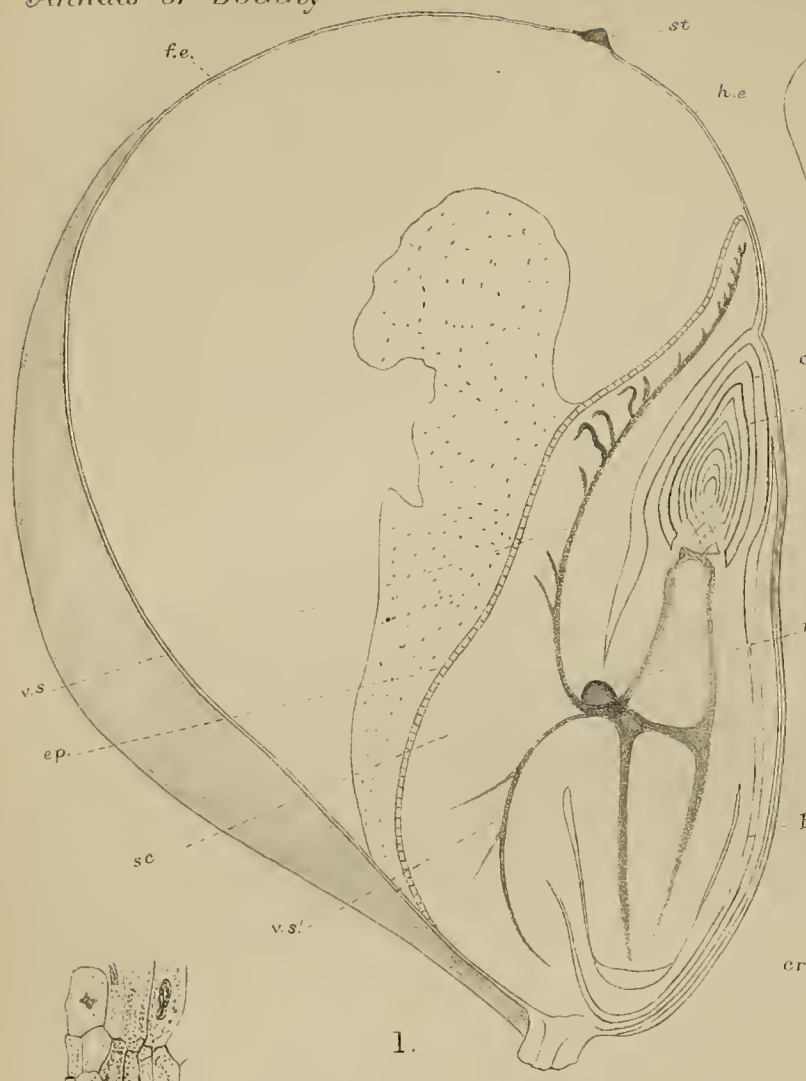

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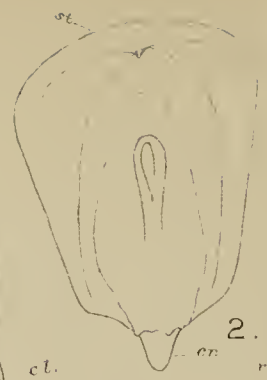

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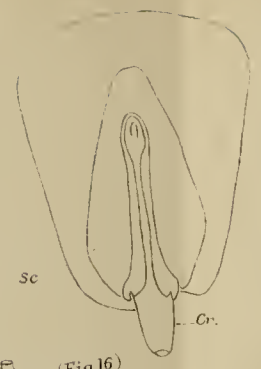

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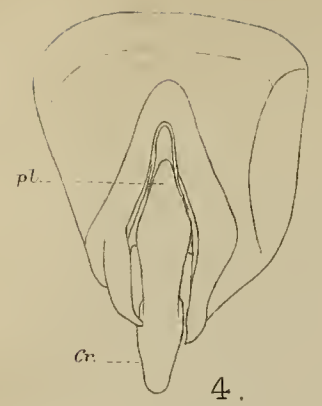

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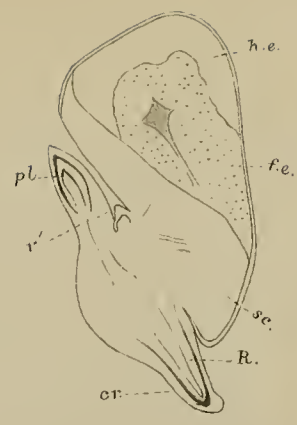

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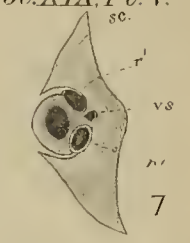

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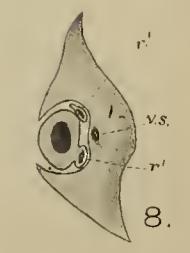

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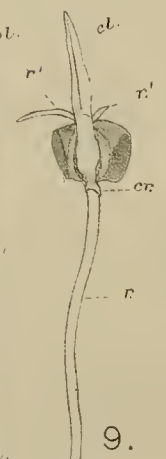

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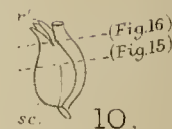

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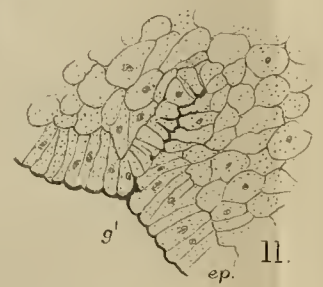

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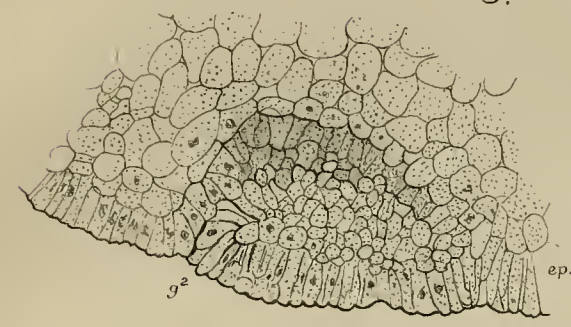

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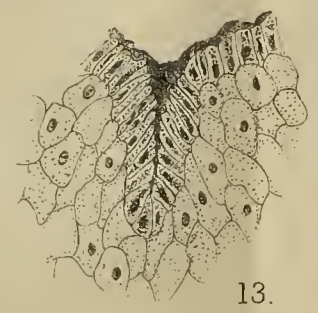

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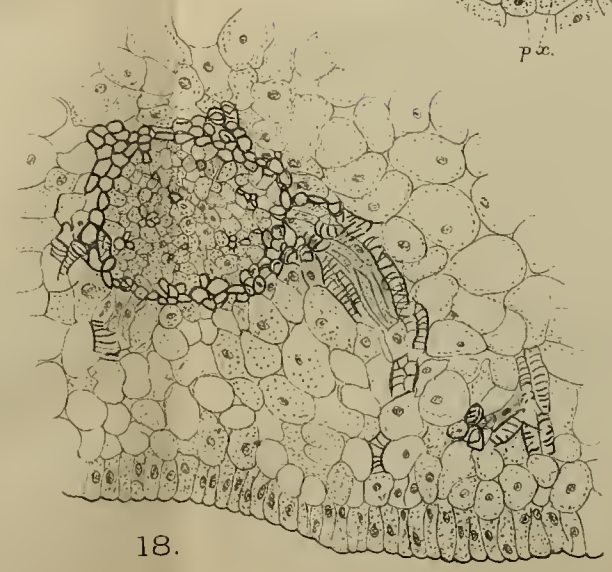

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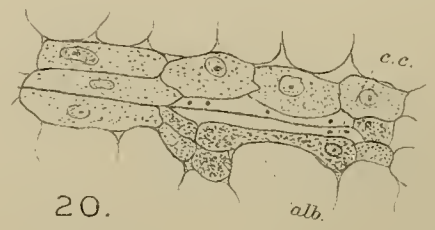

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Further Cultural Experiments with 'Biologic Forms' of the Erysiphaceae [1].

BY

ERNEST S. SALMON, F.L.S.

Introductory.

IN a recent paper (1) I have described certain methods of culture by means of which the conidia of 'biologic forms' of *Erysiphe Graminis* DC. can be induced to infect leaves of host-species which normally are immune to their attacks. In the present paper further methods are described by which the same result can be obtained. A series of experiments has also been carried out with the object of ascertaining the infection-powers of the conidia of the first generation of the Fungus produced on leaves rendered susceptible by certain treatments, as well as the infection-powers of the conidia of succeeding generations. In the first part of the paper the various methods of culture which have been used will be briefly mentioned, as well as the results obtained concerning the infection-powers of the conidia. The second part of the paper will describe more in detail the experiments carried out.

Part I.—The methods, which I have previously described, of inducing susceptibility in a leaf normally immune to the attacks of the 'biologic form' of the Fungus used, have consisted in affecting the vitality of the leaf by cutting out a piece of its tissue or by injuring the leaf by touching it with the red-hot point of a knife (see (1), pp. 108, 111). Conidia of 'biologic forms' which are unable to infect normal uninjured leaves of certain host-species proved able to do so when sown at these injured places.

This method of culture of removing by a cut with a razor a small piece of leaf-tissue was again successfully employed in the preliminary experiments of the present series (see *Part II*, sect. *a*). Further, the results of several experiments have demonstrated the fact that the ascospores of a 'biologic form' are able, like the conidia, to infect such injured leaves, although they proved, in experiments previously carried out and here again repeated, to be unable to infect uninjured leaves of the host-species used.

[1] From the Jodrell Laboratory, Royal Botanic Garden, Kew. Read before the Botanical Section of the British Association, Cambridge, August, 1904.

[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

In one experiment in which a leaf was pricked with a pin, a slight susceptibility[1] was induced. The susceptibility was restricted to the cells at the edge of the holes caused by the pin.

In further experiments an injury to the leaf was caused by stamping out with a cork-borer a circular piece of the leaf-tissue 4 mm. in diameter. Conidia sown on the cells at the edge of the hole were able to cause good infection, and produced eventually little patches of mycelium bearing some hundreds of conidiophores. Ascospores, also, sown at the same place, proved able to cause infection.

Experiments were then made with leaves which had been injured by having large pieces eaten out of them by slugs. Conidia and ascospores were sown over the surface of such injured leaves, and proved able to infect the cells at the edge of the bitten places, producing on these cells little patches of mycelium bearing conidiophores, while elsewhere on the leaf they were not able to cause infection.

In a large number of experiments leaves were injured by pressure in such a way that patches of bruised cells were produced. In some cases the end of a vertical wooden rod (with a flat surface, 7 mm. across) was placed on a leaf laid on a glass slide, and a pressure exerted for a certain time by means of a weight attached to the rod. Bruises were made, also, by pressing hard, with the hand, the rounded end of a glass rod on the leaf placed on a glass slide; and also by nipping the leaf hard at the margin with a pair of forceps. In all the methods the bruised cells themselves (unless they were killed), proved susceptible, and also the cells immediately surrounding them.

The effect of injury caused by the action of narcotics was then tried.

An exposure to ether vapour for 1½ or 2 minutes was found to render the leaf susceptible. In some cases the susceptibility induced was very marked, the leaf presenting, a few days after inoculation, the appearance of being almost fully infected over the whole of the inoculated area.

Exposure to chloroform vapour for 10 seconds, also, induced some degree of susceptibility.

Immersion in a mixture of alcohol and water, and also exposure to vapour of alcohol, rendered leaves in many cases remarkably susceptible. Leaves immersed for 2–22 hours in a 10% mixture of alcohol and water were rendered, in several instances, susceptible to such a degree that the conidia sown produced over the inoculated area abundant patches of mycelium bearing many hundreds of conidiophores. The same marked susceptibility was induced in leaves exposed for 3 minutes to vapour of alcohol.

The effect of injury by heat was then tried. Leaves were placed in

[1] i.e. to the attacks of a 'biologic form' which is unable to infect uninjured leaves of the species.

cold water, and the water heated slowly to 50° C.; the leaves were then taken out, dried and inoculated. A marked susceptibility was shown by the leaf after this treatment, and in many cases inoculation was followed by the appearance of almost full infection. Ascospores as well as conidia were used in these experiments. Marked susceptibility, shown by the production of minute powdery *Oidium*-patches, was also induced by immersing leaves for 1 minute in water at the temperature of 49·5° C. or 50° C. The same result was obtained when leaves attached to plants growing in a pot were immersed for 1 minute in water at the temperature of 50° C.

We see, then, from the results of the above experiments, that not only mechanical injuries, such as wounds from cuts, bruises, attacks by slugs, &c., but also injuries due to the action of narcotics and heat, cause a leaf to become susceptible to a 'biologic form' of a Fungus to which it is normally immune.

To describe cases where a form of a Fungus which is specialized to certain host-plants and confined to them under normal circumstances proves able to infect injured parts of a strange host, I propose the terms *xenoparasite* and *xenoparasitism*[1]. In the case of the specialized Fungus when on its proper host under normal conditions the terms *oecoparasite* and *oecoparasitism* may be used.

It is obvious that mechanical injuries quite similar to those produced by cutting, bruising, &c., described in the above experiments, are constantly being inflicted on plants in nature—by animals, and by frost, hail, wind, &c. In the case of cereals the agricultural operation of rolling seedling plants causes a number of leaves to be torn or bruised. An experiment carried out during the present spring has demonstrated that bruised places on leaves produced by rolling are rendered susceptible in the same way as those caused artificially by pressure in the experiments mentioned above (see, also, *Part II*, Section ϵ). At the beginning of last May I collected from a field of young barley which had just been rolled a number of bruised leaves. The soil of the field was stony and 'steely,' and in the operation of rolling about 30 % of the leaves had been bruised more or less severely. Ten of these injured barley leaves were placed in a Petri dish on damp blotting-paper, and each inoculated over the bruised cells with conidia taken from wheat leaves. On the fourth day several of the leaves bore at the inoculated place numerous small straggling mycelial patches, and on the seventh day these patches, in the case of four of the leaves, bore several little tufts of a few clustered conidiophores. A number of saprophytic Fungi were now growing vigorously at the injured places, and stopping the further growth of the *Oidium*.

[1] The same term may be applied to a Fungus when growing on parts of its normal host which are immune when uninjured. Thus, the *Oidium* on *Euonymus japonicus* is normally unable to infect the old leaves of this plant, but proves able to cause infection when sown at an injured place.

Through the work of Janczewski (7) and of Sorauer (8) proof has been given that the action of frost causes injuries to plants which render them susceptible to attacks by certain saprophytic Fungi which are not able to infect them under normal circumstances. Injuries caused by hail, &c., affecting plants in the same way, have been described by Hartig (10) and Sorauer (9).

In the hypothesis advanced in my previous paper (1, p. 113) to account for the susceptibility shown by leaves injured by a cut or burn, it was assumed that in consequence of the vitality [1] of the leaf-cells being affected by the injury, either the protective enzymes or similar substances normally present are destroyed or become weakened, or the production of them by the protoplasm is interfered with, in the cells in the neighbourhood of the injury. This hypothesis may still be advanced to account for the same susceptibility being shown when leaves are injured by the action of alcohol, ether, chloroform, or heat, since it is known that protoplasmic functions are temporarily inhibited by anaesthetization, and that a high temperature may partially or entirely inhibit the process of the secretion of an enzyme (3).

Attention may be directed to the fact that these cases of the loss of immunity, brought about by causes which affect the vitality of the leaf, find their exact parallel in the recorded instances of induced susceptibility in animals to certain diseases caused by bacteria. The decrease of vitality caused by fatigue, action of drugs, abnormal food, or environment has been proved to induce susceptibility to certain bacteria in the case of animals which are immune under normal circumstances (4, 5).

In a recent paper published by Ray (6) the statement is made that plants of maize exposed to ether vapour or to heat were rendered by this treatment more susceptible to the attacks of a yeast-form of *Ustilago Maydis* which had been grown saprophytically for some time. According to Ray, the increased growth of the Fungus here shown was due to the injury to the plant having caused a change in its metabolism. The change assumed is that a certain plastic substance, which serves as food for the Fungus, accumulates in consequence of its non-transference by an enzyme of the host-plant.

A series of experiments was carried out with the object of ascertaining the infection-powers of the conidia of the first generation produced on leaves injured by the action of alcohol, ether, and heat respectively. Leaves of barley thus treated were sown with conidia taken from wheat. The conidia produced on the treated barley leaves were sown simultaneously on

[1] Until our knowledge of the physiology of the cell has progressed further, it is necessary to use the general term vitality to express the sum of the individual physiological processes at work in the cell. External factors which affect the normal balance in the working of the individual physiological processes increase or decrease the vitality of the plant.

a normal uninjured leaf of barley and of wheat. In the eleven cases in which the barley leaves, on which the conidia were produced, had been treated previously with alcohol, in the four cases in which heat had been used, and in the one case in which ether had been used, the conidia proved totally unable to cause any infection on the barley leaf, while producing in every case full, and usually virulent, infection on the wheat leaf. In a single case [1], only, the conidia produced on a barley leaf previously treated with alcohol, when sown simultaneously on a normal uninjured leaf of barley and of wheat, gave rise on the barley leaf to a small patch of mycelium bearing a few conidiophores. The Fungus, at an early stage, however, showed evident signs of being unable to develop fully, and soon died away. On the wheat leaf full infection resulted, and the Fungus persisted until the death of the leaf.

In order to see, in these cases in which the conidia produced on the treated barley leaves infected wheat leaves, whether the infection-powers of the Fungus would vary in subsequent generations, conidia of the successive generations produced on wheat, up to the sixteenth generation, were cultivated. In these experiments 211 leaves of barley and of wheat were inoculated, and the conidia infected fully, and in most cases virulently, the wheat leaves, while never producing any sign of infection on the barley leaves.

These experiments demonstrate the fact that the infection-powers of a 'biologic form' are not altered by its residence for one generation on a strange host-plant treated in the manner described, a fact which gives some evidence in favour of the idea of the hereditary nature of the infection-powers of some 'biologic forms.' The results of other experiments which I have recorded (2) show, however, that the infection-powers of some 'biologic forms' may be considerably influenced by the effect of a new host-plant [2].

The results obtained in the present series of experiments may thus be summarized :—

(1) Susceptibility can be induced not only by various kinds of mechanical injury, but also by such interference with the normal functions of the cell as follows the application of anaesthetics and heat.

(2) The conidia of the first generation produced on leaves of a strange host-plant previously subjected to the action of alcohol, ether, or heat retain the power of infecting their original host, and do not acquire the power of infecting normal leaves of their temporary host.

[1] See page 145, Exper. No. *a* 99.

[2] Thus, the conidia of the form of *E. Graminis* growing on *Bromus racemosus* are not able to infect *B. commutatus*. When this form, however, is sown on *B. hordeaceus*, the conidia of the first generation prove able to infect *B. commutatus*. Normal (untreated) leaves of the host-species were used in these experiments. Presumably, the change of infection-powers in this case is connected with the change of nutrition.

Part II.—In the Experiments described below, the Fungus used in inoculation was the 'biologic form' of *Erysiphe Graminis* DC. on wheat.

a. Injury by 'cuts[1].'

In four experiments (Exper. Nos. *a* 17, *a* 22, *a* 46, *a* 065) conidia were sown on thirty-one 'cut' barley leaves[2], on the cells of the mesophyll tissue exposed by the cut. Infection resulted on sixteen of these leaves, minute patches of mycelium bearing numerous conidiophores appearing in nine to fourteen days.

In one experiment (No. *a* 45) a sharp razor was drawn longitudinally along the blade of the leaf, making a fine slit-like cut, which extended to, but did not cut through, the lower epidermis. A cut of this nature, 1·5 cm. to 2 cm. long, was made on five leaves. In the case of four leaves infection resulted along the edge of the cut, where numerous patches of mycelium were produced, and on three leaves a few scattered conidiophores were produced by a few of the patches of mycelium bordering the cut.

Uninjured barley leaves were then inoculated with ascospores. In the first experiment (No. 1) two wheat leaves and two barley leaves, both attached to seedling plants in pots, were introduced into a Petri dish, at the bottom of which wheat leaves bearing ripe and bursting perithecia were placed. The four leaves were fixed horizontally at a little distance above the perithecia, and exposed for three days to inoculation by the ascospores which were being continuously thrown up. At the end of this time the two pots of wheat and barley with the four inoculated and marked leaves were placed under a bell-jar. On the fifth day (November 28) the two wheat leaves bore very numerous (fifty to eighty) minute flecks of mycelium. No trace of infection appeared on the barley. On the seventh day many of the patches on the wheat leaves bore groups of ripe conidiophores; the nine control leaves were all free. On the tenth day the two wheat leaves were covered almost continuously on the upper surface for two-thirds of their length with densely powdery *Oidium*-patches; the controls were still all free. No signs of any infection appeared on the barley[3].

In the next experiment (No. 2) leaves of wheat and barley were removed from plants and inoculated with ascospores, with the object of seeing if the injury to the barley leaf caused by its removal from the plant would bring about susceptibility. Two wheat leaves and two barley leaves were exposed for three days to ripe, bursting perithecia. On the tenth day the two

[1] The manner in which the cut was made has been described in (1), p. 108.

[2] In all the experiments the first leaf of seedling plants was used.

[3] A dozen pots of seedling plants of barley were allowed to stand for four months in a green-house, among pots of wheat plants virulently infected with *Oidium*, and were constantly inoculated by conidia blowing on to the leaves and stems. No trace of any infection appeared on the barley, notwithstanding that the health of the plants became much impaired by unfavourable conditions of growth.

wheat leaves were covered, over the inoculated surface, with little powdery *Oidium*-patches, about thirty on each leaf. No signs of any infection were visible on the barley, although the surface of both leaves was covered with many hundreds of germinating ascospores which had been ejected from the perithecia. The number of the ascospores was so great that here and there, at places where hundreds had germinated together, a whitish-looking patch had been produced. There was not the slightest sign of any infection, however.

Experiments were then made to see whether ascospores would be able to infect 'cut' leaves of barley.

In the first experiment (No. 3) three barley leaves were removed from the plant and 'cut' on the upper surface; they were then suspended over ripe perithecia for 48 hours with the 'cut' surface upwards. On the fourth day several germinating ascospores were visible opposite the 'cut' on one leaf, and formation of a lobed haustorium from several of the appressoria had taken place. On the fifth day three vigorously growing patches of mycelial hyphae proceeding from germinating ascospores were visible on this one barley leaf, on the epidermal cells opposite the 'cut.' On the seventh day a few young conidiophores were produced, and on the ninth day ripe conidiophores. On the tenth day all the leaves were yellow and translucent. No infection was visible on two leaves; on the third leaf three patches of mycelium, each bearing conidiophores, were visible opposite the 'cut'; also two small patches of mycelium, each proceeding from a single ascospore and each bearing two or three conidiophores, were visible at a distance of 1 mm. beyond the region opposite the 'cut.' On the remaining parts of this same leaf could be seen hundreds of ascospores which had been ejected from the perithecia, and which had germinated, but, being on cells out of reach of the influence caused by the injury, had failed to produce any infection.

In the second experiment (No. 4) four 'cut' barley leaves were inoculated with drops of water containing ascospores (obtained by crushing ripe perithecia in the water). The drop was placed on the uninjured epidermis opposite the 'cut' in the case of two leaves, and on the cut surface of the other two leaves. On the eighth day a vigorous patch of mycelium with a few young conidiophores was visible on the cut surface (i.e. on cells of the exposed mesophyll) of one leaf. On the twelfth day this leaf bore a vigorous radiating mycelial patch with several conidiophores.

In the remaining two experiments (Nos. 5, 7) six barley leaves were removed from plants and 'cut,' and were then exposed for 48 hours to inoculation from ripe, bursting perithecia. Two leaves were exposed with the 'cut' surface downwards (i. e. facing the perithecia), and four with the 'cut' surface upwards. At the end of the forty-eight hours germinating ascospores, with appressoria formed, were visible on all the leaves at the

'cut' places, and on one leaf penetration from the appressorium, and the formation of a haustorium in an epidermal cell opposite the 'cut' had taken place. By the next day the haustorium had developed lobed processes. On the seventh day young conidiophores were produced, on little patches of mycelium, opposite the 'cut' on two leaves, and on the tenth day mature conidiophores with ripe conidia. On the fourteenth day the leaves were translucent, and hundreds of ascospores were visible which had been sown over the surface of the leaf exposed to the bursting perithecia. The ascospores had germinated, and produced appressoria, but, except at the 'cut' place of two leaves (as noted above), had been unable to cause infection. In two cases, however, at a distance of 2 mm. beyond the region opposite the 'cut,' an ascospore had germinated, and produced a lobed haustorium, and a few short, thick hyphal branches from the appressorium.

β. Injury by pin-pricks.

In the first experiment (No. 6) twelve pricks were made in a barley leaf with a pin (causing holes 0·5–0·75 mm. in diameter), at a distance of 3 mm. from each other, in two parallel lines across the width of the leaf. Conidia were sown round all the holes, and in the region between the two lines of holes. On the seventh day minute flecks of mycelium proceeding from germinating conidia were visible at the edge of two of the holes. On the ninth day a few conidiophores appeared round these two holes. On the fourteenth day a few conidiophores were still present round one of the holes. The leaf was now nearly dead, and translucent at the inoculated places. No production of conidiophores took place in the region between the lines of pin-pricks, but two conidia, each at a distance of 0·75 mm. from the nearest hole, germinated and produced a small patch of barren mycelial hyphae.

In other experiments (Nos. 6, 10) nine pricks were made with a needle, at a distance of 3 mm. from each other, in a single row on a barley leaf. No infection followed the inoculation of this leaf. The same negative result was obtained when a circle, 4 mm. across, of seven pin-pricks was made on a barley leaf, and conidia sown on the cells in the centre of the circle.

Ascospores were then sown on barley leaves, in which nine to fourteen pin-pricks, in a longitudinal row, at a distance of 2 mm. from each other, had been made. No infection resulted; the ascospores germinated vigorously, and produced appressoria, but were not able to form any mycelial hyphae.

γ. Injury by cork-borer.

In one experiment (No. 6) a circular hole, 4 mm. in diameter, was stamped out of a barley leaf with a cork-borer. Conidia were sown, on December 5, and on the fourth day clear signs of infection were visible on

the cells at the edge of the hole. By the seventh day flecks of mycelium, bearing young conidiophores, were produced round the margin of the hole. By the ninth day about a hundred mature conidiophores were visible, all close to the edge of the hole. On the fourteenth day small but vigorous mycelial patches, bearing conidiophores, were visible round the hole ; this part of the leaf was now yellow, translucent, and nearly dead. A few of the conidia which had been sown at a little distance from the hole produced a few short mycelial hyphae.

In another experiment (No. 9) two uninjured wheat leaves, and one barley leaf in which a circular hole 4 mm. in diameter had been made with a cork-borer, were exposed for 48 hours to inoculation from ripe, bursting perithecia. On the seventh day (December 19) the two wheat leaves bore numerous minute flecks of mycelium ; the barley leaf bore small flecks of mycelial hyphae proceeding from germinating ascospores at the margin of the hole, at places where the epidermal cells had been dragged away in the process of stamping out the hole. On the tenth day each of the wheat leaves was covered, for the whole length (6 cm.) which had been exposed to the bursting perithecia, with very numerous, small, powdery *Oidium*-patches. No infection of the barley leaf had occurred except at the very edge of the hole, where at three places little patches of mycelium, bearing two to three conidiophores with chains of ripe conidia, were formed, and also several barren patches of mycelium. This leaf was decolourized, and on examination was found to have been inoculated with ascospores all over its surface ; these spores had germinated everywhere, but except at the injured place had been unable to cause any infection.

8. Injury caused by Slugs.

Experiments were made in which barley leaves injured by the attacks of slugs were inoculated, conidia being used in the first two experiments, and ascospores in the last two.

In the first experiment (8*) barley leaves were exposed for twelve hours to the attacks of some slugs kept in captivity, at the end of which time the leaves were much injured by having large pieces eaten out of them. The leaves were inoculated over their whole upper surface. After thirteen days infection was visible here and there along the edges of the bitten places, where vigorous little patches of mycelium bearing a few conidiophores were formed. By this time the leaf-cells in the neighbourhood of the injured places were yellowish, transparent, and nearly dead. It was noticeable that infection occurred most frequently at the places where the leaf had been bitten nearly to shreds. In another experiment (No. 12) one barley leaf, treated similarly to the above, bore on the sixth day several vigorous little patches of mycelium at the edge of the bitten places. These patches

were, however, barren, with the exception of one patch which bore four conidiophores.

In the first experiment (No. 10) in which ascospores were used, one wheat leaf and one barley leaf, both just removed from seedling plants, were placed by the side of a barley leaf out of which slugs had eaten large pieces[1]. The three leaves were exposed for 48 hours to inoculation from ripe, bursting perithecia. By the third day the leaf which had been injured by slugs was yellow and translucent in its upper half, and hundreds of ascospores could be seen germinating on its surface. On the sixth day the wheat leaf was covered with very numerous powdery *Oidium*-patches; the uninjured barley leaf was covered with hundreds of ascospores which had germinated but failed to produce infection; the barley leaf injured by slugs was infected with numerous vigorous little patches of mycelium, some of which bore a few young conidiophores. The fertile patches were all situated along the edges of the bitten places; at a distance of 1–2 cm. from the places of injury several little barren patches of a few straggling hyphae occurred.

In the second experiment (No. 11) four barley leaves, out of which slugs had eaten portions here and there, thus producing numerous irregularly shaped holes, were exposed for 48 hours to ripe, bursting perithecia. On the sixth day two of the leaves bore each several little patches of mycelium proceeding from germinating ascospores situated close to the edges of the bitten places. The patches were mostly barren, but a few had produced one to three conidiophores. On one leaf an ascospore situated at a distance of 2·5 cm. from the edge of a hole had germinated and produced a few mycelial hyphae and two young conidiophores.

ϵ. Injury by Pressure.

In the first series of experiments the following apparatus was used. A glass tube was supported vertically in the clamp of a stand placed on a table. A wooden rod of a slightly smaller diameter than the tube was passed through the latter, and thus kept upright. The upper end of the rod carried a cork disk, on which the weight used was placed; the lower end had a flat surface of 7 mm. diameter, with a rounded edge to avoid cutting the leaf. The leaf was placed flat on a glass slide on the table, and the end of the wooden rod rested on it.

Three experiments were made in which the weight[2] used was 9·5 oz.; the pressure was applied to three barley leaves, attached to growing plants, for 24 hours. The leaves were then cut off, and placed in a Petri dish; conidia were then sown on the bruised place. In the first experiment

[1] The two holes made by the slugs measured 2·5 cm. × 3 mm., and 1·5 cm. × 1 mm.

[2] Including that of the rod and cork disk.

(No. *i*) vigorous patches of mycelial hyphae, proceeding from the sown conidia, were visible on the fifth day, on the semi-translucent tissue which had been bruised by the pressure of the weight. On the seventh day a small cluster of nearly mature conidiophores was visible on the bruised place. On the thirteenth day there were several little clusters of conidiophores [1] on the bruised part, which was now yellowish and translucent. On the seventeenth day patches of conidiophores were still visible, scattered over the bruised place.

In the second experiment (No. *p*) conidia were sown not only on the bruised part but also at a distance of 1·5 cm. from the edge of the bruise. On the seventh day (Jan. 16) the central part (which measured 2·5 mm. across) of the bruised tissue was formed of translucent, dead, and disorganized cells; round this the cells were slightly yellowish, and nearly translucent, and here numerous little patches of mycelium were formed. On the twelfth day several little clusters of conidiophores, on patches of radiating mycelial hyphae, occurred round the bruise, at distances up to 2 mm. from the edge of the bruise. Two of the conidia sown at a distance of 0·75 cm. from the bruise produced a minute patch of mycelial hyphae and a few conidiophores; some of the conidia sown at a distance of 1·5 cm. produced minute barren patches of mycelial hyphae.

In the third experiment (No. *a* 2) a few patches of mycelial hyphae, bearing a few conidiophores, were produced on the bruised cells.

In the next three experiments, pressure, using a weight of 9·5 oz., was applied for 2 hours only.

In experiment No. *k*, conidia were sown on the bruised place and also on either side at a distance of 1 cm. By the tenth day five little patches of mycelium, each with a few conidiophores, were visible on the bruised place; no infection occurred at the inoculated places 1 cm. distant. In the two other experiments (Nos. *a* 21, *a* 25) no infection resulted on the slightly bruised places.

In six experiments barley leaves were injured by pressure applied by hand in the following way. The barley leaf was laid flat on a glass slide, and the end of a glass rod with rounded edges was pressed firmly down so as to crush a group of cells. By this means a circular bruise, 4·5 mm. in diameter was made. The crushed cells were rendered more or less translucent, and if the bruise was severe soon died; the surrounding tissue for a little distance became discoloured by the expression of cell-sap from the injured cells into the intercellular spaces, and so rendered more or less translucent by transmitted light and opaque by reflected light.

In the first experiment (No. *m*) eight barley leaves were bruised rather lightly on the upper surface. On the sixth day small vigorous mycelial

[1] There were ten of these clusters, each bearing about twenty conidiophores; so that, roughly speaking, about 200 conidiophores, each bearing six to ten conidia in a chain, were produced at the bruised place.

patches, with large lobed haustoria, were observed, on three of the leaves, proceeding from conidia sown on the bruised cells themselves. On the fifteenth day one leaf bore several mycelial patches with a few weak scattered conidiophores; on the two other leaves only a few small barren mycelial patches had been formed. No infection occurred on the other leaves. It was evident that the injury inflicted was not sufficient, in most cases, to render the leaf susceptible.

In the next experiment (No. *a* 1) three barley leaves were bruised more severely. Inoculation was made on the bruised cells on the upper surface of two leaves, and on the lower surface, on cells opposite the bruise, in the third case. On the fourth day numerous vigorous little patches of mycelial hyphae were visible on the upper surface of the two leaves, on the bruised cells themselves. On the sixth day several of these patches bore a few conidiophores. On the eighth day one leaf bore numerous vigorous little tufts of conidiophores on the bruised cells themselves; a few conidiophores had been formed, also, on the bruise on the second leaf; on the third leaf, on the lower surface, no infection had occurred. On the thirteenth day the three leaves were translucent throughout; the two leaves inoculated on the upper surface bore vigorous little conidiophore-bearing patches of mycelium on the bruised cells and on those immediately surrounding them; no infection resulted from the conidia sown at distances of 2 mm. and 3 mm. from the bruise. The leaf inoculated on the lower surface, opposite the bruise, bore one little barren patch only, of a few mycelial hyphae.

In the third experiment (No. *a* 13) three barley leaves were bruised on the upper surface, and a space 0·5 cm. wide was marked off at either side at a distance of 0·5 cm. from the edge of the bruise. Inoculation was made both on the bruised part and on the spaces marked off on either side. On the sixth day infection had resulted on the bruised place on all the leaves, both on the bruised cells themselves and on those immediately surrounding them. On the eighth day small vigorous mycelial patches, mostly bearing a few conidiophores, occurred on all the leaves. No infection occurred at the marked places 0·5–1 cm. distant from the bruise.

In a further experiment (No. *a* 8) one barley leaf was severely bruised and inoculated over the bruise. By the tenth day a few conidiophores and several patches of mycelium were visible on the cells immediately surrounding the bruise.

In one experiment (No. 12) a leaf attached to a seedling plant of barley, about three weeks old, was bruised. The leaf was inoculated at the bruised place, and laid on damp blotting-paper at the bottom of a Petri dish, the lid being placed over it. The roots of the seedling plant were kept in water. On the sixth day the bruised place was covered with very vigorous but barren mycelial patches. By the tenth day the mycelial

patches were comparatively large and vigorous, but still remained barren. The latter fact was perhaps due to the circumstance that several saprophytic Fungi were beginning to invade the injured part of the leaf.

A narrow longitudinal bruise, 3 cm. long and 1 mm. wide, was made on three barley leaves in one experiment (No. *a* 15). By the sixth day infection had taken place on all the leaves at the injured place, vigorous mycelial patches being visible on the injured translucent cells. On the eighth day a few conidiophores were visible. On the tenth day all the leaves bore numerous vigorous mycelial patches bordering the sides of the bruise. Many of the patches bore numerous conidiophores, which in several cases were closely clustered.

In three experiments (Nos. *a* 5, *a* 9, *a* 26) forty-five barley leaves were bruised with the end of a glass rod, as before, and then exposed to ripe, bursting perithecia. In four to six days more or less vigorous patches of mycelium (with vigorous lobed haustoria), proceeding from germinating ascospores, were formed at the edge of the bruise on ten of the leaves. At the end of this time the injured tissue of the leaves became more or less completely covered by a vigorous growth of saprophytic Fungi, the spores of which had been sown with the ascospores.

Five experiments (Nos. *a* 57, *a* 63, *a* 31, *a* 52, *a* 020) were made in which barley leaves were nipped hard at the margin with a pair of forceps, causing a severe bruise about 4 mm. long and 1 mm. wide. Twenty-one leaves were thus treated, and inoculated on the upper surface. Fourteen leaves became infected at the bruised place. On all the fourteen leaves vigorous mycelial patches were produced in five to seven days, and after seven to thirteen days these bore numerous conidiophores which in some cases formed little clustered tufts.

ζ. Injury caused by Narcotics, etc.

Experiments were now made in which leaves were exposed to the action of ether, chloroform, and alcohol respectively, and then inoculated.

a. Ether.

Barley leaves were cut off from seedling plants and exposed to ether vapour in a closed Petri dish. In three experiments nine barley leaves and three wheat leaves were exposed for 20, 10, and 3 minutes; all the leaves proved eventually to have been killed by this treatment.

In experiment No. *a* 55, three barley leaves and two wheat leaves were exposed for 2 minutes to ether vapour, and then inoculated over the greater part of the upper surface. On the twelfth day the two wheat leaves were completely covered, over the inoculated part, with continuous patches of densely powdery *Oidium*. On one barley leaf minute patches of mycelial hyphae were visible, one patch bearing two conidiophores. On

the fifteenth day the wheat leaves bore a continuous dense powdery *Oidium*-patch 2 cm. long; two of the barley leaves bore a few mycelial patches, one or two of which, on each leaf, bore a little cluster of conidiophores. No infection occurred on the third leaf.

In experiment No. *a* 50, four barley and four wheat leaves were exposed, two leaves of each for 2 minutes, and two for 1½ minutes. The leaves were then exposed to the air for 1 hour, after which they were inoculated and placed at the bottom of a closed Petri dish, as usual. On the eighth day it was found that, of the leaves exposed for 2 minutes, the two barley leaves and one of the wheat leaves had been killed; the remaining wheat leaf was killed in places here and there, and in the living parts was virulently infected and bore powdery *Oidium*-patches. Of the leaves exposed for 1½ minutes, the wheat leaves were covered with dense mycelial patches, here and there powdery with ripe conidiophores and conidia; no infection was apparent at this date on the barley. On the thirteenth day the wheat leaves exposed for 1½ minutes bore extended and continuous densely powdery *Oidium*-patches, pinkish in colour, consisting of very vigorous conidiophores bearing conidia in abnormally long chains. One barley leaf was now seen to be infected. This leaf bore, scattered over the inoculated surface, vigorous mycelial patches of interwoven hyphae, and numerous well-grown patches of clustered conidiophores. Altogether several hundreds of conidiophores were produced on this barley leaf, and the appearance was that of almost full infection. The other barley leaf was not infected.

In another experiment (No. *a* 54), in which two barley leaves and two wheat leaves were exposed for 1½ minutes, both the wheat leaves became virulently infected, but no trace of infection appeared on the barley.

In experiment No. *a* 44, three barley leaves and two wheat leaves were exposed for 1 minute. On the ninth day the two wheat leaves were covered continuously for a distance of 4 cm. with a powdery *Oidium*-patch; at this date there were no signs of infection on the barley. On the sixteenth day one barley leaf bore a few minute patches of mycelium, which in two cases bore a few conidiophores. On the twentieth day the one barley leaf bore numerous vigorous little patches of mycelium, most of which bore little groups of clustered conidiophores; another barley leaf bore one comparatively large patch of mycelium bearing a few conidiophores. No infection occurred on the third barley leaf. Conidia taken from the infected barley leaf and sown on an uninjured leaf of barley and of wheat proved able to infect only the wheat.

b. Chloroform-vapour.

Exposure of wheat and barley leaves for 30 seconds proved fatal to all the leaves used. In experiment No. *a* 08, three barley leaves were exposed for 10 seconds. Two of the leaves showed on the ninth day

a few minute patches of mycelium, bearing a few small clusters of conidiophores.

c. Alcohol.

Barley leaves immersed in a 2 % mixture of alcohol and water for 1 hour, 2½ hours, and 4 hours showed no signs of being rendered susceptible.

A 10 % mixture was then used. Immersion in this for 42 hours proved fatal to barley leaves. In one experiment (No. 71) three barley leaves were immersed for 22 hours. On the eleventh day one leaf bore three little clusters of a few conidiophores and a few minute mycelial patches; the two other leaves were killed. In one experiment in which six barley leaves were immersed for 16 hours all the leaves were killed. In another experiment (No. *a* 052), however, in which four barley leaves were immersed for 19 hours, two leaves only were killed. The two remaining leaves were rendered susceptible, and on the eleventh day bore over the upper inoculated surface abundant mycelial patches, with hundreds of conidiophores. These conidia were sown on an uninjured leaf of barley and of wheat, and proved able to infect only the wheat.

In two experiments immersion for 5 hours was given. In the first (No. *a* 84) six barley leaves were used, and three control barley leaves were also inoculated with conidia from the same source. On the ninth day two of the treated leaves bore very numerous mycelial patches over the sown area, but no conidiophores were produced. The latter fact was perhaps due to a sudden spell of very cold weather which lasted during the close of the experiment. No trace of infection occurred on the controls. In the other experiment (No. *a* 06) six leaves were inoculated. On the tenth day five of the leaves bore very numerous vigorous mycelial patches bearing hundreds of clustered conidiophores. These conidia when sown on three uninjured leaves of barley and four uninjured leaves of wheat infected only the wheat leaves.

In six experiments immersion for 4 hours was given, and in every case some at least of the treated leaves were rendered susceptible, the infection induced being in some cases very pronounced.

In one experiment (No. *a* 66) two of the three barley leaves inoculated showed on the sixth day (Feb. 26) clear signs of being infected, by the presence of numerous flecks of mycelium, with hundreds of conidiophores, over the inoculated area. On the ninth day one of the leaves presented the appearance of full infection, the upper surface being covered for a distance of 2 cm. with small, scattered, powdery *Oidium*-patches. On the second leaf vigorous little patches of conidiophores appeared scattered over the surface of the leaf for a length of 4 cm. No infection was visible on the third leaf. On the fifteenth day the two barley leaves still presented the same appearance of full infection; on the third leaf a few mycelial patches

bearing a few conidiophores were visible. In this experiment the leaves used were rather old. The conidia produced on the barley leaves were sown on four leaves of barley and of wheat, and proved able to infect only the wheat.

In experiment No. *a* 77, three barley leaves were again used. On the ninth day two of the leaves appeared fully infected, and bore hundreds of conidiophores in little clusters scattered over the inoculated surface; on the third leaf only a few scattered almost solitary conidiophores appeared. In this experiment again oldish leaves were used. Conidia taken from the two barley leaves and sown on two uninjured leaves of barley and of wheat proved able to infect only the wheat.

In experiment No. *a* 78, three barley leaves were again used, and all became apparently fully infected, bearing on the eighth day numerous little clusters of conidiophores and patches of mycelium over the sown area.

In experiment No. *a* 82, three barley leaves were used, and two of these on the tenth day presented the appearance of being fully infected. In this experiment three control leaves (rather old) of barley were inoculated. On two of these a few isolated single conidiophores appeared.

In experiment No. *a* 09, twelve barley leaves were rubbed lightly in distilled water to remove the adherent film of air; six were then immersed in the alcohol for 4 hours. After being dried, these six leaves were inoculated, together with the other six leaves, and all were placed side by side at the bottom of two Petri dishes. On the ninth day, in one Petri dish, two of the leaves which had been treated with alcohol bore a number of conidiophores, and one control leaf bore four isolated conidiophores. In the second Petri dish, all the treated leaves bore the appearance of being fully infected, the inoculated area of each leaf bearing many hundreds of conidiophores. No trace of infection occurred on the three control leaves. On the fourteenth day one of the treated leaves in the second Petri dish was covered over its surface for a distance of 3 cm. with many hundreds of conidiophores. No trace of any infection appeared on the three control leaves. Conidia from the treated barley leaves were sown on two uninjured leaves of barley and of wheat, and infected only the latter.

In experiment No. *a* 011, three treated leaves and three controls were inoculated. On the eighth day the three treated leaves bore scattered conidiophores over the inoculated surface. By the thirteenth day the three treated leaves bore numerous conidiophores, sometimes in little groups, over the inoculated place; no trace of any infection appeared on the controls.

Immersion of barley leaves for 2 hours in a 10 % mixture of alcohol was made in one experiment (No. *a* 66), in which three leaves were used. On the ninth day a few scattered, isolated conidiophores were visible on all the leaves. On the fifteenth day two of the leaves bore little clustered groups

of conidiophores. On the seventeenth day one leaf bore numerous vigorous patches of clustered conidiophores; the other two leaves bore only a few isolated sub-solitary conidiophores, just as in cases of 'sub-infection[1].'

A 20 % mixture of alcohol was used in a few experiments. It was found that immersion for 4 hours in alcohol of this strength killed barley leaves. In two experiments barley leaves were immersed for 2 hours. In experiment No. *a* 85, the three treated leaves showed on the seventh day evident signs of injury, the injury often being local and causing the death of patches of cells. On one leaf a patch of numerous clustered conidiophores appeared at the inoculated place. In experiment No. *a* 90, three leaves were treated, and inoculated together with three control leaves. On the eighth day all the treated leaves were killed or injured in places towards their tips; one leaf bore a few little clusters of conidiophores, and several patches of mycelium. No infection occurred on the controls.

The effect of vapour of alcohol was tried in two experiments. In the method employed the barley leaves were suspended on an open cradle over a watch-glass containing absolute alcohol, in a hermetically closed Petri dish. Exposure for 10 minutes was found to kill the leaves. In experiment No. *a* 027, two leaves were exposed for 3 minutes (the air being more or less completely saturated with vapour of alcohol at the commencement). On the sixth day one of the leaves presented all the appearance of being fully infected, and bore vigorous patches of mycelium with thousands of conidiophores at a place towards the tip of the leaf, surrounding a patch of cells killed by the action of the alcohol. Conidia from this barley leaf sown on an uninjured leaf of barley and of wheat proved able to infect only the wheat.

Drops of various poisons (caustic potash, 10 %; copper sulphate (1 part in 100); sulphuric acid, 10 %) were placed on barley leaves, and after the leaves had been washed in water conidia and ascospores were sown on the patches of cells killed by the action of the poisons, and on the cells immediately surrounding, but no infection resulted.

η. Injury caused by Heat.

It was found that barley leaves, heated in water up to 67° C., and left in the water until it had cooled to 50° C., were killed. Also, if barley leaves are immersed in water heated to 48° C., and the temperature is then raised to 65° C., and the leaves left in the water until it cools to 60° C., the leaves are at once killed. Barley leaves can, however, stand immersion in water at a temperature of 50° C., and this treatment induces a marked susceptibility of the leaf. In five experiments barley leaves were placed in cold water in a large glass beaker, and the water heated slowly to 50° C. The leaves were then taken out at once, dried, and inocu-

[1] See 'Beihefte z. Botan. Centralbl.,' xiv. 271.

lated. In experiment No. *a* 86, the three barley leaves thus treated all became infected, at the marked places, by the seventh day. On one leaf numerous mycelial patches and hundreds of conidiophores, often in little clusters, were produced; on the two other leaves a few scattered, subsolitary conidiophores appeared among the sown conidia. In experiment No. *a* 07, four barley leaves were gently rubbed in water to remove the adherent film of air. One leaf was then put into a vessel of cold water, and the water heated slowly to 50° C.; the leaf was then taken out, dried, and inoculated. The three other leaves were at the same time removed from the cold water, dried, and inoculated. On the ninth day the heated leaf bore, over the inoculated surface, thousands of scattered conidiophores; none of the controls was infected. In experiment No. *a* 010, six barley leaves were rubbed in water to remove the air-film, and three were then slowly heated in water to 50° C.; all the six leaves were then dried and inoculated. On the ninth day the three heated leaves all bore very numerous conidiophores, and presented the appearance of being fully infected. On one control leaf three isolated conidiophores appeared. In experiment No. *a* 023, three barley leaves were put straight into cold water, and heated slowly to 50° C. The leaves were then taken out, dried, and inoculated. The heating took 1 hour 25 minutes. During this time three control barley leaves stood immersed in a vessel of cold water; these also were dried and inoculated at the same time. On the eighth day numerous scattered conidiophores, and patches of mycelium, were visible on the three heated leaves; no signs of infection were visible on the controls at this date. On the thirteenth day the controls had turned yellowish, and were completely translucent; on two leaves a conidium was visible, which had germinated and produced a few short spreading hyphae, and in one case two conidiophores had been produced from the hyphae.

In experiment No. *a* 0382, six barley leaves were cut off from strong flowering plants, 3 feet high, and 5 months old, growing in the open. Three leaves were immersed in cold water, and the water heated slowly to 50° C. All six leaves were cut into pieces about 8 cm. long, inoculated, and placed in a Petri dish. On the seventh day two of the treated leaves bore many scattered little patches of mycelium, several of which bore a few clustered conidiophores. No infection occurred on the controls.

In one experiment (No. *a* 036) ascospores were used. Six barley leaves and two wheat leaves were used; three of the barley leaves were put straight into cold water, and the water heated gradually to 50° C. The leaves were then taken out and dried, and together with the three control barley leaves and the wheat leaves, were exposed for 48 hours to ripe, bursting perithecia, ejecting ascospores. On the eighth day the two wheat leaves bore numerous powdery *Oidium*-patches scattered over the inoculated surface; no signs of any infection appeared as yet on the barley leaves.

On the tenth day a few tiny flecks of mycelium bearing a few conidiophores were visible on the three barley leaves which had been heated; no trace of any infection occurred on the three control barley leaves. On the thirteenth day the three heated barley leaves bore numerous little scattered clusters of conidiophores. Conidia from these leaves were sown in two cases on uninjured leaves of barley and of wheat, and proved able to infect only the wheat.

In experiment No. *a* 012, six barley leaves were rubbed in water to remove the film of air adherent to their surface, and then immersed for 1 minute in water at the temperature of 50° C. The leaves were then plunged into cold water for a minute, then dried, and inoculated with conidia. No trace of infection resulted on any of the leaves.

In another experiment (No. *a* 022) twelve barley leaves were cut off from seedlings of the same age, growing in one pot; six leaves were immersed for 1 minute in water at the temperature of 50° C., and then dried rapidly by blotting paper; all twelve leaves were then inoculated. On the eighth day three of the leaves which had been heated bore numerous little clustered groups of conidiophores; on one control leaf three isolated conidiophores were visible among the sown conidia. On the fourteenth day one of the leaves which had been heated bore numbers of scattered, whitish, quite powdery little clusters of conidiophores over the inoculated place. The conidia were sown, in fair quantity, on an uninjured leaf of barley and of wheat, and proved able to infect only the wheat. In one experiment (No. *a* 018) twenty seedlings of barley, growing in a pot, were dipped for 1 minute into water at a temperature of 50° C. in such a way as to immerse all the leaves. All the leaves were then inoculated abundantly with conidia. A control pot of barley seedlings after inoculation was placed by the side. On the fifth day several of the leaves of the treated plants bore numerous flecks of mycelium with a few young conidiophores. On the ninth day six leaves bore minute flecks of mycelium, bearing small patches of clustered conidiophores, in a few cases forming a small powdery *Oidium*-patch; many barren mycelial flecks were present on several other leaves. No trace of any infection appeared on the control plants. On one leaf the little *Oidium*-patch kept powdery until the sixteenth day, producing successive crops of conidia. (These conidia were sown on two uninjured leaves of barley and on one uninjured leaf of wheat, and proved able to infect only the wheat.) All traces of the primary infection, however, gradually disappeared by the end of the third week of the experiment, and no signs of any secondary infection from the conidia produced were observed.

In experiment No. *a* 05, three barley leaves were immersed for 1 minute in water at 49·5° C. On the fifth day all the leaves presented the appearance of being fully infected, the Fungus having produced very

numerous flecks of mycelium with young conidiophores. On the seventh day all the leaves bore the appearance of being fully infected; in one case the conidia, produced in little powdery masses on the clustered conidiophores, were so numerous that they could be removed in little heaps with the blade of a scalpel. On the twelfth day this one leaf still bore quite powdery little clusters of conidiophores. Conidia were twice taken from the barley leaves and sown on two uninjured leaves of barley and of wheat; they proved able to infect only the wheat.

In experiment No. *a* 049, three barley leaves, after being immersed in a 10 % mixture of alcohol for 4 hours, were put into cold water and heated slowly to 50°C. All the leaves were killed.

Oat leaves were used in three experiments. In the first (No. *a* 032) six oat leaves were slowly heated in water up to 50° C. On the tenth day two of the leaves bore a few small radiating mycelial patches, one of which, on each leaf, bore two conidiophores; small barren mycelial patches occurred on two of the other leaves. This experiment was repeated (No. *a* 0367). On the seventh day four of the leaves bore numerous, little, spreading mycelial flecks, all barren, except two, on one leaf, which bore five and three conidiophores. In experiment No. *a* 062, six oat leaves were heated slowly to 55° C.; the leaves were killed by this treatment.

Leaves of *Agropyron repens* were used in one experiment (No. *a* 089). Three oldish leaves were slowly heated in water to 50°C. They were then cut transversely into half, inoculated, and placed at the bottom of a Petri dish. On the seventh day most of the leaves were flaccid and dying or dead. One leaf, however, was not killed, and was still rigid, and bore numerous mycelial patches and clusters of conidiophores, presenting, in fact, the appearance of being nearly fully infected[1]. On two other nearly dead leaves patches of barren mycelium were visible.

In experiment No. *a* 097, four pieces of the underground rhizome of *Elymus arenarius* were heated in water to 51° C. A small piece of tissue was then cut away from the side of each piece, and inoculation made on the cut surface and also over the uninjured surface of the rhizome. On the third day minute patches of mycelial hyphae were visible on two of the pieces of rhizome, and on the fifth day a few conidiophores. On the seventh day, many hundreds of conidiophores were visible on one piece of the rhizome, close by the sides of the cut, but not on the surface of it.

[1] Conidia from wheat are not able to infect normal leaves of *A. repens* (see 'Beihefte z. Botan. Centralbl.,' xiv, 308, Tab. 10).

EXPERIMENTS WITH CONIDIA OBTAINED BY SOWING CONIDIA FROM WHEAT ON BARLEY LEAVES RENDERED SUSCEPTIBLE BY THE ACTION OF ALCOHOL, ETHER, AND HEAT RESPECTIVELY.

(1) ALCOHOL.

(*a*) *Conidia produced on barley leaves previously immersed for* 4 *hours in* 10% *alcohol.*

EXPERIMENT No. 77*. Conidia produced on the fourteenth day were sown, at a marked place, on a leaf of barley and of wheat[1]. By the seventh day a little powdery *Oidium*-patch was produced on the wheat leaf, at the marked place; no trace of any infection appeared on the barley.

EXPERIMENT No. *a* 92. Conidia produced on the fourteenth day were sown, at a marked place, on two leaves of both barley and wheat. On the seventh day the two wheat leaves were fully infected, at the marked place, with small vigorous *Oidium*-patches; no trace of any infection appeared on the barley.

EXPERIMENT No. *a* 99. Conidia produced on the sixteenth day were sown, at a marked place, on a leaf of barley and of wheat, and a control leaf of each placed by the side. On the fifth day both the barley and the wheat leaf bore, at the marked place, a small patch of mycelium bearing a few young conidiophores, the patch on the wheat being a little further developed than that on the barley. By the eighth day the wheat leaf had produced two small, contiguous, very powdery *Oidium*-patches; the mycelial patch on the barley leaf had not developed further, except that it now bore five conidiophores which were beginning to wither. On the eleventh day a few conidiophores were still visible on the now hyaline barley leaf, but no further growth of mycelium had taken place. The control leaves of barley and wheat remained quite free. Conidia were taken from the wheat leaf and sown in considerable quantity on a barley leaf, but no trace of any infection resulted.

EXPERIMENTS Nos. *a* 100 and *a* 015. Conidia produced on the tenth day were sown in each experiment, at a marked place, on a leaf of barley and of wheat. On the tenth day a large powdery *Oidium*-patch was produced, at the marked place, on the wheat leaf; no trace of any infection occurred on the barley.

EXPERIMENT No. *a* 019. Conidia produced on the twelfth day were sown, at a marked place, on a leaf of barley and of wheat. By the ninth day a large vigorous and powdery patch of *Oidium* was produced at the

[1] In all these experiments the leaves used were cut off from seedlings of barley and wheat, and placed at the bottom of a Petri dish on damp blotting-paper.

marked place, on the wheat leaf. The powdery mass of accumulated conidia was removed from the wheat leaf. On the twelfth day the Fungus bore, at the same spot, on the wheat leaf another powdery crop of conidia. No trace of any infection occurred on the barley.

Experiment No. *a* 033. Conidia produced on the twelfth day were sown, at a marked place, on a leaf of barley and of wheat. As in the above experiment, successive crops of powdery masses of conidia were produced, at the marked place, on the wheat on the ninth and thirteenth day. No trace of any infection appeared on the barley.

Experiment No. *a* 043. Conidia produced on the fourteenth day were sown, at a marked place, on a leaf of barley and of wheat. Each leaf was made slightly damp with distilled water over the area of the marked place. Barley leaves bearing some hundreds of conidiophores in little scattered groups were then laid for a second over the marked place, so that hundreds of conidia were deposited on each leaf. By the eleventh day numerous powdery *Oidium*-patches occurred on the wheat leaf at the place of inoculation; no trace of any infection appeared on the barley leaf.

(*b*) *Conidia produced on barley leaves previously immersed for* 5 *hours in* 10% *alcohol.*

Experiment No. *a* 047. Conidia produced on the ninth day were sown, at a marked place, on a barley and a wheat leaf. By the sixth day two little powdery *Oidium*-patches were produced on the wheat leaf, at the marked place; no trace of infection resulted on the barley.

Experiment No. *a* 054. Conidia produced on the tenth day were sown on two barley leaves and three wheat leaves. The barley and wheat leaves were damped on their upper surface, and then this surface was laid for a second on a barley leaf bearing a large number of scattered tufts of conidiophores. By the seventh day all the wheat leaves were fully infected; one leaf bore over twenty small, vigorous, powdery patches of *Oidium*, the second leaf bore nine patches, and the third leaf three patches. By the tenth day two of the wheat leaves bore almost continuous densely powdery *Oidium*-patches extending for a distance of 2·5 cm. No trace of any infection appeared on the two barley leaves.

(*c*) *Conidia produced on barley leaves previously immersed for* 19 *hours in* 10% *alcohol.*

Experiment No. *a* 088. Conidia produced on the eleventh day were sown, at a marked place, on a leaf of barley and of wheat. By the seventh day several little powdery *Oidium*-patches were visible, at the marked place, on the wheat; no trace of any infection appeared on the barley.

(*d*) *Conidia produced on barley leaves previously exposed for* 3 *minutes to alcohol vapour.*

EXPERIMENT No. *a* 061. Conidia produced on the tenth day were sown at a marked place on a leaf of barley and of wheat. On the seventh day a little powdery *Oidium*-patch was produced on the wheat; no trace of any infection appeared on the barley.

(2) ETHER.

Conidia produced on barley leaves previously exposed for 1 *minute to ether vapour.*

EXPERIMENT No. *a* 91. Conidia produced on the twenty-first day were sown, at a marked place, on a leaf of barley and of wheat. On the ninth day the wheat leaf bore, at the marked place, two small quite powdery *Oidium*-patches. No trace of any infection appeared on the barley leaf.

(3) HEAT.

(*a*) *Conidia produced on barley leaves previously immersed for* 1 *minute in water at the temperature of* 49·5° C.

EXPERIMENT No. *a* 029. Conidia produced on the twelfth day were sown at a marked place on a leaf of barley and of wheat. The conidia used for inoculation had been produced in sufficient quantity to be scraped off the barley leaves with the point of a scalpel, and deposited in a little heap on each leaf. On the seventh day a large, vigorous, very powdery *Oidium*-patch was produced, on the marked place, on the wheat. The powdery mass of accumulated conidia was removed with a scalpel. On the tenth day another crop of densely powdery masses of conidia was produced, borne by an *Oidium*-patch 7 mm. long. The conidia were again removed. On the fourteenth day a small powdery *Oidium*-patch was visible, at the marked place, on the wheat leaf, which was now nearly dead. No trace of any infection appeared on the barley leaf.

(*b*) *Conidia produced on barley leaves previously immersed in cold water and heated slowly to* 50° C.

EXPERIMENT No. *a* 034. Conidia produced on the twelfth day were sown, at a marked place, on a leaf of barley and of wheat. By the tenth day little powdery *Oidium*-patches were produced, at the marked place, on the wheat; no trace of infection occurred on the barley.

EXPERIMENT No. *a* 036**. Conidia produced on the fifteenth day were sown, at a marked place, on a wheat leaf. On the twelfth day a little powdery *Oidium*-patch was produced at the marked place.

(*c*) *Conidia produced on barley leaves* (*attached to plants growing in a pot*) *previously immersed for* 1 *minute in water at a temperature of* 50° C.

Experiment No. *a* 040. Conidia produced on the ninth day were sown in considerable quantity, at a marked place, on two barley leaves and on one wheat leaf. By the eighth day a vigorous powdery patch of *Oidium* was produced on the wheat leaf; no trace of any infection occurred on the two barley leaves.

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The Proteases of Plants (II).

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IN the interval that has elapsed since the publication (April, 1904) of my last paper on this subject (1) in this periodical, various facts have come to light that contribute not a little to the interest and to the intelligent apprehension of it: although progress in so large a field has not been rapid, for the number of investigators exploring it is still relatively small.

Papaïn.

This material has been more fully investigated than any other in the vegetable kingdom, and yet it cannot be said to be by any means fully understood. The occasion of my reverting to the subject was the reading of a paper on it by Emmerling (2), who found that papaïn digested fibrin most actively in a slightly alkaline liquid; and that, although the amount of amido-acids, &c., produced was relatively small, its action was 'specifically tryptic.'

As his results do not altogether agree with those published by me (3), I have repeated some of my former experiments and have made some under the conditions adopted by Emmerling. His method was somewhat as follows:—1,000 grms. of dry fibrin were covered with feebly alkaline water, but the degree of alkalinity is not stated: 20 grms. of Merck's papaïn were added, as also some toluol. After fourteen days in the incubator at 37°C., 10 grms. of papaïn were added; and after fourteen days more, further 10 grms. of papaïn. At the close of this prolonged digestion, the products were found to be much albumose and peptone, and small quantities of arginin, tyrosin, leucin, asparaginic acid, glycocoll, glutaminic acid, alanin, and phenyl-alanin. No account is given of any experiments made with acid or neutral liquid.

Apart from the alkaline medium, the conditions of Emmerling's experiments differed from mine in that he used Merck's preparation of papaïn, whereas I used Christy's preparation; and further, in that he used toluol, and I chiefly HCN, as the antiseptic. Moreover, the relative weight of the fibrin to that of the papaïn was very much larger in his than in mine.

[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

I felt sure that the points of divergence between his results and mine—such as the slowness of the digestive action on fibrin that he observed, and the relatively small production of amido-acids and hexon-bases—could be accounted for by some or all of these differences of method; and experiment has largely realized this anticipation. The method I adopted was to institute comparative experiments with papaïns derived from several sources, with acid and alkaline liquids, and with toluol and HCN as antiseptics. The quantity of fibrin used was both relatively and absolutely small. The following description of an experiment will make the method clear.

Fibrin-Digestion. Three samples of papaïn were used, obtained respectively from Messrs. Christy, Finkler, and Merck.

4 grms. of each sample of papaïn were mixed with 160 c.c. of distilled water, and after standing for some time were strained through muslin: the liquids obtained were turbid, and gave no tryptophane-reaction; the Christy and Merck liquids were slightly acid, the Finkler liquid neutral.

The 160 c.c. of each papaïn-liquid were put into four bottles, 40 c.c. in each, and were further treated as follows:—

No. 1, added toluol to 1%, and citric acid to 0·5 %
„ 2, „ „ „ Na_2CO_3 „ 0·75 %
„ 3, „ HCN to 0·2%, „ citric acid „ 0·5 %
„ 4, „ „ „ Na_2CO_3 „ 0·75 %

The contents of Nos. 2 and 4 were distinctly alkaline, and remained so throughout the experiment. To each bottle $\frac{1}{2}$ grm. moist fibrin was added. There were thus twelve bottles in all put to digest in the incubator at about 40° C.

After 18 hours' digestion, the effect upon the fibrin was as stated below:—

	(1) *Toluol acid.*	(2) *Tol. alk.*	(3) *HCN acid.*	(4) *HCN alk.*
Christy	not gone	not gone	gone	gone
Finkler	not gone	not gone	not gone	nearly gone
Merck	not gone	not gone	partly gone	gone

Four hours later the fibrin had completely disappeared in the Finkler bottle No. 4.

After 24 hours' digestion the state of the fibrin was:—

	No. 1.	*No.* 2.	*No.* 3.
Christy	not gone	not gone	—
Finkler	not gone	not gone	nearly gone
Merck	not gone	not gone	nearly gone

after 44 hours' digestion the results were:—

	No. 1.	*No.* 2.	*No.* 3.
Christy	nearly gone	nearly gone	—
Finkler	going	unaltered	gone
Merck	going	unaltered	gone

after 68 hours' digestion :—

	No. 1.	*No.* 2.
Christy	gone	gone
Finkler	nearly gone	unaltered
Merck	gone	unaltered

after 92 hours' digestion the final results were :—

	No. 1.	*No.* 2.
Finkler	gone	unaltered
Merck	—	swollen, but not digested.

From these observations it may be concluded that :—

(1) the action on fibrin of these different samples of papaïn was not uniform, Christy's proving to be the most active ;

(2) the action was much slower in the presence of toluol than in the presence of HCN ;

(3) in the presence of HCN, the action was on the whole more rapid in the alkaline than in the acid liquid ; whilst in the presence of toluol it was more rapid in the acid than in the alkaline.

These conclusions afford the explanation why Emmerling found the process of digestion to be so slow in his experiments. It was slow (1) because he used Merck's papaïn and in relatively small quantity ; (2) because he used toluol as the antiseptic ; and (3) because the reaction of the digesting liquid was alkaline. It is clear, from the observations given above, that this was a singularly unfortunate combination of conditions, inasmuch as Merck's papaïn, though active enough under other circumstances, proved to be altogether inert in an alkaline liquid containing toluol, although in my experiment the amount of fibrin to be digested was relatively small.

The other point noted by Emmerling—the smallness of the amount of amido-acids, &c., formed in his digestion-experiments—remains to be considered. With this in mind, I made some observations by means of the tryptophane-method from time to time during the progress of the fibrin-digestion, with the following results :—

Tryptophane-reactions.

After 20 hours' digestion :—

	No. 1.	*No.* 2.	*No.* 3.	*No.* 4.
Christy	—	—	marked	distinct
Finkler	—	—	—	distinct
Merck	—	—	—	faint

after 44 hours' digestion :—

Christy	—	—	marked	distinct
Finkler	—	—	distinct	strong
Merck	—	—	distinct	distinct

after 68 hours' :—

	No. 1.	*No.* 2.	*No.* 3.	*No.* 4.
Christy	faint	distinct	—	—
Finkler	distinct	marked	—	—
Merck	none	distinct	—	—

The tryptophane-test was applied when the fibrin had disappeared in each bottle, except in the case of those No. 2 bottles (Finkler, Merck) where it did not disappear at all.

Seeing that the liquids gave no tryptophane-reaction to begin with, it follows (1) that all the samples of papaïn tested proved capable of effecting complete proteolysis, or at any rate peptolysis, in various degree ; (2) that on the whole their action was more vigorous in the presence of HCN than in the presence of toluol ; and (3) that the action was on the whole stronger in the alkaline than in the acid liquids, though the difference was not great. The explanation of Emmerling's result is that probably his sample of Merck's papaïn, like mine, did not actively peptolyse in alkaline liquid containing toluol ; and, more certainly, that the quantity of papaïn used by him was too small in proportion to the fibrin.

But the evidence of the tryptophane-reactions given above is not conclusive, and is even to some extent paradoxical. For instance, the Finkler bottle No. 2 (toluol-alk.) gave almost as good a reaction as the Finkler No. 4 bottle (HCN-alk.), though in the latter case the fibrin had been digested and in the former it had not. It is obvious, therefore, that these tryptophane-reactions do not necessarily indicate the complete proteolysis of the fibrin supplied for digestion in the experiment; on the contrary, they indicate, in some cases at any rate, the proteolysis of some proteid other than fibrin, and one that is more readily proteolysable. The probable explanation is that all specimens of papaïn contain, in addition to protease, more or less proteid matter. In order to obtain some idea of the extent of this self-digestion, I made a series of experiments without any added proteid ; and, to connect them with my earlier observations (3), I included a series of bottles with sodium fluoride (NaF) as an antiseptic. The experiments may be summarized as follows:—

Autolysis-Experiments.

The method adopted was to put 0·5 grm. of papaïn in each bottle with 40 c.c. distilled water (=1·25 %), containing the antiseptic (toluol 1 %, or HCN 0·2 %, or NaF 1 %): then the contents of the bottles were made either acid (citric acid 0·5 %), or alkaline (Na_2CO_3 0·5 %), or were left at their natural reaction, which was slightly acid in the case of Christy's and Merck's samples, neutral in Finkler's. The mixtures thus prepared of Christy's and Finkler's gave no tryptophane-reaction: but the fresh sample of Merck's used in these experiments (but not in the one previously described)

gave a distinct reaction before digestion. There were thus nine bottles in each experiment.

The application of the tryptophane-test in comparative experiments requires some care in order to ensure that the maximal reaction is really obtained. The reaction of the liquid must be acid; and then the chlorine-water should be added gradually until a vol. equal to that of the liquid to be tested (I usually took 5 c.c.) has been used: after standing some minutes a second vol. of chlorine-water should be added, and if necessary a third vol., until further addition does not intensify the reaction, but only weakens it. I found that the maximum was generally obtained with about 2 vols. of chlorine-water: but sometimes with less, sometimes with more.

The duration of the digestion was 48 hours: the Na_2CO_3 bottles remained alkaline throughout the experiment. The terms used in describing the intensity of the tryptophane-reaction are, as in previous papers, the following: faint, distinct, marked, strong, very strong.

It must also be explained that the sample of Merck's papaïn used in this and subsequent experiments was not the same as that used in the previous experiments (see p. 150), but a fresh and apparently much more active sample.

The resulting tryptophane-reactions were as follows:—

A. Christy's Papaïn.

	Acid.	*Alkaline.*	*Natural.*
Toluol 24 hours	none	faint	none
„ 48 „	none	faint	none
HCN 24 „	distinct	none	distinct
„ 48 „	marked	none	distinct
NaF 24 „	faint	none	none
„ 48 „	faint	none	faint

B. Finkler's Papaïn.

	Acid.	*Alkaline.*	*Natural.*
Toluol 24 hours	faint	strong	distinct
„ 48 „	distinct	strong	distinct
HCN 24 „	marked	faint	distinct
„ 48 „	marked	faint	distinct
NaF 24 „	faint	distinct	faint
„ 48 „	faint	marked	distinct

C. Merck's Papaïn.

	Acid.	*Alkaline.*	*Natural.*
Toluol 24 hours	distinct	distinct	faint
„ 48 „	marked	distinct	distinct
HCN 24 „	marked	faint	distinct
„ 48 „	marked	faint	distinct
NaF 24 „	marked	distinct	distinct
„ 48 „	marked	distinct	distinct

The autolysis-results bring out once more the specific differences in activity between the three samples of papaïn. The most striking tryptophane-reaction was given by Finkler's papaïn, on account, probably, of its containing the largest amount of readily proteolysable proteid. With respect to the reaction of the liquid, Christy's and Merck's samples gave better results in acid than in alkaline liquids, whilst Finkler's was more active in alkaline than in acid. As to the antiseptics employed, Christy's papaïn was active in the presence of HCN, but its action was almost entirely inhibited by both toluol and NaF: Finkler's and Merck's showed about the same activity with all three antiseptics, Finkler's being especially active in the presence of toluol (alkaline). It must be remembered that the Merck solution gave a tryptophane-reaction to begin with.

With the object of obtaining further information as to the relative peptolytic activity of the three samples under various experimental conditions, an experiment was prepared in which such a quantity of readily peptolysable proteid was added—in the shape of Witte-peptone—as to render negligible, on the whole, whatever amount of proteid may have been originally present in the papaïn.

I desired, further, that the experiment should be such as to permit of some comparison between the fibrin-digesting and the peptolysing activity of the papaïn in each case. In previous observations (3) I had found that these two processes do not necessarily run parallel, and that the one may take place without the other. I therefore now added fibrin, as well as Witte-peptone, to the bottles in the following experiments.

Fibrin-digestion and Peptolysis.

The general method adopted was to extract 8 grms. of papaïn for 2–3 hours with 400 c.c. distilled water, and filter. To the filtered liquid 6 grms. of Witte-peptone were added, causing a considerable precipitate in every case. To 120 c.c. of this liquid toluol was added to 1 %: to 120 c.c., HCN to 0·2 %; and to 120 c.c., NaF to 1 %. 40 c.c. of each of these liquids were put into each of three bottles, to which were added respectively either citric acid to 0·5 %, or Na_2CO_3 to 0·5 %, or nothing: in the last case the reaction was neutral or slightly acid. Finally 0·3 grm. fibrin was added to each bottle. The effect of digestion on the fibrin, and the tryptophane-reactions were:—

A. Christy's Papaïn.

	Acid.	*Alkaline.*	*Natural.*
Toluol 24 hours fibrin	unaltered	gone	unaltered
„ „ „ tryptophane	distinct	distinct	faint
„ 48 „ fibrin	not gone	gone	gone
„ „ „ tryptophane	distinct	marked	faint

				Acid.	*Alkaline.*	*Natural.*
HCN	24	hours	fibrin	gone	gone	gone
„	„	„	tryptophane	very strong	marked	distinct
„	48	„	fibrin	gone	gone	gone
„	„	„	tryptophane	very strong	marked	strong
NaF	24	„	fibrin	gone	gone	nearly gone
„	„	„	tryptophane	faint	distinct	faint
„	48	„	fibrin	gone	gone	gone
„	„	„	tryptophane	faint	marked	distinct.

B. Finkler's Papaïn.

				Acid.	*Alkaline.*	*Natural.*
Toluol	24	hours	fibrin	unaltered	nearly gone	unaltered
„	„	„	tryptophane	none	none	distinct
„	48	„	fibrin	unaltered	nearly gone	unaltered
„	„	„	tryptophane	faint	distinct	faint
HCN	24	„	fibrin	gone	gone	gone
„	„	„	tryptophane	distinct	faint	faint
„	48	„	fibrin	gone	gone	gone
„	„	„	tryptophane	distinct	faint	faint
NaF	24	„	fibrin	unaltered	partly gone	unaltered
„	„	„	tryptophane	none	none	none
„	48	„	fibrin	partly gone	gone	unaltered
„	„	„	tryptophane	faint	faint	faint

C. Merck's Papaïn.

				Acid.	*Alkaline.*	*Natural.*
Toluol	24	hours	fibrin	gone	gone	gone
„	„	„	tryptophane	very strong	distinct	marked
„	48	„	tryptophane	very strong	marked	marked
HCN	24	„	fibrin	gone	gone	gone
„	„	„	tryptophane	strong	distinct	distinct
„	48	„	tryptophane	strong	marked	strong
NaF	24	„	fibrin	gone	gone	gone
„	„	„	tryptophane	very strong	distinct	marked
„	48	„	tryptophane	very strong	strong	marked

The maximum reactions, in this case, were not obtained until 3–4 vols. of chlorine-water had been added.

This sample of Merck's papaïn proved itself to be so exceptionally active that there were but slight differences in the results. With the object of obtaining clearer differentiation, I repeated the experiment, using an extract of half-strength (4 grms. papaïn to 400 c.c. distilled water).

D. Merck's Papaïn (half-strength).

			Acid.	*Alkaline.*	*Natural.*
Toluol	24 hours	fibrin	nearly gone	gone	nearly gone
,,	,, ,,	tryptophane	strong	strong	strong
HCN	24 ,,	fibrin	gone	gone	gone
,,	,, ,,	tryptophane	marked	distinct	distinct
NaF	24 ,,	fibrin	gone	gone	gone
,,	,, ,,	tryptophane	very strong	strong	strong
Toluol	48 ,,	fibrin	gone	gone	gone
,,	,, ,,	tryptophane	strong	very strong	very strong
HCN	,, ,,	,,	strong	distinct	marked
NaF	,, ,,	,,	very strong	strong	strong

The results were still insufficiently differentiated, although some differentiation was indicated in the HCN bottles. Following this up, I made a further experiment with still weaker extract (1 grm. to 200 c.c. water) in the presence of HCN, with striking results; the other details were as before.

E. Merck's Papaïn (quarter-strength).

			Acid.	*Alkaline.*	*Natural.*
HCN	24 hours	fibrin	gone	gone	gone
,,	,, ,,	tryptophane	marked	none	faint
,,	48 ,,	,,	marked	faint	distinct

It will be observed that the results of the experiments *C*, *D*, *E*, with Merck's papaïn, differ in degree only: in all the HCN bottles the alkaline one was that which gave the least marked, and the acid that gave the most marked tryptophane-reaction.

No detailed analysis of the foregoing results is necessary to show that the various samples of papaïn experimented upon differed widely in their general proteolytic activity, and in their relation as well to acid and alkali as to the various antiseptics employed. Moreover, they account for the conflicting and sometimes contradictory observations not only of Emmerling and myself, but also of earlier observers such as Mendel (4), Martin (5), and Wurtz (6). One thing, at any rate, is made clear, that the last word as to the properties of papaïn will not have been pronounced until a series of careful observations shall have been made with perfectly fresh material, so as to avoid all those modifications that must necessarily accompany the preparation of the varieties of dried papaïn which have hitherto been used in experiments. Here is a subject for research that might well engage the attention of one of the botanical laboratories in the tropics.

This somewhat negative conclusion is not, however, the only or the most important one to be drawn. The results show that, as I have already

pointed out, fibrin-digestion and the peptolysis of Witte-peptone are not always similarly affected by the various experimental conditions provided. For instance, Christy's papaïn readily digested fibrin in the presence of NaF, whilst there was at the same time little or no peptolysis; and the same was the case with Finkler's papaïn and with weak Merck's papaïn (*E*), in the presence of HCN. The converse does not come out quite so clearly: but there are indications, as for instance in the toluol-acid experiment with Christy's papaïn (p. 154), of some degree of peptolysis with little or no digestion of fibrin. These facts are susceptible of two interpretations:— (1) that a single protease is present, capable of both fibrin-digestion and peptolysis, and that one or other of these activities may be more or less inhibited by the antiseptics: or (2) that two proteases are present in papaïn, which may respond differently and independently to the action of antiseptics. Of the two alternatives, the former is the one that seems to offer the greater difficulties. It is not easy to imagine how one part of the work of the protease could be arrested without the other: it is more natural to conclude that if the protease were affected at all, the whole of its functional activity would be interfered with. If, then, the second alternative be accepted, the interesting conclusion is arrived at that papaïn contains a fibrin-digesting, but not peptolytic, protease of the nature of a pepsin; as well as a peptolytic, but not fibrin-digesting, protease of the nature of an erepsin. If this be so, it will be the first clear demonstration of the existence of a pepsin in the Vegetable Kingdom.

I do not claim that my experiments suffice to establish this conclusion beyond the possibility of doubt. But they at least indicate a method by which a physiological analysis of possible mixtures of enzymes may be effected. It is, I believe, by the application of this method to the fresh juices of the Papaw that the question will be settled for that plant. In the meantime I am applying it to the investigation of other plants, and have already obtained some confirmatory results in the case of the Hyacinth-bulb, of the Pine-apple, and of Yeast, of which I hope to give an account in a subsequent paper. An investigation of animal trypsin along these lines would, I believe, yield results of considerable interest.

Digestion by Leaves.

In a previous paper (7) I have given an account of some experiments upon the proteolytic action of the foliage-leaves of various plants, viz.: *Spinacia oleracea*, *Dahlia*, *Mirabilis Jalapa*, *Tropaeolum majus*, *Prunus Laurocerasus*, *Ricinus communis*, *Helianthus tuberosus*, *Pelargonium zonale*, *Brassica oleracea*, *Holcus mollis*, *Phalaris canariensis*, *Apium graveolens*, *Scolopendrium vulgare*, *Lactuca sativa*. The conclusion to be drawn from those experiments was that the leaves could peptolyse but not peptonise; in other words, that they contain an erepsin but no fibrin-digesting protease.

Since then I have made further investigations in this direction, and I avail myself of this opportunity to place them on record. I have confirmed my previous results in many of the cases mentioned above, and have extended them to the Lime (*Tilia vulgaris*), the Rhubarb (*Rheum officinale* and *undulatum*), and *Phytolacca decandra*. The observations on *Rheum* and *Phytolacca* present features of sufficient interest to justify special mention.

Rheum officinale and undulatum.

These leaves were selected with the object of testing the digestive activity of tissues known to be strongly acid.

In preparing the leaf for the experiment, the petiole and the lamina were kept separate: on grinding in the mincing-machine, a quantity of clear acid liquid was obtained from the petioles; a watery extract was made of the lamina, and strained through muslin: the liquids were strongly acid. The earlier experiments gave a purely negative result: e. g.—

40 c.c. of petiole-liquid were put into each of two bottles: to the one 0·2 grm. of fibrin was added, to the other 0·5 grm. of Witte-peptone; the antiseptic was toluol 1 %; two similar bottles of lamina-extract were prepared. After digestion for about 70 hours the fibrin was unaffected, and no tryptophane-reaction could be detected in any bottle.

It then occurred to me that possibly digestive action had been inhibited by the acidity of the liquids. I therefore made an experiment in which excess of $CaCO_3$ had been added to the liquids, but the results were still negative.

I repeated the experiment with the modification that the tissue was extracted with water for several hours after mincing: the effect was that the bottles containing Witte-peptone gave more or less distinct tryptophane-reaction. The inference to be drawn seemed to be that the protease is not readily to be extracted from the tissues of the Rhubarb. Acting on this, I made experiments in which the minced tissue itself was used, with complete success, e. g.—

250 grms. of lamina were minced, a little distilled water was added, and the mixture left to stand for 20 hours; the liquid was then strained through muslin; about 200 c.c. were obtained, giving no tryptophane-reaction. Four bottles, of 100 c.c. each, were then prepared as follows:—

No. 1. 100 c.c. strained liquid + 1 grm. Witte-peptone;
„ 2. „ „ „ „ + 5 grms. $CaCO_3$;
„ 3. 10 grms. lamina, 100 c.c. distilled water + 1 grm. Witte-peptone;
„ 4. „ „ „ „ „ + 5 grms. $CaCO_3$.

Toluol was added to 1 %.

After 22 hours in the incubator, the tryptophane-reactions were:—

No. 1, no reaction (liquid acid); No. 2, no reaction (liquid neutral);
„ 3, strong „ „ ; „ 4, marked „ „ .

Hence it appears that peptolysis is effected readily by the tissue itself, whether the reaction be acid or neutral, whilst extracts may be inert. It should be added that I failed to detect any digestion of fibrin, whether extracts or tissue were used; so that this leaf, like so many others, seems to contain only erepsin.

Phytolacca decandra.

The interest attaching to the observations on the leaves of this plant is that they afford the first instance of fibrin-digestion by ordinary foliage-leaves, as distinguished from the leaves of carnivorous plants (*Nepenthes*, &c.), on the one hand, and the leaves of laticiferous plants (e. g. the Fig) on the other

70 grms. of fresh leaves were minced with the machine and were extracted with 150 c.c. distilled water containing 1 % toluol for 22 hours: the liquid was then strained off through muslin: 40 c.c. liquid were placed in each of four bottles, to which was added—No. 1, nothing; No. 2, ½ grm. Witte-peptone; No. 3, ½ grm. casein; No. 4, 0·2 grm. fibrin.

After 21 hours' digestion the fibrin had disappeared in No. 4. The tryptophane-reactions were—in No. 1, distinct; No. 2, strong; No. 3, marked; No. 4, distinct.

In another similar experiment, 50 c.c. of leaf-extract digested ½ grm. fibrin within 48 hours, the liquid then giving strong tryptophane-reaction.

Ficus Carica.

It is well known that the latex of the Fig digests proteids (see my paper 8): but I thought that the investigation of the leaves from this point of view might yield interesting results, especially if experiments were made at different times of the year.

The first experiment was made on June 4, when the leaves were young, and did not seem to contain any milky latex.

Experiment 1. 60 grms. fresh leaves were minced with the machine, the material being then extracted for a short time with 200 c.c. distilled water, containing 1 % toluol. 50 c.c. of the strained liquid were put into each of two bottles: to the one was added ½ grm. of fibrin, to the other ½ grm. of casein. At the end of 48 hours in the incubator the fibrin remained unaltered, and neither bottle gave any tryptophane-reaction.

The second experiment was made on August 11, when the leaves contained latex abundantly; digestion was then rapid.

Experiment 2. 90 grms. of leaves were extracted with about 200 c.c. distilled water with toluol 1 %. 50 c.c. of the strained liquid were put into each of two bottles; to the one was added ½ grm. fibrin, to the other ½ grm. Witte-peptone.

After 23 hours' digestion the fibrin had disappeared in the one bottle, and the contents gave strong tryptophane-reaction; the contents of the other bottle also gave a strong reaction.

It would appear from these results that the protease is contained in the latex and not in the tissues. In connexion with Experiment 2 I incidentally made an observation of some interest that has a bearing upon the foregoing conclusion. On testing the reaction of the leaf-extract, I was surprised to find that it was not acid, as is generally the case with vegetable extracts. It seemed to be somewhat alkaline, and further inquiry proved it to be amphoteric. The latex dropping from an injured leaf was strongly acid. However, at the close of the digestion-experiments the reaction of the liquid had become distinctly acid.

Asparagus officinalis.

Though the material for these observations consisted not of leaves but of shoots, they may be conveniently introduced here. Their interest lies in the fact that they afford yet another instance of fibrin-digestion by plants.

(June). On mincing a number of shoots with the machine, enough juice was obtained for the purpose of experiment. It was an acid, turbid liquid; when further acidified with acetic acid, boiled and filtered, it gave a marked tryptophane-reaction on the addition of 2–3 times its volume of chlorine-water. Since the liquid gives the tryptophane-reaction to begin with, it cannot be used for experiments on peptolysis.

50 c.c. of the expressed juice, with toluol added to 1 %, were put into each of three bottles, with $\frac{1}{2}$ grm. fibrin; to No. 1, nothing further was added; to No. 2, Na_2CO_3 to 1 %; to No. 3, HCl to 0·16 %.

After 22 hours in the incubator, the fibrin was found to be mostly digested in Nos. 1 and 3, and apparently unaltered in No. 2; 24 hours later it had disappeared in Nos. 1 and 3, and was partly digested in No. 2.

Cucurbita Pepo var. ovifera.

I may also include some observations on the digestion of fibrin by the Vegetable Marrow. I have not always succeeded with this material: but the following are the details of a successful experiment.

Part of a green, not quite ripe, Marrow, with the rind, was minced, and from the tissue 300 c.c. of expressed juice were obtained: 50 c.c. of it were put into each of five bottles with 0·2 grm. fibrin, and there was further added—to No. 1, nothing; to Nos. 2, 3, 4, 5, HCl to 0·1, 0·2, 0·5, 0·5 % respectively, and to No. 5 some toluol. After 22 hours in the incubator, the fibrin had disappeared in all the bottles; and their contents all gave marked tryptophane-reaction.

It may be useful to those interested in the investigation of proteolysis in plants if I append a chronological summary of all the known cases, which have been adequately examined from the chemical point of view, of the digestion of fibrin or albumin—cases, that is, which indicate the presence of a protease other than erepsin. The dates given are those of the publication of papers.

Germinating seeds: von Gorup-Besanez ('74), Green ('86, '90), Neumeister ('94), Vines (Wheat-Germ; '03), Weis ('03).
Nepenthes: von Gorup-Besanez ('76), Vines ('77, '97, '98, '01), Clautriau ('00).
Carica Papaya (papaïn): Wurtz ('79), Martin ('84, '85), Vines ('01, '03, '05), Mendel and Underhill ('01), Emmerling ('02).
Ficus Carica (Fig): Hansen ('86, '87), Mussi ('90), Vines ('02, '05).
Myxomycetes: Krukenberg ('79), Greenwood ('85, '87).
Bacteria: Bitter ('87), Lauder-Brunton and McFadyen ('89), Fermi ('90, '91), Emmerling and Reiser ('02).
Moulds (*Aspergillus*, &c.): Bourquelot ('93), Malfitano ('00), Butkewitsch ('02).
Yeast (*Saccharomyces*): Beyerinck ('97), Hahn and Geret ('98, '00), Vines ('02, '04).
Basidiomycetous Fungi (Mushrooms, &c.): Hjort ('97), Vines ('03, '04).
Fruits: *Ananas sativus* (Pine-apple): Chittenden ('91, '94), Vines ('02, '03).
Cucumis Melo var. *utilissimus*: Green ('92).
Cucumis Melo (Melon): Vines ('03).
Cucumis sativus (Cucumber): Vines ('03).
Cucurbita Pepo var. *ovifera* (Vegetable Marrow): Vines ('05).
Asparagus officinalis (shoots): Vines ('05).
Phytolacca decandra (foliage-leaves): Vines ('05).
Bulbs: *Hyacinthus orientalis* and *Tulipa* sp.: Vines ('03, '04).

For the sake of completeness I may add that I have found evidence of the presence of erepsin in a great variety of plants: so many indeed that it may be assumed that this protease is present in some part, or most parts, of every plant at one stage or other of its development. In two cases (Yeast and Mushroom) I have satisfied myself of the simultaneous presence of erepsin and a fibrin-digesting protease (1).

In conclusion, I would draw attention to the striking fact that the apparently universal distribution of erepsin in the tissues of plants that I have demonstrated, is paralleled by a similar distribution in the tissues of animals. This important discovery has recently been made by my friend and colleague, Dr. Vernon. His paper on the subject will shortly appear in the Journal of Physiology; in the meantime I have his permission to make use of the notes with which he has kindly supplied me. He has found that glycerin-extracts of the different organs of various animals, both vertebrate and invertebrate (e. g. frog's liver, pancreas, and ovary; cat's ovary, liver, lung, spleen and kidney; rabbit's kidney and liver; pigeon's kidney and liver; eel's kidney and liver; sheep's liver; lobster's muscle, liver, and kidney; Anodon's kidney, &c.), have no action on fibrin, but peptolyse Witte-peptone with various degrees of activity, tryptophane being always formed in the process. The protease is clearly erepsin. It acts more vigorously in dilute alkaline (0·1 % Na_2CO_3) than in dilute acid (0·1 % acetic) liquids, differing in this respect from the erepsin of plants, which acts most vigorously, as I have shown, in liquids of the natural degree

of acidity. But Dr. Vernon's results indicate a remarkable and interesting convergence between the tissue-erepsins of animals and of plants in this respect, inasmuch as he finds the protease of the lower animals to be relatively more active in acid liquids than that of the higher. For instance he determined the ratio of the activity of glycerin-extract of cat's kidney in alkaline to that in acid liquids to be 76 : 1 ; cat's liver 12 : 1 ; rabbit's kidney 42 : 1 ; pigeon's kidney 5 : 1 ; frog's liver 1·8 : 1 ; eel's kidney 1·4 : 1 ; lobster's liver 8 : 1 ; Anodon's kidney 2 : 1. It is not inconceivable that, with a more extended range of observation, animals will be found whose erepsin, like that of plants, is more active in acid than in alkaline liquids.

In this connexion I may quote an interesting passage from von Fürth's recent work on the chemical physiology of the lower animals (9). Comparing the proteolysing secretions of the Invertebrates with the gastric juice of the Vertebrates, the author states that 'so far the presence of free acid in the digestive secretions has not been demonstrated in the case of any Invertebrate. In certain cases, where the matter was especially investigated, it was clearly ascertained that the acid reaction was due to the presence of acid salts, and that accordingly the proteid-digestion was rather tryptic than peptic.' This agrees in a remarkable manner with the view, first expressed by Fernbach and Hubert with regard to malt, that the natural acidity which is so favourable to the activity of the vegetable proteases is due to the presence of acid salts such as monobasic phosphate of potash (see my paper, No. 1, p. 297).

List of Papers referred to.

1. Vines : The Proteases of Plants ; Annals of Botany, vol. xviii, 1904, p. 289 (April).
2. Emmerling : Ueber die Eiweissspaltung durch Papayotin ; Ber. deutsch. chem. Ges., vol. 35, p. 695, 1902.
3. Vines : Proteolytic Enzymes in Plants (II) ; Annals of Botany, vol. xvii, 1903, p. 606 (June).
4. Mendel and Underhill : Observations on the Digestion of Proteids with Papaïn ; Trans. Connecticut Acad. of Arts and Sciences, xi, 1901.
5. Martin : Papaïn Digestion ; Journ. of Physiol., v, 1884, p. 213 : also, Nature of Papaïn, and its Action on Vegetable Proteids ; Journ. of Physiol., vi, 1885, p. 336.
6. Wurtz : Recherches cliniques et chimiques sur la Papaïne ; Paris Médical, 1879.
7. Vines : Proteolytic Enzymes in Plants ; Annals of Botany, vol. xvii, p. 249, 1903 (January).
8. Vines : Tryptophane in Proteolysis ; Annals of Botany, vol. xvi, p. 7, 1902 (March).
9. von Fürth : Vergleichende chemische Physiologie der niederen Thiere ; 1903, p. 254.

NOTES.

ALGOLOGICAL NOTES. VI. THE PLANKTON OF SOME ENGLISH RIVERS.—Unfortunately I have been unable to continue my investigations of Thames Plankton this year, although a few remarks on one or two samples which were taken in April and May will be found at the end of this note. The year has been a particularly favourable one for such work, as I imagine that the season has been almost absolutely normal. The object of this note is to describe some samples of Plankton taken from the Cam at Cambridge and the Trent at Nottingham during the month of August; as both were collected in the same week, they may be regarded as representing corresponding periodical phases in the Plankton of the two rivers. I was, however, unable to gather material in the Thames at the same time of the year, and consequently have had to fall back on samples collected in August, 1902, for purposes of comparison [1]. In respect of the strength of the current the Thames occupies a position almost midway between that of the other two rivers concerned, the rate of flow being markedly less than that of the Trent. The comparison of the Plankton of the three rivers is therefore an interesting one from this point of view alone. Zacharias [2] and Zimmer [3] have both shown that the rate of flow of a stream has a considerable influence on the quality of the Plankton. The latter finds that 'das Potamoplankton sich dem Plankton eines Teiches seiner Zusammensetzung nach um so mehr nähert, je langsamer der Fluss fliesst.' The slow-flowing Cam therefore should possess a Plankton approximately like that of a pond. Before proceeding to discuss this point in detail, reference must be made to the table, which shows the comparative composition of the Plankton of the Trent, Thames and Cam.

The samples of Plankton were collected from an ordinary rowing-boat. The Trent material was collected on the stretch of river between Trent Bridge and the Great Central Railway's bridge over the river at Nottingham; the current was a strong one, and it was no easy matter to row against it with the net out, and consequently part of the material was collected from a stationary boat with the tow-net playing out into the current [4]. The samples contained a very considerable percentage of mud, and a certain number of the Diatoms were dead and represented only by the empty frustules, although living specimens of all the species mentioned in the table were to be found; in these respects the Plankton recalled that of the

[1] Cf. Fritsch, Algol. Notes, No. III. Preliminary report on the Phytoplankton of the Thames; Annals of Botany, vol. xvi, 1902, table.

[2] Das Potamoplankton; Zoolog. Anzeiger, No. 550, 1898, p. 46.

[3] Das Plankton des Oderstromes; Plöner Forschungsber., Teil 7, 1899, pp. 4, 7.

[4] The samples obtained in this latter manner were, however, not nearly so satisfactory as those collected from the moving boat.

Table, illustrating comparative constitution of the Plankton of the Trent, Thames and Cam during the month of August[1].

	Trent. 25, viii. t = 14° C.	Thames. 19, viii. t = 18° C.	Cam. 29, viii. t = 16° C.
Scenedesmus quadricauda (Turp.), Bréb.	rc.	r.	—
,, *acutus*, Meyen, var. *obliquus*, Rabh.	rr.	—	vr.
Pediastrum Boryanum (Turp.), Men.	r.	c.	r.
,, *pertusum*, Ktz.	vr.	r.	—
Chlamydomonas spec.	vr.	vr.	—
Eudorina elegans, Ehrb.	—	c.	—
Pandorina morum, Ehrb.	vr.[2]	r.	—
Volvox globator, L.	vr.[2]	—	—
Coelastrum microporum, Naeg.	vr.[2]	—	—
Closterium acerosum (Schrank), Ehrb.	vr.	—	—
,, *moniliferum*, Ehrb.	rc.	c.	rc.
,, *lunula* (Müll.), Nitzsch	vr.	—	—
Cosmarium margaritiferum, Men.	—	rc.	rc.
,, *granatum*, Bréb.	vr.	—	—
Melosira arenaria, Moore[3]	—	c.	r.
,, *varians*, Ag.	rc.	vc.	c.
Cyclotella operculata (Ag.), Ktz.	rc.	rc.[4]	—
Campylodiscus noricus, Ehrb.	vr.	r.	vr.
Surirella splendida (Ehrb.), Ktz.	vr.	r.	—
,, *ovata*, Ktz.	vr.	—	—
Cymatopleura Solea (Bréb.), Sm.	rr.	rc.	—
,, *elliptica*, Bréb.	vr.	rc.[5]	—
Himantidium faba, Ehrb.	—	r.	—
Cymbella gastroides, Ktz.	rr.	c.	rc.
Amphora ovalis, Ktz.	—	rc.	—
Cocconeis Placentula, Ehrb.	r.	rc.	rc.
Fragilaria virescens, Ralfs	rc.	vc.	rc.
,, *mutabilis* (Sm.), Grun.	—	rc.	—
Synedra Acus, Ktz.	rc.	r.	—
,, *Ulna*, Ehrb.	rc.	rc.	c.
Nitzschia sigmoidea (Nitzsch), Sm.	rr.[5]	rc.	—
Navicula gracilis, Ehrb.	—	rc.	—
,, *exilis*, Grun.?	—	—	c.
,, *lanceolata*, Sm.	—	—	vc.
Pinnularia viridis (Ehrb.), Rabh.	—	r.	—
Pleurosigma attenuatum (Ktz.), Sm.	—	c.	r.
Tabellaria fenestrata, Ktz.	—	rc.	rr.
Bacillaria paradoxa, Gmel.	vr.[2]	—	—
Merismopedia glaucum (Ehrb.), Naeg.	vr.	—	—
Microcystis spec.	r.	—	—
Oscillaria spec.	r.	—	r.
Euglena viridis, Ehrb.	vr.	rc.	—
Phacus pleuronectes, Nitzsch	vr.	—	—
Synura Volvox, Ehrb.	—	r.	—
Ceratium hirundinella, O. F. M.	vr.	—	—

[1] vc. = very common ; c. = common ; rc = rather common ; rr. = rather rare ; r. = rare ; vr. = very rare.

[2] Only one individual seen.

[3] I take this opportunity of correcting an incorrect determination. The species described as *Melosira moniliformis*, Ag., in the former papers is really *M. arenaria*, Moore.

[4] On looking through old preparations of Thames Plankton from Maidenhead I found this species present in some amount.

[5] Occasionally with epiphytic individuals of *Amphora minutissima*.

Thames during the winter months[1]. There is an abundant growth of Algae along the banks of the river (mainly *Cladophora* with epiphytic *Oedogonium*).—The Plankton of the Cam was gathered three days previously, and the samples were for the most part taken from the part of the river immediately below Cambridge. The current was scarcely noticeable, and the material obtained was practically free from mud. Large numbers of aquatic plants (*Sagittaria*, *Oenanthe*, *Potamogeton*, *Lemna*, &c., especially the first of these) grow in the river, and from this point of view a Thames backwater is recalled; these plants are covered with a more or less dense investment of Algae, whilst small floating masses (a mixture of Conjugates and *Oscillaria*) occur quite commonly on the surface of the water. These two points make Plankton-collecting a difficult matter, for it is almost impossible to prevent the net's coming in contact with the water-plants, and consequently to prevent the enclosure of some of the attached Algae in the sample[2]. I do not therefore regard my collections from this river as perfectly pure Plankton, although I consider it very probable that all the true Plankton forms develop amid the protection of the aquatic plants occurring in such a river (cf. below). For the sake of comparison I have chosen samples of Thames Plankton collected between Maidenhead and Cookham on August 19, 1902, i. e. from a part of the river sufficiently far removed from the estuary as to put a probability of marine influence out of the question[3].

Comparing in the first place the Trent with the Thames, it is noticeable that as regards the number of different species there is little to choose between the two rivers; but if we look at the constitution of the Plankton from the point of view of number of individuals, we find that eight species occur commonly (c) or very commonly (vc) in the Thames, whereas in the Trent one is not able to talk of any species as common. Altogether, a glance at the table will show at once that the majority of species are rarer than in the Thames, the exceptions being the two species of *Scenedesmus*, *Synedra Acus*, Ktz., and a few forms (e. g. *Volvox globator*, *Bacillaria paradoxa*, *Ceratium hirundinella*) which were not observed in the Thames. This quite agrees with the observations which have been hitherto made on the Plankton of rivers; for in the rapidly-flowing Danube Brunnthaler found a Plankton very poor in number of individuals[4]. If the commoner species of the Plankton of the Trent (i. e. those designated rather common) are picked out, we shall find that a number of the common forms in the Thames Plankton are not included; thus *Scenedesmus quadricauda*, *Closterium moniliferum*, *Melosira varians*, *Cyclotella operculata*, *Fragilaria virescens*, *Synedra Acus*, *S. Ulna* may be called the dominant forms of the Trent Plankton, of which only the first and last but one play no important part in the Thames at the corresponding time of the year. Yet to make the list of dominant forms in the Thames complete we must add *Pediastrum Boryanum*, *Eudorina elegans*,

[1] Cf. Fritsch, Further observations on the Phytoplankton of the River Thames; Ann. of Bot., vol. xvii, 1903, pp. 633, 634.

[2] The leaves of the *Sagittaria*, for instance, are covered with a mass of the species of *Navicula* observed in the Plankton.

[3] The distance of Maidenhead from the estuary of the Thames is approximately the same as that of Nottingham from the mouth of the Trent.

[4] Cf. Brunnthaler, Plankton-Studien. I. Das Phytoplankton des Donaustromes bei Wien; Verhandl. d. k. k. zool.-bot. Gesellsch. in Wien, Jahrg. 1900, p. 309.

Melosira arenaria, *Cymbella gastroides* and *Pleurosigma attenuatum*, of which three were not observed in the Plankton of the Trent at all. These remarks will suffice to show the difference in the constitution of the Plankton both as regards quantity and quality in the two rivers under discussion.

We must now consider the resemblances between the Plankton of the Thames and Trent, and these are very marked. In the first place, the filamentous Diatoms, *Melosira* and *Fragilaria*, are important constituents in both cases. Further, both rivers have a number of species characteristic of Potamoplankton in common, viz. the species of *Synedra* and of *Cymatopleura*, *Cyclotella operculata*, *Nitzschia sigmoidea*, *Surirella splendida*, *Campylodiscus noricus*, *Pediastrum Boryanum* and *Scenedesmus quadricauda*. Apart therefore from a certain difference in composition and a general decrease in number of individuals, the Trent may be said to possess a typical river Plankton[1], the nature of which is similar to that of the Thames. Lower stretches of the latter river (i. e. those at Richmond or Teddington) of course show more marked differences, owing to the influence of the tide[2]; and it will be interesting to compare the lower portions of the Trent with them.

A few interesting forms were found in the Trent Plankton. *Ceratium hirundinella* has for the first time been observed in the Plankton of English rivers, as also *Volvox globator*. The individuals of the former species were provided with one upper and three lower processes, of which the middle one was the longest, whilst the lateral ones were of unequal length. The occurrence of *Bacillaria paradoxa* in the Trent is of considerable interest, as I also observed it last year in the Thames near Teddington[3]; it would seem as though this species could live in perfectly fresh water, although the number of individuals found in the two rivers is very small.

We now come to the Plankton of the Cam, and in considering it we must bear in mind that we are dealing with a slow-flowing river, which is only tributary to the main stream, the Ouse. Owing to the inconsiderable current large numbers of aquatic plants are able to develop (cf. p. 165), and this point has already led me to compare the Cam with a Thames backwater. In such a river all the Plankton probably develops on the leaves, &c., of the aquatic plants (which are for instance covered with a sediment of those Diatoms which occur so commonly in the Plankton); the rate of flow is probably not sufficiently strong to interfere with their development. In 1903 I was able to study the Plankton of a number of backwaters of the Thames, and in looking through the Cam material I was at once struck by the great similarity of the Plankton from some points of view. As in the backwaters, the quantity of individuals is much greater, although the number of different species (Cam 16, Thames 30, Trent 32) is markedly less than in a main river like the Thames or Trent. Diatoms, however, are by far the most dominant forms in the Cam, although

[1] i. e. the Plankton is dominated by the Diatoms, only a few green forms being present in at all sensible numbers.

[2] Cf. the table in my algological note III. From all that I have seen it seems that the influence of the tide is perceptible considerably above Teddington Lock; the Plankton even at Hampton Court is not so rich in green forms as in the higher reaches of the river. This is only one of the many problems that the Thames and other big rivers present.

[3] Cf. Fritsch, Further observations, &c.; *loc. cit.* pp. 638, 639.

Closterium moniliferum and *Cosmarium margaritiferum* occur in sensible numbers. The Plankton of the Cam, therefore, agrees with those Thames backwaters which, although richer in number of individuals, still have the Diatom element predominant (small backwater at Walton, backwater at Shepperton[1]). Yet, as I was led to conclude in the case of the Thames backwaters, the Plankton of the Cam is still rather far from resembling that of a pond, and, however different from the Plankton of a big stream, still shows the essential characters of a river Plankton.

I may just add a few remarks on the Plankton of the Thames, based on three samples collected in April and May of this year. In correspondence with the mildness of the season, the number of individuals was already very considerable at this time of the year, and, as is to be expected, the green forms had reached a greater degree of development than at the corresponding season last year. Further, I was only able to recognize the *Melosira*-stage in one of the three samples (from Cookham), the other two having apparently already passed on to the *Synedra*-stage. It thus appears possible that some important changes in the periodicity of the Thames Plankton may take place according to the character of the season—a point which I hope to settle by periodical observations extending over a number of years.

F. E. FRITSCH.

University College, London.
September 28, 1904.

ON A BRILLIANT PIGMENT APPEARING AFTER INJURY IN SPECIES OF JACOBINIA (N. O. ACANTHACEAE).—(Abstract.)[2]—Shoots of certain species of *Jacobinia*[3], when bruised and extracted with water, yield a beautiful purplish liquid. Liebmann discovered these species while travelling in Central America about half a century ago, and found the Indians using them for dyeing purposes. Thomas[4], while in Mexico, submitted the colouring principle of *Jacobinia Mohintli* to a brief examination. Since then these plants seem to have received no further investigation, and their peculiarity is apparently little known to botanists. The object of the present paper is to direct attention to this conspicuous example of pigment-formation, and to give a few details concerning the chromogen and the colouring matter resulting from it. The author hopes to make a full investigation later. So far, the observations have been made on the two very similar species, *Jacobinia tinctoria* and *Jacobinia Mohintli*. The peculiar behaviour of the former plant was brought to the writer's notice, when in Ceylon, by Mr. Willis, the Director of the Royal Botanic Gardens, Peradeniya.

The pigment does not exist as such in the living plant, but appears only on death. Leaves, however, killed by boiling water remain green and do not darken. Hence the pigment most likely arises through enzymic action. Slight alkalinity hastens its appearance. Oxygen is also necessary for its formation. It is readily soluble in water and gives a fluorescent solution, purple to violet by transmitted and blood-red by reflected light. A trace of acid robs the solution of most of its colour. The original tint reappears on neutralization. Alkali turns it bluer, and if strong

[1] Cf. Fritsch, Further observations, &c., pp. 639–646.

[2] Read before the Botanical Section of the British Association, Cambridge, August, 1904.

[3] *Jacobinia tinctoria*, *J. Mohintli*, *J. incana*, *J. neglecta*, and *J. verrucosa*.

[4] Journ. de Pharm. et de Chimie, 1866, sér. iv. t. iii. p. 251.

changes it to green, eventually destroying it. Light does not alter it. All parts of the plant except the flower can produce the pigment.

Such a reducing agent as stannous chloride decolorizes an aqueous solution of the pigment. Micro-organisms can also readily bleach it, when oxygen is excluded. On allowing air to enter, the original colour at once returns.

The whole phenomenon bears some resemblance to the way in which indigo arises in plant tissues. The chromogen of *Jacobinia* is probably a glucoside. In the living cell this substance and its enzyme may be differently situated, perhaps one in the protoplasm and the other in the sap. On the destruction of the cell the two come in contact. The first result is the formation of a colourless body. Then this through the oxygen of the air, possibly assisted by an oxidase, is changed into the pigment.

This behaviour of the *Jacobinia* is perhaps only a striking instance of a common feature of plant-juices, viz. their tendency to darken on exposure to the air.

J. PARKIN.

CAMBRIDGE.

ON THE STRUCTURE AND AFFINITIES OF FOSSIL PLANTS FROM THE PALAEOZOIC ROCKS.—V. ON A NEW TYPE OF SPHENOPHYLLACEOUS CONE (SPHENOPHYLLUM FERTILE) FROM THE LOWER COAL-MEASURES.—(Abstract.)[1]—The class Sphenophyllales, of which the fossil described is a new representative, shows on the one hand clear affinities with the Equisetales, while on the other it approaches the Lycopods; some botanists have endeavoured to trace a relation to the Ferns. The nearest allies among recent plants are probably the Psilotaceae, which some writers have even proposed to include in the Sphenophyllales.

The new strobilus appears to find its natural place in the type-genus *Sphenophyllum*, as at present constituted, but it possesses peculiar features of considerable importance, which may probably ultimately justify generic separation. The specimen, of which a number of transverse and longitudinal sections have been prepared by Mr. Lomax, is from one of the calcareous nodules of the Lower Coal-Measures of Lancashire, and was found at Shore Littleborough, a locality rich in petrified remains, now being opened up by the enterprise of the owner, Mr. W. H. Sutcliffe.

The close affinity of the strobilus with *Sphenophyllum* is shown by the anatomy of the axis, which has the solid triarch wood characteristic of that genus, and by the fact that the whorled sporophylls are divided into dorsal and ventral lobes, as in all other known fructifications of this class. But whereas, in all the forms hitherto described, the lower or dorsal lobes are sterile, forming a system of protective bracts, while the ventral lobes alone bear the sporangia; in the new cone, dorsal and ventral lobes are alike fertile, and no sterile bracts are differentiated. On this ground the name *Sphenophyllum fertile* is proposed for the new species.

Each lobe of the sporophyll divided palmately into several segments, the sporangiophores, each of which consisted of a slender pedicel, terminating in a large

[1] Read before the Royal Society, Dec. 1, 1904.

peltate lamina, on which two pendulous sporangia were borne. In the bi-sporangiate character of the sporangiophores and in other details of structure, *Sphenophyllum fertile* approaches the *Bowmanites Römeri* of Count Solms-Laubach, while in the form and segmentation of the sporophylls there is a considerable resemblance to the Lower Carboniferous genus *Cheirostrobus*.

The wall of the sporangium has a rather complex structure, the most interesting feature in which is the well-defined small-celled stomium, marking the line of longitudinal dehiscence.

The spores, so far as observed, are all of one kind; they are ellipsoidal in form, with longitudinal crests or ridges; their dimensions are 90–96 μ in length by 65–70 μ in width.

The most characteristic point in the structure of the new cone—the fertility of both dorsal and ventral lobes of the sporophyll—is regarded as more probably due to special modification than to the retention of a primitive condition.

D. H. SCOTT.

Kew.

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Vol. XIX. No. LXXIV. April, 1905. Price 14s.

ANNALS OF BOTANY

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London
HENRY FROWDE, AMEN CORNER, E.C.
Oxford
CLARENDON PRESS DEPOSITORY, 116 HIGH STREET
1905

Printed by HORACE HART, *at the* CLARENDON PRESS, *Oxford.*

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The Proteases of Plants (III).

BY

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

Sherardian Professor of Botany in the University of Oxford.

IN the series of papers (Nos. 1–5) that I have contributed during the last three years to the Annals of Botany, I have given the results of a large number of experiments which suffice to prove that proteases are very widely distributed both in the body of the individual plant and in the vegetable kingdom: so widely, indeed, that further investigation may be expected to show, in the course of time, that they are present in every part of every plant at some stage or other of its development.

With regard to the nature of the proteases, I have shown that they are not, as had been previously thought, necessarily such as can act upon the higher proteids (fibrin or albumin). Though I have been able to add to the number of the plants whose juices can effect fibrin-digestion (see 5, p. 161), a more important contribution to the knowledge of the subject is the discovery of a protease which is without action on the higher proteids but proteolyses the simpler proteids, notably albumoses and peptones, as indicated by the application of the tryptophane-test (2, p. 262). This protease thus belongs to the group of the ereptases, finding its nearest analogue in the erepsin of the animal body: but with this difference, that whilst animal erepsin is most active in a slightly alkaline medium, the erepsin of plants is most active in a slightly acid medium (see 4, p. 314). The erepsin is of more general occurrence in plants than the protease that digests fibrin; as is shown by the fact that in many cases the juices or prepared extracts of different parts of various plants that were found to proteolyse albumoses or peptones actively had no digestive action on fibrin. No case was, however, observed of the digestion of fibrin, whether by the juice, an extract, or the tissue of a part of a plant, without the occurrence of tryptophane among the products. In other words, whilst peptolysis can take place without fibrin-digestion (peptonization), fibrin-digestion has not yet been found to take place without peptolysis. This justifies the statement that the peptolytic enzyme may occur independently, whereas there is no evidence of the independent existence of a purely

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fibrin-digesting (or peptonizing) enzyme: a statement that raises the important question of the nature of the latter enzyme, to the discussion of which this paper is devoted.

The Nature of the Fibrin-digesting Protease.

The original view, propounded on the discovery of proteid-digestion by plants (1874), was that the active enzyme is allied to the pepsin of the higher animals. For instance, Darwin (6), speaking of *Dionaea*, says: 'When a leaf closes on any object, it may be said to form itself into a temporary stomach; and if the object yields ever so little animal matter . . . the glands on the surface pour forth their acid secretion, which acts like the gastric juice of animals.' Similarly, Sir Joseph Hooker (7) said, with regard to *Nepenthes*: 'From the observations it would appear probable that a substance acting as pepsin does is given off from the inner wall of the pitcher, but chiefly after placing animal matter in the acid fluid': and von Gorup-Besanez and Will (8) described the pitcher-liquid of *Nepenthes* as 'a vegetable solution of pepsin.' Von Gorup-Besanez did not actually use the word 'pepsin' in the account of his discovery (9) of a peptonizing ferment in germinating seeds (Malt, Vetch, &c.), but his observations suggest that this was his idea of its nature.

This view was a fair inference from the fact that the higher proteids had been observed, in these cases, to be digested by an acid liquid; and was supported by the discovery, made by von Gorup-Besanez, that peptones are formed in the process. Not only did he discover this, but he went on to inquire whether or not the peptones so formed underwent further change (peptolysis). In an experiment with extract of Vetch seeds acting on fibrin swollen with 0·2 % HCl, he looked for, but failed to find, leucin, tyrosin, asparagin, or asparaginic acid among the products of digestion. This negative result must have been a disappointment to the investigator, since the object of his research was to account for the presence of leucin and asparagin in seedlings, and it had occurred to him that these substances might possibly be formed in the germinating seed by a peptolytic process analogous to the pancreatic digestion of proteids in the animal body. However, the idea seemed untenable in the face of the above experiment, so von Gorup-Besanez contented himself with simply recording the presence in these seeds of 'peptonbildende Fermente.'

Within a comparatively short time, the pepsin-theory of the nature of the fibrin-digesting protease had to make way for the trypsin-theory by which it was replaced. The first step in this direction was the observation made by Wurtz (10), in his investigation of papaïn, that this substance could digest in neutral and alkaline as well as in acid media; and on this account he regarded it as allied rather to the pancreatin (i.e.

trypsin) than to the pepsin of animals. This view was further supported by Martin's discovery (11) of leucin and tyrosin among the products of papaïn-digestion. On this ground Martin concluded that 'papaïn is a proteolytic ferment acting almost exactly like trypsin.' In a subsequent paper (12) Martin made the following interesting remark: 'Why, indeed, there should be a pepsin-like ferment in some plants and a trypsin-like in others is as much a problem as why there should be the two forms in mammals.' To what extent this problem has now been solved will appear in the sequel. In the meantime it may be generally asserted that the result of nearly all subsequent investigation has been to establish the 'tryptic' action of vegetable enzymes. Without quoting every individual case, it may be mentioned, for instance, that Green found (13) in the seed of the Lupin (*L. hirsutus*) 'a proteolytic ferment, working in an acid medium, capable of converting fibrin into peptone, leucin, and tyrosin.' With regard to the protease of the Pineapple (*Ananas sativus*), Chittenden wrote (14): 'The results indicate that the ferment is more nearly related to trypsin than to pepsin, in that not only are proteoses and peptone formed by its action, but also leucin and tyrosin.' In 1902 I summarized the conclusion to be drawn, as well from the researches of others as from my own, as follows (1, p. 19):—'The additional instances that I have now given of the production of tryptophane, selected as they are from various classes and from different parts of plants, bear out my previously expressed opinion that the proteolytic enzymes of plants in general are essentially "tryptic." This statement will at any rate hold good until definite evidence is adduced to prove the existence of a "peptic" enzyme.'

The trypsin-theory was not, however, generally accepted without some demur. Thus Clautriau affirmed (15) that the enzyme of the pitcher-liquid of *Nepenthes* is a pepsin: but in my reply (16) I pointed out that this assertion must be incorrect, inasmuch as I had detected tryptophane among the products of *Nepenthes*-digestion. Again, Mendel and Underhill (17) made the statement that their observations indicate 'that papaïn belongs to a class of enzymes which differs somewhat in type from the two proteolytic enzymes that have received most careful investigation in the past, viz. pepsin and trypsin. While the products of the papaïn-digestion of proteids resemble quite closely those of pepsin, so far as these have been examined in detail, the enzyme differs from ordinary animal pepsin in that it acts readily in both neutral and alkaline media. On the other hand, although papaïn is comparable with trypsin in exerting a solvent action in fluids of various reactions, the failure to form leucin, tyrosin, and tryptophane in appreciable quantities—at least under conditions in which they are readily formed in large quantities by other tryptic enzymes—places it in a class of its own for the present.' In the course of special experiments to investigate these results (3, p. 605), I ascertained that the

reason why leucin, tyrosin, and tryptophane were not found by Mendel and Underhill was that sodium fluoride (NaF 1 %), which they employed as the antiseptic, prejudicially affects the digestive activity of most samples of papaïn in that it retards or inhibits peptolysis but not peptonization. I was thus able to confirm the accuracy of Mendel and Underhill's observations, though I showed that they had been somewhat misinterpreted.

Their observations struck me as being of singular importance; in fact they suggested to me those further investigations into the nature of plant-proteases which have led me to the new view that I have already expressed with reference to certain special cases (see 5, p. 157), and that I now propound more comprehensively and in fuller detail as an advance upon the trypsin-theory.

The actual starting-point of the train of thought was the discovery of erepsin, to which I have already alluded (see p. 171). The fact that this protease was found to be present in various parts of different plants, unassociated with a fibrin-digesting enzyme, suggested the possibility that in the cases in which a juice or an extract both peptonized and peptolysed, this complete proteolysis might be effected, not by a single 'tryptic' enzyme, as was generally supposed, but by two distinct proteases; the one essentially peptolytic, erepsin in fact; the other purely fibrin-digesting or peptonizing, a pepsin. In a word, my idea was that the assumed 'vegetable trypsin' might be a mixture of an ereptic with a peptic enzyme. The known facts of digestion could be accounted for equally well on either hypothesis: the process and the products of digestion would be the same in either case. What was wanted for a decision between the two hypotheses was some means of analysing this supposed mixture, so as to separate the two proteases, either actually or, at any rate, in their action; and I seemed to have obtained a clue to the latter mode of analysis in the observations of Mendel and Underhill as explained by the results of my re-examination of them. The behaviour of papaïn in presence of NaF may be explained, on the assumption of a single 'tryptic' enzyme, by supposing that this protease is so acted upon by NaF that its peptolysing activity is inhibited; its peptonizing, fibrin-digesting, activity remaining unimpaired. But it may be urged against this supposition, and with considerable force, that when a protease is prejudicially affected by any agent or condition, presumably all forms of its activity suffer equally. This supposition is, in fact, less reasonable than the assumption that two distinct proteases are present, and that the arrest of the peptolytic action of papaïn by NaF is due to the inhibition of the peptolytic enzyme (erepsin). This clue I have endeavoured to follow up.

In the first place I sought for other instances of the differential action of various antiseptics, but without adequate success; papaïn is so far the only clear case of the kind that I have observed. I next tried the method

of solubility: that is, I prepared extracts of various plants and parts of plants, either with distilled water or with 2 % solution of common salt (NaCl), and compared their digestive activities. This method, which is, of course, only applicable when solid material has to be dealt with, gave interesting results in the case of Yeast (*Saccharomyces Cerevisiae*) and of the Mushroom (*Agaricus campestris*). I found in both cases (4) that a rapidly prepared watery extract could not digest fibrin, though it peptolysed Witte-peptone: on the other hand, a rapidly prepared extract made with NaCl-solution digested fibrin within 24 hours, and also peptolysed Witte-peptone. From these facts I inferred that there are two proteases in these plants: one, readily soluble in water, digesting peptone but not fibrin: the other, less soluble in water, digesting fibrin. The former cannot well be anything but erepsin: as to the latter, the method gave no certain indication whether it were a pepsin or a trypsin, inasmuch as the NaCl-solution dissolved out not only the peptonizing enzyme, but the erepsin as well. A decisive result, on this method, could only be attained by ensuring the removal by water of the whole of the erepsin, leaving the peptonizing enzyme behind alone: but this I had not succeeded in doing to my satisfaction. Consequently, I was content to regard the peptonizing enzyme, provisionally, as a trypsin, in accordance with the prevalent view (4, p. 315).

I then sought for another method that should be more decisive in its results and of more general application. In the before-mentioned experiments with Yeast and the Mushroom, I had incidentally observed that peptolysis and fibrin-digestion were effected in much the same manner, but not to the same degree, by the reaction of the liquid, whether acid, alkaline, or neutral: it appeared, in fact, that a physiological analysis might be effected in this way. I applied this method, in the first instance, to the investigation of papaïn (5), with results that led me to the conclusion that this substance contains two proteases, an erepsin, and a fibrin-digesting but not peptolytic enzyme which can only be regarded as of the nature of a pepsin. Since writing that paper, I have applied the method to the Pineapple, Yeast, the Mushroom, Malt, Hyacinth-bulb, and the pitcher-liquid of *Nepenthes*; in fact, to most of the plants which I knew to be capable of digesting fibrin, and in every case with confirmatory results.

The following is a selection from the very numerous experiments: for the sake of completeness I introduce some made with papaïn, although I have already dealt with that substance (5). I may explain that an important feature in my method is the simultaneous presentation of fibrin and Witte-peptone for digestion. Moreover, the essential point for a successful experiment is to add acid or alkali in due proportion to the enzyme-strength of the digesting liquid. When the reaction was artificially varied, HCl was the acid used, and Na_2Co_3 the alkali. The antiseptic

employed in each case was that which I had found by previous experience to affect proteolysis as little as possible: in some cases the antiseptic was varied in different experiments for the sake of comparison.

Carica Papaya.

4 grms. of papaïn (Christy) were extracted for a couple of hours with 200 cc. distilled water: 4 grms. of Witte-peptone were added, and after standing for 3 hours the liquid was decanted from the undissolved residue: the liquid was faintly acid: HCN to 0·2% was added as the antiseptic. 40 cc. of the liquid were put into each of 5 bottles with 0·2 grm. of fibrin, and treated respectively as follows: No. 1, nothing added; No. 2, Na_2CO_3 to 0·5 % (alkaline); No. 3, Na_2CO_3 to 1 %; No. 4, HCl to 0·2 %; No. 5, HCl to 0·3 %.

After 24 hours' digestion in the incubator at 38° C. the fibrin had disappeared in all the bottles: the tryptophane-reactions were:—

1.	2.	3.	4.	5.
distinct	distinct	distinct	strong	strong

Another experiment, in which half the quantity of papaïn was used, gave similar results.

Conclusion: fibrin-digestion not materially affected by difference of reaction: peptolysis diminished by neutral or alkaline reaction.

In the following experiment no Witte-peptone was added, but the quantity of fibrin was increased to ½ grm., and the limits of alkalinity and acidity were extended: in all other respects the conditions were as in the preceding experiment: the bottles were:—No. 1, natural reaction (nearly neutral); No. 2, Na_2CO_3 to 1·5 %; No. 3, HCl to 0·2 %; No. 4, HCl to 0·5 %.

After 24 hours' digestion the fibrin had disappeared in all the bottles except No. 4, where it did not seem to have been materially reduced: the tryptophane-reactions were:—

1.	2.	3.	4.
distinct	none	marked	none

Conclusion. The results given by bottles Nos. 1–3 confirm the conclusion drawn from the preceding experiments: it appears that the addition of Na_2CO_3 to 1·5 % inhibited peptolysis but did not materially affect fibrin-digestion. With regard to bottle No. 4, HCl to the extent of 0·5 % completely arrested both processes.

These experiments demonstrate the differential effect of alkalinity on the activity of papaïn, diminishing or arresting peptolysis but not fibrin-digestion. I interpret this to mean that papaïn contains a fibrin-digesting enzyme having a wide range of action, limited in one direction by 0·5% HCl, and in the other by a greater amount than 1·5 % Na_2Co_3; as well as a peptolytic enzyme of narrower range, limited by 0·5 % HCl on the one hand and by 1·5 % Na_2Co_3 on the other. I may add that these experimental results agree on the whole with those previously recorded (5, p. 155;

3, p. 605). It is not surprising that the peptolytic enzyme should have been found to be less active in neutral or alkaline liquid than in acid, when it is remembered that the latex and the juices of the Papaw are acid.

I have not attempted to determine the limits of alkalinity and acidity for other samples of papaïn: no doubt they would be found to vary considerably. But I know from previous experiments that the nature of the differential effect is essentially the same in all, under the same conditions.

Ananas sativus.

40 cc. of the expressed juice of a ripe Pineapple were put into each of 5 bottles, 2 % of Witte-peptone having been previously added: 0·3 grm. fibrin was put into each bottle. To 2 of the bottles Na_2CO_3 was gradually added to the extent of 7·5 %, when a definitely alkaline reaction was attained: the contents of 2 bottles were left at natural acidity: to the fifth bottle HCl 0·2 % was added. To a naturally acid bottle and to an alkaline bottle HCN 0·2 % was added: to a similar pair of bottles NaF 1 % was added: there were thus 4 bottles containing an antiseptic, but no antiseptic was added to the HCl bottle.

After 24 hours' digestion in the incubator at 38° C. the fibrin had disappeared in all the bottles: the tryptophane-reactions were:—

HCN acid.	*HCN alk.*	*NaF acid.*	*NaF alk.*	*HCl.*
marked	none	strong	none	strong

Conclusion: peptolysis, but not fibrin-digestion, completely inhibited by alkalinity.

A corresponding experiment made with juice diluted with equal volume of distilled water gave results of the same nature. In yet another experiment in which Na_2CO_3 was added to 2·5 % (reaction still acid) and to 5 % (reaction slightly alkaline), a faint tryptophane-reaction was observed, showing that peptolysis was not altogether inhibited.

The effect of alkalinity upon peptolysis is further demonstrated by the following experiment, in which three degrees of alkalinity were compared, and in which Witte-peptone was not added until after the completion of fibrin-digestion.

50 cc. of juice were placed in each of 4 bottles with 1 % toluol and ½ grm. fibrin: No. 1, contents of natural acidity; No. 2, slightly alkaline by addition of Na_2CO_3 to 4 %; No. 3, more alkaline, Na_2CO_3 6 %; No. 4, strongly alkaline, Na_2CO_3 8 %.

After 24 hours' digestion the results were:—

	1.	2.	3.	4.
fibrin	nearly gone	gone	gone	attacked
tryptophane-reaction	faint	faint	none	none

after 48 hours' digestion:—

fibrin	gone	—	—	nearly gone
tryptophane-reaction	faint	faint	none	none

to each bottle 0·3 grm. Witte-peptone (about 1 %) now added: after 24 hours' further digestion :—

	1.	2.	3.	4.
fibrin	—	—	—	gone
tryptophane-reaction	strong	distinct	none	none

These results are in agreement with those obtained with papaïn, but are even more striking in the differential effect of alkalinity upon fibrin-digestion and peptolysis: the former process was fairly active after the addition of 8 % Na_2Co_3, whilst the latter was only slightly active with 4 % Na_2Co_3, a degree of alkalinity not far from neutralization.

Saccharomyces Cerevisiae.

The experiments were made with the dried, granulated Yeast mentioned in a previous paper (4, p. 293).

10 grms. of the dried Yeast, ground fine, were rubbed in a mortar with about 200 cc. of 2 % NaCl-solution, and after standing for an hour or so, the mixture was put upon a filter. After 3 hours' filtration an acid liquid was obtained giving no tryptophane-reaction: to 200 cc. of the liquid 2 grms. of Witte-peptone were added, and toluol to 1 %: 40 cc. were now put into each of 5 bottles treated as follows:— No. 1, left at natural acidity; No. 2, added Na_2CO_3 to 1·5 % (alkaline); No. 3, Na_2CO_3 to 2 %; No. 4, HCl to 0·2 %; No. 5, HCl to 0·5 %: 0·2 grm. of fibrin was added to each bottle.

After 24 hours' digestion the results were :—

	1.	2.	3.	4.	5.
fibrin	gone	unaltered	unaltered	unaltered	unaltered
tryptophane	very strong	very strong	strong	strong	faint

after 48 hours' digestion :—

fibrin as before; tryptophane-reaction still faint in No. 5.

Conclusion: peptolysis active through wide range of reaction, limited in the acid direction by 0·5 % HCl: fibrin-digestion inhibited by all the variations from natural acidity.

These results are in general agreement with those recorded in the paper already referred to (4, pp. 299, 304), allowing for the fact that the two sets of experiments were made with two different samples of dried Yeast, of which the one used in the above experiment showed itself to be the more active. Combining the two sets of results, the limits of proteolytic activity of a 5 % Yeast extract (NaCl) are approximately—for peptolysis of Witte-peptone, from about 3 % Na_2Co_3 to about 0·5 % HCl; for fibrin-digestion, from about 1 % Na_2Co_3 to about 0·1 % HCl; so that the range of reaction is much more extended for peptolysis than for fibrin-digestion.

The chief interest of these results with Yeast lies in the comparison of

them with those obtained with papaïn and with the Pineapple. Reference to those results shows, in the first place, that whilst in those cases it was possible to arrest peptolysis, in the case of Yeast it was possible to arrest fibrin-digestion. This is an important piece of evidence in the inquiry into the nature of 'vegetable trypsin': the arrest of one of the two processes would have been a suggestive fact, but the arrest of both of them, in different material, is a weighty argument. Again, in papaïn and in the Pineapple, peptolysis was found to have a narrower reaction-range than fibrin-digestion; whereas in Yeast the contrary is the case. I will defer any discussion on this point to the end of this paper. The experiments with other plants that yet remain to be described give results agreeing in the main with those of Yeast.

Agaricus campestris.

The material used consisted of dried Mushrooms ground to fine powder.

3 grms. of the powder were extracted with 120 cc. of 2 % NaCl-solution, and the mixture was placed on a filter for some hours in the cold. The filtrate was a dark, slightly acid liquid, giving no tryptophane-reaction: to it was added 1 grm. Witte-peptone, and toluol to about 1 %. 40 cc. were put into each of 3 bottles: the contents of No. 1 were left at natural reaction; those of No. 2 were made alkaline with Na_2CO_3 0·5 %; those of No. 3 were acidified with HCl 0·1 %: to each bottle 0·2 grm. fibrin was added.

After 24 hours' digestion in the incubator at 39° C. the results were:—

	1.	2.	3.
fibrin	gone	unaltered	unaltered
tryptophane	marked	strong	distinct

after 48 hours' digestion:—

fibrin	—	unaltered	unaltered
tryptophane	—	—	marked

Conclusion: peptolysis somewhat retarded by acid, promoted by alkali: fibrin-digestion arrested by deviation from normal reaction, whether acid or alkaline.

Here again, as in the case of Yeast, it is fibrin-digestion that is the more affected by increased acidity or by alkalinity, the differential action being well marked.

Hordeum sativum.

In view of Weis's (18) important investigation of the proteolysis of Malt, I took the opportunity of making some experiments upon it. It offers this disadvantage, that the extracts give a tryptophane-reaction to begin with, as might be expected. By previous experiment I had

ascertained that NaCl-extracts digest fibrin more actively than watery extracts.

200 grms. of green Malt (11 days' germination) were ground to pulp in the machine, and were extracted for 3 hours with 500 cc. of 2 % NaCl-solution, and then left on the filter in a cold room for several hours. The filtered liquid was distinctly acid, and gave a distinct tryptophane-reaction. To 200 cc. of the liquid 4 grms. of Witte-peptone were added, as also toluol to about 1 %. 40 cc. of the extract were placed in each of 6 bottles: bottle No. 1 contained extract with toluol only; bottles Nos. 2–6, extract with Witte-peptone and toluol; into each bottle was put 0·3 grm. fibrin. No further additions were made to Nos. 1 and 2: to No. 3, Na_2CO_3 was added to 1 % (alkaline reaction); to No. 4, Na_2CO_3 to 2 %; to No. 5, HCl to 0·1 %; to No. 6, HCl to 0·2 %.

After 24 hours in the incubator at 45° C., a temperature selected in accordance with Weis's observations, the results were:—

	1.	2.	3.	4.	5.	6.
fibrin	nearly gone	unaltered	unaltered	unaltered	nearly gone	unaltered
tryptophane	marked	very strong	marked	marked	strong	very strong

and after 48 hours:—

fibrin	gone	gone	unaltered	unaltered	gone	unaltered
tryptophane	marked	—	very strong	very strong	—	—

Conclusion: fibrin-digestion inhibited by alkalinity, and by 0·2 % HCl: peptolysis active within the same range of reaction, though it must be remembered that the extract gave a distinct tryptophane-reaction to begin with.

I observed that the reaction of the Na_2CO_3 2 % bottle in this experiment changed from alkaline to acid, as commonly occurs in vegetable extracts, and consequently makes it difficult to neutralize them or to make them alkaline permanently. In order, therefore, to confirm and extend the result as to the effect of alkalinity upon the peptolytic activity of Malt-extract, I made this further experiment, using a weaker extract and larger percentages of Na_2CO_3.

50 grms. of Malt ground to pulp were extracted with 250 cc. of 2 % NaCl-solution, and filtered, the whole process occupying about 6 hours: the acid filtrate gave a distinct tryptophane-reaction: to the 160 cc. of filtrate obtained, 3 grms. of Witte-peptone were added, and HCN to 0·2 %: 40 cc. were put into each of 4 bottles, as follows:—No. 1, left at natural acidity; to No. 2, Na_2CO_3 to 2 % was added; to No. 3, Na_2CO_3 to 3 %; to No. 4, Na_2CO_3 to 4 %; the contents of all the bottles (except No. 1) were more or less strongly alkaline. After 24 hours' digestion at 40° C. the reactions of Nos. 2, 3, and 4 were still alkaline: the tryptophane-reactions were:—

1.	2.	3.	4.
strong	distinct	distinct	marked

After 24 hours' further digestion the reaction of No. 2 had become slightly acid, those of Nos. 3 and 4 remaining alkaline: the tryptophane-reactions were:—

1.	2.	3.	4.
strong	strong	strong	marked

After 24 hours' further digestion the reaction was still alkaline in Nos. 3 and 4: the tryptophane-reaction had become very strong in Nos. 1 and 2, and was unchanged in Nos. 3 and 4.

Conclusion: peptolysis not inhibited by distinct alkalinity, though certainly retarded; nor is the intensity of the tryptophane-reaction diminished by long-continued digestion with alkali.

The second experiment confirms the first as regards peptolysis. It appears that though alkalinity retards peptolysis to begin with, the process eventually attains considerable activity.

Taking the results of the two experiments together, it is clear that peptolysis has a wider reaction-range than fibrin-digestion: in fact the latter process is confined, as in Yeast, to about natural acidity.

The comparison of my results with those of Weis raises points of considerable interest. Our methods of experiment differed in almost every respect: Weis worked with a strong aqueous extract, I with a more dilute NaCl-extract: he employed mainly vegetable proteids (glutin and legumin) as the material for digestion, I used fibrin and Witte-peptone of animal origin; and the means of estimating digestive activity were altogether different. Yet we both come to the conclusion that two distinct proteases exist in Malt. His statement is that two stages can be distinguished in the proteolysis effected by Malt, a peptic stage and a tryptic stage; and that these two stages are the result of the action of two enzymes, a peptase and a tryptase, since they are diversely affected by external conditions. I regard the two enzymes as being respectively a peptase and an ereptase (not a tryptase): but the difference between us is more apparent than real. For, on another page of his work (18, p. 234), Weis discusses the action of the tryptase, and raises the question whether or not it can peptonize as well as peptolyse. On the analogy of animal trypsin, this might, he admits, be the case: but he expresses a doubt if animal trypsin be really a single protease: so he leaves the question open. However, in a foot-note, he alludes to Cohnheim's discovery of erepsin in a manner suggesting that he thinks his 'tryptase' may be an enzyme of that nature.

We agree, not only in this cardinal point, but also generally in the conclusion that alkalinity retards peptolysis: though my experiments go somewhat further than his, and show that in an alkaline liquid peptolysis is eventually active. The reason of this lies in the difference in the relative duration of our experiments. Weis's determinations were made after only 2 hours' digestion; mine after at least 24. Had his experiments been more prolonged, his results would, no doubt, have been in accordance with mine.

Hyacinthus orientalis.

In a previous paper (2) I stated that the bulb of the Hyacinth can peptolyse Witte-peptone (p. 251), and can digest fibrin in an alkaline medium (p. 254). More recently (4, p. 316) I have shown that both peptolysis and fibrin-digestion were effected by a 2 % NaCl-extract of the bulb, but less actively in a liquid to which 0·1 % HCl had been added than in a liquid of natural acidity or slightly alkaline. I now give the results of new experiments made with the view of inducing differential effects.

It may be explained that strong NaCl-extracts (3 parts of water to 1 of bulb) are so active, in both peptolysis and fibrin-digestion, that it is convenient to prepare them more dilute.

50 grms. of mashed bulb were extracted for a couple of hours with 200 cc. 2 % NaCl-solution: the liquid was then strained off through muslin, and was found to be slightly acid and to give a faint tryptophane-reaction. 4 grms. of Witte-peptone were added to it, and toluol to 1 %. 40 cc. of the mixture were put into each of 5 bottles, treated as follows:—No. 1, nothing added; No. 2, Na_2CO_3 to 1·5 %; No. 3, Na_2CO_3 to 2 %; No. 4, HCl to 0·2 %; No. 5, HCl to 0·4 %: to each bottle 0·3 grm. fibrin was added.

After 24 hours' digestion (40° C.) the results were:—

	1.	2.	3.	4.	5.
fibrin	gone	gone	gone	partly gone	unaltered
tryptophane	very strong	strong	strong	marked	faint

The fibrin remained unaltered in No. 5 after further digestion for 48 hours: the tryptophane-reaction had then become distinct.

These results show that an addition of HCl to 0·4 % is about the limit of acidity for both fibrin-digestion and peptolysis for an extract of this degree of concentration: but as the differentiation was inconclusive, I made another experiment with more dilute extract, and with stronger alkali.

30 grms. of bulb were extracted with 200 cc. 2 % NaCl-solution, to which 4 grms. of Witte-peptone were added, and HCN to 0·16 %: 40 cc. were put into each of 5 bottles, with 0·2 grm. fibrin, as follows:—No. 1, natural acidity; No. 2, Na_2CO_3 to 2 %; No. 3, Na_2CO_3 to 3 %; No. 4, Na_2CO_3 to 4 %; No. 5, HCl to 0·2 %.

After 24 hours' digestion the results were:—

	1.	2.	3.	4.	5.
fibrin	gone	gone	gone	unaltered	unaltered
tryptophane	strong	strong	marked	distinct	marked

after 48 hours' digestion the results were:—

fibrin	—	—	—	partly gone	unaltered
tryptophane	very strong	strong	strong	strong	very strong

Conclusion: the limit of alkalinity for peptolysis had not yet been reached, but its retarding effect is clear: the limits of alkalinity and of acidity for fibrin-digestion

are indicated: the reaction-range of peptolysis is wider than that of fibrin-digestion, though they both are wide.

Even in this experiment the differentiation is not so marked as might be desired: but it suffices to show that here, as in the case of Yeast, fibrin-digestion is more readily affected than peptolysis by increased acidity or by alkalinity.

Nepenthes.

In a previous paper (16, p. 570) I showed that pitcher-liquid not only digests fibrin in acid reaction (0·3 % HCl), but also effects peptolysis as indicated by the tryptophane-reaction. The following are some recent experiments made with a view to differential action. The liquid used was obtained from various species, and was neutral.

To 60 cc. of the liquid 1 grm. of Witte-peptone was added, and HCN to 0·2 %: 20 cc. were put into each of 3 bottles, with 0·2 grm. fibrin, treated as follows:—No. 1, Na_2CO_3 0·5 %; No. 2, HCl 0·2 %; No. 3, HCl 0·5 %.

After 24 hours' digestion (40° C.) the results were:—

	1.	2.	3.
fibrin	unaltered	partly gone	gone
tryptophane	faint	distinct	faint

0·2 grm. Witte-peptone now added to each bottle, and after 24 hours' further digestion the results were:—

	1.	2.	3.
fibrin	unaltered	gone	gone
tryptophane	faint	distinct	marked

Bottle No. 1 was further digested for 96 hours, when a marked tryptophane-reaction was obtained; the fibrin remaining unaltered.

This experiment brought to light the new and important fact that *Nepenthes*-liquid can effect peptolysis in an alkaline liquid. With a view to confirmation the following experiments were made.

20 cc. of pitcher-liquid were put into each of 2 bottles, to each of which were added HCN to 0·2 %, Witte-peptone 0·4 grm., and 0·1 grm. of fibrin: the contents of both bottles were made alkaline to the extent of 0·25 in the one case, of 0·15 in the other. After 48 hours' digestion the results were:—

	0·25 Na_2CO_3.	0·15 Na_2CO_3.
fibrin	unaltered	unaltered
tryptophane	none	none

after 48 hours' further digestion:—

fibrin	unaltered	unaltered
tryptophane	faint	distinct

In another similar experiment, I obtained distinct tryptophane-reactions in liquids containing 0·5 %, 1 %, and 1·5 % Na_2CO_3 after 144 hours' digestion.

The following experiment may be given in full. 50 cc. of neutral pitcher-liquid were made alkaline by the addition of Na_2CO_3 to 0·4 %: 1 grm. of Witte-peptone was added, and HCN to 0·2 %. 10 cc. of the liquid were boiled and put into a separate bottle as a control. After 96 hours' digestion the boiled and the unboiled liquids were separately evaporated to half-bulk: the unboiled liquid then gave a marked tryptophane-reaction, the boiled liquid only a faint reaction.

Conclusion: the pitcher-liquid peptonizes fibrin much more rapidly than it peptolyses Witte-peptone: fibrin-digestion inhibited by alkalinity, promoted by acidity; the limit of acidity was not reached: peptolysis, slow in any case, much retarded by alkalinity, but not inhibited by small percentage of alkali that arrested fibrin-digestion.

The fact that fibrin-digestion is so much more active than peptolysis, suggests at once the presence of two proteases; for, were there a single 'tryptic' protease, both digestive processes should be equally active: and further, that the ereptase is present in relatively small quantity. In its general proteolytic action, the liquid does not agree exactly with any of the other juices or extracts: in its reaction-range for fibrin-digestion it resembles the extracts of Yeast, Mushroom, and Malt, though with a higher HCl-limit: in its reaction-range for peptolysis it resembles papaïn, Pineapple juice, and extracts of Hyacinth-bulb. The subject requires further investigation: but for lack of a supply of pitcher-liquid I cannot pursue it at present, though I hope to do so later in the year. However, the results now given show a divergence in the reaction-ranges for fibrin-digestion and peptolysis sufficiently marked to suggest the presence of two proteases.

Summary and Conclusion.

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. In endeavouring to bring the facts together, it must be recognized that they are not capable of close comparison on account of the different chemical composition of the juices and extracts, and, more especially, of the difference of their initial reactions, two only being neutral (papaïn, *Nepenthes*-liquid), the rest more or less strongly acid. Nevertheless, it is possible to make a few general statements. Taking peptolysis first, it appears that it always took place within a range extending from distinct alkalinity to a degree of acidity beyond the natural: the difference between the individual cases is thus one of degree only. Fibrin-digestion, on the other hand, was much less uniform, showing such wide and striking differences that it is possible to arrange the individual cases into two groups, thus:—

(*a*) those in which it was limited to acid reaction: Yeast, Mushroom, Malt, *Nepenthes*.

(*b*) those in which it also occurred with alkaline reaction: papaïn, Pineapple, Hyacinth-bulb.

Moreover, in the latter group, fibrin-digestion proceeded when the alkalinity was relatively strong: papaïn, 1·5 % Na_2CO_3; Pineapple, acid liquid to which 8 % Na_2CO_3 had been added; Hyacinth, acid liquid to which 3–4 % Na_2CO_3 had been added. Out of all this diversity one inference of fundamental importance can immediately be drawn, namely this, that in every case it is at natural acidity that *both* fibrin-digestion and peptolysis are active; this is their point of coincidence.

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 in the introduction (see p. 174) 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. I do not term the ereptic enzyme simply 'erepsin,' or the fibrin-digesting enzyme 'pepsin,' because these terms have been specifically applied to the proteases of animals, and because the properties are not identical in either case: thus 'vegetable erepsin' differs from animal erepsin in that it is active through a wider range of both acid and alkaline reaction, whilst the latter (at least in the case of the higher animals) is inactive in the presence of acid; and 'vegetable pepsin' can, in certain cases at least, act in an alkaline medium, whilst animal pepsin cannot. Neither the erepsins nor the pepsins of animals and plants are identical. It is convenient to regard the different kinds of erepsin as members of the group of *ereptases*, as being, as it were, species of the genus: similarly the pepsins form the group of *peptases*. Ereptases and peptases belong to the larger group of *proteases*, as being enzymes capable of acting upon proteid substances.

The fact that the ereptase occurs alone, so far as I have been able to discover, in many plants, notably in leaves, makes, as I have already urged, in favour of the view that all peptolytic action in plants is due to this enzyme. The position would be materially strengthened if, in the course of investigation, cases of the independent existence of the peptase were likewise to be brought to light. But this has not yet been done; and the fact that wherever fibrin-digestion has been observed it has always been found to be accompanied by peptolysis, remains to provide an argument of some weight against the view of the autonomy of the two enzymes that I have been led to adopt. The only immediate reply to this objection is the fairly obvious one, that a merely peptonizing enzyme alone would be

of little use to the plant. The significance of the proteases in the economy is that they facilitate the translocation of organic nitrogen in the form of readily diffusible substances. This end is fully attained when the proteolysis is such as to produce from indiffusible proteids bodies like leucin, tyrosin, &c., but only imperfectly when it goes no further than the formation of albumoses and peptones. These facts and considerations also supply the material for an answer to Martin's question on p. 173, and, it may be added, are as applicable in the case of animals as in that of plants.

The duality of the enzymes is the main point to be determined, but it is by no means the only point: the wide differences in reaction-range both of peptolysis and of fibrin-digestion exhibited by the various juices and extracts have yet to be considered. How is the fact to be accounted for that peptolysis is retarded in the case of papaïn, Pineapple juice, and *Nepenthes*-liquid by a degree of alkalinity that in the other cases has little or no effect; or the fact that fibrin-digestion is practically limited to acid reaction in the case of *Nepenthes*, Yeast, Mushroom, and Malt, whilst it is actively carried on in alkaline liquid by papaïn, Pineapple juice, and extract of Hyacinth-bulb? A possible explanation would be that the proteases are not of the same kind in the two sets of cases. On the hypothesis of a single 'tryptic' protease it might be supposed that there exist several varieties of the protease. Similarly on the hypothesis of two proteases, an ereptase and a peptase, it might be supposed that varieties of ereptases and peptases exist in the different plants. But this is not the only possible explanation: another may be suggested, based not upon qualitative but on quantitative differences. I have found, in previous experiments with Yeast (4), that differences in the zymotic strength of an extract are accompanied by differences in its reaction-range: that, for instance, a degree of alkalinity that sufficed to inhibit fibrin-digestion by a 5 % Yeast-liquid had little or no effect upon a 10 % liquid. Quantity is therefore a factor in the problem: but, in view of the foregoing experimental results, the quantitative explanation is only applicable on the hypothesis of two proteases. Although the available facts are perhaps insufficient to settle finally the question as between the qualitative and the quantitative explanations—for these researches do not amount to more than pioneer-work—yet in some respects they distinctly support the latter. Thus the peptolytic results are on the whole so uniform, with a common range extending from distinct alkalinity to distinct acidity, that—if allowance be made for the incidental differences in the chemical composition of the various juices and extracts—there is no sufficient reason for attributing the observed differences in reaction-range to a distinct ereptase in each case. These differences may be more reasonably ascribed to variations in the quantity of one and the same ereptase: the reaction-range

may be taken as indicating the relative amount of ereptase in the individual cases.

The results of fibrin-digestion, being so divergent, are more difficult to deal with. As I have already pointed out (see p. 184), these results fall naturally into two groups: those obtainable through a wide range of reaction from alkalinity to acidity; those limited to acidity. It does not appear possible, at present, to attribute this marked divergence wholly to variations in the quantity of one and the same peptase: it seems to indicate difference in kind rather than in degree. It may be that there are in plants two varieties of peptases more adapted respectively to acid or to alkaline reaction. The decision must be left to more extended and exact experiments than I have yet been able to make.

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.

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Nuclear Division in the Pollen Mother-cells of Lilium canadense.

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With Plates VI, VII, VIII, and IX.

THE investigations to be described in the present paper were carried on upon the pollen mother-cells of *Lilium canadense*, L., which is abundant in this vicinity. Some study, for purposes of comparison, was made of the pollen mother-cells of *L. tigrinum*, Andr., and *L. longiflorum*, Thunb. So far as they went, my results with these species were entirely in harmony with those obtained from the first-named species, in which alone, however, a complete series of stages was studied. All the figures and descriptions have reference to *L. canadense*.

I shall speak, for the most part, only of the behaviour of the nuclear substances, with especial reference to the history of the chromosomes. Some of the more important results have already (Allen, '04) been briefly announced. The process of spindle-formation, which I hope to describe in a future paper, agrees in general, though with some interesting variations in detail, especially in the second division, with the course of events as I have ('03) observed it in the pollen mother-cells of *Larix*.

Methods.

A number of fixing fluids were tried; the best results were obtained from Flemming's stronger chrom-osmic-acetic acid solution, and from the slight modification of this formula used by Mottier ('97). There seemed to be little choice as between the two. Some good preparations were obtained from material fixed in Flemming's weaker solution, but as a rule this fluid produced more or less plasmolysis and distortion of the cell.

Material was fixed in the field from day to day during the season of development and division of the pollen mother-cells. Anthers containing

[Annals of Botany, Vol. XIX. No. LXXIV. April, 1905.]

pollen mother-cells may be found by the end of the first week of June; and stages in their division during the rest of June and the first week of July. The anthers were removed from the bud, usually cut transversely in half, and immersed at once in the fixing fluid. Collections were made during each of five summers, from 1899 to 1903 inclusive.

Sections were cut from three to thirty micra in thickness, and before staining were immersed from fifteen to thirty minutes in a solution of hydrogen peroxide. For the study of the cytoplasmic structures and of the processes connected with spindle-formation, sections of 5–6 μ thick, stained with Flemming's triple stain, were found of most use. For the study of the chromatin, the best results were obtained from sections from twelve to thirty micra in thickness, stained with Heidenhain's iron-alum-haematoxylin. The two staining methods, however, supplement each other, and a comparison of sections from the same anther, some treated in one way, some in the other, is always helpful.

DESCRIPTION OF OBSERVATIONS.

The Heterotypic Division.

The Period of the Nuclear Reticulum.

The divisions in the sporogenous tissue of the anther sac which precede the formation of the pollen mother-cells go on very rapidly, accompanied by growth of the cells and by a corresponding increase in size in all dimensions of the anther itself. A section taken at any stage during this series of divisions shows cells and nuclei in all stages of division. Even when the majority of the cells in the sac have entered upon the long period of growth which characterizes the pollen mother-cells, occasional figures may be found showing stages in the last preceding division. But soon these isolated divisions are completed, and the sporogenous tissue then consists of a cylinder, from six to eight cells in diameter, all the cells being in substantially the same stage of development. A section taken at any time from this period down to the appearance of the multipolar spindle-figures shows a striking uniformity as to stage of development among all the mother-cells of a sac. Apparently the processes concerned in the development of the cell and in the preparation for the heterotypic division go on very slowly and at about the same rate in all the cells of a sac. In some cases a certain amount of progress may be noted in passing from one end of a sac to the other; more often there is a sort of rhythmical variation, so that at two or three regions of the sac the greatest progress has been made, while in moving from one to another of these points we pass through a region showing gradually earlier, and then gradually later stages,

until we reach the second point of greatest progress. The variation within one sac is, however, extremely small. The same general statement is true as to a uniformity between different sacs of the same anther, and different anthers of the same flower, but in these cases there is a somewhat greater range of variation as to the stages represented.

I have attempted to follow carefully the processes concerned in the reconstruction of the daughter-nucleus of the last pre-heterotypic division, and its growth to the size characteristic of the nucleus of the pollen mother-cell. The daughter-chromosomes of the mitosis just mentioned aggregate into a dense mass at either pole of the spindle. After the formation of the new nuclear membrane, nuclear sap appears within it, and the mass of chromatin becomes less dense. The chromosomes are now seen to have lost their regular outline and to have become reticulated, so that it is impossible to trace in the newly-formed nucleus the outline of any individual chromosome. Fig. 1, Pl. VI, shows the nucleus of a young pollen mother-cell at the time when cell-division has just been completed by the splitting of the cell-plate and the formation of a thin, orange-staining wall. The old spindle-fibres (not shown in the figure) are still attached to the nucleus, and on the side to which they are attached (the lower side in the figure) the outline of the nucleus is flattened and still quite irregular, while on the opposite (polar) side it is convex and more evenly rounded. The nucleus, as a whole, is still small and considerably flattened, its shortest diameter coinciding with the axis of the spindle. The chromatic material is massed on the equatorial side of the nucleus. It is broken up into irregular masses, the shape of some of which suggests that of the chromosomes which were their source. The larger masses show an affinity for the safranin stain, and are connected by blue-staining fibres of varying thickness, down to that of very fine threads. The blue-staining fibrous material also forms short threads attached to various portions of the red-staining masses, the whole effect being that of a very ragged and irregular reticulum occupying the greater part of the nuclear cavity, especially on the equatorial side. The colours of the constituents of this network vary, of course, with the time of exposure to the different stains, also to some extent with the time of exposure to the bleaching reagent and with the degree of penetration of the fixing fluid. Preparations may be obtained in which the whole reticulum is violet, others in which it is all stained orange-red. Even in these cases, however, a certain difference is observable between the affinity of the substance of the larger masses and that of the fibres for the different stains, and by careful regulation of the times of exposure a fairly distinct differentiation may be secured. The substance of the larger masses also shows a stronger affinity for the haematoxylin than does that of the fibres. This construction of the nuclear reticulum, out of rather red-staining masses and blue-staining fibres of

uneven thickness, is retained, as will be seen, until the time of the formation of the spirem. There is no such stage in the history of the pollen mother-cells of this species as is found by Miss Sargant ('97) and Mottier ('97) in other species of *Lilium*, in which there appears a fine network of linin fibres, containing imbedded chromatin granules.

The two differently staining substances that I have described are certainly not to be considered respectively as the chromatin and linin, which may be sharply differentiated later in the spirem ; it is more probable that the larger masses, which may correspond to the 'net knots' described by Flemming ('78, '80, '82) in the resting nucleus, contain both chromatin and linin, remaining for the present in these clumps derived from the chromosomes of the preceding division, and destined later to be distributed more uniformly along the spirem. This notion is confirmed by the fact that in iron-haematoxylin preparations of nuclei at the stage shown in Fig. 8, the knots are sometimes seen to be composed of dark granules imbedded in a more lightly stained substance. I have seen nothing at all comparable to Miss Sargant's ('97) 'amorphous chromatin.'

An apparently similar construction of the nuclear reticulum is described and figured by K. and A. E. Schreiner ('04) in the primary spermatocytes of *Myxine*. In this case, however, each knot of the reticulum is formed by one of the fifty-two chromosomes, which is connected with its neighbours by linin fibres. This condition in *Myxine* becomes transformed, by the spreading out of the chromatin along the linin fibres, into a fine reticulum.

The amount of nuclear sap is still (at the stage of Fig. 1) relatively small. Masses of nucleolar matter are already present, varying in number and shape. In the figure two of these are shown, and they are quite rounded, but in many cases they are more irregular in outline. In this case, too, the nucleoles appear on the polar side of the nucleus, as though the material from which they have been re-formed had entered the nucleus from the side opposite that to which the spindle-fibres are attached. This is the position usually occupied by the nucleolar masses at a very early stage in the reconstruction of these nuclei. The nucleoles are readily distinguishable from the chromatic knots, not only by their size, but also by their staining properties. In preparations which have been exposed to the hydrogen peroxide for but a brief period, the nucleoles show little affinity for the safranin, but remain orange or yellow. If they have been bleached somewhat longer, they take on a bright red colour, which is still quite different from the dull red of the knots of the reticulum. The staining properties of the nucleoles seem also to be affected by the degree of penetration of the fixing fluid.

The nucleus soon rounds itself up, as the old spindle-fibres disappear, and increases in size, apparently by the taking in of additional sap, since the amount of chromatic material increases slowly if at all, while its consti-

tuent parts are separated more widely and form a rather uniformly distributed but very ragged and irregular reticulum (Fig. 2). There is a tendency for the material of the reticulum to aggregate about the periphery, but this tendency is not very marked as yet, and a considerable amount of it is distributed through the central portion of the nuclear cavity. The nucleolar material has considerably increased in amount and is gathered into large, often very irregular bodies, in contact with which is a considerable amount of the material of the reticulum, resulting in one or more large, ragged masses in the interior of the nucleus. The outlines of the nucleole can, however, always be distinguished in such a mass. The number of nucleoles in a nucleus is variable, two, three and four being common numbers. Fig. 3 shows a small portion of the reticulum, as seen in a tangential view of the nucleus. It will be seen that there is as yet no suggestion of a spirem-thread, but instead, irregular knots of all sizes, connected by narrower strands and fibres, the whole system branching and anastomosing in the most varied fashion.

As the nucleus increases still further in size, the nucleolar masses round up and separate themselves to a large extent from the reticulum (Fig. 4), the material of the latter tending more and more to take up a peripheral position. During the period of growth of the nucleus, the cell has also been growing, though somewhat more slowly. The cells are separated by rather thin, orange-staining walls.

After the chromatic material of the nucleus has become located almost wholly in the peripheral region (Fig. 5), there begins a period of rapid growth of the chromatin, evidenced by a marked increase in size of the knots; and this growth continues until just previous to the formation of the spirem (compare Figs. 4, 5, and 7). The relation between fibres and knots, and the ragged appearance of the whole reticulum, are as yet (Fig. 5) little changed. Fig. 6 shows a tangential view of a portion of the reticulum at this stage. The nucleoles (usually two or more) are now large, approximately spherical bodies of quite regular outline, lying in the nuclear sap, either entirely free from the reticulum or in contact with small portions of it. The nucleoles quite constantly at this stage show a number of rounded, lightly-stained areas in their interior. This 'vacuolated' appearance is first noticed after the rounding up of the nucleole and its nearly complete separation from the reticulum. Since these vacuoles appear so early in the prophases, at a time when the nucleole itself has just assumed its typical size and form, it seems hardly likely that their presence, in this or later stages, is indicative of the giving up of material by the nucleole toward the formation of the chromosomes.

The nucleus, as a whole, now grows rapidly until it has reached nearly or quite its final size; the knots of the reticulum (Figs. 7 and 8), having likewise grown considerably, lose their ragged look, because of the dis-

appearance of the short, fine threads, and show a tendency to an elongation in the direction of the connecting fibres. These fibres also become more uniform in thickness. All these changes are in the direction of the formation of a spirem; but, as may be seen from Fig. 8, the whole structure is still plainly a reticulum. At this stage there appears not infrequently a pairing of the fibres, due to the fact that two fibres, sometimes short, sometimes of a length equal to half the diameter of the nucleus, run close together and approximately parallel, terminating at either end in the same knot. Instances of this sort appear in Fig. 8, and a particularly good illustration in Fig. 9. Cases of paired fibres at this stage, however, are hardly numerous enough to attract special attention, except in view of subsequent events.

The nuclear membrane, which in the earlier stages has appeared in section as a deeply blue-stained line, is now much less easily distinguishable, though it can always be followed. The difference in appearance seems to be due to some change which takes place at this stage that affects the affinity of the membrane for stains. The nucleus in its growth has become in general longer in one diameter, whereas in previous stages it was nearly or quite spherical. It is sometimes located toward one side of the cell, but its position is still usually central.

Synapsis.

The transformation of the reticulum into a spirem goes on rapidly. The fibres increase in length, tending more and more toward a uniform thickness; the whole intra-nuclear system is now (Fig. 10) seen to be composed of rather slender blue-staining fibres or strands, still interrupted in many places by red-staining bodies of irregular size and shape. It appears as though the material of the knots were being drawn out along certain of the fibres of the former reticulum, and in the process were assuming the staining properties of the fibres. Cases of the pairing of the fibres now become gradually more frequent, due, apparently, to an approximation of the individual strands during or after their formation from the knots; and at the same time there appears a tendency toward a heaping up or aggregation of the nuclear materials, a tendency which is first noticeable in occasional nuclei at a stage similar to that shown in Fig. 8. The density of this aggregation, which is the beginning of the synapsis stage, increases (Figs. 10, 11, 12), about in proportion as the pairing of the fibres becomes more frequent; and it is impossible to avoid the impression that the heaping up of the whole system is closely related to the approximation of the fibres in pairs. Almost simultaneously with the first occurrence of the aggregated condition (Fig. 10), the greater part of the nucleolar material appears as one or more masses flattened against the

nuclear membrane; and this flattening of the nucleole or nucleoles becomes more pronounced as the aggregation of the other nuclear materials becomes closer. The aggregation is at first more commonly in the central portion of the nuclear cavity, and has no apparent relation to the eccentric position of the nucleole; but later the whole mass moves over to one side of the nucleus (Figs. 12, 14), usually to that side already occupied by the nucleole, and then the appearance is as if the nucleole were pressed against the membrane by the synaptic mass.

As the material of the reticulum becomes more and more densely aggregated, the fibres grow in length at the expense of the knots, which finally disappear entirely (Fig. 12); the fibres become quite uniform in thickness. The nuclei represented in Figs. 10 and 11 are from sections respectively twenty and thirty micra in thickness; the nuclei in question were uncut, and the free ends of fibres which appear in them are not due to the cutting of strands. At this time, therefore, a continuous spirem has not been formed. But at the stage shown in Fig. 12, no such free ends are to be observed in an uncut nucleus. It is impossible to say with certainty that no free ends are present in such a dense mass as is shown in this figure; but the fact that they never appear in any of the strands that pass out from the peripheral portion of the mass makes it extremely probable that there is now present a continuous spirem.

The most important fact to be noted in connexion with the spirem at this time (Figs. 12–14) is that it is plainly composed of two slender threads lying side by side. This double nature of the spirem is best distinguished in relatively thin tangential sections of the nucleus, such as that shown in Fig. 13, rather lightly stained with iron-haematoxylin. Often the two threads run closely together for a considerable distance, sometimes loosely twisted about each other; at other times they are in contact and appear to be fused; in some places they diverge more or less widely. Fig. 12 represents a tangential view of a nucleus whose chromatic materials are massed at the surface turned toward the observer. Here on account of the density of the mass the construction of the spirem-thread is not so easily determined; but all the portions which are disengaged from the main mass have the same double structure as appears in Fig. 13; and the same structure is occasionally visible even in the interior of the synaptic mass. For the most part the paired threads within the mass are in close contact, if not actually already fused, giving the appearance of a single strand of double thickness. In a thin median section, such as that shown in Fig. 14, less evidence appears of the double nature of the spirem; though even in such a section the loops of the spirem, which occasionally run out from the synaptic mass into other portions of the nuclear cavity and back again (as the one shown at *a*), are almost invariably seen to be composed of two parallel or twisted threads. From what has been said of the process of

transformation of the reticulum into the spirem, it is plain that the double nature of the latter is not due to a longitudinal splitting of a single thread; but that the two threads are formed independently of each other out of the materials of the reticulum, and approach each other while they are in process of formation. From such appearances as that of Fig. 9, it seems that the approximation of the materials of the two threads may begin during the period of the reticulum, resulting, perhaps, in a fusion of some of the knots in pairs. But Figs. 10 and 11 make it plain that this approximation is brought about for the most part while the material of the knots is being distributed along the strands of the future spirem, and that the approximation of these strands has some causal relation to the massing of the whole system in the synaptic figure.

There seems to be no rule as to the side of the nuclear cavity which shall be occupied by the synaptic mass. In a longitudinal section of an anther sac, there are no more cases of nuclei in which the massing is on any particular side—e. g. toward one end of the sac—than there are in which it is toward any other side. As has been said, the greater part of the nucleolar material is flattened against the nuclear membrane, nearly always in the immediate neighbourhood of the aggregated spirem. Sometimes two or three flattened nucleoles appear in the same nucleus. All stages may be found in the transition from the rounded to the flattened form, so there can be no doubt that these bodies are the same as the nucleoles of the earlier stages. The vacuolated appearance is also often retained, even in cases of considerable flattening. The nucleoles shown in Figs. 11, 12, and 14, represent only moderate instances of the flattening, which often continues until the nucleole is an extremely thin plate, just inside the membrane, and extending, in section, around a quarter or even a third of the periphery of the nucleus. Seen in surface view, this plate has an irregular, lobed outline, and is perforated by occasional irregular holes. The nuclear membrane is still very lightly stained, though plainly present.

Fig. 14 represents a median section of a cell whose nucleus is in the condition just described. Both cell and nucleus have reached approximately their final size. The nucleus has, in most cases, taken up an eccentric position within the cell. In case the cell is longer in one diameter, the nucleus is commonly nearer one end. The long axis of the nucleus generally, though not always, coincides with that of the cell. The cells are still angular, united into a tissue, with orange-staining walls of about the same thickness as in younger stages. The tapetal cells of the inner layer, bounding the mass of spore mother-cells, are very much vacuolated and often shrunken, the majority of them with their nuclei and the remaining cytoplasm at the end of the cell which is in contact with the spore mother-cells.

Even at the stage represented in Figs. 13 and 14, the parallel threads

are in many places in close contact and seemingly fused. They now approximate much more closely throughout their length, twist tightly about each other and fuse into what appears to be a single thread. Although the close approximation of the threads occurs quite early in the synapsis period, the completion of their fusion is a matter of considerable time. During the process all stages of fusion may be observed in the same nucleus. In places the two strands are parallel but not in contact; some portions of the thread show their double nature only by a lighter line in the middle; in other places the two threads are twisted about each other, and in still others there appears a single homogeneous thread. After a considerable time all signs of a double nature disappear, and the synaptic mass contains apparently a single relatively thick thread (Fig. 19). After the fusion and during the continuance of synapsis, the spirem shortens and thickens to some extent, but the thickness of the thread at the close of synapsis, as may be seen by comparing Figs. 19–21 with Figs. 12 and 13, is not much greater than its thickness immediately after the fusion. The diameter of the thread, as the figures show, is at no time perfectly uniform. The synaptic aggregation still persists (Figs. 19), and the mass of the strands composing it is about the same as at its first appearance, but the cluster is looser, and it is easier to follow a single strand for a considerable distance. The looser arrangement is partly accounted for by the fusion of two threads into one, and the consequent lessening by one-half of the total length involved, partly by the shortening and thickening just noted; but there is also already evident a tendency of the thread to disentangle itself and to become distributed more uniformly within the nuclear cavity.

In the most favourable preparations of material in the stages just described, lightly stained with iron-haematoxylin, there appears very clearly the differentiation into chromatin and linin already mentioned as occasionally visible at the stage of Fig. 8. The thread in such a preparation is seen to be composed of a lightly-stained ground substance in which darker bodies are imbedded. By careful washing in the iron alum, the ground substance may be almost entirely decolorized, so that the thread at first sight seems to consist merely of a row of granules (Figs. 15–18). In preparations stained with the triple stain, the chromatin bodies are blue, the linin is red. If the section is over-stained with safranin, the chromatin bodies are dark red, the linin being much lighter.

This differentiation within the spirem thread appears most plainly in cells at or near the ends, especially the cut ends, of the sections, where the fixing fluid first came in contact with the tissues, and where, therefore, its effect may be supposed to have been strongest. In these regions, both cells and nuclei are often shrunken, and the cytoplasm has the peculiar granular appearance which is a characteristic effect of exposure to a too strong solution of osmic acid. The spirem shows little or no change.

Something of the same differentiation in the spirem may often be made out in cells which appear in other respects more normal than those just described, and which are, on the whole, much better fixed; this fact is evidence that the appearances described within the spirem are not artefact, but that they correspond to an actual distinction between the substances of which the thread is composed. This distinction is best preserved by a strength of the fixing fluid which is less favourable for the fixation of the cell as a whole, and especially of the cytoplasmic structures.

Many observers, beginning with Balbiani ('76, '81), have noted in the spirems of both plant and animal nuclei a similar differentiation into darkly-staining granules or discs and a lighter ground substance. The application of Flemming's ('80) term 'chromatin' was restricted by Pfitzner ('81) to the substance of the darker bodies, the 'chromomeres' of Fol ('91), which E. Zacharias ('82) found to be composed of nuclein; and Schwarz ('87) proposed for the lightly-staining ground substance the name 'linin.'

Fig. 15 shows, at a higher magnification than the previous figures, a portion of the two parallel threads before fusion, at the stage of Figs. 12 and 13; in Fig. 16 is shown the beginning of the fusion of the threads, some portions of the two threads being still separate, others showing a fusion of the linin, the chromomeres being distinct, and others showing the chromomeres also fused. The chromomeres are very irregular in outline, each seeming to be made up of a number of smaller granules. They vary considerably in size. In general the threads approximate in such a way that the chromomeres come together in pairs; but this is not an invariable rule, for in some places there seems to be a chromomere in one thread which does not find a mate in the other. All stages in the fusion of these bodies in pairs may be found; often a dumb-bell-shaped figure is formed first, but eventually the two fuse into an approximately spherical mass, still with an irregular outline and the appearance of being composed of a number of smaller granules. Fig. 17 shows the structure of the thread in a somewhat older nucleus; here the threads have fused along their whole length, but the chromomeres in some parts are separate, and all stages of their fusion may still be observed. It will be noticed that in some cases successive chromomeres, especially some of the smaller ones, on the same half of the fusion thread are in contact or very close together; and such a series of two or more may be in contact with a similar series on the other half-thread. It is quite possible that this appearance of a fusion of successive chromomeres is connected with the shortening of the thread as a whole, whereby is brought about a combination of the smaller bodies into progressively larger ones.

Fig. 18 (from a nucleus at about the stage of Fig. 19) shows the fusion more nearly completed; but even here there is occasionally a pair of chromomeres which have not yet united. I have not found instances of

complete fusion of all the chromomeres of the thread until after the close of the synaptic period. Fig. 24, Pl. VII, from a nucleus in the stage of the uniformly distributed spirem (shown in Fig. 23), shows such an apparently complete fusion. There now remains no evidence that the thread ever was anything other than a single structure, containing a single row of chromomeres. In Fig. 24 there appears occasionally a small chromomere located near one edge of the thread, and it may be that these represent the occasional chromomeres of one fusing thread, already noted, which failed to find mates in the other thread.

The chromomeres are imbedded in the linin, and seem to project from it slightly at the edges of the thread. To the projecting points a fine, short linin fibre can sometimes be seen to be attached. These fine fibres are not often easily distinguishable in iron-haematoxylin preparations, but in those stained with the triple stain (Figs. 19–21), they are more plainly visible. In the cells represented in Figs. 19–21, the internal structure of the thread is not apparent, but there appear on the surface of the thread two rows of external swellings or granules, usually quite small, but occasionally (as in Fig. 19) having a diameter equal to a third or even a half of that of the thread itself; these swellings stain blue in preparations in which the thread, as a whole, is red, dark blue when the body of the thread is light blue or purple. The two rows lie on opposite sides of the thread, and for the most part are so arranged that each swelling in one row is opposite one in the other row. In preparations which show the internal differentiation of the thread into linin and chromatin, the position of these pairs of external swellings, where they can be made out, corresponds with that of the chromomeres. The swellings, therefore, are the projecting points of the chromomeres. These projections are not always differentially stained, even with the triple stain, and then do not show except as they give to the thread an irregular outline; but they are frequently very noticeable, especially on portions of the thread which lie comparatively free. From each one may be traced a very fine fibre extending out perpendicularly to the surface of the spirem thread, usually extremely short, but sometimes of considerable length. Where the attached fibres are of some length they may be seen, even in preparations in which the swellings are not differentially stained. I have not been able to distinguish these swellings nor the regular attachment of the fibres to the spirem before the fusion of the parallel threads; but after the fusion they are of regular occurrence.

Miss Sargant ('96, '97) seems to have considered these external swellings to be the chromatin bodies themselves. She finds that at a very early stage the threads of the nuclear network contain a single row of dots; this row becomes double during the transition to synapsis, as a result, she thinks, of a fission of each dot of the single row. The spirem is formed, therefore, with two rows of dots on opposite margins, which are separated

later by the splitting of the thread. From some of her figures ('96, Figs. 15–18), it seems possible that the dots she describes may be the chromomeres; but others ('96, Figs. 13*a*, 13*b*; '97, Figs. 2 *a*, 3*b*, 4, 5) make it plain that the 'chromatin dots' are merely the external swellings on the thread. The description of Farmer and Moore ('95) also seems to refer to these swellings. They find that, before synapsis, the much-convoluted linin filament 'becomes charged with chromatin granules which are especially arranged along the parallel edges of the somewhat flattened thread.' After synapsis, as the thread thickens, the chromatin becomes more abundant in it, and its distribution along the edges is then very apparent. Splitting occurs in the middle line between the rows of chromatin material. In a more recent paper, however, Farmer and Moore ('03) give figures of the longitudinally split spirem which show a row of what appear to be the real chromomeres in each daughter-spirem.

From my preparations it is apparent that the external dots or granules are not the chromatin bodies themselves, but merely the portions of these bodies which project beyond the linin in which they are imbedded. The appearance of the two rows of 'dots' is not, therefore, a preliminary to longitudinal splitting, since the dots of a pair are simply the external projections of a single chromomere.

After the fusion of the parallel threads, and at about the time that the spirem first shows signs of a looser arrangement, the 'sickle-shaped' nucleoles begin to lose their extremely flattened appearance and to return to a more or less spherical shape. All stages in this transition may be found. On regaining the rounded shape (Fig. 19), the nucleoles again show the vacuolated condition characteristic of earlier stages. The nuclei from this time onwards resemble those of the pre-synaptic period in the size of the nucleoles and in their varying number, usually two or three, sometimes more, appearing in each nucleus. Nucleoles may now be found in any part of the nucleus, but for the most part they lie either close to, or within, the still closely crowded coils of the spirem. The nuclear membrane again displays a decided affinity for stains, and from this time onwards it stands out in triple-stained sections as a fairly thick, dark blue line.

While these processes are going on in the nucleus, the cell as a whole has been undergoing a change of shape. At a very early period, even before the completion of the fusion of the parallel nuclear threads, the cell walls connecting the pollen mother-cells into a tissue begin to dissolve, and the cells round up. By the time of the return of the nucleoles to their spherical shape, the cell walls have for the most part disappeared, and the cells have separated and rounded up, each surrounded only by a plasma membrane. In many places, however, two or more cells are directly in contact with one another by their plasma membranes, their outlines being flattened along the surfaces of contact. The size of the cavity of the

anther-sac has been increased to allow room for the rounding up of the cells, partly by the collapse of the bounding layer of tapetal cells, partly by the increase in size of the anther as a whole. In the fluid filling the sac, in which the cells now float, there are numerous drops of a substance which stains bright red in the triple stain, and deep black in the haematoxylin. There now begins to be formed about each mother-cell a new independent cell wall, which, while it is still very thin, is often demonstrable in slightly plasmolyzed cells. There is considerable variation in the thickness of the new walls of different cells at the same stage of development. At the close of synapsis the wall is often still very thin (Fig. 20), but in some instances it has attained a considerable thickness (Fig. 21); in the latter case its thickness is usually unequal on different sides of the same cell.

The series of stages which I have described under the general term 'synapsis' (Moore, '95*a*) is marked by the continuous presence of an aggregation of the chromatic materials within the nucleus, at first approximately central, but soon becoming eccentric. This period is an extremely long one, as is evidenced by the large proportion of anthers in material fixed from day to day through a considerable period, the cells of which show synaptic figures. It seems to me certain that these stages in the lily extend over a period of some days at least, very probably of a week or more. The peculiar aggregation of the nuclear thread at this period seems to have been first observed by Tschistiakoff ('75). He gives (in his Fig. VI) a very good representation of a synaptic nucleus in the 'pollen grain' of *Cupressus*. The figure is plainly that of a pollen mother-cell. Synaptic figures were observed in various pollen mother-cells by Tangl ('82), Strasburger ('82), Heuser ('84), and Guignard ('84), all of whom, however, thought the peculiar appearance due to the action of reagents. Tangl and Strasburger observed flattened nucleoles at the same period, and the latter interpreted them as secretion products, the real nucleole having, as he thought, disappeared at an earlier stage. Strasburger also noted the slight affinity of the nuclear membrane at this period for stains. E. Overton ('91) observed a similar stage in the prophases of the first division in the embryo-sac of *Lilium*. Van Beneden and Julin ('84) found that the chromatin strand in the nucleus of the primary spermatocyte of *Ascaris megalocephala* originates from a nucleolus-like mass; a similar aggregation was found by Kultschitzky ('88), O. Hertwig ('90), and Brauer ('93). Brauer's description makes it plain that this mass is of the same nature as the synaptic aggregation in plant nuclei. Hertwig also observed flattened nucleoles at this or an immediately preceding stage. It is worth noting in this connexion that Winiwarter ('00), whose description of the processes occurring during synapsis is similar to mine, finds that in the primary oöcyte of the rabbit the nuclear membrane is very indistinct during synapsis.

These and numerous other observations seem to show that the peculiar

phenomena connected with synapsis are remarkably uniform throughout the plant and animal kingdoms; and though some of these phenomena, such as the behaviour of the nucleoles and the changes in the affinity of the nuclear membrane for stains, are difficult to account for at present, it is possible that they may ultimately be shown to have a very important relation to the transformations going on in the substance of the spirem.

The Period of the Uniformly Distributed Spirem.

There now occurs a set of simultaneous processes which affect the arrangement of the spirem thread and the position of the nucleus within the cell. The rearrangement of the spirem (Figs. 20, 21) is a further manifestation of the tendency, already noted during the latter part of the synaptic period, toward a loosening of the coils of the spirem and its more even distribution throughout the nuclear cavity. The thread is quite uniform in thickness, showing very regularly in triple-stained preparations the two rows of dark-staining granules, to which short fibres are attached. The effect is, in triple-stained material, to give to the thread a somewhat ragged look; in material stained with haematoxylin this is much less apparent, owing to the lack of differentiation of the granules in question, and to the general failure of the fibres to take up the stain. In the latter mentioned preparations the outline of the thread appears irregularly wavy, but not ragged. The thread, as shown by cross sections, is practically cylindrical. The nuclear membrane stands out sharply.

During the rearrangement of the thread, there occurs a change in the location of the nucleus as a whole, so that, instead of the position it has for some time maintained near one side or one end of the cell, it comes to be approximately central. The final result is such a symmetrical arrangement of the various parts of the cell as is shown in Fig. 22, Pl. VII.

The nuclear thread is now very uniformly distributed throughout the whole nuclear cavity. Portions of it are in contact at many points with the nuclear membrane. The course of the thread may best be studied in sections cut thick enough to include the whole or the greater part of a nucleus, stained with iron-haematoxylin and washed out in such a way as to decolorize the cell wall and cytoplasm, leaving the intra-nuclear substances still black. Fig. 23 is from such a preparation twenty-four micra in thickness. Even at this thickness the most favourably situated nucleus will be sectioned in one or both of the cutting planes, but enough is left to give an adequate notion of the course of the thread. The free ends observable in the figure are all on one or the other of the cut surfaces, and I have never found a free end except at a cut surface, or at so short a distance from the surface as to be easily accounted for by a slight displacement in cutting. A section thirty micra thick includes some entirely uncut

nuclei, and from a study of such sections it is quite certain that the thread at this stage is continuous.

I have been able to find no regularity whatever in the course of the thread, except that it seems to be as evenly distributed as possible throughout the nuclear cavity. Sections sometimes give the impression of a greater massing toward the centre of the nucleus, but careful study shows this effect to be due to the greater number of thicknesses of the thread to be seen in looking through the middle part of the spherical nucleus. The portions of the thread which reach the periphery and then run back into the interior of the cavity might be described as loops, but in the arrangement and course of these loops there seems to be no regularity. There is no apparent relation between the number of loops at this stage and the number of chromosomes which are to be formed later. There are certainly many more than twelve peripheral portions of the thread, and in some cases I have been able to count more than twenty-four, although the exact determination of the number in any nucleus is extremely difficult; but as yet there seems to be no relationship between any definable portions of the thread and the individual segments into which it is later to be divided. Neither at this nor at any later stage of this division have I been able to detect any evidence, in the arrangement of the spirem or of the chromosomes, of a polarity of the nucleus, such as was described by Rabl ('85) for the prophases of nuclear division in animal cells, and as was found by Flemming ('87) in the spermatocytes of *Salamandra.* In this respect the pollen mother-cell of the lily offers a striking contrast to its immediate ancestors; in these, the spirem, before segmentation, has a very uniform arrangement, corresponding closely with that of the daughter-spirem of the previous division. Strasburger ('04), however, finds that in the pollen mother-cell of *Tradescantia* the spirem is arranged in a very regular spiral; and both Strasburger ('88) and E. Overton ('91) have described an arrangement of the chromosomes and the nucleole in pollen mother-cells with reference to a 'Polfeld' and a 'Gegenpolseite.'

The external projections of the chromomeres show best in thinner sections stained with the triple stain, such as the one represented in Fig. 22. It is quite conceivable that the fine fibres, which run out for a short distance from these projections, may belong to a system which connects all parts of the thread with one another, and possibly with the cytoplasm as well; in such a case the greater part of the fibrous connexions may be either too delicate to be differentiated and distinguished with our present technique, or may have been destroyed in some of the processes involved in making the preparations. I have seen no direct evidence of such connexions in the lily; but similar systems of fibrous connexions between the chromomeres of various parts of the spirem have been found by Brauer ('93) in the spermatogonia and the primary spermatocytes of *Ascaris*, by Winiwarter ('00) in

the primary oöcytes of the rabbit and of man, and by Schreiner ('04) in the primary spermatocytes of *Myxine* and *Spinax*. Such a system of linin connectives between all the chromatin bodies in the nucleus, if shown to be of general occurrence, would more closely relate the spirem stage to that of the reticulum, and might help to solve some of the problems connected with the migrations and changes in form of the intra-nuclear substances. Under favourable conditions, the internal differentiation of the thread into chromatin and linin is still apparent (Fig. 24); the fusion of the individual chromomeres brought together by the fusion of the parallel threads seems to be complete. Each chromomere appears to be made up of many smaller granules, and of course the observed fusion of the larger masses tells us nothing of what, if anything, has taken place between the smaller bodies of which they are composed.

In Fig. 23 two nucleoles are shown; the number is usually more than one, commonly two or three, not infrequently more. The nucleus is now located (Fig. 22), as nearly as may be, in the centre of the cell. The cell itself has assumed a generally spherical or elliptical form; in the latter case its long axis commonly coincides with that of the anther sac. Cells are still often in contact in two's or three's, and then are flattened on the sides of contact. The form of the nucleus is approximately that of the cell, so that the thickness of the cytoplasmic layer is very uniform. The cell wall is generally quite thick, often unevenly so; in the latter case, the thickest portions are most commonly at the ends, if the cell is longer in one diameter.

Longitudinal Splitting.

While the spirem is in the evenly distributed condition just described, it undergoes longitudinal fission into two threads. Its fission is preceded by that of the chromomeres (Fig. 27), which is not simultaneous in all parts of the thread. The division of the chromomeres seems to be followed quite rapidly by that of the portion of the thread in which they lie, so that parts of the thread may be found split, with the halves more or less divergent, while contiguous portions are still undivided and contain a single row of large chromomeres. The resultant figures are strikingly similar to those found much earlier at the time of the fusion which produced the single spirem (compare Fig. 27 with Figs. 15–17, Fig. 25 with Fig. 13). This similarity in appearance gives some ground for supposing that the threads which we now see separating from each other may be identical with those which formerly united. As to this possibility I have no direct evidence to offer, since the fusion has been to all appearances complete and has continued over a long period. In the pollen mother-cell of the lily, dark-staining bodies embedded in the spirem, and dividing before the splitting of the thread, have been described and figured by Guignard ('85, '91), Mottier ('97),

and Grégoire ('99). Mottier's figures of the chromatin bodies in the pollen mother-cells of *L. Martagon* and *Helleborus foetidus* are very similar to those which I have observed.

Fig. 25 shows a tangential view of a portion of the longitudinally split spirem, in which the course of the threads is easily traceable. Fig. 26 represents a thin median section of a nucleus in the same stage, in which the threads are frequently cut and occasionally displaced by the knife. In general the two threads are more or less tightly twisted about each other, in places so tightly that it is difficult or impossible to determine more than a single strand of double thickness; in some places they are more widely divergent, and in others they run parallel for a distance without any twisting.

The general course of the double thread and its distribution in the nuclear cavity are at this stage the same as before the fission. The external projections of the chromomeres, with the attached fibres, do not show in the figures, but in material more favourably stained they are plainly present. These projections appear on what was, before the splitting, the outer surface of the thread, the inner surface of the daughter-threads (i. e. the plane in which the splitting has occurred) being quite smooth and evenly stained. Very shortly after the completion of the longitudinal fission, there is manifest a tendency for the thread to become aggregated in the central portion of the nuclear cavity, leaving fewer loops which reach to the nuclear membrane. The nucleole shown in Fig. 26 is very irregular in outline. This, however, is exceptional. Somewhat elongated nucleoles are occasionally seen, but the great majority are nearly or quite spherical, and they are seldom vacuolated, differing in this respect from their appearance in earlier stages.

Chromosome Formation.

In nuclei in which the tendency toward a central aggregation of the spirem first becomes apparent there occasionally appear free ends of the thread; as the spirem is double, these ends are always in pairs. They occur in sections thick enough to include a whole nucleus, so that the break in the thread cannot be due to cutting. The free ends observable are usually at or near the periphery of the nucleus. Sometimes two pairs of ends lie very close together, and in some such cases they can be seen to be connected by a very lightly-staining substance. It is evident that at these places the transverse segmentation of the double thread is occurring or has just occurred. The number of free ends, if any, to be distinguished in a nucleus at this time is very small; and since, if segmentation were simultaneous in all parts of the thread, twenty-four pairs of ends would be present, it follows that the segmentation is successive. Whether any

particular order is followed in this matter, such as a breaking of the thread first into two parts, each of these into two or three, and so on, I have been unable to determine.

The massing of the thread toward the centre becomes more and more marked, until the condition shown in Figs. 28 and 29 is reached. The close and apparently tangled mass at the centre includes a considerable part of the spirem, together with one or more rounded nucleolar masses. Another nucleole, or nucleoles, may frequently be seen in the peripheral region of the nucleus. It is while the nuclear contents are in this condition that segmentation, for the most part, occurs, although, as has been said, the first breaks may appear before the tendency toward massing has become so marked. In Fig. 28, showing a comparatively early stage of the massed condition, few free ends of the thread are to be seen, and these are all at or near the periphery of the nucleus; for the most part, the portions of the thread in the peripheral region consist of loops which originate in and return to the central aggregation. At this stage also two pairs of the free ends commonly lie close together (as at *a* and *a'*, *b* and *b'*, Fig. 28). As time goes on, fewer of the continuous loops are visible, and correspondingly more free ends. Fig. 29 shows one of the later stages, at which a considerable number of free ends are visible, all in the peripheral region. The general effect of a section of such a nucleus is that of strands radiating like the spokes of a wheel from the central mass, each spoke consisting of two separate threads more or less twisted about each other. The exact number of free ends at this stage is very difficult to determine with accuracy; but in favourable cases enough may be counted to make sure that nearly all at least of the twenty-four pairs which would be present at the time of complete segmentation are located in the peripheral region. It follows from the facts described that the central massing of the spirem is due to a rearrangement of the twisted, unsegmented double thread in such a way as to form twelve loops, continuous with each other by means of the centrally massed strands, and with the bend which produces each loop located in the peripheral region of the nucleus; and that transverse segmentation occurs in the peripheral portion of the loops, so that each of the chromosomes so formed has its ends at the periphery and its central portion in the central region of the nucleus.

Miss Sargant ('96, '97) has observed a similar stage of aggregation in both the pollen mother-cells and the embryo-sac mother-cells of *Lilium Martagon*. She describes this 'second synapsis' as occurring just after the completion of segmentation, and finds in it the phenomena characteristic of the earlier stage to which the term 'synapsis' is more generally applied—namely, a clustering of the chromosomes about an amorphous nucleolar mass against one side of the nuclear membrane, and the apparent

disappearance of the latter. In each of these respects her description is inapplicable to *Lilium canadense*. The transition to the synapsis-like figure occurs here before segmentation, or when that process has barely begun. The nuclear membrane remains distinct, the tangled mass is always central instead of peripheral, and the nucleoles retain in general their rounded form. Occasionally a nucleole is seen that is elongated or somewhat irregular in outline, but never at all approaching the curious 'sickle shape' of synapsis. The nucleoles sometimes appear vacuolated, sometimes homogeneous. I have seen no evidence in this or in any other stage of any genetic connexion between the material of the nucleoles and that of the chromosomes.

Ernst ('02) also describes and figures a stage of aggregation following segmentation in the macrospore mother-cells of *Paris* and *Trillium*, but he confuses it with the much earlier synapsis, properly so-called, which stage he seems not to have observed.

Schaffner ('97) finds, in the macrospore mother-cell nucleus of *Lilium philadelphicum*, that after longitudinal fission the chromatin thread becomes arranged in twelve loops, the 'head' of each loop being near the nuclear membrane. Segmentation occurs by the breaking apart of these loops; that is, the transverse breaking occurs in the central part of the nucleus, so that the peripheral portion of each loop becomes the central portion of one of the newly-formed chromosomes. The same arrangement of the spirem and the same method of segmentation are described as characteristic for the heterotypic divisions of both animals and plants in a recent paper by Farmer and Moore ('03). My figures agree both with those of Schaffner and with those of Farmer and Moore as to the formation of the loops; my series of preparations, however, makes it certain that one of the loops does not represent that part of the spirem which is destined to form a chromosome, but that, on the contrary, the peripheral portion of the loop marks the region in which the separation between two adjacent chromosomes is to occur. While the general arrangement of the segmenting spirem is as I have just described, it is subject to occasional variation. For instance, in Fig. 29 one newly-formed chromosome (*aa*) lies comparatively free from the central mass and can be traced throughout its length. A single chromosome from a nucleus at about the same stage is shown in Fig. 33; but on account of the closeness of the central mass and the usual arrangement of the chromosomes with reference thereto, it is only in exceptional cases that a chromosome can be so followed for its entire length. Between the time of longitudinal fission and that of segmentation there is little if any increase in the thickness of the thread; but as soon as the free ends begin to appear, the process of thickening seems to go on more rapidly, as well as somewhat unevenly, so that a decided difference in thickness becomes apparent, even between different parts of the same

segment. A comparison of Figs. 28 and 29 with Fig. 26 shows that for the most part the thickness is little greater at the time of segmentation than at that of longitudinal fission, although some portions of the thread have already thickened considerably. Fig. 33 shows a marked difference in thickness between different portions of the same chromosome.

The chromosomes, formed as above described, now enter upon a period of shortening and thickening, two stages in which are shown in Figs. 30 and 32. Quite early in this period, usually (Fig. 30), the chromosomes take up a position close to the nuclear membrane. This peripheral arrangement, however, may not be effected until a considerable thickening has occurred; in such cases the massing at the centre persists, sometimes nearly to the stage of Fig. 32, with relatively short, thick chromosomes radiating toward the periphery. The various chromosomes do not shorten and thicken at the same rate; and it is common to find in the same nucleus long, slender chromosomes, and other much thicker ones, not more than half as long. Chromosomes *a* and *b*, Fig. 32, illustrate this difference, which is often much greater than in this instance.

Figs. 33–54 show individual chromosomes at various stages, from segmentation to a time immediately before their arrangement on the equatorial plate. Such a series shows that the original longitudinal split, which is plainly apparent at the time of segmentation (Figs. 28, 29, 33), remains distinct until the chromosomes have reached their mature form. The chromosomes are always plainly double, and their double nature does not follow, as the figures show, from a folding over of a single chromosome and the approximation of its ends. The plane of separation between the daughter chromosomes on the equatorial plate is that of the original longitudinal split. The notion of the origin of the double appearance by a folding has found support in the shape of many of the chromosomes immediately after their formation, when, owing to the fact that their length is greater than the diameter of the nucleus, they are necessarily more or less curved, bent, or even looped. The chromosome shown at *aa*, Fig. 29, is bent into a loop at a point not very far from its centre; and the chromosome at the same stage, shown in Fig. 33, is curved into a rather shallow U, the curve following in this case pretty closely the concavity of the nuclear membrane. As the chromosomes shorten they usually straighten out more or less (Figs. 34, 36, 37, 38); but in some cases (Figs. 40, 44, 53, 54) one or both daughter chromosomes remain curved down to a very late stage.

Another point upon which stress has been laid as evidencing the formation of a double chromosome by the bending of a single one is the fact that the two parts are commonly continuous or fused at one end. It is true that the daughter chromosomes often lie closely in contact at one or both ends; and sometimes it is difficult to make out a line of demarca-

tion between the ends so in contact. This is especially true in triple-stained sections; but in material carefully stained with iron haematoxylin, in which the colour is washed out of everything except chromosomes and nucleoles, it is possible in the majority of cases to determine that the ends of the daughter chromosomes, even though in contact, are really separate. In some cases it is impossible to distinguish the separate ends, and there appears, at least, to be a more or less complete fusion. The chromosomes represented in Figs. 43 and 46 show such an apparent fusion, and in those shown in Figs. 36, 38, and 42 the fusion is less complete. In Fig. 42 the daughter chromosomes are in close contact at both ends, and it may be, though from their position it is impossible to determine, that a similar partial fusion has occurred at both ends. The daughter chromosomes in Fig. 52 are closely in contact at one end, but there is no evidence of fusion.

Whether this fusion at one or both ends be real or apparent, it is certain that its occurrence in *Lilium canadense* is much less common than has been described for many species of this and other genera. The phenomenon is not noticeably more frequent at any one stage than at any other, although I have never found it before a certain amount of shortening has occurred; and at any period the great majority of the chromosomes are plainly two-parted throughout their length.

At the time of segmentation the halves of each mother chromosome are several times twisted about each other (Fig. 33); they are closely appressed in most parts, and there is seldom any considerable divergence at any point. As the process of shortening and thickening proceeds, its natural result is a gradual lessening of the number of twists. The course of untwisting may be followed in Figs. 33, 34, 37, 38 and 41. A common effect of this untwisting is a lessening of the closeness of contact between the daughter chromosomes, and a divergence from each other at one or both ends or in the middle. Some retain a considerable twist in the mature form, e. g. Fig. 50. Perhaps the commonest case is the retention of a quarter- or half-twist in the middle portion, with the ends more or less diverging (Figs. 35, 40, 45, 47, 51). Occasionally the twist is entirely lost, and the daughter chromosomes remain in contact only at the centre of each; thus such a peculiar form may arise as is shown in Fig. 44, where each daughter chromosome is bent into a U, the two U's lying in planes perpendicular to each other. In some cases the daughter chromosomes lie close to each other and approximately parallel, with little or no twisting; instances of this sort are shown in Figs. 36, 42 and 52. They may remain in contact at one end, diverging widely at the other (Fig. 43); or they may be in contact at or near both ends, with the centres diverging (Figs. 49, 53). In these various ways, as a result of the untwisting of the daughter chromosomes and of their adhesion or divergence at various

points, arise the many shapes described for the heterotypic division: I's, J's, X's, Y's, V's, U's, and O's, as well as aberrant forms which are difficult of classification.

As a rule, the daughter segments of any mother chromosome shorten and thicken at about the same rate; but this is not always the case. A marked instance of unequal shortening at an intermediate stage is shown in Fig. 35. The difference in length between chromosomes of the same nucleus or of different nuclei at about the same stage, due to variations in the rate of shortening, persists down to the time of spindle formation. When the chromosomes are being pulled into place on the equatorial plate some of them have attained nearly or quite their final form, while others are still comparatively long and slender.

At the time of segmentation the distinction between chromatin and linin may still be detected in some cases, though I have not obtained any preparations of this or later stages in which this differentiation is as well marked as in some of the earlier figures. Shortly after segmentation there appear, in a few of my preparations, chromosomes in which the differentiation is fairly clear, and which show a double row of chromomeres in each daughter chromosome, an appearance already noted at this stage in the lily by Grégoire ('99) and Strasburger ('00), and in the pollen mother-cells of *Naias* by Guignard ('99), and interpreted by these authors as an early indication of the second longitudinal splitting. Miss Sargant ('96, '97) also finds a double row of 'chromatin dots' in each daughter chromosome; but her figures indicate that, as in the earlier stages, these dots are merely the external projections of the chromomeres. Fig. 31 represents, on a larger scale, two chromosomes at the same stage as that of Fig. 30, in which appears this differentiation between chromatin and linin. A few short fibres are also seen attached to the chromomeres. It is not uncommon to find a slight forking of the ends of the daughter chromosomes (as at *a*, Fig. 31); and rarely there appears (*b*, Fig. 31) a partial split in the interior of the daughter chromosome. There can be no doubt, I think, that the appearance of the two rows of chromomeres and the occasional partial fission of the daughter chromosomes really indicate the initiation of the second longitudinal split which is to be completed after the separation of the daughter chromosomes in the metaphases. As the chromosomes shorten still further, the visible distinction between chromatin and linin entirely disappears, and with it all suggestion of a longitudinal fission of the daughter chromosomes. From this time on the substance of the chromosomes appears perfectly homogeneous.

The fine fibres attached to the chromosomes remain visible, and the ragged appearance of the chromosomes increases with their shortening, partly from the crowding together of the points of attachment of the fibres, partly from a growth of the latter in length and thickness. There is thus

a gradual increase in the total amount of fibrous material in the nucleus down to the time of the disappearance of the nuclear membrane. An indication of this increase of fibrous material appears in Fig. 32, drawn from iron haematoxylin material; but it is much more noticeable in preparations treated with the triple stain. The fibres are granular and usually crooked or wavy. Some of the longer ones are now seen to connect separate chromosomes; others run from the chromosomes to the nuclear membrane, and often seem to be continuous through the membrane with cytoplasmic fibres. Very short fibres are sometimes attached to the nucleoles, but the latter commonly appear quite detached from other nuclear substances. The chromosomes now stain distinctly red in the triple stain, and, leaving out of account the blue-staining fibres, which cause the ragged appearance mentioned, each chromosome has a somewhat uneven or undulating outline (Figs. 33–54).

The nucleoles remain generally spherical, sometimes appearing elongated, and, as time goes on, not uncommonly showing one or more small bud-like attachments (*n*, Fig. 32), plainly of nucleolar matter. In stages still later than that of Fig. 32 the number of intra-nucleolar vacuoles increases; the nucleoles show a decreasing affinity for the safranin and an increasing affinity for the orange. In the latter part of the period of chromosome development, but before the initiation of the cytoplasmic processes which directly result in the formation of the spindle, the nuclear membrane begins to lose its smooth, even appearance, becoming more or less granular and irregular or wavy in outline. Just before the disappearance of the nuclear membrane, the two or three nucleoles usually present in the nucleus up to this time break up into a much larger number of bodies, which appear as rounded droplets scattered through the nucleus. If the nucleole has retained its affinity for the safranin up to the time of its fragmentation, these small nucleoles appear at first red; but, as shown by preparations in which a succession of stages appears in the same anther sac, their staining power often gradually diminishes.

When the nuclear membrane gives way, and the cytoplasmic fibres push into the nuclear cavity, the chromosomes aggregate into a close, irregular mass at the centre of the cell (Fig. 55, Pl. VIII). At the same moment the small nucleoles already spoken of disappear, and there appear in all parts of the cytoplasm a great number of still smaller, rounded, usually red-staining bodies. It seems quite certain that these granules result from a second fragmentation of the nucleolar material and its extrusion, perhaps in a state of solution, into all parts of the cell. Up to this time the chromosomes have retained their ragged appearance, due to the attached fibres; but from the beginning of the transformation of the multipolar into the bipolar spindle, the chromosomes appear with smooth outlines and with fibres attached to them only in bundles and

at definite points. The change is not in the outline of the chromosome itself, but in the attached fibrous material. It would seem that all of this material formerly present within the nucleus is made use of in the construction of the spindle.

At the time of the disappearance of the nuclear membrane, the chromosomes have not, as a rule, shortened to their final form; and, as in earlier stages, there is, between individual chromosomes, often considerable difference as to the amount of contraction that has occurred. The shortening is completed, however, by the time of their arrangement upon the equatorial plate (Figs. 56, 57). Even yet, as appears best in polar view (Fig. 57), there is a considerable diversity in their length, due for the most part to an actual difference in mass between individual chromosomes; and as to their arrangement on the spindle, their degree of curvature, and the relation to each other of the daughter segments, there is, as many writers have noted, a remarkable diversity.

While the multipolar spindle is being transformed into the bipolar form the individual chromosomes become separated from the close mass in which they appear immediately after the collapse of the nuclear membrane. By the time of the formation of the 'multipolar diarch' figure the chromosomes are scattered irregularly along the whole length of the spindle. The majority of them lie with their long axes parallel, or nearly parallel, with the axis of the spindle; and a chromosome so arranged is often seen to have a bundle of fibres attached at the end nearest the equatorial plane. Others lie transversely to the spindle.

The chromosomes do not reach the equatorial plane at the same time; and the separation into daughter chromosomes of those first arriving in this plane begins while the tardier ones are still scattered along the spindle. It is also not uncommon, before a chromosome has reached its position in the plate, to find its daughter segments diverging at one end toward the poles of the spindle, with a bundle of fibres connecting each diverging end with the corresponding pole. By the time of the completion of the plate, therefore (Fig. 56), it is a regular thing to find that the separation of some at least of the daughter chromosomes has already begun; but the process of separation is for a time quite slow, even after the plate is fully formed.

The chromosomes of the equatorial plate are arranged nearly in a single plane; they seem to be somewhat crowded (Fig. 57), so that often one or more may be slightly above or below the plane of the rest. The apparent divergence from a single plane shown by the chromosomes in Fig. 56 is due in part to the fact that the plane of the section is not quite perpendicular to the equatorial plane of the spindle.

The chromosomes still show much the same variations in shape that have been noted at earlier stages. Each chromosome is composed of two

short, thick bodies, so closely appressed to one another that the double nature of the whole is often quite difficult of detection. In the best iron haematoxylin preparations, however, the daughter segments can always be distinguished. In no case have I found any evidence of a splitting of the daughter chromosomes at this stage, such as Strasburger ('00) describes in *Lilium Martagon*. Figs. 57 and 59 show their appearance in polar view; Figs. 56 and 60–67 represent them as seen when the line of vision is practically in the equatorial plane. The chromosomes commonly retain something of their original twist (Figs. 59, 60, 63–67, and most of those shown in Fig. 57); they are gradually untwisted by the separation of the daughter chromosomes. Others appear simply as pairs of straight or curved rods in contact at their middle portion or throughout their length (*a*, Fig. 57, Fig. 61). I have found none which were in contact at the ends and divergent in the middle, excepting in such cases as Fig. 67, in which the opening in the middle is plainly due to the beginning of the separation of the daughter chromosomes. Rarely the daughter chromosomes appear to be completely fused at one end (Fig. 62).

Fig. 57 shows the arrangement of the chromosomes in the equatorial plate as seen in polar view. I have found no variations from the typical number (twelve) in any case in which the section was thick enough to exclude the possibility of one or more being missing. The majority of the chromosomes are arranged about the periphery of the plate, with a few, commonly two or three, lying at various angles in the central region. Of those which lie in the periphery, the usual orientation is radial, as the figure shows; but it is not uncommon to find a peripheral chromosome which lies tangentially to the spindle.

Each radially arranged peripheral chromosome (Fig. 56) has a bundle of spindle fibres attached to the inner end of each daughter segment, the outer end then extending out perpendicularly from the spindle into the cytoplasm. To this statement as to the attachment of the fibres to the radial chromosomes I have found no exception. In viewing the spindle from the side (as in Fig. 56), these radially arranged chromosomes present quite different views, according as they lie in the central portion of the figure, and are then seen from the end, or as they lie at the sides, and are seen laterally. In the former case, the ends of the two daughter chromosomes turned toward the observer are usually seen to be in close contact and often flattened against each other. These two ends may lie side by side in the equatorial plane (Fig. 56, second from right), or in a plane parallel with the spindle axis (Fig. 64), or in any intermediate position. This variation in the relative position of the outer ends is accounted for by the different degrees to which the daughter chromosomes are twisted about each other, and by the varying amounts of untwisting which they

have undergone as they are being pulled apart. In some cases (Fig. 64) there is an appearance which may be interpreted as that of four ends turned toward the observer, especially when viewed under medium magnification. But careful study of such a figure with a higher power shows that two of the four parts are at a higher plane and are really the ends of the daughter chromosomes, while the other two are in a lower plane and represent simply lateral protrusions of the daughter chromosomes due to their twisting about each other. Fig. 64 shows how such a four-parted appearance may arise. As already remarked, I have never found an instance of an actually four-parted chromosome (i. e. one showing the second longitudinal split) in the equatorial plate.

In case the radially arranged chromosomes are seen laterally (Figs. 60–63), their two-parted nature is even more plainly evident. In this position we can note the variations in form already described; the daughter chromosomes may be somewhat twisted about each other, with their outer ends either diverging (Fig. 60), in contact (Fig. 63), or apparently fused (Fig. 62); or the daughter chromosomes may be simply in contact, with little or no twisting (Fig. 61).

As has been said, the majority of the chromosomes are of the type already described, namely radially arranged and attached by their inner ends to the periphery of the spindle. A few, however, which lie peripherally are tangential to the spindle. These tangentially arranged chromosomes are always, so far as I have observed, of the twisted, closely appressed type. The attachment of the spindle fibres to the daughter chromosomes in such a case is not necessarily at the end; it may be at either end, or at or near the middle, or at any point between the middle and either end. Figs. 65–67 furnish a series, showing this variety in the point of attachment of the spindle fibres. The attachment to the daughter segments of any parent chromosome is always, however, at corresponding points.

The chromosomes lying in the interior of the equatorial plate (*b*, *c*, *d*, Fig. 57), like the tangential chromosomes, typically consist of twisted, closely appressed segments; and, again like the tangential chromosomes, the attachment here is not at any definite point, but may be anywhere along the length of the segment.

To sum up the condition of the chromosomes as regards their attachment to the spindle: in a large majority of cases the fibres are attached at, or very near, one end of each daughter chromosome; but they may be attached at the middle, or at any point between the middle and either end. It will be shown that the differences between the forms exhibited by the daughter chromosomes in the metaphases and anaphases result from this variation in the point of attachment of the spindle fibres.

The Separation of the Daughter Chromosomes.

My observations as to the orientation of the chromosomes in the equatorial plate, and their behaviour in the metaphases and anaphases, agree essentially with recent descriptions of Strasburger ('00) and Mottier ('03).

In case of the attachment of the spindle fibres to one end of each daughter chromosome, the separation is completed by the untwisting of the daughter chromosomes, if any twist remains, and their gradual pulling apart, the separation beginning, of course, at the end to which the fibres are attached, and progressing toward the other end. The last points of contact between the daughter chromosomes are, therefore, at the end of each which was originally unattached to the spindle, in the case of the radial chromosomes at the outer end, this final contact occurring in the equatorial plane. Figs. 68 and 69 show the progress of this separation, as seen when looking at the spindle in the equatorial plane. These figures represent the chromosome viewed in the same way as in Fig. 64, but at later stages. The relative position of the outer ends of the daughter chromosomes, it is seen, changes with the degree of untwisting; in Fig. 69, the end of one daughter chromosome is turned outward, that of the other toward the interior of the spindle.

Figs. 70–72 show similar stages, the chromosomes being viewed laterally. Figs. 70 and 71 show about the same amount of twisting of the daughter chromosomes as appears in Figs. 60 and 63; Fig. 72, in which there is no twisting, is comparable with Fig. 61. Fig. 72 illustrates the often-observed phenomenon of an elongation or stretching of the separating portions of the daughter chromosomes, due to their plasticity. This plasticity of the chromosome material is also frequently shown by the appearance of a projection (Fig. 70) at the point to which the spindle fibres are attached; the remaining substance of the attached end of the chromosome lags behind, as it were, giving to the daughter chromosome a slightly hooked form (Fig. 70), which is retained in the anaphases. From the more common radial arrangement of the chromosomes in the equatorial plate, and the attachment of the spindle fibres to the inner end of the daughter chromosomes on the side toward the pole, it is plain that this short hook will in most cases be turned toward the interior of the spindle, and so will not ordinarily be visible if the chromosome be viewed from the side turned outward; either a lateral view, or one of that side of the chromosome turned toward the interior of the spindle, will be necessary to show the presence of the hook.

At about the stage represented by Fig. 69, namely, that at which the daughter chromosomes remain in contact only by their equatorial ends, a longitudinal split (Figs. 58, 74–77) appears in each daughter chromosome. The occurrence of this split seems to be very sudden, and when first seen

it usually extends the full length of the daughter chromosome, excepting at its polar end. Fig. 73 represents the only instance I have found in which an early stage of the split was visible. In this case a fission appears in the middle portion of one daughter chromosome, the equatorial end still showing no evidence of division. This fission of the daughter chromosomes is usually in a plane which passes through the chromosome and the central axis of the spindle; it would seem, therefore, to represent a split at right angles to the original one which produced the daughter chromosomes. But on account of the twisting and untwisting which the chromosomes have undergone, it is impossible to be certain upon this point.

The newly separated granddaughter chromosomes immediately diverge at their equatorial ends, giving to each daughter chromosome the form of a V, with the apex toward the pole. All four equatorial ends of the V are usually turned outward (Figs. 74, 75, 77); in Fig. 77 the chromosomes are shown as seen from the interior of the spindle, the equatorial ends then being turned away from the observer. Fig. 76 shows an unusual case, in that the two V's lie in planes perpendicular to each other; the four equatorial ends are in this case also turned outward. The upper daughter chromosome in this figure shows some evidence of being split throughout its length, the polar end being apparently double.

As the V-shaped daughter chromosomes finally separate from each other and approach the poles of the spindle (Figs. 79, 88), they commonly contract more or less, losing the long-drawn-out appearance which they have frequently presented during the process of separation, and regaining about the same length that they had in the equatorial plate.

It is not uncommon for single chromosomes to remain attached in the equatorial plane, even after their fellows have reached, or nearly reached, the poles. Fig. 78 represents two V-shaped daughter chromosomes which have undergone longitudinal splitting; one arm of one has separated from the corresponding arm of the other, but the remaining two arms are still in contact, and indeed are so closely attached as to appear fused. In this case the angles of the V's have been pulled nearly to the poles of the spindle. A similar case is shown in Fig. 58. These are instances of the possibility of a fusion, or apparent fusion, between two separate portions of the chromosome substance when closely appressed—a possibility which was illustrated in an earlier stage by the occasional fusion of the daughter chromosomes at their ends. It is not surprising that such a fusion of two masses of this very plastic substance should occur; the striking fact is rather that it occurs so seldom. That in such cases there is no real fusion, but only a temporary adhesion, is suggested by the history of the chromosomes, which, in the nucleus of the animal sperm, form a seemingly homogeneous mass, but which reappear as separate entities before the fusion of the sexual nuclei.

Such appearances as those of Figs. 79 and 88 would lead to the conclusion that the newly-formed segments of the daughter chromosomes remain attached at the angle of the V—in other words, that the second longitudinal split is not completed, at least in this mitosis. This is the regular appearance if the chromosomes are viewed, as is the case in these figures, from the exterior of the spindle. But a very different impression is gained (Fig. 80) by observing that side of the chromosomes turned inward. It will be remembered that the slight hook which is commonly formed upon the polar ends is usually turned inward. The hooked shape of the daughter chromosomes is shown in Fig. 80, and it is plain that each is split throughout its whole length, the polar end of each granddaughter chromosome being turned inward, its equatorial end, as we have seen, turned outward. It would seem that this inward hooking of the polar ends, with often more or less overlapping of the hooked ends of the granddaughter chromosomes, obscures the view of the splitting from the outer side, and gives the appearance of a continuity between the two arms of the V at the angle. At the angle of each V in Fig. 79 there is a slight indentation, which is doubtless an indication of the completed splitting. It is not always the case that an interior view shows the completed splitting so plainly as does Fig. 80, but such figures are so common that there is no doubt that the complete separation of the granddaughter chromosomes in the anaphases of this division is the general rule. It will be seen that the splitting of the daughter chromosomes shown in Fig. 80 has been accompanied by the separation of the spindle fibres into distinct clusters, one cluster attached to each granddaughter chromosome.

The appearances just described are characteristic for the large majority of the chromosomes; but in cases of the attachment of the spindle fibres otherwise than at the ends of the daughter chromosomes (Figs. 65–67) the resulting figures are somewhat different. Figs. 81 and 83 represent later stages in the separation of such chromosomes; and it will be seen that, as the daughter chromosomes retreat toward the poles, a bend occurs at the point of attachment; that is, the more common hooked form is exaggerated, and each daughter chromosome consists of two arms whose relative length depends upon the point of attachment of the fibres. The result of the splitting of such daughter chromosomes is shown in Figs. 82 and 86; the derivation of Fig. 82 from such a form as is shown in Fig. 81 is plain; and, on the other hand, Fig. 82 differs from the commoner type (Fig. 80) only in the greater length of the hook at the polar end of each granddaughter chromosome. If the fibres are attached near the middle, the two arms of each daughter chromosome, and, after the second longitudinal split, those of each granddaughter chromosome, will be of about equal length; each daughter chromosome will then (Fig. 85) consist of two V's with their angles in contact and turned toward the pole, and with their arms diverging.

Fig. 84 shows an early stage in the longitudinal split; one end of each V-shaped daughter chromosome is here double, the other end still undivided. Fig. 85 is therefore to be compared with Fig. 76, the difference in appearance between the two figures being due solely to a difference in the place of attachment of the spindle fibres. Fig. 87 shows two pairs of daughter chromosomes from the same spindle, each pair showing the completed longitudinal fission; in one case the granddaughter chromosomes are only slightly hooked, in the other they are V-shaped. In this case, comparison shows that the total length of the two arms of the V-shaped granddaughter chromosomes is about equal to that of the single arm, with its short hook, of the commoner form.

Fig. 88, Pl. IX, shows the daughter chromosomes approaching one pole; in Fig. 89 they have reached the diaster stage. Twelve V's are shown in Fig. 88; in Fig. 89 the daughter chromosomes are so close together that it is impossible to distinguish them at their angles; twenty-two ends turned away from the pole are visible, and in the preparation two others may be observed underneath those shown in the figure. In each of these cases, therefore, all of the chromosomes were originally attached to the spindle at their ends, and the longitudinal split of each daughter chromosome resulted in a single V. Fig. 90 (from a section too thin to contain all of the chromosomes) represents a slightly later stage than Fig. 89; the chromosomes have been drawn closely together at the poles. A large number of 'extra-nuclear nucleoles' are still present, and they show a tendency to accumulate in the equatorial region, in which the new cell-plate is to be formed.

The Reconstruction of the Daughter Nuclei.

When the daughter chromosomes reach the polar region they are drawn tightly together, as we have seen, the free ends of the V's radiating outward and curving toward the equatorial region. These free ends now come closer and closer together, the ultimate result being an extremely dense mass (Fig. 91), in which it is difficult to trace the outline of any individual chromosome. At this stage a membrane appears about the daughter nucleus. After the membrane is formed, the nucleus begins to grow in size (Fig. 92), and the chromosomes become separated once more by clear spaces filled with nuclear sap. This growth of the nucleus is contemporaneous with the formation of the cell-plate and the new cell-wall. After the appearance of the nuclear membrane, the extra-nuclear nucleoles are most numerous in the neighbourhood of the daughter nuclei (Figs. 91–93); many of the nucleoles are larger than during the continuance of mitosis, as though some of the smaller ones had united. I have never seen any nucleolar substance within the daughter nucleus, however, at this or at later stages. This seems to be generally the case in the daughter

nuclei of the first division in the pollen mother-cells of Angiosperms. In *Podophyllum* and *Helleborus*, however, according to Mottier ('97), nucleoles appear in these nuclei.

The nucleus lies very close to the cell-wall; its outline, when the nuclear membrane first appears (Fig. 91), is irregular, especially on the side toward the equatorial plane of the spindle; as the nucleus grows it becomes more rounded (Fig. 92), but remains always flattened in the axial plane of the spindle.

While the nucleus is growing, the chromosomes seem to stretch out, becoming longer and slenderer (Figs. 92, 93). Their free ends, extending toward the equatorial plane of the spindle, become curved in such a way as to bring the end of one arm of a V into contact with the end of an arm of another V; these ends then apparently fuse. At the same time, the ends by which the granddaughter chromosomes were in contact at the angles of the V's also seem to have fused, the final result being a single continuous closed spirem. Fig. 92 shows the spirem, viewed laterally, at the time of the formation of the cell-plate; Fig. 93 shows an oblique view of the nucleus after the completion of cell-division. From these figures it appears that, although the strands of the spirem become somewhat bent and curved, they still retain the general form which results from the joining of the V-shaped chromosomes in the manner just described. Looking at the nucleus, therefore, as in Fig. 93, one sees a sort of rosette, formed by the arms of the V's diverging from an open space about what was formerly the polar region, the spirem lying almost entirely in the peripheral region of the nucleus. The nucleus never passes into anything resembling a resting condition. A few achromatic fibres are sometimes seen attached to the spirem strands; but the latter retain their distinct outlines and show no sign of breaking up or reticulation. I do not find, as did Mottier ('97) at the time of the formation of the cell-plate, a shortening of the spindle fibres and a closer approximation of the daughter nuclei; in *Lilium canadense* the nuclei remain near the cell-wall in about the positions occupied by the poles of the first spindle.

The Homoeotypic Division.

The Prophases.

While the processes involved in the division of the pollen mother-cell nucleus extend over a very long time, the succession of events in the division of the daughter nuclei is extremely rapid. Practically the whole series of stages may often be traced within a single anther sac.

The first evidence of a preparation for the new division is seen in a further increase in size of the nucleus, accompanied by a loosening

and spreading apart of the spirem thread, which is now seen to be composed of segments (Figs. 94, 95), with their ends often close together or in contact, as though they had just broken apart from one another. Each segment has the shape of a V with its arms more or less curved. The angle of the V is on what was in the former division the polar side of the nucleus, and its free ends are on the side toward the partition-wall between the daughter-cells (the equatorial plane of the first division figure). Fig. 94 shows this arrangement of the V's, the closed angles being on the upper side of the figure; a few free ends are visible on the lower side. In some cases an end of one V lies directly over the end of a neighbouring V, and it is impossible to determine whether they are separate or attached. A comparison of Fig. 94 with Figs. 89 and 92 can leave no doubt, I think, that the V-shaped chromosomes which appear in the prophases of the second division are identical with the daughter chromosomes of the first division. Their arrangement is the same, except for the bending and curving due to their being gathered into the rounded cavity of the daughter nucleus. They are somewhat longer and thinner, a result of the elongation which, as has been mentioned, occurs after the formation of the daughter nucleus. The V-shaped chromosomes in Fig. 94 seem to be closed at their angles; but certain of the V's in Fig. 95, which gives a more oblique view of the nucleus than does Fig. 94, lie in such a way that it is evident that the two arms (the granddaughter chromosomes of the former division) are only in contact, but not fused, at the angle of the V. In the nucleus shown in Fig. 95, as in that of Fig. 94, there are twelve V's.

The nucleus continues to increase in size, becoming at the same time less flattened, so that it is, at the time of the disappearance of the nuclear membrane, nearly spherical. The chromosomes remain in the peripheral region, the central portion being occupied by nuclear sap.

As the membrane breaks down and the cytoplasmic fibres enter the nuclear cavity (Fig. 96), the chromosomes are crowded into a close mass, as in the previous division. No material change has taken place in the appearance of the chromosomes since the segmentation of the spirem. When they are being pulled into place on the spindle it is often apparent that the two arms of each are really separate. Figs. 97–100 represent individual chromosomes at this stage. The arms remain in contact at the end at which they were in contact in the anaphases of the previous division; but at other points they may be variously in contact or divergent, resulting in figures which remind one of many of the various forms of the chromosomes of the first mitosis. They differ, however, from the heterotypic chromosomes in that there is never any twisting of the two parts about each other.

Immediately after the disappearance of the nuclear membrane, as in the first division, the chromosomes lose the ragged fibres which have

been attached to them ; these fibres seem to be used in the formation of the spindle. Each daughter chromosome has now only a single bundle of fibres attached, in the majority of cases, at, or close to, its end.

The Equatorial Plate.

Fig. 101 shows the equatorial plate in lateral view ; in Figs. 104–106 are represented individual chromosomes similarly viewed. The majority of the chromosomes are attached to the spindle at, or very close to, the end at which the daughter chromosomes are in contact with each other. To each daughter chromosome a bundle of fibres is attached, running toward the corresponding spindle-pole. In general the chromosomes lie in a direction which approximates more or less closely that of the long axis of the spindle. The two daughter chromosomes may be nearly parallel with each other, pointing toward the same pole (Fig. 104); or they may diverge toward opposite poles (Fig. 106). Less commonly, the chromosomes stand out nearly or quite at right angles to the axis of the spindle (Fig. 105).

The arrangement of the chromosomes in the equatorial plate, as well as their number, are best determined in a polar view (Figs. 102, 103). Usually the chromosomes are attached at the periphery of the spindle; it is not common to find any of them in the interior of the equatorial plate, as is regularly the case in the first division, although this occasionally happens, as appears in the lower of the two cells represented in Fig. 103. The general appearance of the chromosomes in polar view is that of V's, usually considerably foreshortened on account of the orientation just described. The inner end of one arm of a V is directly superposed in such a view over the inner end of the other arm, as follows from the manner of attachment to the spindle shown in Figs. 104–106. The chromosome as a whole, then, lies in a plane nearly perpendicular to the equatorial plane.

The plate shown in Fig. 102 contains fourteen V's, instead of twelve, which, as has been said, is the number of chromosomes that, so far as I can determine, constantly occurs. The discrepancy in this case seems to be accounted for by the fact that in two instances (at *a* and *b*) two V's are in close contact with each other. Furthermore, as appears especially well at *a*, each V lies in the plane of the equatorial plate, instead of nearly at right angles to that plane as is ordinarily the case. The two V's at *a*, Fig. 102, are, therefore, in all probability, to be thought of as corresponding respectively to the two arms of the V-shaped chromosome at *c* (same figure). One V-shaped daughter chromosome at *a* is superposed over its sister segment in exactly the same way that one of the rod-shaped daughter chromosomes at *c* is superposed over its sister. In other words, the varying methods of attachment of the chromosomes to the spindle fibres in the heterotypic division reappear in the homoeotypic division ; and,

just as was the case with the granddaughter chromosomes of the previous division, so with the same segments (now daughter chromosomes) in the present division—the majority are attached to the spindle at or close to their ends, resulting in giving them the shape of a straight or hooked rod, while a few (usually not more than one or two in a cell) are attached at some point between the two ends, resulting in the shape of a V, whose arms may or may not be of equal length. The same fact is very well illustrated in Fig. 103, which represents a polar view of the equatorial plates of two sister-cells. The chromosomes *a* and *b* in the upper cell, *a'* and *b'* in the lower, consist each of two V-shaped daughter segments, one superposed upon the other, while each of the other chromosomes in either cell consists of two rod-shaped segments similarly superposed one upon the other. From the fact that in each sister-cell appears the same number of chromosomes of each form, and the further fact that chromosomes *a* and *b* in the upper cell correspond very closely in position with chromosomes *a'* and *b'* in the lower, it seems quite certain that *a* and *a'*, *b* and *b'* are respectively sister chromosomes of the previous division, comparable with those shown in Fig. 85; and the conclusion is strongly suggested that the method of attachment of each individual chromosome persists from one division to the next. All the evidence furnished by the shape and point of attachment of the chromosomes of the homoeotypic division, and by the general numerical proportion of the different forms at various stages, confirms this notion of a persistence in the point of attachment. Such a persistence is hardly conceivable, it seems to me, without supposing also that some of the kinoplasmic fibres have a continuous existence; and so the facts just described afford some confirmation for the doctrine of fibrillar persistence.

The Metaphases and Anaphases.

The separation of the more numerous rod-shaped daughter chromosomes begins, as a result of the method of their attachment to the spindle, at the ends which have been so long in contact. As these ends are pulled apart (Fig. 107), the unattached, originally more or less divergent, ends are frequently brought into contact, and the final separation of the daughter chromosomes is then at the junction of these originally free ends in the equatorial plane. The ends which are now turned toward the poles are hooked (as was the case in the previous division), due to the lagging behind of some of the substance of the attached end of the chromosome, or to a bending at the point of attachment if this is not quite at the end, so that the daughter chromosome has the shape of a J. Some are not hooked, but appear as straight rods.

The same variations in shape of the daughter chromosomes which

were noted in the equatorial plate appear during their separation. At *a* and *a'*, Fig. 107, appear two, evidently sister chromosomes, showing a form intermediate between that of a hooked rod and a V ; and at *b* and *b'* (same figure) are two V-shaped sister chromosomes, each having one arm somewhat shorter than the other. Similar forms appear in later stages in the separation of the chromosomes, and during their passage toward the poles.

As the chromosomes approach the poles, they become considerably straightened out, the rod-shaped ones then often reaching nearly from the pole to the equator. After reaching the pole, however, they seem to become shorter and thicker (a process which was also noted in the previous division). The equatorial ends then become curved, and finally all the chromosomes are bent and closely compacted together. The extra-nuclear nucleoles, which were numerous in the prophases, become less numerous, and frequently almost entirely disappear by the time of the formation of the mature spindle. They reappear, however, in considerable numbers during the separation of the daughter chromosomes, and at first are especially numerous in the equatorial region. After the formation of the cell-plate, as in the preceding division, they gather in the neighbourhood of the reforming daughter nuclei, and often some of the smaller ones seem to fuse into larger bodies. The reconstruction of the daughter nuclei is closely parallel to the corresponding process in the preceding division.

Discussion.

The Visible Idioplasmic Structures.

It is well established that in both plants and animals, as first shown by van Beneden ('83) for *Ascaris*, the union of the sexual nuclei involves no fusion of their chromosomes ; the latter pass, apparently unchanged, into the fusion nucleus. We must also, as the case stands at present (see Boveri, '04), accept the notion of the general persistence of individual chromosomes, and the consequent reappearance in the division of any somatic nucleus of the same chromosomes which were present in the anaphases of the division immediately preceding. New evidence of this general fact, derived from a study of plant nuclei, has recently been published by Rosenberg ('04 *c*).

The acceptance of the doctrines just stated involves the further notion that the chromosomes received respectively from the germ-cells of the male and female parents remain separate throughout the life-history of the offspring. Every nucleus of the new generation is therefore double, in the sense that it contains two uncombined sets of chromosomes, and therefore

(on the assumption that the chromosome substance is the bearer of the hereditary characters of the organism) the basis for two separate and more or less different sets of individual qualities. A considerable amount of evidence has been brought forward, especially by Rückert ('95) and Häcker ('97, '02), tending to show that not only do the parental chromosomes retain their individuality, but, at least in the earlier divisions, present a distinct separation into two groups; and Häcker finds that at various stages in the ancestry of the germ-cells, even down to the appearance of the primary oöcyte and primary spermatocyte, the nuclei show, by their two-parted, two-lobed or flattened form, and by the possession of two nucleoles and occasionally of two spirems, evidences of their twofold nature. On the other hand, Moenkhaus ('04), who succeeded in obtaining hybrids between two species of fishes having chromosomes differing in form and size, finds that, although the chromosomes retain their morphological individuality, they lose their original grouping after the first two cleavage divisions. The fact that in these later stages Moenkhaus found bilobed and binucleolated nuclei, as well as a grouping of chromosomes into two (mingled) groups, shows that while the phenomena discussed by Häcker may result from the continued independence of the two parental substances, they are not sufficient evidence for the acceptance of the view that these substances are isolated in different regions of the nucleus.

It should be added 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, by which during the life of the organism some chemical or other change is effected in the constitution of the chromosomes themselves. All that seems reasonably certain is that the chromosomes remain as distinct entities, and that (at least in the direct ancestry of the germ-cells) they undergo no visible modifications except those attendant upon their alternate growth and division from one cell generation to another.

In connexion with the constancy of the chromosome number and the persistence of individual chromosomes, the question arises whether all the chromosomes in a given nucleus are essentially similar to one another, or whether they differ in structure and function. That the chromosomes received by the organism from either parent are sufficient for its complete development is shown by Boveri's ('89) experiments on the fertilization of enucleated egg-fragments, and by recent work on artificially-induced parthenogenesis (Loeb, '99; Wilson, '01). The nucleus of the sexually-produced individual, then, contains at least two complete sets of hereditary substances, either set capable of inducing the development of a complete individual. Boveri's ('02) experiments on double-fertilized sea-urchin eggs, which divide simultaneously into three or four cells containing varying numbers of chromosomes, go far toward showing a qualitative

difference between the individual chromosomes derived from either germ-nucleus.

In harmony with this evidence as to a difference in function is the fact that the chromosomes of a single nucleus often differ greatly among themselves as to size and shape. Such differences have been noted, for example, by Guignard ('99) in the pollen mother-cells of *Naias*, and by Boveri ('04) in sea-urchin eggs. I have already spoken of the variations in size of the chromosomes of the lily in both the heterotypic and homoeotypic divisions. Strasburger ('00) finds in the pollen mother-cells of *Funkia* two sorts of chromosomes—some short, hardly longer than broad, and others several times as long as the former. Perhaps the most remarkable observations of similar phenomena are those of Sutton ('02) on the spermatogonial chromosomes of *Brachystola*. For nine cell generations preceding the formation of the primary spermatocytes he finds in each nucleus twenty-three chromosomes of varying size; of these, one is the 'accessory chromosome.' The remaining twenty-two may be arranged, according to their size, in eleven pairs, those of each pair being approximately equal in size, but differing in this respect from those of any other pair; and the eleven pairs form a progression from smallest to largest, such that the proportional difference in size between any two pairs in one nucleus is practically the same as that between the corresponding pairs in any other nucleus. It seems very natural, at least, to suppose that such constant differences in appearance between the individual chromosomes correspond in some way to the functional differences which Boveri's experiments indicate. Sutton's observations also seem to make it certain that of each pair of similar chromosomes in a somatic nucleus, one was ultimately derived from each parent; the two elements of a pair are, therefore, in a sense, homologous, and are probably to be thought of as covering the same portion of the field of individual development. Very similar size relations have been found by Montgomery ('04) in the spermatogonia of *Plethodon* and *Desmognathus*.

Somewhat analogous phenomena are those which consist in the appearance of one or two chromosomes differing greatly in size from the other elements within the nucleus. To this class belongs the peculiar body called by McClung ('99) the 'accessory chromosome,' which seems to have been first discovered by Henking ('91) in the spermatocytes of *Pyrrhocoris*. McClung ('02) has suggested a possible function for this particular element, namely, that it determines the production of male reproductive cells in the individual in which it occurs. Montgomery ('04) has classed this and similar aberrant chromosomes under the general term 'heterochromosomes.' Schreiner ('04) also finds marked differences in size between the chromosomes in the spermatogonia and spermatocytes of *Myxine*. In the spermatogonia two chromosomes are especially distinguished by their larger size from their fellows.

Not only do the chromosomes in all probability differ among themselves in structure and function; but, in view of the extremely complicated facts of heredity, as well as of the pains taken in somatic divisions to ensure an exact longitudinal halving of each chromosome, we are forced to think of these elements as by no means simple in structure, but rather as having a characteristic and complex organization. Observation has shown the chromosomes, or at least the spirem-thread before segmentation, to be made up as a rule of chromatin bodies (chromomeres) embedded in a less chromatic ground-substance (linin). Strasburger ('82) found that the chromomeres are formed by a progressive fusion of much smaller granules, and this observation has been confirmed in many cases. The chromomeres, as I have observed them in the lily, seem quite plainly to be aggregates of smaller bodies. Little has been done toward a determination of the exact number of either the chromomeres or their constituent parts, largely because the differential staining power of the two substances in the spirem is commonly lost as the contraction of the thread proceeds, and the chromosomes appear homogeneous. An elaborate organization of the chromosomes, which are subdivided into 'chromomeres,' and these into 'chromioles,' has been described by Eisen ('00) for the spermatogenetic divisions of *Batrachoseps*.

It was first observed by Pfitzner ('81) and Flemming ('81) that the longitudinal splitting of the spirem is preceded by a fission of the chromomeres; and the common assumption has been that this process involves also a fission of each of the smaller granules of which the chromomere is composed. This assumption is usually incapable of proof by direct observation; but it is supported by Brauer's ('93) discovery of a fission of each of the chromatin granules in the thread; these granules are very small at this stage, and later combine into a much smaller number of chromomeres.

Weismann ('92) identified the darkly-staining chromomeres with his hypothetical 'ids,' or 'ancestral germ plasms,' each of which contains within itself all of the qualities of the species, but differs from its fellows by the possession of a certain ancestral combination of individual characters. This notion of the identity of the 'id' with one of the visible chromatin bodies was accepted by Strasburger ('94). He considered the 'id' as made up of a number of smaller chromatin granules, together with more or less linin. Brauer ('93) thought that the chromatin granules, rather than the chromosomes, are the elements which retain an individual existence from one mitosis to the next. From the facts that have been mentioned regarding the formation of the chromatin bodies in the spirem by an aggregation of smaller granules, it is plain that the use of such terms as 'id,' 'chromomere,' or 'chromiole' cannot imply any certain homology between the structures to which the same name is applied in different species. As

the contraction of the spirem proceeds, constantly larger aggregates of granules result; and it is not improbable that the 'chromomeres' observed in two different cases may represent very different degrees of aggregation. Nor is there any reason to suppose that the smallest visible granules represent either the ultimate units (if such there be) or, in different cases, homologous combinations of such units. Therefore the notion that a longitudinal splitting of the spirem involves a fission of ultimate hereditary units, while it is in consonance with observed facts, does not at present rest upon any sort of direct proof.

Outward Manifestations of Idioplasmic Activity.

In an experimental study of heredity we have to deal, at least in large part, with the reappearance or non-reappearance of the offspring of certain parental characters. The reappearance of one such character seems in many cases to be independent of the presence or absence in the same individual of any other character; in other words, the hereditary endowment of an individual is at least in part analyzable into separate qualities which seem to act as independent units. For such a unit quality Bateson ('02) has proposed the name 'allelomorph.' Whether the whole hereditary endowment of an individual is capable of analysis into unit qualities is as yet undetermined. Further, there is evidence that in some cases a correlation exists between these simple allelomorphs, so that two or more of them may form a sort of compound unit; in such a case, one quality always appears accompanied by the correlated quality. For instance, Bateson and Saunders ('02) found, in their *Matthiola* experiments, that a correlation always exists between green seeds and purple or claret flowers; and similar instances are common in the experience of plant and animal breeders. In other cases, there may perhaps be an apparently simple character, which, however, is really compound, and which, although normally transmitted as a unit, under certain conditions may split up into its components. Bateson and Saunders have shown that the conception of such 'hypallelomorphs,' which may break up into simpler units as a result of cross-fertilization, offers at least a convenient hypothesis for the explanation of some apparently aberrant phenomena.

These conceptions, derived from experimental study, of simple units (allelomorphs) and units of a higher order (correlated allelomorphs and hypallelomorphs), seem to find an analogy in the structure of the spirem-thread, containing, as it does, a series of chromomeres, which in turn are composed of smaller bodies. It is impossible to avoid the notion that this structure of the hereditary substance in some way corresponds to the compound nature noted in the character of an organism. More than this it is probably not safe to say at present. The smallest visible chromatin

granules may themselves well possess an inconceivably complex structure; and it is not yet practicable to attempt to identify the lowest observable order of elements in the spirem-thread as the bearers of the apparently simple unit qualities manifested by the organism.

If the parents, the transmission of whose qualities to their offspring is to be studied, are of the same race, and so differ from each other only in the minor respects which fall within the range of individual variation, the study of heredity offers serious difficulties. It is, therefore, when the parents differ from each other by characters of a racial or of a higher order that the inheritance or non-inheritance of special parental qualities is most easily determined; and consequently, the experimental facts which are of most value for correlation with, and elucidation of, the results of cytological study are, in general, those derived from a study of cross-breeding between individuals of different races, varieties, species, or even genera.

It has been shown that the parental hereditary substances (idioplasms) to all appearances remain separate during the life of a sexually-produced individual, so that each cell of such an individual contains the physical basis for two complete sets of individual qualities. As to any particular quality, then, with respect to which the parents differed—e.g. shape of leaves—there are in the offspring two different, and therefore more or less conflicting, hereditary tendencies. As a consequence of the joint action of these two tendencies, some resultant quality must appear in the offspring, whose relation to the corresponding qualities of the parents may be shown in one of two ways—either the resultant quality may be intermediate between the parental qualities; or one parental quality may entirely predominate over its opponent, and the offspring in this respect exactly resemble one parent. Observation shows that both of these hypothetical cases are actually realized.

A well-known instance of a blending of parental characters is furnished by the hybrid *Drosera obovata*, whose leaves are intermediate in shape between those of the parents, *D. rotundifolia* and *D. longifolia*. Mendel ('65) found that in various *Pisum* hybrids the time of flowering was almost exactly intermediate between the times of the parents. Macfarlane ('90) observed in numerous cases that the blending extends to matters of cellular structure; for example, in a hybrid *Hedychium* the starch-grains were intermediate as to size and shape between those of the parents. A different sort of blending, apparently clearly due to the independent existence within the cell of the parental idioplasms, is that noted by Hildebrand ('89) in a hybrid between two species of *Oxalis*; in this instance both forms of hairs characteristic of the parent species arise from a single epidermal cell.

What may perhaps be considered a special case of the blending of parental characters is that in which the resultant quality appears to be,

not intermediate, but quite different from that of either parent. This occurs frequently with reference to qualities which may be taken as indicating an increased or decreased vigour in the offspring; as, for instance, Burbank's walnut hybrid (Swingle and Webber, '98), a cross between *Juglans regia* and *J. Californica*, which grows twice as fast as the combined growth of both parents. In the same category is a result obtained by Mendel ('65), who found that crossing a long-stemmed and a short-stemmed variety of pea gave a hybrid with a stem longer than that of either parent. The flower colours of several of Correns' ('02) *Mirabilis* hybrids are analogous phenomena; for instance, crosses between a white-flowered (*M. Jalapa alba*) and a yellow-flowered race (*M. Jalapa flava*) have red flowers. In the appearance of a hybrid character entirely different from the corresponding character of either parent, there is, as Bateson ('02) suggests, something analogous to the result of a chemical reaction. Since the idioplasm is composed of a complex chemical substance, or, more properly, substances, and since, as I have pointed out, the possibility of some interaction between two parental idioplasms within the same nucleus is not excluded by anything that we know at present, and since, further, the determination of cell characters by the idioplasm undoubtedly involves a series of chemical reactions, it is not surprising that we should obtain results of this character in many instances of the combined action of two different idioplasms.

In discussing such cases as those last mentioned it is necessary to be certain that, with respect to the quality in question, both parents were pure-bred; otherwise, the appearance of a character different from the corresponding character of either parent may be simply the reappearance of a quality of a more remote ancestor, and not at all the result of a combination of the two parental qualities. Cases of the appearance of a character different from that of either parent are, therefore, unless the pedigree of the parents is fully known, liable to the suspicion that they are merely due to the reappearance of a latent or recessive allelomorph.

On the other hand, a conflicting action of the parental idioplasms may result in a quality which is in no sense intermediate, but which exactly resembles the corresponding character in one parent. It was the observation of such phenomena that led Mendel ('65) to the conception of parental qualities as either 'dominant' or 'recessive.' When, for instance, he crossed two varieties of *Pisum*, one having green, the other yellow, cotyledons, all the offspring had yellow cotyledons; in other words, the character of possessing yellow cotyledons is dominant, that of possessing green cotyledons is recessive. In the same way, the possession of round, smooth seeds is a dominant quality, that of angular, wrinkled seeds is recessive. The so-called 'law of dominance' was never stated by Mendel as a general law; and cases of intermediate character similar to those

I have just mentioned show that, although in many instances it holds with greater or less exactness, it is very far from being universal. It should be noted also that even in cases which are classed as illustrations of dominance, as Correns ('03 *a*, '03 *b*) has pointed out, the dominant quality by no means always appears in the offspring in the intensity which was characteristic of the dominant parent. Thus, Mendel says that 'the hybrid character resembles that of one of the parental forms so closely that the other either escapes observation completely or cannot be detected with certainty.' This is illustrated by the colour of the embryo in the *Matthiola* crosses of Bateson and Saunders ('02), in which green usually appeared to be the dominant colour, but was often much diminished in the crosses. A series of such facts might be collected, which would show every conceivable gradation between the complete dominance of one parental quality and the appearance of an exactly intermediate character in the offspring. Nevertheless, the frequently-occurring fact of dominance, taken in connexion with the reappearance of the recessive character in the next generation, is important as showing that the physical bases for two more or less antagonistic qualities may exist side by side in the same nucleus without any such interaction as materially affects the nature of either.

Considering the cross-bred individual as a whole, it constitutes a complex of qualities, some of which may approach, or be identical with, the corresponding qualities of the father; others bear a like relation to those of the mother, and still others are intermediate between, or even entirely different from, the corresponding qualities of either parent. As a result of the summation of all these various qualities, the offspring occupies, in general, a position intermediate between the respective parents. In the simplest possible case—that, namely, in which the parents differ in only one quality—if dominance occurs with respect to that quality, the offspring exactly resembles one parent. The same result may follow in more complicated cases, provided all the characters of one parent in respect to which the parents differ happen to be dominant. This, however, becomes less probable with an increase in the number of differences between the parents. The possibility of an exact resemblance between the hybrid and one parent may be illustrated by the much-discussed 'false hybrids' of Millardet ('94). The fact that these hybrids breed true may be due to what we may term a permanent dominance, as distinguished from the ordinary (Mendelian) form; or it may result from a complete disappearance of one set of characters, due to something like a destruction or extrusion of one parental idioplasm.

It will naturally be expected that whatever balance may be struck in one case as the result of the combined action of two idioplasms will appear in other cases in which, under substantially similar conditions, the same or similar idioplasms come into a like relation. This implies that

the individual resulting from a particular cross will display throughout life the same combination of congenital qualities; and also that different individuals resulting from the same cross will closely resemble each other. These expectations also are in general realized by the facts presented by hybridization experiments. It appears, however, not unnaturally, that the balance which may be struck between the more or less conflicting idioplasms is a matter of delicate adjustment, subject to modification by various influences, such as differences of nutrition, and perhaps in many cases by internal causes which cannot at present be traced.

As to the general constancy during ontogeny of the characters of a hybrid individual, it is a recognized rule that, for instance, the colour, shape, and size of the flowers of a hybrid plant are as constant as in a pure-bred individual. In the case of the *Drosera* hybrid already mentioned, the characteristic leaf-form prevails throughout ontogeny. The same is true regarding the various characters studied by Mendel in his *Pisum* hybrids.

But it is not difficult to find exceptions to this rule, which illustrates the comparative instability of the balance between the parental idioplasms. One class of exceptions includes the 'mosaic' hybrids, several of which were described by Darwin ('68). Among these are Lecoq's crosses between different coloured varieties of *Mirabilis*, some of which produced flowers half of one colour, half of another; and the case of a mongrel dog, part of whose skin was hairy and part naked, the parts being distinctly separated. The flowers of crosses between the carnations Scott and McGowan (Swingle and Webber, '98) show, side by side, the colours of the two parents. A frequently cited instance is *Cytisus Adami*, occasional branches or single flowers of which revert to the type of either parent. De Vries ('03), who considers this plant a true hybrid, and Lotsy ('04) suggest that the reversion of certain members may be due to a qualitatively unequal nuclear division interpolated among the normal somatic divisions, so that certain cells receive only one parental idioplasm in nearly or quite pure form. Correns ('03 *b*) and Halsted ('04) have obtained hybrid maize-grains whose endosperm displays in different parts of the same grain the distinctive characters of the two parents.

A very different class of exceptions to the same rule consists of those hybrids which undergo changes during ontogeny. In this class Darwin ('68) cites Gärtner's hybrids between *Tropaeolum minus* and *T. majus*, whose flowers were at first intermediate, but some of which later in the season produced flowers in all respects like those of one parent. Cannon ('03 *a*) mentions several analogous cases. Whether such exceptional behaviour is due to environmental influences, or to local internal conditions, it accords well with the conception of the presence throughout the life of the hybrid of two separate, independent idioplasms.

The rule as to the similarity among individuals of the same cross is by no means of so general application as that of the constancy of individual characters during ontogeny. Nägeli (quoted by Swingle and Webber, '98) finds that 'in general the hybrids in the first generation vary the less the more distantly related the parent forms are; that is, the specific hybrids vary less than the varietal hybrids, the former often being characterized by great uniformity, the latter by great diversity of form.' And Swingle and Webber note the general fact that 'races of cultivated plants, even though very diverse, produce very variable hybrids in the first generation, while usually by crossing wild species closely resembling each other hybrids are obtained which are constant in the first generation.' Similarity between hybrids of the same parentage is illustrated by most of the cases already considered, notably by Mendel's *Pisum* hybrids. Instances of variation are furnished by the *Hieracium* hybrids produced by Mendel ('69); also (Swingle and Webber, '98) by the two different crosses obtained by Gärtner between *Nicotiana quadrivalvus* and *N. macrophylla.*

Satisfactory evidence upon this point is, however, difficult to obtain, for the reason that the parents of any particular individual were themselves sexually produced; each of them received from its parents two different idioplasms, and the generation with which we are directly concerned is affected by the unequal distribution among its members of these grandparental idioplasms. In determining the amount of variation among the individuals produced by a given cross it is, therefore, necessary to be certain that the parental idioplasms were pure as respects the particular qualities under consideration. Absolute certainty upon this point is probably always impossible, and even approximate certainty is out of the question unless, as is usually not the case, we know the pedigree of the parents for many generations. In cases of variation among hybrids of the same cross, therefore, it is necessary always to take into consideration the possibility that the variation is due to ancestral influences, a possibility which is especially strong in the case of cultivated races, and which, no doubt, accounts for much of the variation noted among hybrids of such races. It is very possible that, if perfect certainty with respect to ancestry were attainable, uniformity among offspring of the same cross between pure-bred parents would be found much more nearly universal than at present it appears to be.

A special case, to be classed with those just discussed, is afforded by the fact that, in most cases, it makes no difference in which direction the cross takes place—that is, a cross between *A* male and *B* female gives the same results as one between *A* female and *B* male. This seems to imply that differences in the source of the respective idioplasms do not, by differences in their relative position within the nucleus or otherwise, affect their capacity for determining or influencing external characters. Occasional

notable exceptions to this rule are known, in the form of differences between reciprocal crosses, e. g. the mule and hinny, Gärtner's and Focke's (Swingle and Webber, '98) *Digitalis* crosses, the *Nymphaea* hybrids of Caspary, and some of the grape hybrids studied by Millardet.

In the foregoing paragraphs I have attempted to point out what seems to me the remarkable parallel existing between the data obtained by the experimental study of plant and animal breeding on the one hand, and, on the other, such results of cytological investigation as the constancy of the chromosome number, the continuous separation between the parental elements within the nuclei of the offspring, the individual persistence and distinctive appearance of the chromosomes, and the finer details of their structure. I shall return later to a consideration of the experimentally-derived facts and of the light that they may throw upon the problems with which the present paper is especially concerned.

The Reduction of the Chromosome Number.

The conception of the individuality of the chromosomes, together with the fact of the general constancy of their number in any particular species, involve, as Boveri ('88, '90) early pointed out, the necessity, at some period in ontogeny, of a reduction of their number to one-half, in order that that number may not be doubled in each generation by the fusion of the sexual nuclei. We now know that such a reduction does occur at a definite point in the life-history of every sexually-produced individual, at least among the higher animals and the higher plants; that in some way two successive nuclear divisions, differing from the ordinary type, are concerned in this numerical reduction ; and that the reduced number of elements (whether at this stage involving a real or only an apparent reduction) is first to be observed in the prophases of the earlier of these two peculiar divisions. We know, too, that in the Metazoa the divisions concerned in chromosome reduction are the two immediately preceding the formation of the definitive sexual cells ; and that in the Spermaphytes, Pteridophytes, and Bryophytes, these divisions are the two which result in spore-formation, and which, therefore, determine the transition from the sporophyte to the gametophyte generation.

The prophases of the first of the two divisions just referred to (the heterotypic division) are characterized by their unusually long duration, and by the appearance of a stage in which the nuclear constituents are aggregated into a more or less compact mass, usually in contact with one side of the nuclear membrane. This stage of aggregation, to which Moore ('95 *a*) gave the name ' synapsis,' is regularly found, so far as we now know, only in the prophases of this division. Although this condition had been described by various writers, Brauer ('93) seems to have been the first to

suggest that it is in some way connected with the formation of only half the usual number of chromosomes; and Moore ('94, '95 *a*) first definitely maintained that it is during synapsis that the numerical reduction occurs.

Those observers who have found what they consider reduction divisions in Weismann's sense have for the most part interpreted their results in essential accordance with those of Rückert ('92 *a*, '92 *b*, '94), Häcker ('90, '92, '93, '95, '99), and vom Rath ('92, '93); that is, they find that in the prophases of the heterotypic division the chromosomes are bivalent, consisting of two somatic chromosomes attached end to end; and that in one or the other of the two succeeding divisions these two somatic chromosomes are distributed to different nuclei. This method of reduction has been described especially among the Arthropods. Montgomery ('01) suggested that the two chromosomes so joined end to end are always derived from different parents; synapsis is, then, a process of conjugation of the maternal and paternal elements, its object being a 'rejuvenescence' of the chromosomes. He thought it probable that there is an exchange of substances during this temporary contact.

Cannon ('02, '03 *a*) suggested, as a cytological basis for Mendel's law of the purity of the germ-cells, that 'the chromosomes derived from the father and the mother unite in synapsis and separate in the metaphase of one of the maturation divisions, and also a single longitudinal division occurs, so that the end is attained that the chromatin is distributed in such a way that two of the cells receive pure paternal and two cells pure maternal chromosomes, and no cells receive chromosomes from both the father and the mother.' Cannon's notion of the purity of the germ-cells at this time was plainly quite different from that of Mendel, for the sort of division postulated by Cannon could not possibly result in new combinations of parental qualities such as Mendel actually found. This difficulty was recognized by Sutton ('03), and later by Cannon himself ('03 *b*). Sutton proposed to modify Cannon's conception by assuming that it is a matter of chance whether one or the other chromosome of a pair goes to a particular daughter nucleus; and this notion has been accepted by Boveri ('04). The result of the operation of chance in this respect would be that in a sufficiently large number of germ-cells from the same individual every possible combination of the parental chromosomes will occur. The number of possible varieties to be found among the offspring would depend, accordingly, upon the number of chromosomes characteristic of the species. Sutton showed that in cases of the occurrence of the commoner chromosome numbers the amount of variation thus made possible is relatively great; for instance, if, as in the lily, the reduced number of chromosomes is twelve, the number of possible combinations that may occur in the germ-cells of one parent is 4,096; and, as the result of the fusion of these germ-cells with those from a similar parent, the number of possible combinations of parental characters

in the offspring is 16,777,216. On the other hand, the reduced number of chromosomes in the pea, as Cannon ('03 *b*) finds, is seven; and he has pointed out that on Sutton's hypothesis there can be only seven groups of characters, each of which groups can be transmitted independently of any other. Now, Mendel found in varieties of the pea seven separate characters which were transmitted independently of each other; and if Sutton's notion be correct, each of these characters must be one of a set which corresponds to one of the indivisible chromosomes; and Mendel could not possibly, therefore, have found an eighth character which would follow his law of the separation of parental qualities. To suppose that Mendel so completely exhausted the possibilities of the case is probably giving him more credit than even his most devoted followers would be inclined to allow.

Montgomery's notion, that any two chromosomes which conjugate in synapsis are derived respectively from the male and the female parents, was supported by his observation ('01) that when, as in *Protenor*, *Peliopelta*, and *Zaitha*, certain spermatogonial chromosomes are distinguishable from the others by their size, these peculiar chromosomes pair with each other in synapsis. A striking case of the same sort is furnished by *Brachystola*, according to Sutton ('02). Here all the spermatogonial chromosomes, as already mentioned, can be arranged according to their varying sizes in pairs; and each bivalent heterotypic chromosome is formed by one of these pairs of hitherto separate elements. Similar phenomena to those found by Sutton are described by Montgomery ('04) for the spermatogenesis of *Plethodon* and *Desmognathus*, and for the oögenesis of *Ascaris megalocephala bivalens*.

Häcker ('02, '04) has observed phenomena in the oögenesis of *Cyclops brevicornis*, which he interprets as a conjugation of paternal and maternal chromosomes. In the germinal vesicle the bivalent chromosomes are arranged in two groups, representing respectively, he thinks, the maternal and paternal elements. The first maturation division is equational, so that the chromosomes passing to the secondary oöcyte may be represented by *ab*, *cd*, . . . (male), and *no*, *pq*, . . . (female). In the secondary oöcyte nucleus, a conjugation in pairs occurs between these chromosomes—e. g. *ab* (male), which is bent at its middle, comes into contact at the point of bending with the similarly bent *no* (female). In the second maturation division, a transverse division of each chromosome occurs at the point of bending, so that the separation in this division may be represented thus:

a (male)	*n* (female) . . . . .
↕	↕
b (male)	*o* (female) . . . . .

There is thus effected a thorough mixing of the parental elements, and each germ-cell receives an equal number of chromosomes originally derived from each parent.

These attempts to explain chromosome reduction by a separation of entire chromosomes are based, as has been said, upon what appear to be the facts particularly among the Arthropods. For those cases in which a double longitudinal splitting is observed, and in which, therefore, both heterotypic and homoeotypic divisions seem to be equational, it has been found much more difficult to frame a satisfactory hypothesis. Such a double longitudinal splitting was described by Brauer ('93) in the spermatogenesis of *Ascaris*; and a similar process has been found by various authors in widely divergent groups of plants and animals, particularly among the Vertebrates (Flemming, '87; Moore, '95 *b*; Meves, '96; McGregor, '99; Kingsbury, '99, &c.); and among the Seed Plants (Strasburger, '95, '00; Mottier, '03; Guignard, '99; Grégoire, '99, and others).

O. Hertwig ('90) conceived of the fusion of the sexual cells as involving a fusion of the parental idioplasms in such a way as to give rise to a new and different idioplasm characteristic of the new individual. The divisions resulting in the formation of the germ-cells, he thought, are equation divisions, and the germ-cells formed by the same individual are all similar. Strasburger ('94) thought that in the prophases of the heterotypic division each 'id' derived from one parent fuses with an 'id' derived from the other parent, the result being a new idioplasm which is equationally divided among the germ-cells. Brauer seems to have considered that no such fusion is necessary, but that the two parental idioplasms remain and are simply reduced in mass by two equation divisions. Montgomery ('01), Sutton ('03), and Boveri ('04) have suggested that the phenomena in Vertebrates may be harmonized with those in Arthropods by supposing that in the former the chromosomes conjugate side by side instead of end to end, and that one of the apparent longitudinal splittings is, therefore, really a separation of two independent somatic chromosomes. This suggestion is an approximation to the facts as I have described them in the lily; but the fusion of the two spirems into a single thread, as I have observed it, is a very different process from a temporary attachment of the chromosomes end to end; and the two methods may be expected, if they actually occur in different groups of organisms, to lead to very different results as regards the transmission of hereditary characters.

It seems quite possible from the figures of Brauer ('93) that the occurrences in *Ascaris* are really very similar to those in the lily. He finds that at about the beginning of the synaptic period certain strands of the nuclear network appear double, while at the close of synapsis there is present a continuous doubly-split spirem. He thinks it probable that the two longitudinal fissions are nearly or quite simultaneous, and that, owing to their fineness in the earlier stages, the strands appear double when they may really be four-parted. The double longitudinal splitting of the spirem certainly occurs earlier in *Ascaris* than in the lily; but

between the first appearance of double strands and that of a four-parted spirem a considerable time evidently, from Brauer's figures and descriptions, elapses; and it is at least conceivable that the occurrence of a double thread at the very beginning of synapsis is the same phenomenon that I have observed at the same stage in the lily, and that in *Ascaris* also these two threads actually fuse into one, which soon after is doubly split. Miss Sargant ('96, '97) and Farmer and Moore ('95) also describe two rows of 'chromatin dots' in the nuclear threads of the lily previous to synapsis; but, as I have pointed out, there is some doubt as to the real nature of these bodies.

Sabaschnikoff ('97) attempts to harmonize the occurrences in the oögenesis of *Ascaris* with Weismann's notion of a reduction division. He thinks that, during synapsis and previous to the formation of a spirem, the chromatin granules become arranged into groups of four, not by a double fission of single granules, but by the approximation of originally separate bodies. The tetrad groups are connected with one another by fine linin fibres; they approach each other and become arranged to form a spirem, which is thus composed from the start of four threads. Sabaschnikoff's view is not very different from that of a conjugation of separate spirem threads, or at least of their constituent chromatin granules; but it involves no notion of a fusion; the grouping of the granules is simply preparatory to their ultimate distribution into different germ nuclei.

Winiwarter ('00) seems to have been the first actually to observe a fusion of parallel threads in the prophases of the heterotypic division. He finds this process to occur during synapsis in the oögenesis of the rabbit and of man, and his figures are very similar to mine. The result of the fusion is a comparatively thick moniliform thread (whether continuous or not at this period he does not determine), which later splits longitudinally. A complete fusion in pairs of all the chromomeres seems not to occur; the double nature of the thread remains always apparent in places. Winiwarter considers the longitudinal splitting to involve merely a separation of the threads which previously fused. Schoenfeld ('01) finds in bovine spermatogenesis a similar fusion of slender threads in pairs.

In a recent preliminary paper, A. and K. E. Schreiner ('04) describe a like series of processes in the spermatogenetic divisions of *Myxine* and *Spinax*. According to these authors, the material of the daughter chromosomes of the last spermatogonial division becomes distributed upon fine linin fibres; there is no continuous spirem, but in synapsis the strands aggregate in that half of the nuclear cavity nearest the centrosphere; they lie parallel to each other, or converge slightly toward the pole. The strands now approach each other and gradually fuse in pairs. Somewhat later a longitudinal splitting appears, which the authors seem to consider

as a separation of the original threads, which have been for a time in contact. Each of the parallel threads now contains a row of granules substantially uniform in size. In this stage in *Myxine* there is sometimes present also an indication of a second longitudinal split at right angles to the first. As a result of shortening and thickening, and of different degrees of separation of the daughter segments, the mature chromosomes appear variously as rings, 8's, loops, V's, X's, or pairs of parallel rods; they also often plainly show the second split. The separation in the first mitosis, as in the lily, is in the plane of the first split; each daughter chromosome is two-parted as a result of the second split, and the two parts are separated in the next mitosis.

The Schreiners accept Sutton's notion of a pairing of homologous paternal and maternal chromosomes, and their subsequent separation in such a way that it is purely a matter of chance whether a particular chromosome shall pass to one or the other daughter nucleus. During the time of contact of the two chromosomes of a pair, there occurs a more or less intimate fusion (conjugation or copulation). These authors attempt to harmonize the facts of chromosome reduction in the Arthropods with their own observations on Vertebrates by assuming that in the former group there occurs first a parallel arrangement of two chromosomes, then a separation at one end, the chromosomes remaining attached at the other, so that when they are finally separated in the metaphases of one division or the other the appearance is that of a transverse separation. This supposition is supported by the various appearances of the chromosomes in *Spinax*. Here the most common form is that of a closed ring, resulting from the continued attachment of the chromosomes of a pair at both ends; but a separation may occur at one end, leading to all possible variations in form from that of a ring broken at one point to that of two straight rods attached at one end and making various angles with each other. If the latter form should become common, the appearance of the chromosomes would be similar to that described for many Arthropods.

De Vries, largely upon hypothetical grounds, has recently ('03) concluded that, shortly before the separation in the heterotypic division, the chromosomes become arranged side by side in pairs, the arrangement being such that each paternal chromosome is paired with a homologous maternal chromosome, and each paternal pangen, or group of pangens, is opposite the corresponding maternal structure. In this condition of intimate contact between the parental idioplasms, a mutual interchange of some of their pangens occurs, in such a way that when the chromosomes later separate, each set, whether paternal or maternal, contains a complete set of pangens, but some of the latter are paternal, some maternal, in origin. This interchange of pangens is determined entirely by chance, so that, if the same process is repeated in a sufficiently large number of cells, every possible

combination of a complete set of paternal and maternal pangens will result. The separation of the chromosomes after this interchange results in the appearance of a longitudinal splitting of the chromosomes—the 'first longitudinal fission,' which has been observed in many cases, especially in plants. It is true that in the case of many animals this separation seems to be transverse; but de Vries suggests, as do the Schreiners, that after the longitudinal fission the chromosomes may remain temporarily in contact by their ends, their final complete separation then producing the effect of a transverse breaking. In the case of the offspring of two individuals of the same race or variety, the two idioplasms resemble each other exactly as to the number and general nature of their pangens; the process just described, then, results in an exchange of like elements, which differ from each other only in those minute details which correspond to the externally apparent differences between individuals of the same race. In the case of a cross between a variety and its mother species, we have to deal with two idioplasms which differ from one another only as regards a single pangen, which is present in both, but is active in one case, latent in the other. Evidently the active and the latent pangen are as capable of being exchanged as are any of the other pangens, and the two idioplasms may apply themselves to each other as exactly as in the case first mentioned. When we come to a cross between two different species, however, an exact correspondence between the two idioplasms is no longer to be expected; the number of pangens, or their arrangement, may differ, and so the application of the idioplasms to each other and the consequent interchange of pangens is made difficult. If the parents belong to very closely related species, this difficulty may not be a serious one; but as the relationship becomes more distant the difficulty increases until the processes leading to the formation of the germ-cells become impossible, and the hybrid is necessarily sterile.

Lotsy ('04) agrees with de Vries that in those cases in which a double longitudinal fission of the chromosomes has been observed, each chromosome must be considered as bivalent, and as resulting from a lateral apposition of a paternal and a maternal segment. He points out that, depending upon the plane in which the split occurs, a longitudinal division of such a bivalent chromosome may be either equational or qualitative; and he concludes that of the two longitudinal fissions observed to occur, one results in an equation division, the other in a reduction division. He points out that the difference between the two planes of separation is brought about either by the fact that the successive division-spindles lie in planes at right angles to each other, as in the division of animal spermatocytes, or, if the two divisions are to be in the same plane, as in the formation of polar bodies from the animal egg, by a revolution of the chromosomes themselves through an arc of 90°. Lotsy considers that, as

de Vries has suggested, the object of the lateral apposition of paternal and maternal chromosomes is an exchange of pangens, and that as a result of the heterotypic and homoeotypic divisions each germ-cell contains only paternal or only maternal chromosomes.

Very interesting phenomena have been observed by Rosenberg ('04 *b*) in the pollen mother-cells of *Drosera*. He finds that shortly after synapsis the short chromosomes lie side by side in pairs; at first the two of a pair may be some distance apart, but soon they come into close contact with each other. The number of pairs is the reduced chromosome number. A fission of each chromosome occurs early, sometimes before the two of a pair are in contact; this fission may be parallel or at right angles with the plane of contact of the chromosomes. The heterotypic division separates the chromosomes of a pair; and the separation in the homoeotypic division is in the plane of the fission just mentioned. Rosenberg ('03, '04 *a*) has also studied a hybrid between *Drosera rotundifolia* and *D. longifolia*. The reduced number of chromosomes in the parents is respectively ten and twenty; thirty is the number in the somatic cells of the hybrid. In the heterotypic division in the pollen mother-cells of the hybrid twenty chromosomes appear, ten of which are double, ten single. The ten double chromosomes divide in the usual way; the single chromosomes either pass undivided to one or the other pole, or else are left on the spindle and take no part in the formation of the daughter nuclei. From the occurrences in the pollen mother-cells of the pure species, it must be inferred that each double chromosome in the hybrid results from the conjugation of a maternal and a paternal element; while the single chromosomes are those derived from the *longifolia* parent which have failed to find mates among the smaller number from the *rotundifolia* parent. This inference is confirmed by the fact that the chromosomes of *D. rotundifolia* are much larger than those of *D. longifolia*; and that each double chromosome in the hybrid consists of a larger and smaller daughter segment.

Berghs ('04) has studied the heterotypic and homoeotypic divisions in the microsporogenesis of *Allium fistulosum* and *Lilium speciosum*; and his latest results, as reported by Grégoire ('04), agree very closely, as to the fusion in pairs of the strands of the spirem during synapsis, the double longitudinal splitting, and the subsequent history of the chromosomes, with my observations. Both of these authors seem inclined to deny the formation of a single continuous spirem, although Grégoire (p. 308) says that after the completion of the fusion the spirem appears to be unsegmented. They consider the first longitudinal splitting as merely the separation of threads which have been for a time in contact but have retained their autonomy.

The apparent difficulty of reconciling the notion of a double longi-

tudinal splitting with the facts developed in recent experimental studies of hybridization, has led a number of observers to a re-examination of those cases in which the double splitting had been described; and some of these observers have been convinced that such a double splitting actually does not occur. Farmer and Moore ('03), reversing their former ('95) views, now hold that the apparently double chromosomes appearing in the heterotypic mitoses in both plants and animals are formed by a bending upon itself in the middle of each segment of the spirem. The spirem splits longitudinally before segmentation, but the split becomes nearly or quite obscured, to reappear as a fission of the daughter chromosomes during or after their separation in the metaphases. This notion, if correct, would bring the facts in the Seed Plants and Vertebrates more nearly into harmony with those commonly described for the Arthropods. A study of the sporogenous divisions of several species of Ferns by Gregory ('04) gives results similar to those of Farmer and Moore; and Williams ('04), who finds chromosome reduction to occur in the formation of the tetraspores of *Dictyota*, obtains in this material further corroborative evidence as to the method of formation of the chromosomes. Cannon ('03 *b*) has brought forward some (by no means convincing) evidence that, in the ancestry of the microspores of both pure and hybrid races of peas, the chromosomes become arranged in pairs end to end (or, in one case, side by side) in the anaphases of the last pre-heterotypic division. Montgomery ('03) has recently attempted to show that in Amphibia as in Arthropods there occurs a conjugation of chromosomes end to end in pairs, and that the heterotypic division separates the two chromosomes of a pair. He finds, therefore, only a single longitudinal splitting.

Strasburger ('04) has also reversed his former opinion ('00) concerning a double longitudinal splitting in the prophases of the heterotypic division, and comes to substantially the same conclusions as Farmer and Moore. He finds that in the pollen mother-cells of *Galtonia* and *Tradescantia* each chromosome divides transversely into two segments, which are finally separated in the heterotypic mitosis; from the method of their formation, it follows that the segments of a pair are contiguous to each other in the spirem before segmentation. The longitudinal split, which was visible in the spirem at an early stage and then disappeared, reappears in the metaphases and anaphases of the heterotypic division, forming the granddaughter chromosomes which are to be separated in the homoeotypic division. Strasburger finds some evidence, in the arrangement of the chromosomes in the equatorial plate in the heterotypic mitosis of *Tradescantia*, of variations in the arrangement of different pairs of daughter chromosomes which might result in such varying combinations of maternal and paternal segments as is postulated by Sutton and Boveri. Strasburger has also studied the stages preceding those just mentioned, and he finds

during synapsis occurrences which seem to him to throw much light upon the real nature of the heterotypic division. In the synapsis stage in *Galtonia* and *Thalictrum* he finds (as is reported also by J. B. Overton, '04) that the chromatin becomes separated from the linin fibres, and, in the form of fine granules, aggregates into groups whose number corresponds to the reduced number of chromosomes which are later to be formed. The granules of each group unite into a small, dense body (zygosome), which elongates somewhat, becomes constricted in the middle, and then breaks up again into granules; these become distributed along the linin, so forming a continuous spirem thread, which, after the disappearance of the synaptic condition, splits longitudinally. Strasburger considers that the formation of the zygosomes is a means for bringing into intimate contact the chromatin from two hitherto separate homologous chromosomes, one paternal and one maternal; and that the material of each zygosome becomes distributed along the linin thread to form a heterotypic chromosome, whose later transverse division has been foreshadowed in the constriction of the zygosome.

The evidence for these most recently developed views is not yet published except in preliminary form, and it seems very difficult to bring them into harmony with the best-known facts as to the heterotypic figures in the lily. My own preparations certainly show that two longitudinal splittings occur during the heterotypic division. The real significance of these two fissions, however, can be determined only in connexion with the fact that early in the prophases two spirems are formed, which come to lie alongside of each other, and finally fuse into a single thread. Häcker ('02) has been able to show that in *Diaptomus* the double nature of the nucleus may show itself even as late as the prophases of the last pre-heterotypic division, by the formation of a double spirem. The conclusion is inevitable, though, so far as my observations go, not susceptible of direct proof, that the two spirems which fuse in synapsis contain the substances derived respectively from the male and female parents. It is not surprising that the approximation of the two spirems should result in a massing of their substance in a space much less than that which it formerly occupied. The peculiar appearances of synapsis, so far as these concern the material of the spirem thread, thus receive a natural explanation; but the metamorphoses undergone by the nucleolar substance are not so easily accounted for. From my own observations, any transfer of substance from the nucleoles to the spirem or vice versa, at this or any other stage, seems to be excluded. But the constant occurrence in so many species of extremely flattened nucleoles at the time of synapsis may perhaps have a significance of which as yet we can form no conception. In this connexion some observations of Häcker ('02) are of interest. One of the most frequent indications of the 'autonomy of the nuclear halves' is the

appearance in the young nucleus of two nucleoles, which, if a long resting period ensues, commonly fuse into one. In the primary spermatocytes of *Diaptomus* two nucleoles appear in the early prophases and become fused during synapsis. This seems to be the only case he has discovered in which a fusion of nucleoles occurs during the prophases; and this unusual occurrence during this particular mitosis in *Diaptomus* may very well be a manifestation of the same general tendency toward a fusion of two groups of nuclear substances which shows itself, apparently universally, in a rearrangement of the chromatin. The fusion of the two spirems involves a change in the position of the material of one or both; it would be extremely interesting to determine whether there is a mutual approximation, or whether in this respect one is passive, the other active; but upon this point I have no evidence to offer.

The aggregation of the nuclear material begins, as I have said, in the very early stages of the formation of a uniform spirem; the movement, then, is not by the fully-formed spirem threads, but, in part at least, by the knots and fibrous material which constitute the reticulum. This fact also appears from the not infrequent occurrence, in the formation of the spirem, of two short threads ending in the same knot; such figures may well result from a previous fusion of the knots in pairs. But this occurrence is by no means general. The fusion of the two threads is followed by the fusion of the chromomeres in pairs; this fusion is not completed for some time after the threads have come in contact, but finally no evidence remains in the appearance of the chromomeres of their double origin. Occasionally a chromomere seems not to find a mate, but such occurrences are exceptional; and the most striking fact apparent in the approximation of the threads is that they come together in such a way that in general each chromomere in one thread lies opposite a chromomere in the other thread. All appearances indicate that the object of the whole process is the fusion of the chromomeres. The result, therefore, seems to be the bringing into at least intimate contact of the chromatin derived respectively from the two parental germ nuclei. In speaking of the fusion of the chromomeres I do not mean to imply that I have observed any indication of the actual fusion of the smaller granules of which the chromomeres are composed; all that is apparent is a flowing together of two masses of granules into a single mass. How the number of granules present after the union compares with the total number before it is impossible to determine; plainly there are several conceivable possibilities in the case, which will be discussed in another connexion. It is sufficient for the present to say that the fusion of the two spirems effects that intimate association of diverse hereditary substances which was anticipated in the fusion of the parental germ-cells some hundreds or thousands of cell generations previously.

After the completion of the union of the two threads, the now seem-

ingly single spirem gradually distributes itself more uniformly throughout the nuclear cavity. An important difference is manifest between the arrangement of the spirem at this time and its arrangement in the prophases of previous divisions. In the earlier divisions the arrangement of the spirem displayed a distinct polarity, and was always closely similar to its arrangement in the preceding anaphases. This is not the case, so far as my observation goes, at any stage in the prophases of the heterotypic division. It is true that, as already noted, some authors have found a certain polarity in the arrangement of the chromosomes after segmentation, and Strasburger has noted a regularity in the course of the spirem of *Tradescantia*; and it is quite possible that in all cases some order prevails in the course of the unsegmented thread which has not been detected. But such polarity as may exist, at least in the case of the lily, is certainly very much less striking and characteristic than in the somatic divisions. It seems that the transformations undergone by the nuclear material during synapsis have resulted in the obliteration of nearly or quite all traces of its former symmetrical arrangement.

The condition of apparently complete fusion persists, as has been said, for a long time, certainly for several days. There then occurs a longitudinal splitting of the spirem, which is preceded by a fission of each chromomere, and which to all appearances is similar to the splitting that occurs in the prophases of a somatic mitosis. This longitudinal splitting, if we were ignorant of the processes preceding, would naturally seem to be preparatory to an 'equation division'; and so it has been interpreted by most of those who have observed it in the prophases of the heterotypic division, either in animals or plants. But, on the other hand, the figures produced by this splitting are so closely similar to those which were observed in the fusion that produced the single spirem as to suggest that possibly this splitting is really the separation of the two threads which have been for some time in very close combination. This view would make of the splitting a preparation for a separation of the idioplasms which have existed together throughout the life of the sexually-produced individual. The question of the nature of this splitting, and therefore of the heterotypic mitosis, depends upon what has occurred within each individual chromomere since the time of its formation from two separate bodies; and this problem is as yet beyond the reach of observation. One *a priori* argument, however, is of some weight in this connexion—namely, that it is hardly conceivable that the fusion of the threads, with all of the elaborate adjustments that it involves, should have occurred if they are afterward to separate without any sort of interaction or inter-relation between them or their constituent parts. Experimental data, to which I shall refer on a later page, suggest more definite conceptions of the processes involved in the fusion and subsequent splitting of the spirem.

The stages in the heterotypic division which succeed the longitudinal splitting of the spirem thread show many peculiarities, most of which are, I think, explicable in the light of the facts already described. Segmentation of the thread into one-half the somatic number of chromosomes results naturally from the fusion of the two threads side by side. Segmentation occurs at every point in the thread at which two chromosomes are joined ; and there is thus no such difficulty as is involved in the notion of ' bivalent ' chromosomes which result from the segmentation of the thread at only half the points of union between its constituent segments. Each segment of the thread has been formed by a maternal and a paternal chromosome lying side by side ; but whether these parental chromosomes are to be thought of as corresponding respectively to the two visible segments resulting from longitudinal splitting is a question which, as I have said, cannot be answered from direct observation. However this may be, from its method of origin it is to be expected that each heterotypic chromosome in its mature form will have twice the thickness of a somatic chromosome ; and it is well known that the chromosomes in this mitosis are much thicker than those that appear in any other division. They are, in fact, somewhat shorter and thicker, though not greatly so, than would be accounted for by the lateral apposition of two somatic chromosomes ; this is doubtless to be explained by a slight increase in the amount of contraction which the chromosomes normally undergo during the prophases. The process of growth in mass of the nuclear material which occurs during the resting stage is followed during each somatic mitosis by the longitudinal splitting of each chromatic segment. A similar increase in mass occurs, as my figures show, in the stages succeeding the anaphases of the last pre-heterotypic division. As a result of this growth and of the fusion of the threads in synapsis, the spirem must undergo two longitudinal fissions in order to produce segments which shall approximately correspond in size and chromatin content to the daughter chromosomes of a somatic mitosis. This, as we have seen, is what actually happens. The longitudinal splitting which occurs before segmentation seems to be the one necessitated by the fusion of the two threads ; and the splitting which is completed in the metaphases is the one provided for by the growth of the chromatin which has occurred since the anaphases of the last preceding division.

Evidences of the second splitting appear in the lily, as I have shown, shortly after segmentation. In many cases described by other authors, no trace of this fission appears until after the separation of the daughter chromosomes. It is quite possible that further study may show that the fission in these species also appears at the earlier stage ; but, in any event, the thick chromosomes of the heterotypic mitosis are at least potentially four-parted, instead of being, like those of any somatic mitosis, two-parted ; and in order that daughter chromosomes may be produced

which are comparable to those produced by a somatic division, the two mitoses must follow each other without an intervening stage of growth. This is the reason why the condition of distribution of the chromatin—the 'resting' stage—is commonly omitted between the heterotypic and homoeotypic divisions. In cases in which some time elapses between these two divisions (e. g. in the embryo-sac of *Lilium*) the material of the chromosomes shows a tendency to pass into the usual distributed condition; but that no growth occurs at this time is shown by the size of the chromosomes when they appear upon the homoeotypic spindle.

It seems, then, that all of the characteristic peculiarities of the heterotypic and homoeotypic mitoses result from the fact that the fusion of the hereditary substances contained in the parental germ nuclei, instead of being effected by the fusion of the germ-cells, is postponed until the close of the generation which results from the fusion of these cells. The fusion of the hereditary substances necessitates a further set of processes (the heterotypic mitosis) which shall reduce the mass of the chromatin in each segment of the fusion thread to that characteristic of the prophases of an ordinary mitosis. The period of growth which precedes synapsis, therefore, may be thought of as belonging strictly to the prophases of the homoeotypic division, which have been interrupted by the occurrence of the fusion and the further processes necessitated by it.

It must be remembered that we have no direct evidence that all the segments of one of the two daughter spirems produced by longitudinal fission pass finally into the same daughter nucleus. It is possible that it is entirely a matter of chance whether one or the other daughter segment of any particular chromosome shall pass into a particular daughter nucleus. The possibility of such variations, as already mentioned, has been considered by Sutton ('03), and has served as a basis for an attempt to harmonize his conception of chromosome reduction with Mendelian results in hybridization. On the other hand, the regularity of the processes leading to the separation of the chromosomes in this and in other divisions, and the elaborateness of the mechanism devised apparently for the securing of a very definite result, make it seem unlikely that the element of chance should play so large a part in this or in any other mitosis. It seems to me much more probable that each daughter thread resulting from the longitudinal splitting contains the chromatic substance destined for one of the daughter nuclei, and that the phenomena of chromosome reduction in the lily offer an explanation of the experimental facts without involving the hypothesis of a chance distribution of the chromosomes between the daughter nuclei.

The Distribution of Parental Characters to the Germ-cells.

In respect to the nature of the germ-cells produced by the heterotypic and homoeotypic divisions, there is an important difference between the

higher animals and plants. In animals, each of these cells is a sexual cell destined to fuse with another, usually from another individual, and so to give rise at once to a new animal provided with the parental idioplasms. In the case of plants, the cells in question are asexual spores, which give rise to a new generation which in turn produces the sexual cells. Each cell of the generation produced by the germination of the spore is characterized, like the spore, by the presence of the reduced number of chromosomes. The sexual cell ultimately produced by this generation, therefore, since it is descended directly from the spore through a series of cell divisions interrupted by no cell fusions, presumably resembles the spore in its idioplasm content; and therefore the generation produced by the fusion of two such sexual cells resembles the sexually-produced animal in its endowment of two separate idioplasms. Now it happens that in the Spermaphytes, which are the only types of plants to be considered in the present discussion, we are practically confined, so far as the study of hereditary characters is concerned, to the sexually-produced generation, the sporophyte, since the gametophyte, characterized by the possession of the reduced idioplasm, is in this group a very short-lived, few-celled generation. So far, therefore, as concerns the determination of the characters of the predominant phase in its life-history by two independent idioplasms, a Seed Plant essentially resembles one of the Metazoa.

The observations of others, as well as my own, seem to show that the division of the chromatin in the homoeotypic mitosis is essentially similar, except for the reduced number of chromosomes, to that characteristic of somatic divisions. This being the case, it is in the first longitudinal splitting, which determines the grouping of the hereditary materials that are to be distributed in the heterotypic mitosis, that we must seek an explanation of the nature of chromosome reduction.

The fusion of the two spirems and the subsequent splitting of the fusion thread may be imagined to produce any one of several different results, as regards the chromatin bodies within the threads, and these various possibilities will be considered separately.

1. There may be a complete fusion in pairs, not only of the chromomeres and of their visible component granules, but also of the smaller units, if such there be, resulting in the production of a single idioplasm different from that of either parent. The subsequent fission of the thread must then be equational, since any other method of division would result in germ-cells each provided with only a partial set of idioplasmic structures, and hence, so far as we can judge, incapable of giving rise to a complete individual. Such an entire fusion of the parental idioplasms was postulated by Strasburger ('94), and is, indeed, so far as I can see, the only hypothesis which is compatible with the notion of the universal occurrence in all mitoses (including the heterotypic) of an 'equation division.' Hertwig ('90) also

maintained that the two parental idioplasms fuse to form a new idioplasm different from either; but he conceived this process as involved in the fusion of the germ nuclei, instead of being, as we now see it must be, postponed to a much later period.

Such a complete fusion of the parental idioplasms, with a subsequent equational division, would result in the production of only one kind of germ-cells. If the individual producing these germ-cells be a hybrid, then the generation produced by fertilization between this and other hybrids from the same cross will consist of individuals all of one sort. I shall speak of the generation resulting from the inbreeding of hybrids from the same cross as the second hybrid generation. Now, with regard to this second generation, while its members all resemble each other, two possibilities arise as regards a comparison between them and their parents, the hybrids of the first generation. First, the two generations may be exactly similar; in other words, the original hybrids may breed true, and so constitute a variety which, having once appeared, is constant. But this is not, at least theoretically, necessary. We may imagine that the set of characters resulting from the intimate fusion of two idioplasms is something quite different from the set produced by the joint action of the same idioplasms existing separately within the same cell. So the possibility arises that the hybrids of the second generation, while resembling each other, may differ from their parents, the first generation hybrids.

So far as I am familiar with the literature, no instances have been reported which seem to correspond to the latter-mentioned possibility; hybrids which breed true, however, have been described in a number of cases. Well-known instances are the *Hieracium* hybrids of Mendel ('69); those of *Salix* reported by Wichura ('65); de Vries' ('01) hybrid between *Oenothera rubrinervis* and *O. nanella*; and Burbank's cross (Swingle and Webber, '98) between *Rubus ursinus* and *R. crataegifolius*. In this class too may possibly be included Millardet's 'false hybrids' already mentioned. In these there occurs apparently a complete dominance in the first hybrid generation of all the characters of one parent; and this complete dominance of one set of characters persists in the offspring of the second and later generations. Cases of constant hybrids are numerous enough to justify the assumption that a complete fusion of the parental idioplasms is possible; and that, therefore, the heterotypic division may, in certain instances, be an equational one. But their comparative rarity is evidence that this method of chromosome reduction is not usual; although the possibility remains that hybrids do not furnish a fair test in this matter, that a complete fusion may more readily occur between the idioplasms of parents of the same race, and that, therefore, such a fusion with later equational division may be commoner than now seems probable. On the other hand, de Vries ('03) points out that constant hybrids, so far as known, result from the crossing of parents

belonging to separate species. I have already referred to his views as to the difficulty of adjustment of parental idioplasms derived from different species in the heterotypic prophases; and he suggests that this inability of the idioplasms to adjust themselves to one another in some way makes impossible the 'splitting' of parental qualities which occurs in the formation of the germ-cells of varietal hybrids. I confess that I find it impossible to imagine how such a failure of adjustment of the two idioplasms could lead to the production of a constant idioplasm, unless it be supposed that it necessitates an actual complete fusion of the two parental substances; and such a fusion could hardly occur in such a case unless the possibility of fusion is characteristic of the structure of the idioplasms themselves. I am inclined to think, therefore, even though at present the known constant hybrids be all of specific rank, that their occurrence is evidence of the possibility of the perfectly normal occurrence of a more or less complete fusion of the idioplasms in the prophases of the heterotypic division. This conception of de Vries' has been adversely criticized also by Correns ('03 *c*).

2. There may be in part a fusion such as was described above, while certain portions of the two idioplasms remain uncombined and capable of distribution to the germ-cells in varying combinations. This case is a transitional one, and will, therefore, be considered in connexion with the next.

3. There may be more or less mixture of the idioplasms as a result of the contact of the two threads, but no actual fusion. The substance to be separated into two portions by the splitting of the thread is then not a single idioplasm, and, unless we suppose that the actual number of idioplasmic structures may be increased from generation to generation, there cannot be an equation division; but, for the production of functional germ-cells, it seems most likely that there should be a redistribution of the parental materials in such a way that each daughter thread receives an endowment corresponding to a complete individual. But the two resultant idioplasms may contain the materials of the parental idioplasms arranged into entirely new and different combinations. This redistribution of the materials of the parental idioplasms is a process which can occur only at a certain point in ontogeny; it is here, then, if anywhere, that the element of chance may be supposed to enter, especially as its operation will effect the greatest possible amount of variation among the offspring. A similar operation of the law of chance has been suggested by Strasburger ('04) in the separation of the chromatin granules as a result of the division of the 'zygosome.' As a result of the mixing of the parental idioplasms and their redistribution, we should then find, if the cases at hand be numerous enough, every possible combination of different portions of the two idioplasms which is capable of giving rise to a complete set of individual characters. But this is virtually a restatement of Mendel's 'law of the

purity of the germ-cells.' That is to say, the method of mingling and redistribution of the parental idioplasms which we are now considering would naturally lead to a purely Mendelian distribution of parental characters in the pure-bred offspring of the next generation.

A purely Mendelian distribution of parental characters is as yet, however, a purely hypothetical case. The utmost that can be said is that many crosses follow the Mendelian law with reference to certain isolated characters; and that if we can cross two races in which occur two, three, or more pairs of Mendelian 'allelomorphs,' each pair will follow the law independently of the presence of the others, and we shall obtain results with reference to these few pairs of allelomorphs which are reasonably consistent with what the law teaches us to expect. But it has never been shown that, in a cross between two individuals, whether of the same or of different races, which differ from one another in many points, the law of gametic purity holds with respect to all the differing qualities. On the contrary, all of the recorded cases of obedience to the Mendelian law are instances of isolated qualities of plants or animals whose other qualities either have not been shown to follow the law or have been shown to deviate from it in a marked degree.

A priori, Mendel's law can apply to all the qualities of an individual only on condition that the character of the individual is constituted entirely of unit qualities, each of which is represented by an element of the idioplasm which can be transmitted as such without reference to any of the other elements. Such a constitution has not been shown in any case. All that has been shown is that certain qualities may act as though they were such isolated units ; and it seems probable that in certain other cases there may be combinations of simple units (perhaps separable under particular conditions) into units of a higher order. As regards the great mass of the hereditary endowment of any individual, we do not yet know that it is so divisible into unit qualities of any order ; and so the present evidence suffices only to show that, as regards certain portions of the parental idioplasm, a rearrangement and redistribution, without fusion, may occur. In other words, we cannot say as yet that the possibility under consideration of a complete redistribution of the idioplasmic structures of any order ever actually occurs.

Conversely, it is of course possible that further study may show variation in hitherto unnoticed points between the offspring of apparently constant hybrids. So it may be that there are no cases either of complete and perfect fusion of the parental idioplasms, or of their complete analysis into separable units ; and that, therefore, the results of chromatin reduction actually belong always to category (2), (1) and (3) being limiting cases which are seldom or never reached.

4. It is conceivable that such a rearrangement occurs as described

under case (3), but that the chromosomes are the units whose redistribution takes place according to the Mendelian law. This possibility has been discussed in connexion with Sutton's ('03) hypothesis. It is not out of harmony with the facts already referred to of the correlation of individual characters. Thus Mendel ('65) found that in *Pisum* a grey, grey-brown, or leather-brown colour of the seed-coat always occurs in connexion with violet-red blossoms and reddish spots in the leaf-axils. On the other hand, this hypothesis, at least in the form in which Sutton stated it, seems to be inapplicable to the cases of constant hybrids; and it is negatived by the cytological facts in the lily, and in other cases in which a double longitudinal splitting occurs. The possibility remains, as I have suggested, that radically different methods of chromosome reduction prevail in different groups of organisms, although before the fact of such variation can be finally accepted it must be confirmed by much better evidence than is now available.

5. It is possible that portions of the idioplasms which do not fuse, or the idioplasms as wholes, may interact, perhaps chemically, upon each other while in intimate contact in the fusion thread, so that when they are afterward separated any particular portion has not the same hereditary value that it had before. Such interaction might conceivably result in the parental idioplasms, though remaining separate, coming to resemble each other more or less closely; and in this case the results of their separation in the germ-cells, so far as concerns the hereditary endowment of the offspring, might be indistinguishable from the results of a complete fusion and subsequent equation division (hypothesis 1). The reactions that may be imagined to occur in this way belong in the same category as those to which I have referred as not impossible in the somatic nuclei. Such reactions between the substances of the parental idioplasms would in any case produce results extremely difficult to trace in the hereditary characters of the next generation; and in the present state of our knowledge of the facts of heredity it is probably quite impossible to test the question of their occurrence.

6. It is also possible that, after their contact in the fusion thread, the parental idioplasms may be completely separated by the longitudinal splitting, so that each germ-cell receives the pure idioplasm of one or the other parent. This would result in something similar to Cannon's conception of the 'purity of the germ-cells.' Such a complete separation might be a regular occurrence, or it might occur as an individual case under Mendel's law, since one of the possible recombinations of idioplasmic units would be identical with the previously-existing combination. This also is a possibility which, so far as I can discover, has no basis in the observed facts of heredity. It is strongly suggested, as has been said, by the striking resemblance between the appearance of the thread when splitting and its appearance at the previous stage of fusion. But it would be extremely

surprising to find such pains taken to ensure an exact adjustment and fusion of the two threads, if they are to be completely separated again at a later stage. The latter objection might be met, of course, by supposing that the two idioplasms, while remaining distinct in the fusion thread, interact without an interchange of substance, as suggested in hypothesis (5), already discussed.

As a result of the comparison which I have attempted to trace between the results of experiment and those of cytological study, it seems to me safe to say that the intimate contact of the parental idioplasms in the prophases of the heterotypic division, and the subsequent separation of their substance by the longitudinal splitting of the spirem, produce results of different nature in different instances. In a few well-authenticated cases (instances of constant hybrids) there appears to be a thorough fusion of the idioplasms and a subsequent equational splitting; although it remains possible that a more careful analysis of these same cases may show that the fusion is not so complete as the facts now seem to indicate. In a considerable number of other cases there appear to be certain elements of either parental idioplasm which remain distinct, and by the longitudinal fission of the thread are redistributed to the germ-cells according to Mendel's law. In none of these cases has it yet been shown that the whole idioplasm is divisible into units which are capable of a Mendelian redistribution.

It remains possible, therefore, that in every case there is at least a partial fusion of the idioplasms; and that in every case also certain portions remain uncombined and are later separated. The questions whether there ever occurs a complete fusion, or whether in any case no fusion at all occurs, and whether the problem may not be further complicated by some of the other factors that I have mentioned, must be left for future investigation.

The studies whose results are embodied in the foregoing paper were begun at the suggestion of Professor R. A. Harper, and have been carried on under his direction and with the constant assistance of his helpful criticism.

MADISON, WISCONSIN
August 8, 1904.

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DESCRIPTION OF FIGURES IN PLATES VI, VII, VIII, AND IX.

Illustrating Prof. Allen's paper on the Pollen Mother-cells of *Lilium canadense.*

All the figures were drawn with the aid of a camera lucida, and with a Zeiss apochromatic 2 mm. objective, 1.30 apert.: Figs. 15, 16, 17, 18, 24, 27, and 31 with compens. oc. 18; all the others with compens. oc. 12.

PLATE VI.

Fig. 1. Reconstructing nucleus of pollen mother-cell, shortly after close of last pre-heterotypic division. Spindle fibres (not shown) still attached on equatorial side (lower side in figure).

Fig. 2. Somewhat older nucleus, showing increased size; irregular nucleolar masses.

Fig. 3. Tangential view of portion of nuclear reticulum, same stage as Fig. 2.

Fig. 4. Pollen mother-cell at slightly later stage.

Fig. 5. Still older nucleus; peripheral arrangement of chromatic material; rounded, vacuolated nucleole.

Fig. 6. Tangential view of portion of nuclear reticulum, same stage as Fig. 5.

Fig. 7. Median section of still older nucleus, shortly before synapsis; beginning of formation of spirem.

Fig. 8. Tangential view of nucleus, same stage as Fig. 7.

Fig. 9. Portion of nuclear reticulum showing paired fibres; same stage as Figs. 7 and 8.

Figs. 10, 11. Stages in the transition to synapsis.

Fig. 12. Nucleus in synapsis, tangential view; spirem composed of two parallel threads; no free ends; flattened nucleole.

Fig. 13. Portion of double spirem, same stage as Fig. 12.

Fig. 14. Median section of cell with nucleus in synapsis; paired strands at *a*.

Figs. 15–18. Stages in the fusion of the parallel threads; substance of threads differentiated into chromatin and linin.

Fig. 19. Late synapsis stage, with single thick spirem; cell has separated from its fellows and rounded up.

Fig. 20. Cell showing stage in transition from synapsis.

PLATE VII.

Fig. 21. Later stage in transition from synapsis.

Fig. 22. Median section of cell with uniformly distributed spirem.

Fig. 23. Nearly complete nucleus, showing course of spirem, at same stage as Fig. 22. The free ends visible are all in the plane of cutting.

Fig. 24. Portion of thread, stage of Fig. 23, showing single row of chromomeres.

Fig. 25. Portion of tangential view of nucleus, showing longitudinal splitting of the spirem.

Fig. 26. Median section of cell, same stage as Fig. 25.

Fig. 27. Portions of spirem, showing process of longitudinal splitting.

Fig. 28. Early stage in segmentation; a few free ends, all in the periphery.

Fig. 29. Later stage in segmentation.

Fig. 30. Nucleus some time after segmentation; chromosomes mostly in the periphery and somewhat shorter and thicker than in Fig. 29.

Fig. 31. Two chromosomes, same stage as Fig. 30; two rows of chromomeres in each daughter chromosome; traces of second longitudinal splitting at *a* and *b*.

Fig. 32. Nucleus just before formation of multipolar spindle; short, thick chromosomes.

Fig. 33. Chromosome at time of completion of segmentation.

Figs. 34, 35. Chromosomes, showing early stages in the process of shortening and thickening.

PLATE VII.

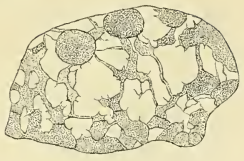
Figs. 36–54. Further stages in the shortening of the chromosomes.
Fig. 55. Cell at stage of multipolar spindle ; extra-nuclear nucleoles.
Fig. 56. Cell at equatorial plate stage.
Fig. 57. Equatorial plate, polar view.
Fig. 58. Metaphase ; longitudinal splitting of daughter chromosomes.
Fig. 59. Polar view of single chromosome from an equatorial plate.
Figs. 60–67. Lateral views of chromosomes from various equatorial plates.
Figs. 68–87. Stages in the separation of the daughter chromosomes.

PLATE IX.

Fig. 88. Daughter chromosomes approaching the pole.
Fig. 89. Daughter chromosomes at the pole (diaster stage).
Fig. 90. Slightly later than Fig. 89 ; shortening of daughter chromosomes.
Fig. 91. Formation of daughter nuclei ; chromosomes densely massed ; beginning of cell-plate formation.
Fig. 92. Reconstructed spirem in daughter nucleus ; cell-plate.
Fig. 93. Oblique view of daughter-cell and nucleus ; cell-division complete.

HOMOEOTYPIC DIVISION.

Figs. 94, 95. Separation of chromosomes in the prophases.
Fig. 96. Multipolar spindle.
Figs. 97–100. Single chromosomes during the prophases.
Fig. 101. Equatorial plate, lateral view.
Figs. 102, 103. Equatorial plates in polar view.
Figs. 104–106. Lateral views of chromosomes from the equatorial plate.
Fig. 107. Metaphase.



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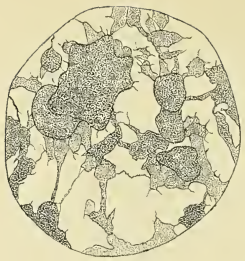

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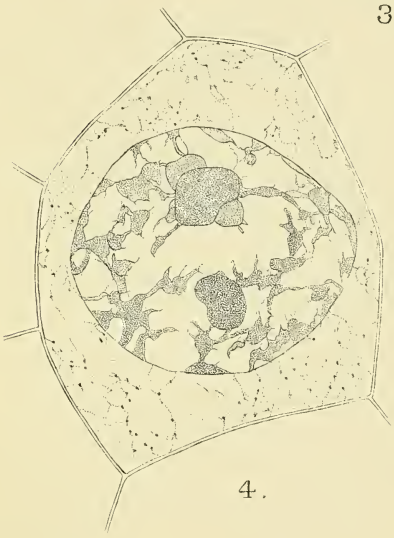

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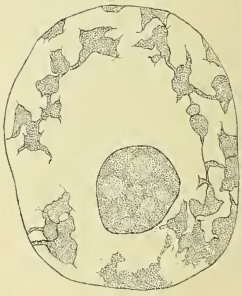

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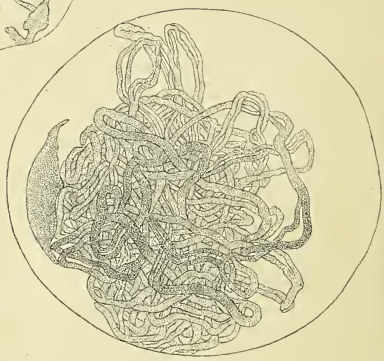

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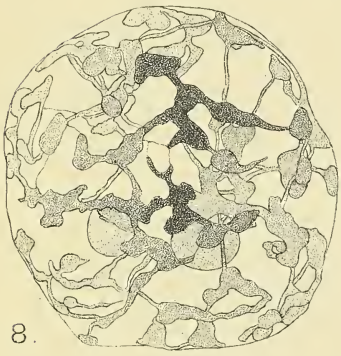

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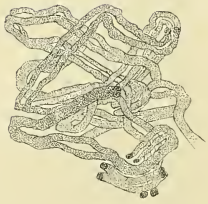

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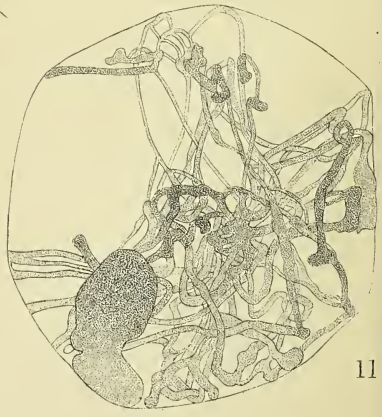

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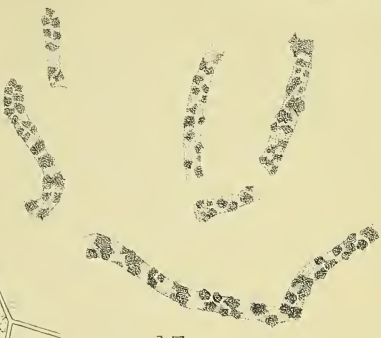

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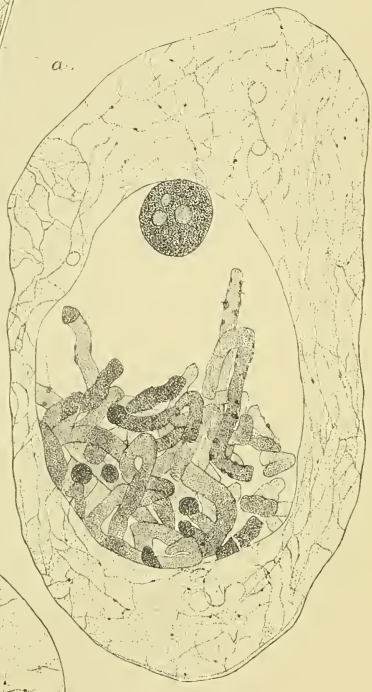

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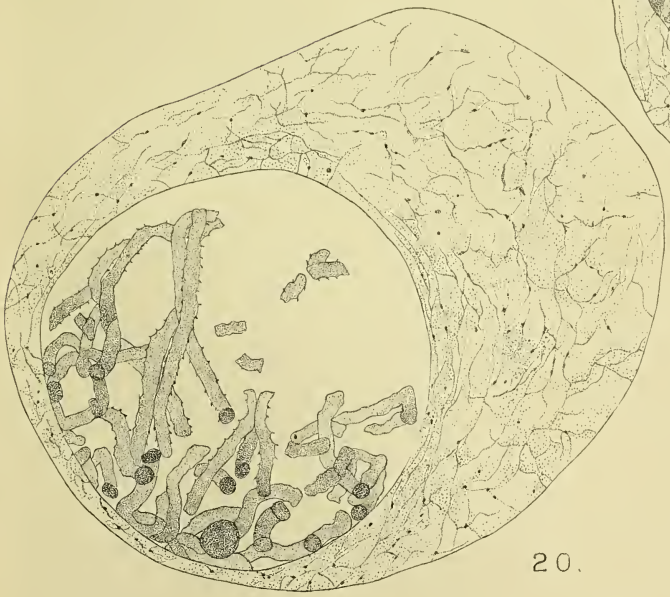

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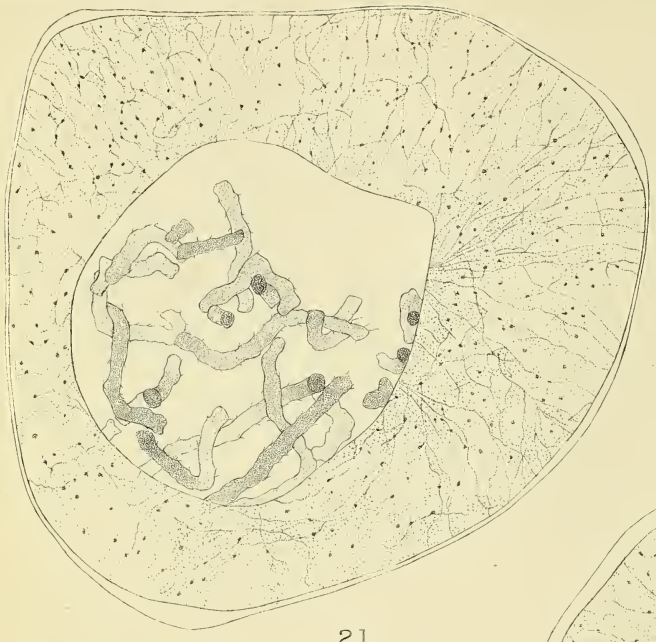

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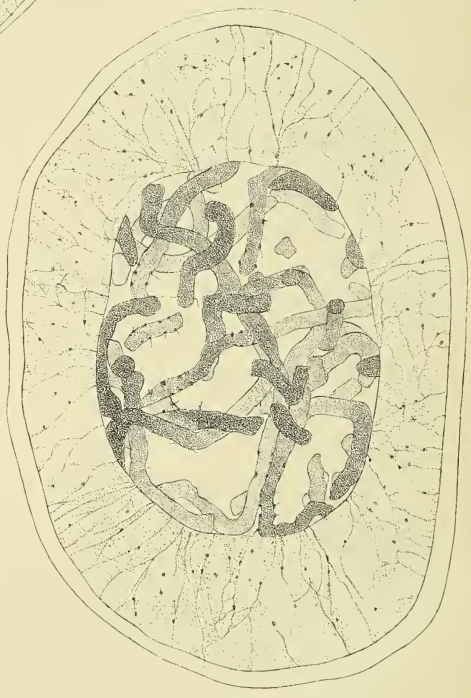

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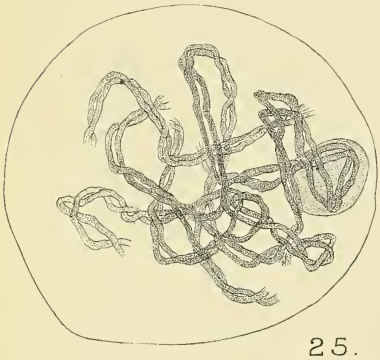

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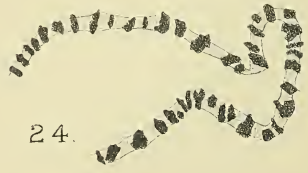

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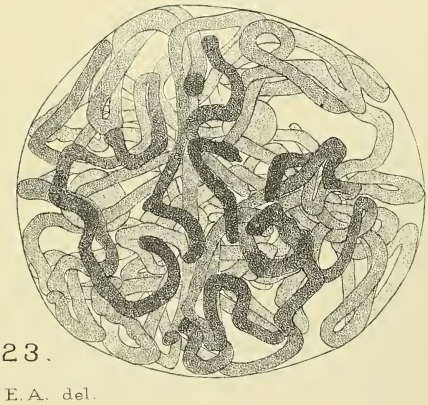

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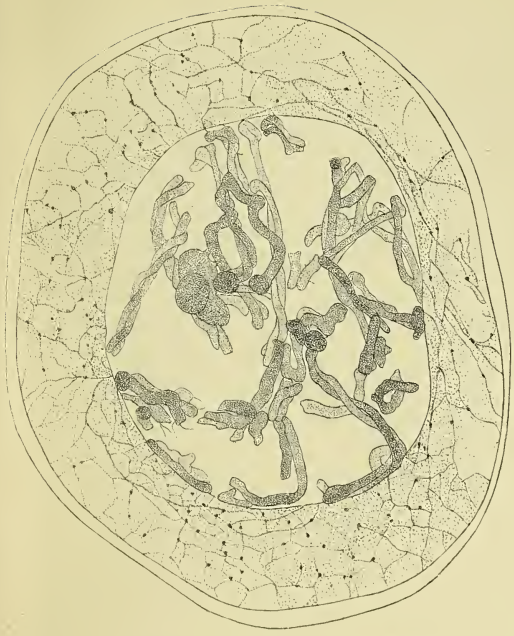

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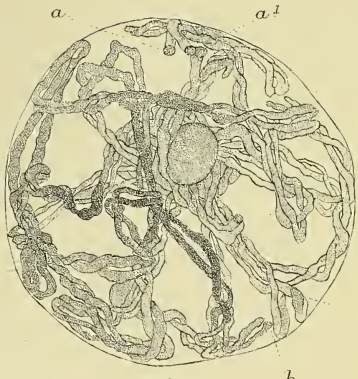

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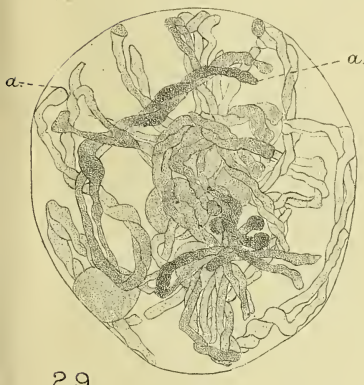

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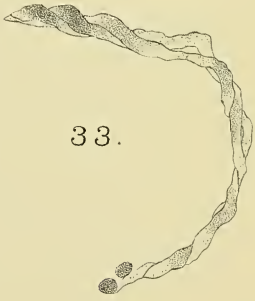

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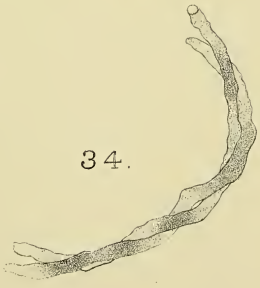

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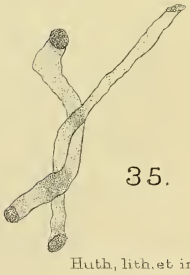

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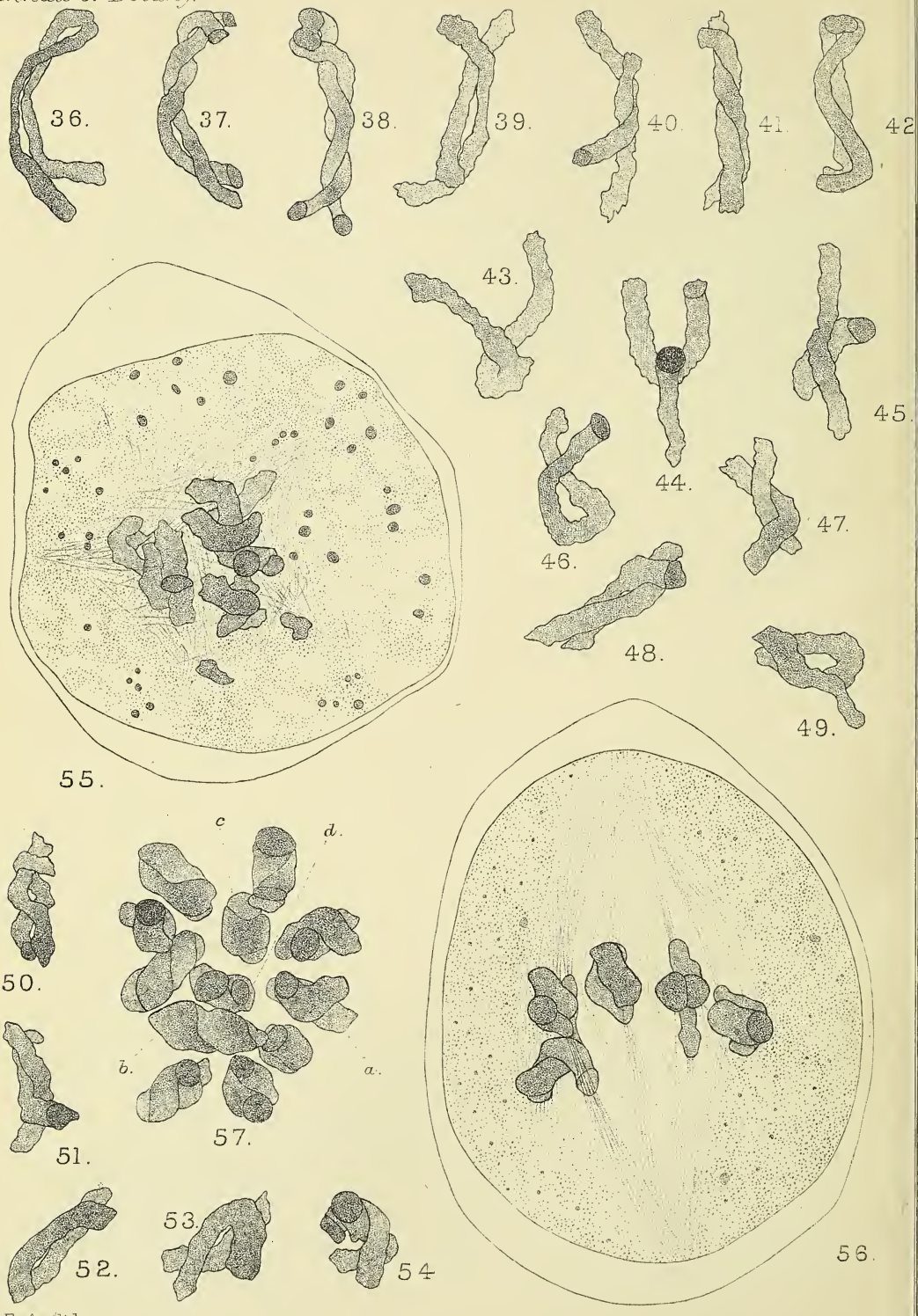

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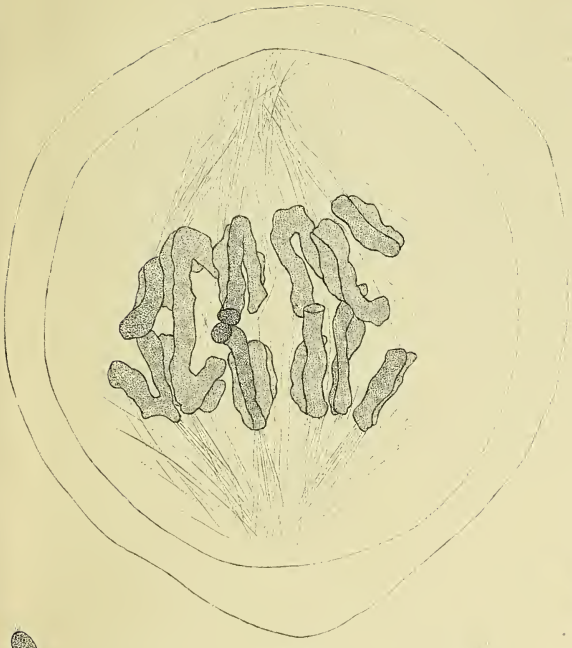

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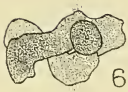

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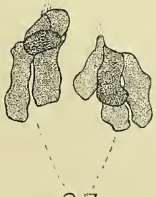

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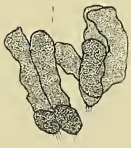

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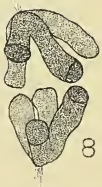

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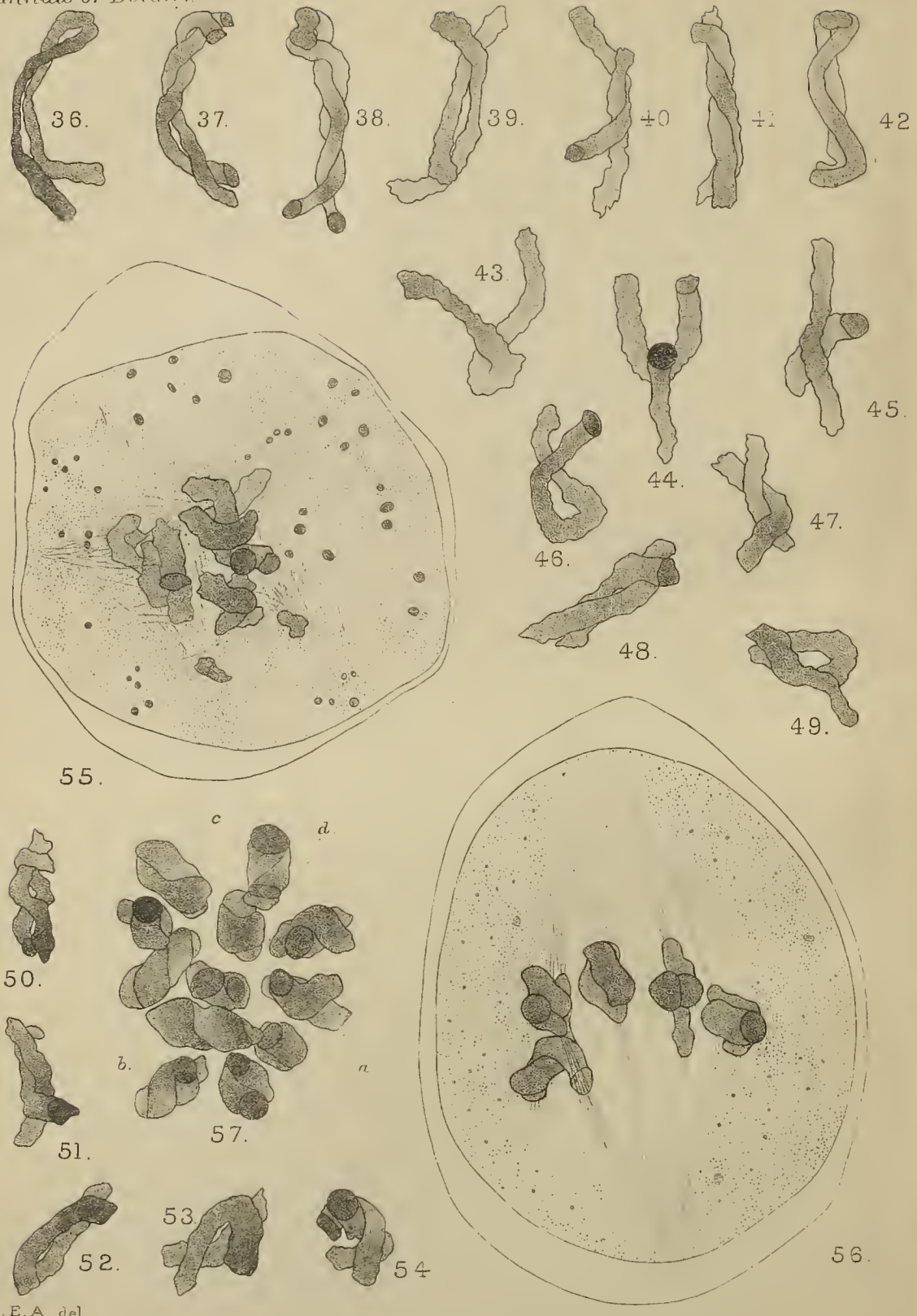

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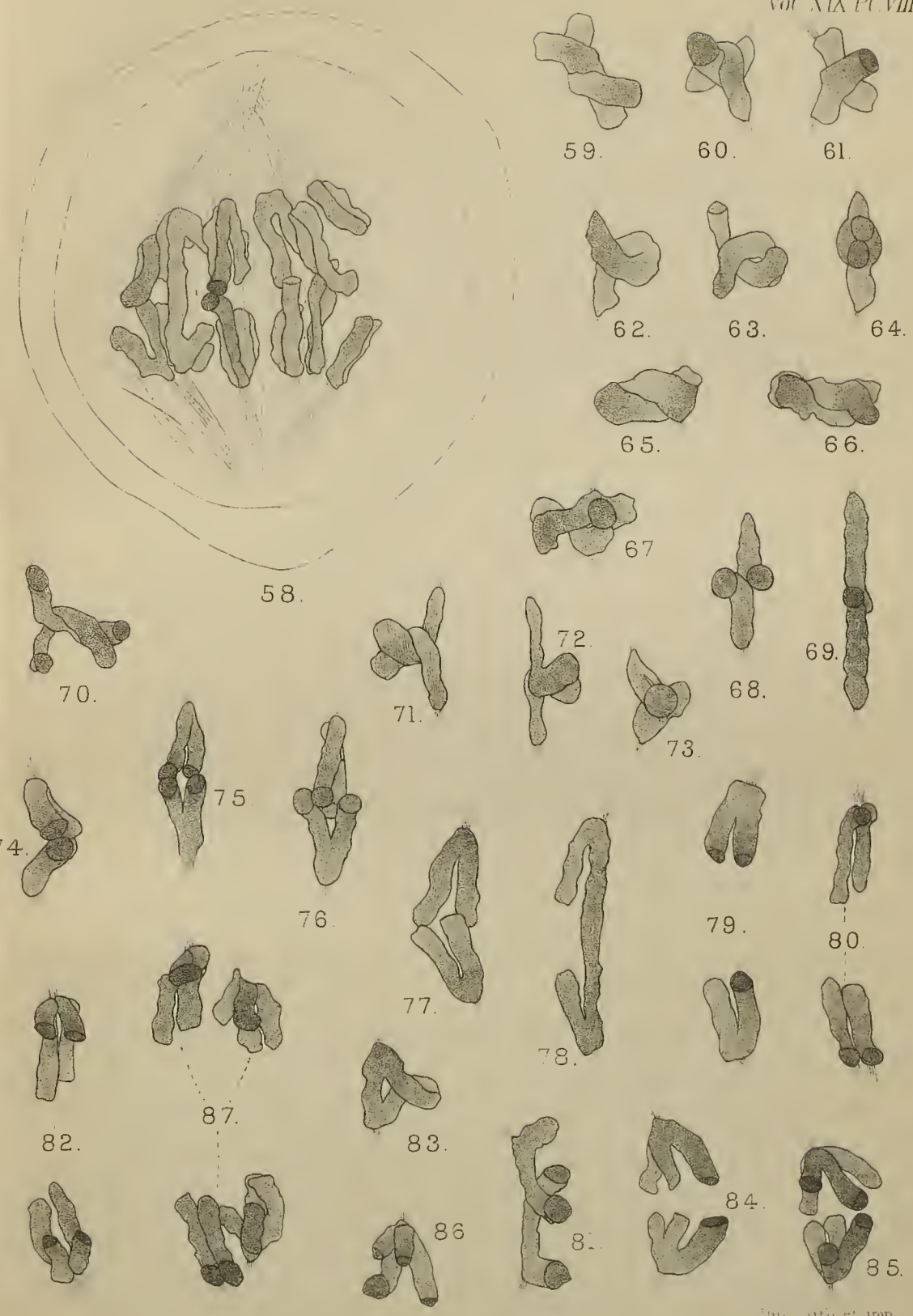

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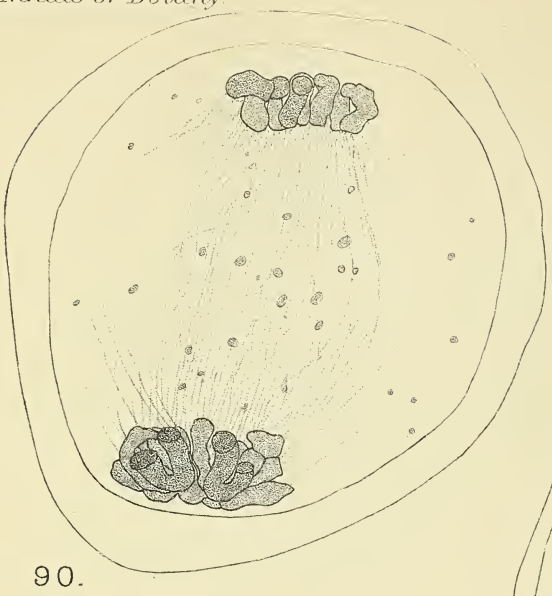

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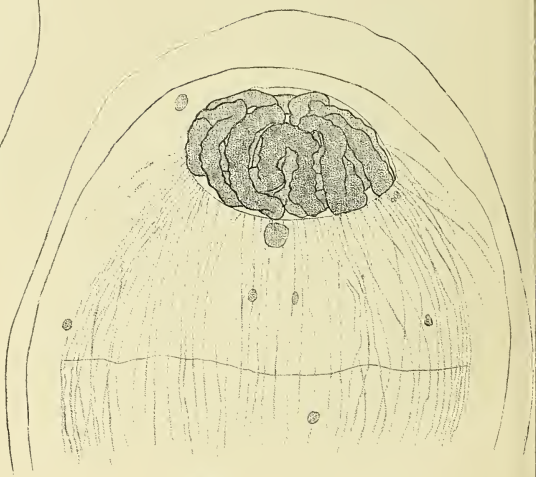

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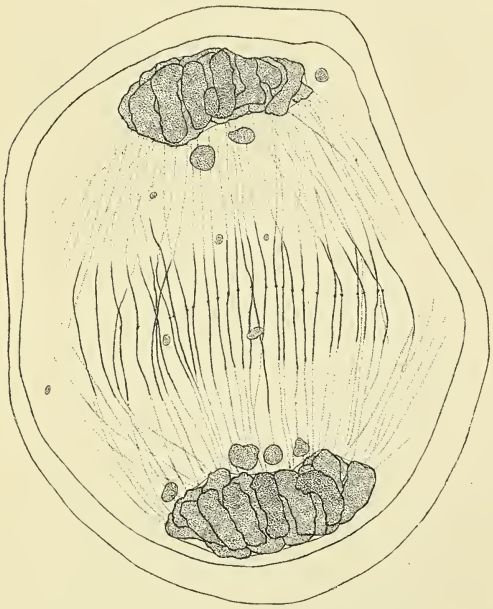

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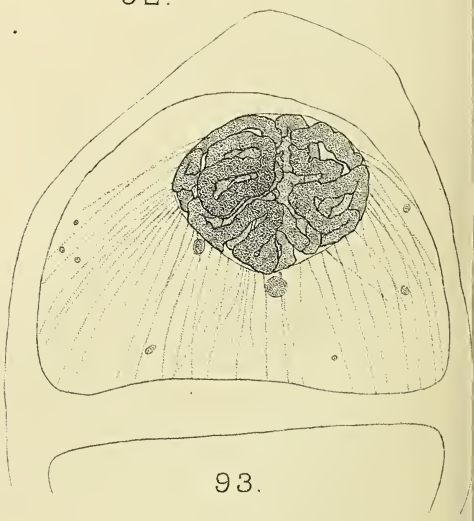

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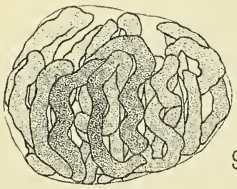

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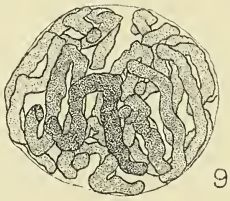

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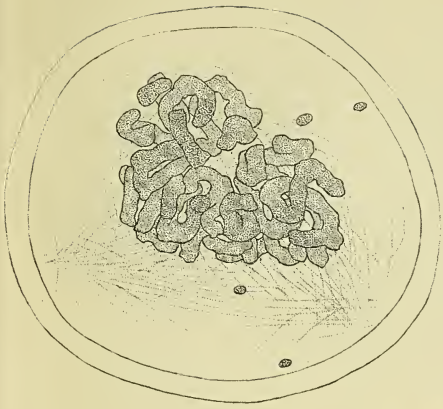

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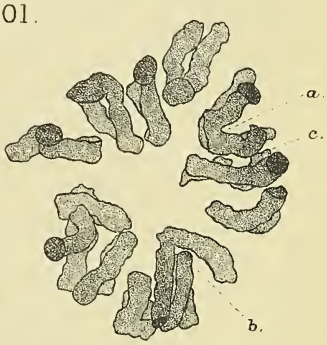

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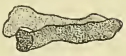

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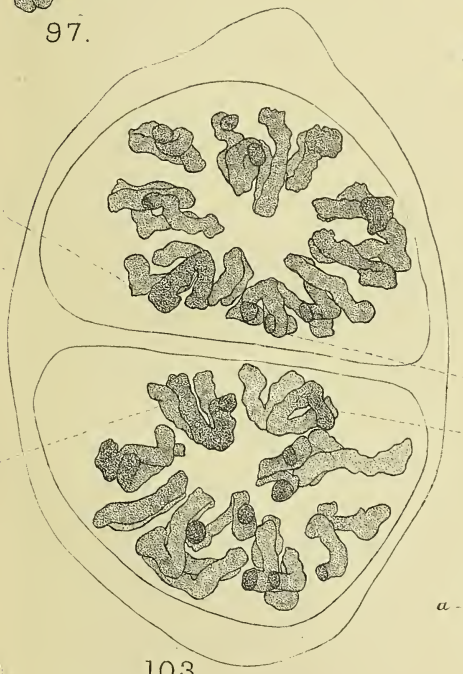

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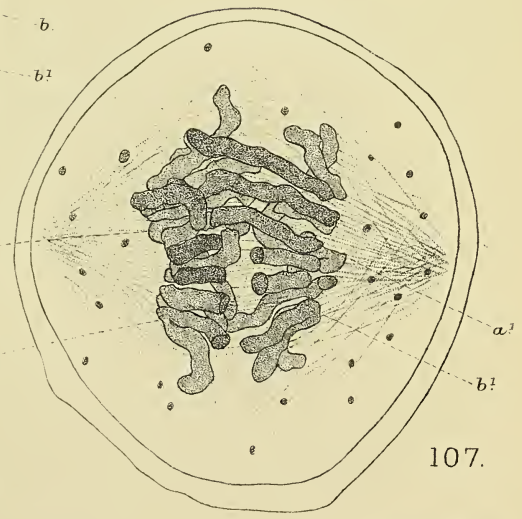

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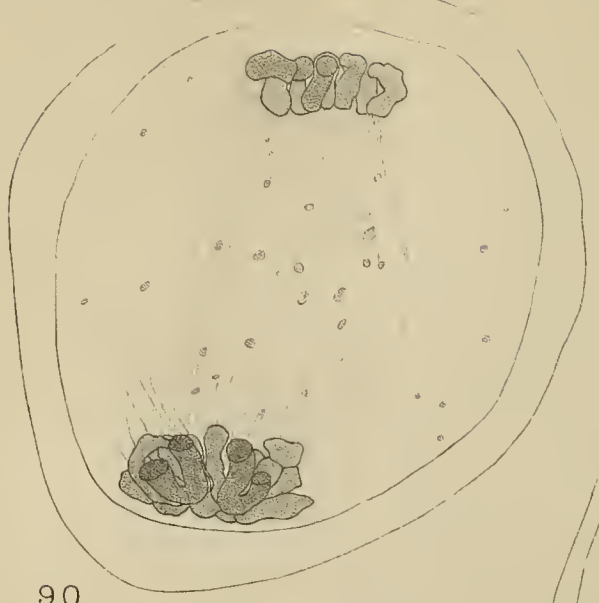

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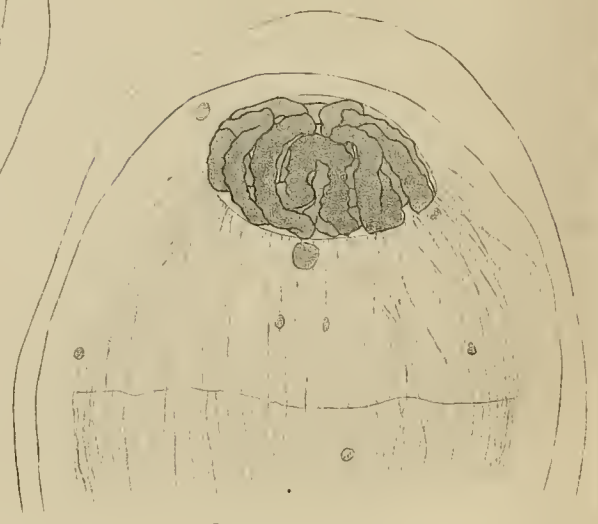

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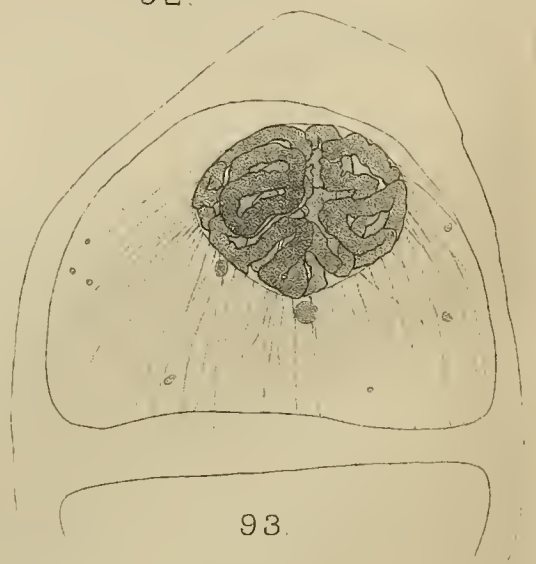

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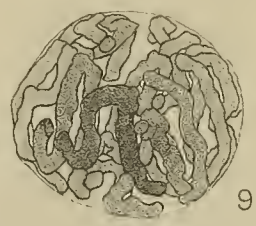

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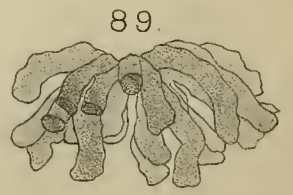

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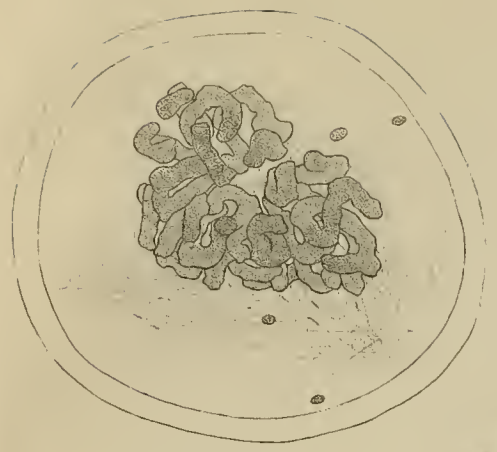

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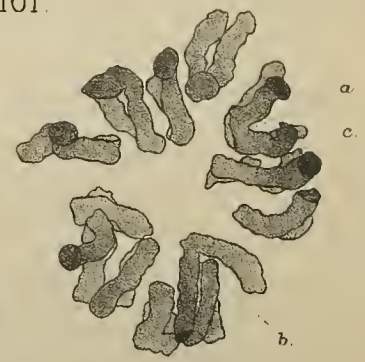
97.

99.



a

c.

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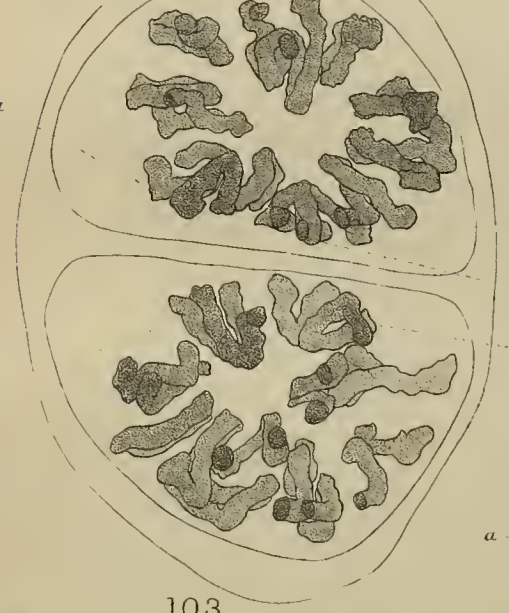

a

a^1

103.

b

b^1

a

b

a^1

b^1

107.

104.

105.

106.

Huth, lith et imp

On the Anatomy of Archangiopteris Henryi and other Marattiaceae.

BY

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With Plate X.

ARCHANGIOPTERIS was established in 1899 by Christ and Giesenhagen as a new genus of the Marattiaceae connecting *Angiopteris* with *Danaea*[1]. The plants upon which the genus was founded, as indicated by the specific name, were discovered by Dr. A. Henry in Yunnan in the south-west of China. The Fern was found in one locality only, in a mountain forest to the south-east of Mengtse. The material upon which the following observations were made was a small piece of a stock preserved in spirit which was presented by Dr. Henry to Professor Bower, who handed it over to me for investigation. Dr. Henry informs me that it was only by a lucky accident that this particular material reached Europe at all. It was left behind in a house at Mengtse when Dr. Henry had to retire from that district in consequence of an upheaval among the natives. During his absence the Chinese soldiers who were put on guard to protect European property looted the houses. They forced an entrance into the houses through trap-doors in the floors, which were built on piles some feet above the ground. Luckily in Dr. Henry's house a heavy box had been left accidentally above the trap-door, and in consequence it escaped loot. A year afterwards the bottle containing the material was brought home by Mr. Miller, of the customs service, who returned to Mengtse after the troubles were over.

A detailed account of the morphology and structure of the leaf of *Archangiopteris* is to be found in the original account given by Christ and Giesenhagen, but nothing has yet been published upon the anatomy of the rhizome and petiole save a passing reference to the latter by Brebner[2]

[1] Flora, Bd. 86, p. 72.

[2] 'On the Anatomy of Danaea and other Marattiaceae,' Annals of Botany, vol. xvi, No. lxiii, pp. 537 and 538, 1902.

[Annals of Botany, Vol. XIX. No. LXXIV. April, 1905.]

in his paper on the anatomy of *Danaea*. The addition of a new genus to an order so limited in the number of its genera as the Marattiaceae is of sufficient interest to warrant the following supplementary observations upon this the only example of the genus hitherto discovered.

The material at my disposal proved upon examination to consist almost entirely of three large leaf-bases inserted so closely together as to appear to arise one upon the other, as shown by Figs. 1 and 2, Pl. X. Further investigation disclosed a fourth young leaf and a small portion of the apical region of the stem lying between the larger leaf-bases. Unfortunately the specimen had not been cut off from the rest of the stock upon which it grew, but torn away, and in consequence the lower end of the stem was in a very lacerated and fragmentary condition. The tissues, however, were in a fairly satisfactory state of preservation. The portion of stock examined does not appear to have been subterranean, but it was impossible to decide whether it grew erect or not. There was, however, no suggestion of dorsiventrality; on the other hand, the arrangement of the leaves and the structure of the vascular system indicated a radial symmetry.

The two leaves belonging to the petioles, the cut ends of which are shown in the figures, also accompanied the specimen. They were about 28 inches in length, including the terminal pinna. Two pairs of shortly stalked, nearly opposite lateral pinnae are inserted upon the upper third of the rachis in each leaf. The basal region of the petiole is enlarged into a conspicuous pulvinus (Figs. 1 and 2), another occurs about halfway up the free petiole, and the bases of the stalks of the pinnae also show a pulvinar structure. The surface of the basal pulvinus is rough, corrugated, and dark brown in colour. This region is thereby sharply marked off from the rest of the petiole, which is yellowish white in colour and, although bearing a few paleae, is smooth and shining. As stated by Christ and Giesenhagen, the upper part of the petiole shows no articulation with the basal pulvinus. The bend seen in the drawings just above the enlarged bases of the petioles has probably been produced in the collecting.

The characteristic stipular appendages of the order are present at the bases of the leaves, and are produced by the prolongation of the margins of the enlarged leaf-base into two broad wings thinning down towards their edges until they become quite membranous (Figs. 3 and 4). Just above the middle of the basal pulvinus the two lateral wings are united across the adaxial surface of their leaf by a plate of tissue forming a transverse intra-axillary commissure, as described by Sachs in *Angiopteris*, *Marattia* and *Danaea*[1]. This cross-piece is abaxially concave, and in the bud it curves over and protects its own leaf, which is circinately coiled up behind it (Fig. 3). The top of the young leaf is covered over by the

[1] Text-book of Botany (English edition), p. 417, 1882.

overlapping upper margins of the wings, which in this region are free from the leaf-base. The lower parts of the stipular wings (below the transverse commissure) enfold the next youngest leaf in front. During the unfolding of the leaf the transverse commissure gets torn across, as shown in Fig. 4. A comparison with the stipular appendages of *Kaulfussia aesculifolia* (represented somewhat diagrammatically by Fig. 5) shows that the protective arrangements in this plant are on a slightly different plan. The upper free portions of the wings are here much larger, and suffice alone for the protection of their own leaf, while the transverse commissure joins the lower parts of the stipular wings in curving over and protecting the next youngest leaf *in front.*

The Vascular System of the Stem.

In order to observe the structure of the stem the larger leaf-bases were first of all removed and the rest of the specimen was then cut up into a series of transverse sections. Unfortunately the insertion of the vascular strands of the first two leaves upon those of the stem could not be satisfactorily followed out on account of the torn and ragged condition of the end of the specimen. The portion of the stem that extended beyond this point was very short and, being so near the meristematic tissues of the apex, it was not very suitable for anatomical purposes. The concrescence of the large leaf-bases with the axis of the stock causes the outline of the latter to be very irregular, and at the same time renders it almost impossible to draw any definite limit between the ground-tissue of the stem itself and that of the adhering leaf-bases. The vascular system of the stem consists of a single dictyostelic ring of two to four small meristeles more or less oval in section. In addition there is usually a small vascular strand lying in the central ground-tissue enclosed by this ring (*int. s.* in Fig. 6). This internal strand may, however, sometimes be absent (Fig. 7). A varying number of entering leaf-traces occur at different levels in the peripheral ground-tissue, which is also traversed by a number of roots passing downwards and outwards. The meristeles of the ring anastomose with each other in a somewhat irregular manner; anastomoses being present other than those which close the leaf-gaps. In one region the meristeles all fuse up to form a single large curved strand.

The small internal strand runs free in the central ground-tissue through the greater part of its course, but from time to time it approaches the dictyostelic ring and fuses with those meristeles that are about to close up the upper ends of a leaf-gap. Soon after it separates off again and passes on across the central ground-tissue to the next leaf-gap above. In one region the central strand ended blindly in the downward direction and for some distance below this point the dictyostelic ring alone was

present (Fig. 7), until another central strand separated off from the inner surface of one of its meristeles.

The roots arise from the external surface and sides of the stem meristeles. A root appears to arise invariably from the point where the central strand fuses with a meristele of the ring. The xylem elements of the root stele spread out over the outer side of the xylem of the stem to form a small attachment-disc of irregular isodiammetric tracheides. These tracheides are differentiated very near the apex—at the same time as the endarch protoxylems of the stem stele itself, and before the intervening metaxylem. The roots have a cortex of their own right up to the stem stele (Fig. 6, *r*), so that there is no 'root-pedicel.' When free from the stem the xylem of the root has seven to ten protoxylem groups, and its centre is occupied by a few cells of thin-walled parenchyma. There is a well-marked endodermis with barred thickenings on its radial walls.

The Vascular System of the Petiole.

The arrangement of the vascular strands in the petiole showed a certain amount of variation in the three leaves in which it was possible to follow their course throughout. On this account it would have been rather difficult to fix with any confidence upon those features which may be held as common to the leaves of the plant in general, were it not for the light thrown upon their interpretation by the structure of the petiole of *Kaulfussia aesculifolia*. Since the number of petioles of *Kaulfussia* examined renders the observations more reliable, it is advisable to describe the arrangement in this plant before going on to that in *Archangiopteris*. Seven vascular strands are given off from the dictyostele of the stem to supply each leaf. They anastomose and divide freely as they pass out, so that a varying number of separate strands are to be found in the base of the petiole. These are arranged in a curve of the form of a widely open arch with its concavity facing directly towards the apex of the rhizome (Fig. 8). The two terminal strands of the arch (marked *x* in all the figures) are somewhat larger than the rest, and as they pass upwards they approach the median plane of the petiole. As they do so they also curve inwards, and at the same time turn round so that their protoxylems face away from each other towards the sides of the petiole (Fig. 9). This rotation is sometimes continued until at a point still further up their protoxylems face almost directly towards the median plane (Fig. 10). At a certain point in the enlarged basal region of the petiole the terminal strands are usually joined together by a transverse suture (*s* in Fig. 9); sometimes, however, it is the two penultimate strands that are thus joined together.

The several strands so far described are all clearly to be regarded as constituting a leaf-trace strictly comparable with the typical leaf-trace of

the Polypodiaceae and Cyatheaceae; the curve that they outline being but a slight variation of the horseshoe design so frequently met with in these two orders. In the enlarged basal region of the petiole, however, a new feature is introduced by the occurrence of one or more internal vascular strands lying within the typical curve (Fig. 8). These internal strands arise as branches from the inside of the median abaxial strands of the curve, but they do not lie in the course of the curve itself, nor can they be considered as forming part of it. Two such strands are usually present (Figs. 9 and 10), but in one case as many as five were observed (*a*, *b*, *c*, *d*, *e* in Fig. 8). They were arranged in an arc concentric with the abaxial part of the typical curve. Two of them (*a* and *d*) ended blindly above, and two others (*b* and *c*) fused together, so that here also only two internal strands eventually remained. As they pass upwards these internal strands in all cases gradually move across the central ground-tissue towards the adaxial side of the petiole, and before the top of the pulvinus is reached they fuse with the two terminal incurved strands of the typical arc (*xx* in Figs. 9 and 10). In a few cases a single strand alone arose from the abaxial strands of the typical curve, but this soon divided into two which behave as before. The protoxylems of the internal strands first of all face outwards, towards the abaxial surface of the petiole (Fig. 8), but as they advance across the internal ground-tissue they gradually turn round so as to face in the same direction as the terminal strands with which they fuse (Figs. 9 and 10). The internal strands may fuse with either end of the terminal strands according as the protoxylems of the latter face towards the sides of the petiole (Fig. 9) or towards its median plane (Fig. 10). In the region above the pulvinus all the strands present in the petiole belong to the typical curve. The two terminal strands sink in towards the centre of the petiole, and at a point higher up they fuse together across the median plane to form a single large internal strand with the protoxylem on its adaxial side, as described and figured by Bertrand and Cornaille [1].

In *Archangiopteris* two vascular strands only are given off from the dictyostele of the stem to supply each leaf. As they pass outwards they divide up into several (8 or 9) separate strands which are arranged at the base of the leaf in a typical horseshoe-shaped curve, entirely similar to that in *Kaulfussia* (Fig. 11). The terminal strands of the curve (*xx* in all the figures) advance towards the median plane of the petiole, and at one point in the pulvinus they fuse together to form a single broad strand the protoxylems of which face abaxially (Fig. 13). Higher up they become separate again, and curving gradually inwards they turn round at the same time so that their protoxylems face towards the sides of the petiole (Fig. 14).

[1] Études sur quelques caractéristiques de la structure des Filicinées actuelles. Mémoires de l'Université de Lille, tom. x, Mém. 29, pp. 151 et seq., 1902.

All the strands hitherto described belong to the typical leaf-trace curve referred to above, but the additional internal strands that occur in the basal pulvinus of *Kaulfussia* are also to be found in the same region of *Archangiopteris*; they are, however, more feebly developed and less regular in their behaviour. In all the leaves examined, and it probably holds true for the leaves of the plant in general, the internal strands invariably arise as branches on the inside of the strands of the abaxial region of the typical curve, and they always pass upwards across the internal ground-tissue to fuse with the median adaxial strands of the curve before the top of the pulvinar region is reached. Only two of these strands were found in any one petiole, and almost immediately after their origin they fuse together to form a single one with its protoxylems on the abaxial side (cf. Figs. 11 and 12). After passing upwards for a short distance they become separate again, and finally fuse with the two terminal strands of the typical curve (cf. Figs. 13 and 14). In one case a single strand only was given off, which passed obliquely across and fused with the terminal strand on its own side.

The internal strands seem to join on indifferently to either end of the incurved terminal strands. In one case the same internal strand fused first with one end of the terminal strand, and then, after separating off again, finally fused up with the other. Sometimes the internal strands are connected with their respective terminal strands by a short suture before they themselves come into actual contact.

All the vascular strands in the petiole above the basal pulvinus belong to the typical horseshoe-shaped curve. The ends of the horseshoe are curved deeply inwards so that the two terminal strands take up a more or less internal position (Fig. 15). They remain separate from each other throughout the greater part of the petiole, but in certain regions they fuse together to form a single large internal strand with the protoxylem facing adaxially (Fig. 16), just as in *Kaulfussia*. In these two plants, therefore, an important distinction has to be made between these more or less included strands of the typical curve and the internal strands that occur in the basal pulvinus which have quite a different origin. In other Marattiaceae, however, the internal strands of the pulvinus are continued into the upper part of the petiole. For instance, in a form of *Marattia fraxinea* that I examined there were, at a point just above the basal pulvinus, two separate concentric rings of internal strands, and also a single strand occupying the centre of the ground-tissue. In the upward direction these strands gradually disappear in precisely the manner that might have been anticipated from the behaviour of the internal strands in the basal pulvinus of *Kaulfussia* and *Archangiopteris*. First of all the central strand fuses with one of the median adaxial strands of the inmost ring. Then those of the inmost ring successively fuse with the adaxial strands of the ring next without, which

in turn disappears by fusing in a similar manner with the outermost ring, or typical curve. All this takes place so slowly that a number of the internal strands continue their course from the pulvinus far up into the region of the rachis. According to Bertrand and Cornaille (l. c.), the same holds true for the internal strands in the petiole of *Angiopteris*.

It is known also that the vascular system of the petiole of *Helminthostachys* is characterized by the presence of an internal strand, and in the light of these facts it becomes necessary to determine the exact manner in which it arises. In this plant the leaf-trace departs from the stem stele as a single circular or elliptic strand with a mesarch protoxylem. In the free petiole the protoxylem becomes endarch, the endodermis disappears, and the leaf-trace divides up into several strands which arrange themselves along a curve almost completely closed adaxially. Then in all the petioles examined I was astonished to find that the terminal strand of one arm of the curve, *and of one arm only*, sinks inwards towards the centre of the petiole, and turns round so that its protoxylem faces adaxially. This internal strand has, therefore, a different origin from those in the basal pulvinus of the Marattiaceae, and can only be compared with the internal strands in the upper part of the petiole of *Kaulfussia* and *Archangiopteris*, the surprising feature about it being that it represents the adaxial terminal strand of one arm only of the curve.

To return again to *Archangiopteris*; the manner in which the pinnae obtain their vascular supply from the rachis is worthy of especial notice. The terminal strands of the petiolar curve are always separate from each other in the neighbourhood of the insertion of the pinnae, and each pinna receives two strands from the rachis. One of them is comparatively large, and comes from the side of the curve (Fig. 17, *p. tr.*); the other is small, and comes from the extremity of the incurved terminal strand on the same side. This small strand travels right across the *central ground-tissue within the curve* to fuse with the adaxial end of the large strand (Fig. 17, *st.*). In my examples the small strand never became quite free from the terminal strand, but moved along in contact with its inner surface.

In the petiole of *Kaulfussia*, also, a strand passes across the internal ground-tissue from the extremity of the incurved terminal strand of the curve to join those that depart from its sides into the branch. Again, Bertrand and Cornaille (l. c.) describe a similar procedure in the petioles of *Marattia fraxinea* and *Angiopteris*. They find that the primary branches are supplied with vascular strands from the internal system (which is continued from the basal pulvinus far up into the rachis) in addition to those they obtain from the outer ring, and this is also confirmed in the *Marattia* that I myself examined.

I believe, with these authors, that this method of vascular supply to the

petiolar branches will probably prove to be characteristic of the Marattiaceae as an order, and I agree with them in regarding it as a distinction between this order and the leptosporangiate Filicineae. As regards the Ophioglossaceae, *Helminthostachys* seems to present a parallel case; for each of the first pair of pinnae certainly receives branches from the internal strand, although the greater portion of it passes out into the fertile spike.

The Structure of the Stipule.

The wings of the stipule of *Archangiopteris* contain a number of small anastomosing vascular strands arranged in a row with their protoxylems facing adaxially towards the median plane of the petiole. There are also a few additional strands in the upper part of the stipule lying on the adaxial side of the main row, and facing in the opposite direction. They are all derived from about three main branches which arise from the strands at the adaxial corners of the petiolar curve. The transverse commissure has no vascular supply at all in *Archangiopteris*, but in *Kaulfussia* it contains a row of strands facing away from the petiole.

In *Archangiopteris* a small group of cells having the appearance of an arrested meristem is to be found at the bottom of a little pit situated at the base of the stipule near the margin of the wing, but slightly over on to the adaxial surface (about the point x in Figs. 3 and 4). A similar group occupying the same position is also present in *Kaulfussia* (x in Fig. 5). It is here rather more highly developed, and sometimes forms a definite projection into the bottom of the pit. In both genera there is a broad curved vascular strand in the stipule which is derived from the corner strands of the petiolar curve and terminates just below this meristematic group. The whole structure clearly belongs to the petiole and not to the stem. These structures are to be found on all the leaves, even the young ones, and they are no doubt to be regarded as arrested apices of dormant adventitious buds, and the 'stipular budding' that is well known to occur in the Marattiaceae is probably to be accounted for by their belated development.

Histology.

Archangiopteris, as regards the minute structure of its tissues, agrees almost exactly with the descriptions given for the related genera by Miss Shove, Farmer and Hill, and Brebner[1]. The protoxylem elements

[1] R. F. Shove, 'On the Structure of the Stem of *Angiopteris evecta*,' Annals of Botany, vol. xiv, No. lv, p. 497, 1900. Farmer and Hill, 'On the Development and Structure of the vascular strands in *Angiopteris evecta*, and some other Marattiaceae,' Annals of Botany, vol. xvi, No. lxii, p. 371, 1902. Brebner, l. c.

are endarch in both stem and petiole. The phloem extends all round the meristeles of the stem, but protophloem occurs only on the outer side of the xylem, and there, as described by Miss Shove in *Angiopteris*, it lies between the xylem and the metaphloem. This extraordinary fact can be observed with perfect clearness in all the strands, external or internal, of both stem and petiole, and even in those of the stipule. The cells of phloem-parenchyma in immediate contact with the sieve-tubes are small and have albuminoid contents. A layer of large cells usually containing starch intervenes between the protophloem and the metaphloem, and two or three layers of similar cells separate the former from the xylem. The two cell-layers immediately surrounding the phloem are slightly different from the rest of the ground-tissue of the stem both in their form and in their reactions towards staining reagents. They might, perhaps, be regarded as a pericycle, but since neither an external nor an internal endodermis could be demonstrated in either stem or petiole, it is impossible to set any definite limit to the vascular strands. As in the rest of the order, there is no sclerotic tissue of any sort in the stem, but in the petiole there is a layer of fibres with colourless lignified walls situated a few layers below the epidermis. In the pulvini this fibrous layer is replaced by a zone of collenchyma. It is, perhaps, also worth mentioning that a certain amount of sclerotic tissue, quite different in nature from that of the fibrous zone, is to be found in the upper part of the rachis in the form of *Marattia fraxinea* that I examined. It consists of short rectangular elements with very thick pitted walls; something like stone cells. They form a very irregular and incomplete sheath around the vascular strands, separated from the phloem by two or three layers of thin-walled parenchyma.

Mucilage canals occur irregularly distributed in the stem, root, and petiole. In the latter they are confined to the tissue within the fibrous zone, except in the pulvini, where they also occur between the collenchyma and the epidermis. Tannin sacs are present in the petiole, but not in the stem. The paleae which occur at the base of the petiole arise from the bottom of deep pits. They are lanceolate in form, with a toothed margin, and run out into a long narrow point.

General Conclusions.

In forming any conclusions based upon this account of the structure of *Archangiopteris* it is necessary to bear in mind that a small portion of a single plant only has been examined, and that, since a comparatively small specimen would naturally recommend itself to a collector as convenient for manipulation and transport, it may quite well be that this particular specimen does not represent the full size attainable by the species. It is possible, therefore, that other specimens may disclose a structure considerably more

complex than that described above. With this reservation the mature stem of *Archangiopteris* may be said to present a simpler structure than that of any of the other Marattiaceae. In fact it still retains at maturity a stage rapidly passed through by the young plants of the other genera. It has been shown that in young plants of *Angiopteris*, *Marattia*, and *Danaea*[1] there is a stage with a single internal strand only, which itself fuses with each leaf-gap margin ; just as in the mature stem of *Archangiopteris*. The mature stem of *Kaulfussia*, although it only contains a single internal strand, is a stage in advance of *Archangiopteris*, for here the internal strand runs freely and continuously through the central ground-tissue, and is only connected with the leaf-gap margin by means of branches.

The vascular system of the petiole is also comparatively simple both in *Archangiopteris* and *Kaulfussia*, the arrangement of the strands being based upon a simple horseshoe-shaped curve comparable to that typical for the Cyatheaceae and Polypodiaceae. It is clear also that the further increase in complexity that occurs is not due to any alterations in the outline of this curve, but to the formation of an altogether new system of internal strands which arise from the inside of those of the original curve.

Miss Shove (l. c.) has stated that in the base of the petiole of *Angiopteris* the internal strands are seen to arise from the outer ring, and the same thing has been said of *Marattia fraxinea* by MM. Bertrand and Cornaille (l. c.). There is very little doubt, therefore, that in these genera also the outer ring of strands represents the same original curve, which remains comparatively simple throughout the order. It is certain also that the internal petiolar strands have no direct connexion with those in the stem. The initial appearance of these strands probably took place in the basal region of the petiole, and it is suggested that *Archangiopteris* and *Kaulfussia* represent an early stage in which they are still confined to the basal pulvinus. The more or less internal strands that occur in the upper part of the petiole of these two plants really belong to the original horseshoe-shaped curve, and are not to be confused with those found in the basal pulvinus, nor with those that occur practically throughout the petiole in *Marattia* and *Angiopteris*. With this exception my observations are thoroughly in accord with the conclusion arrived at by Bertrand and Cornaille—that these internal petiolar strands of the Marattiaceae are not strictly comparable with anything to be found in the petioles of the leptosporangiate Ferns, nor, in my opinion, are they comparable with the internal strand found in the petiole of *Helminthostachys*.

There is no essential difference between the Marattiales and the Filicales as regards the origin and final distribution of the vascular strands

[1] Farmer and Hill, l. c., p. 376. Brebner, l. c., p. 530. Jeffrey, 'The Structure and Development of the stem in the Pteridophyta and Gymnosperms,' Phil. Trans., vol. 195, p. 120, 1902.

in the stem, nor as regards the primitive vascular arrangement in the petiole. At the same time the Marattiales possess a number of distinctive anatomical features of considerable value; such as (1) the centrifugal differentiation of the protophloem, (2) the exceptional nature of the internal vascular strands in the petiole, (3) the special method by which the petiolar branches are supplied with vascular tissue, and (4) the fact that roots arise upon the internal strands in the stem. The first of these characters is quite without parallel in either the Ophioglossales or the Filicales. It is true that a centrifugal development has been observed in the phloem of *Todea* by Seward and Ford [1]; I have also seen the same thing in *Osmunda regalis*, where it is quite clear that the so-called protophloem is still undifferentiated when the sieve-tubes of the rest of the phloem lying within it are fully formed. But this state of affairs is not really comparable with that in the Marattiaceae, because the 'protophloem' in the Osmundaceae is clearly derived from the same layer of meristematic cells which also gives rise to the pericycle; and further, its elements, even when mature, are not vertically elongated like sieve-tubes, but tangentially, like the cells of the pericycle. In fact these elements may be regarded as specialized inner layers of the pericycle, and it may be said that the Osmundaceae have no true protophloem at all. Again, in the phloem of *Aneimia* and *Schizaea* there is no clear distinction into protophloem and metaphloem, but all the sieve-tubes remain functional throughout; in this case, however, *the whole* phloem has more the appearance of protophloem than of metaphloem. In relation to this it is suggestive to note that Boodle [2] is of the opinion that in *Schizaea digitata*, *the whole of the phloem* is derived from the same meristematic layer that also gives rise to the pericycle and endodermis. From some observations that I made on *Aneimia hirta* it appears that the same view holds good for this plant also. It is, indeed, hardly possible at present to decide upon the proper morphological value to be given to these differences in the constitution of the phloem, but at the same time the unique position of the protophloem in the Marattiaceae seems to me to be of so great importance that, until some idea of its real meaning has been obtained, the other anatomical evidence cannot with advantage be brought to bear upon the question of the relationship of the order.

The sori and sporangia on the one fertile leaf of the specimen were examined by Professor Bower, who permits me to add the following account.

'The sporangia correspond very closely to those of *Angiopteris*; they are somewhat more elongated and pointed. Cut transversely, they show the same differentiation of the three- to four-layered wall. Comparing my

[1] 'The Anatomy of Todea,' &c., Trans. Linn. Soc., 2nd Series, vol. vi, p. 237, 1903.

[2] 'Comparative Anatomy of the Hymenophyllaceae,' &c., Annals of Botany, vol. xiv, No. lviii, p. 377, 1901.

figure of *Angiopteris* (Studies, III, Pl. II, Fig. 77) and the description (p. 55), there are the same large, turgid, thin-walled brown cells on the peripheral side ; laterally the same bands of deep prismatic lignified cells ; while the central face, which is thin-walled, bears in a median position the fissure of dehiscence. A transverse section from the distal end of the sporangium shows the narrow bridge of indurated tissue extending from the lateral bands just described across the apex (compare l. c., Fig. 76) ; and thus the arch of indurated tissue or " annulus " is complete over the apex, as it is in *Angiopteris*, and the mechanism of dehiscence will be the same. Longitudinal sections, i. e. vertically through the sorus, show a structure similar to that seen (l. c., Fig. 72) for *Angiopteris*, with a slight difference of outline, *Archangiopteris* being more pointed at the apex.

' There can be no doubt of the near affinity to *Angiopteris*. There may, however, be some difference of opinion as to the phyletic relations of these two Ferns. So far as a conclusion can be drawn from the outline of the sorus, I should be disposed to look upon the long sorus of *Archangiopteris* as a derivative and secondary condition ; while the compact, almost circular sorus of *Angiopteris*, corresponding as it does with most of the modern Marattiaceae, and with the very large majority of Palaeozoic Ferns, would seem to be the more primitive type. It may be that the extension of the sorus to the dimensions now seen in *Archangiopteris* was an incident of relatively late occurrence.'

EXPLANATION OF FIGURES IN PLATE X.

Illustrating Mr. Gwynne-Vaughan's paper on '*Archangiopteris* and other Marattiaceae.'

Figs. 1 and 2. Views of the specimen of *Archangiopteris Henryi* seen from opposite sides (Natural size.)

Fig. 3. Adaxial aspect of the youngest leaf seen in Fig. 2, showing the stipular appendages. *w.*, wings of stipule ; *com.*, transverse commissure ; the mark *x* indicates the points at which the arrested apices may be found.

Fig. 4. Adaxial aspect of the base of the upper leaf seen in Figs. 1 and 2. Lettering as in Fig. 3.

Fig. 5. Diagrammatic representation of the end of a rhizome of *Kaulfussia*, showing stipular appendages. Lettering as in Fig. 3.

Fig. 6. *Archangiopteris*. Transverse section of stem ; the departing traces of two leaves are shown. *s.s.*, meristeles of stem ; *int. s.*, internal strand ; *l.t.*, leaf-trace ; *r.*, root stele. The dotted line indicates the limit of the root cortex.

Fig. 7. *Archangiopteris*. Transverse section of the stem in a region where no internal strand is present. Lettering as in Fig. 6.

Fig. 8. *Kaulfussia.* Represents the vascular system at the very base of a petiole. The letters *a. b. c. d. e.* indicate the internal strands. In this and in all succeeding figures the courses traversed by these strands are indicated by the fine continuous lines. The dotted line joins up the strands that form the typical curve, the terminal strands of which are marked with an *x*. The arrow-heads show the direction of the protoxylems.

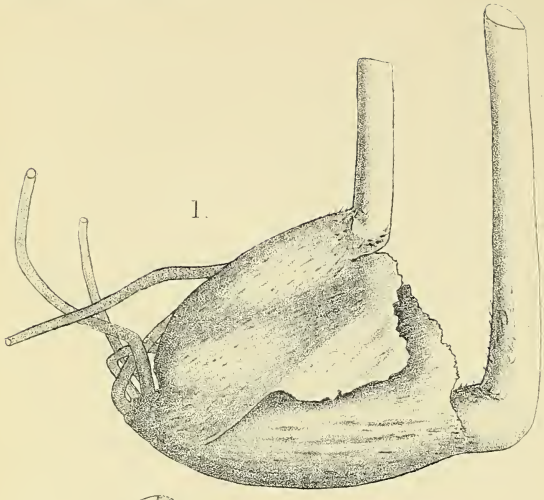
Fig. 9. *Kaulfussia.* The vascular system in the lower part of another petiole. *s.* is the transverse suture passing across between the two terminal strands.

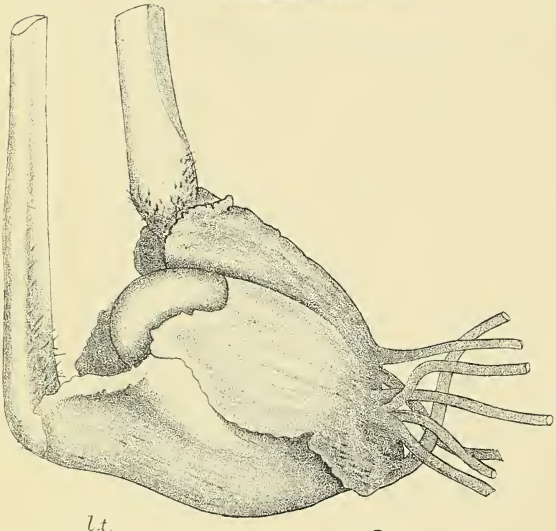
Fig. 10. *Kaulfussia.* The vascular system in the upper part of a third petiole.

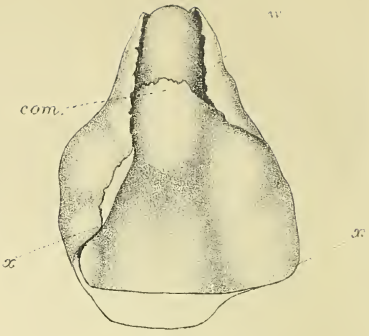
Figs. 11–14. *Archangiopteris.* The vascular system at successively higher levels in the basal pulvinus of the same petiole. *a. b.*, internal strands.

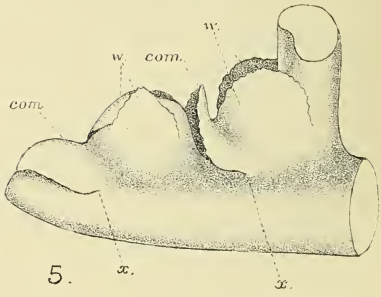
Figs. 15 and 16. *Archangiopteris.* The vascular system at two succeeding levels in the petiole above the basal pulvinus.

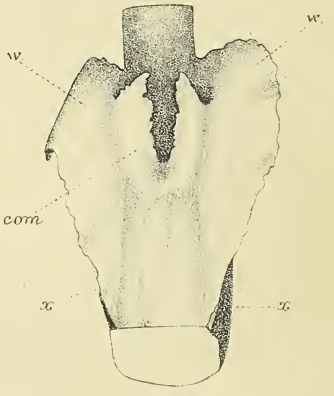
Fig. 17. *Archangiopteris.* The vascular system in the rachis just below the insertion of a pinna. *p. tr.*, main strand for the pinna; *st.*, strand which passes out into pinna from end of terminal incurved strand.

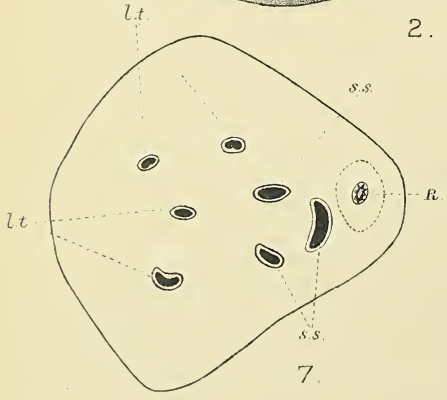


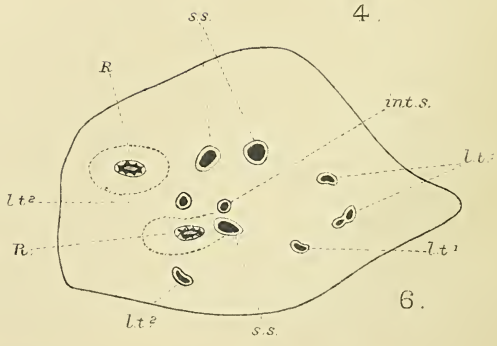












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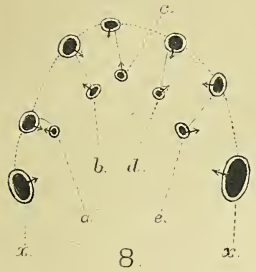
GWYNNE-VAUGHAN,—ARCHANGIOPTERIS & KAULFUSSIA.



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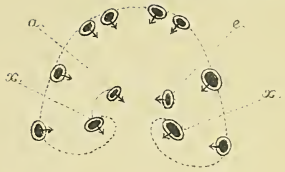

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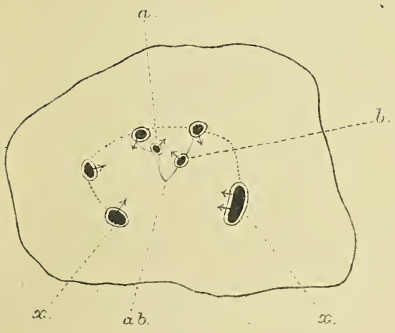

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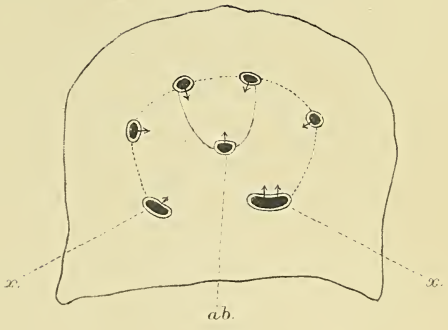

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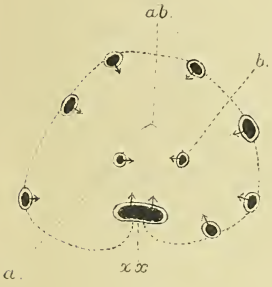

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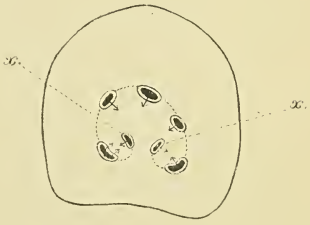

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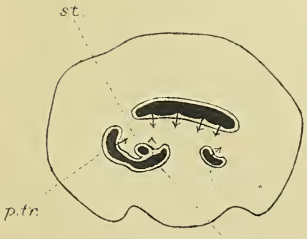

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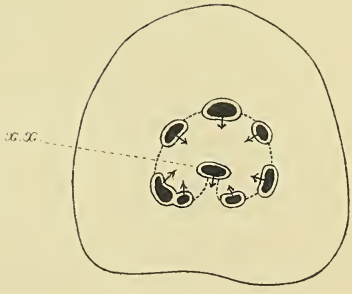


16.

Huth, lith. et imp.

On Two New Specimens of Spencerites insignis.

BY

MISS E. M. BERRIDGE, F.L.S.

With Plates XI and XII and three Figures in the Text.

A SPECIMEN of this fossil strobilus from the Coal-Measures of Yorkshire and Lancashire was first described by Professor Williamson as a *Lepidostrobus*[1], no specific name being given; later accounts were published of this and other specimens under the names of *Lepidostrobus insignis* and *Lepidodendron Spenceri*[2].

In 1897 this type of cone was the subject of a paper by Dr. Scott[3], who showed that the distinctive characteristics of the fossil justified its removal, already suggested by Professor Williamson, from the genus *Lepidostrobus;* Dr. Scott therefore gave a full description of this and an allied species under the names *Spencerites insignis* and *Spencerites majusculus.* Until the latter part of 1903 only four examples of the strobilus *Spencerites insignis* were known. In the summer of that year, however, I found two specimens at Dulesgate, from which some series of sections were cut by Mr. Lomax, among them being the fine radial section which is represented in Pl. XI, Phot. 1. The new examples are chiefly remarkable for the good preservation of the sporophylls; these prove to be more complicated than was previously supposed. They also vary considerably in other respects from the specimens described by Dr. Scott; the following account deals chiefly with these points of difference:—

Axis. The diameter of the axis is 5 mm., which is the maximum measurement given by Dr. Scott for the cones. It is evident from this and other measurements that the new specimens are rather large examples of the fossil.

Within the ring of primary wood there is a well-marked pith, the prosenchymatous cells of which are mostly thin-walled, but a strand of thick-walled cells appears near the centre (Phot. 5, *m*).

[1] 'Organization of the Fossil Plants of the Coal-Measures,' Part IX, Phil. Trans., 1878.

[2] 'Organization,' &c., Parts X, XVI, XIX, Phil. Trans., 1880, 1889, and 1893.

[3] (i) 'On the Structure and Affinities of Fossil Plants from the Palaeozoic Rocks'; (ii) 'On *Spencerites*, a new Genus of Lycopodiaceous Cones.' Phil. Trans. Roy. Soc., vol. 189 B, 1897.

[Annals of Botany, Vol. XIX. No. LXXIV. April, 1905.]

The protoxylems of the woody cylinder are about twenty in number, this corresponding to the number of orthostichies of sporophylls. The prominence of the angles of the stele formed by the protoxylems varies considerably at different levels in the axis. When the section passes near the level at which a whorl of ten leaf-traces leaves the stele, ten angles are much more clearly marked than the other ten alternate with them; this probably accounts for the fact that ten is given as the number of protoxylems in Dr. Scott's paper.

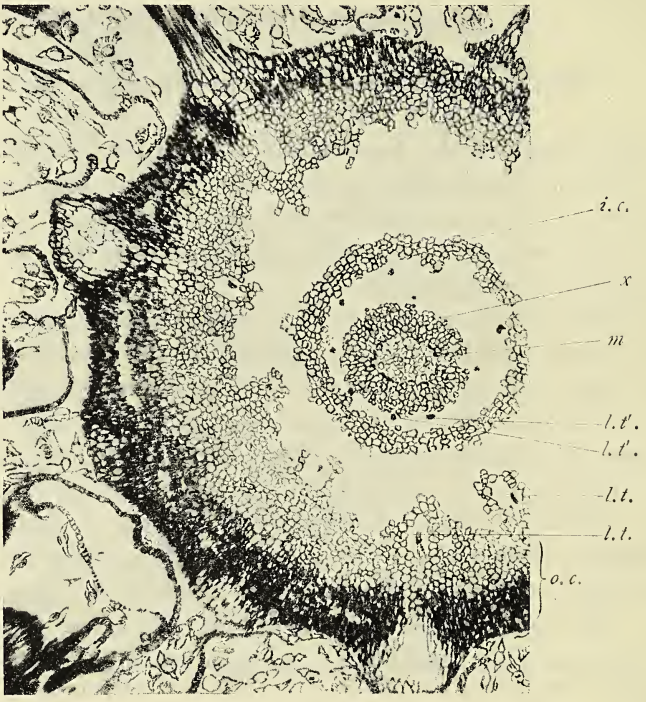


Fig. 2. Transverse section of the axis, showing the two zones of the outer cortex, and two alternating whorls of leaf-traces. *m*, pith; *x*, xylem; *i.c.*, inner cortex; *o.c.*, outer cortex; *l.t.*, *l.t.*, leaf-traces belonging to the outer whorl; *l.t'.*, *l.t'.*, leaf-traces belonging to the inner whorl.

Of the zones of tissue surrounding the wood only the inner and outer layers of the cortex remain, the phloem and middle cortex having perished. The inner cortex consists of thickened, somewhat elongated cells, and is in every respect similar to that of previous specimens.

The outer cortex, however, is evidently very variable in this genus. Dr. Scott has taken the specimens showing Dictyoxylon structure as typical, but he mentions other cases in which 'the cell-walls are considerably thickened throughout the external cortex.' In the present examples it appears to be differentiated into two zones; the outer is uniformly thickened, the Dictyoxylon character being absent; the inner consists of

delicate thin-walled tissue, which, however, shows no trace of the trabecular character of the middle cortex as preserved in certain specimens in the Williamson collection (Phot. 10).

It is evident from the position of the leaf-traces both in the transverse (Fig. 2) and tangential sections (Phot. 2) of the axis, that the sporophylls were arranged in alternating verticils, each whorl consisting of ten sporophylls. Dr. Scott has mentioned this as a probable arrangement in his paper

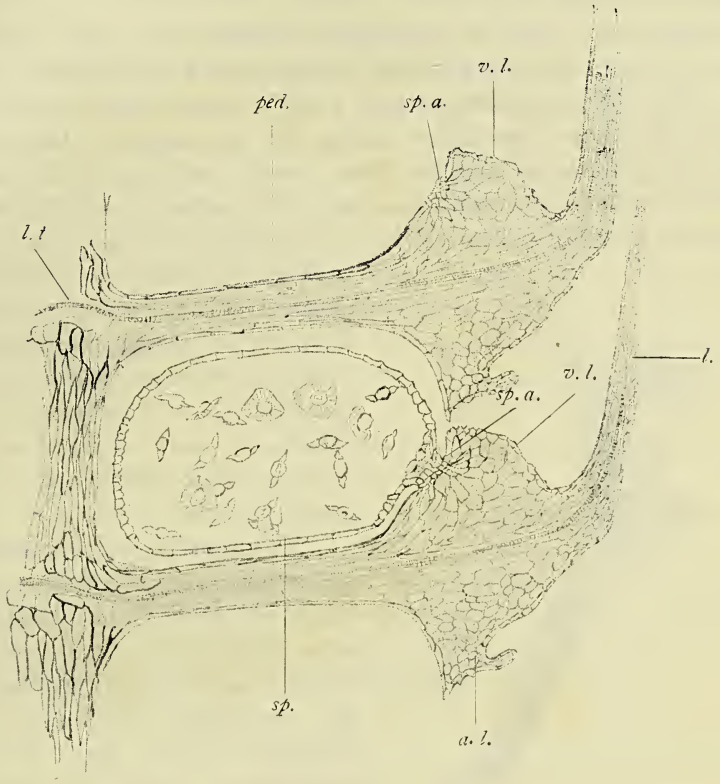


FIG. 3. Diagram showing the probable form of the sporophylls, with one sporangium. *l.*, distal limb; *v.l.*, ventral lobe; *d.l.*, dorsal lobe; *sp.*, sporangium; *sp.a.*, sporangial attachment; *ped.*, pedicel; *l.t.*, leaf-trace.

'On *Spencerites*,' and pointed out to me the clear evidence regarding this character furnished by the new slides. He nevertheless considers the phyllotaxy as somewhat variable, being sometimes spiral and sometimes verticillate. Such variability is often present among recent Lycopods; in 'Die natürlichen Pflanzenfamilien' it is mentioned that both a spiral and whorled arrangement of the leaves is frequently to be found at different levels on the same shoot; this is markedly the case in *L. Selago*.

The whorled arrangement is also evident in Phot. 7, a tangential section of the other specimen, passing through the pedicels of the sporophylls.

The course of the leaf-trace is considerably arched just before it enters the pedicel (Phot. 9). In certain cross-sections, therefore, the same leaf-trace appears twice, as is the case in Phot. 10 (*l.t.*, *l.t.*), giving the appearance of superposed whorls. The traces of each whorl of sporophylls are inserted on the woody cylinder at the level of the whorl next below.

Sporophylls and Sporangia. Some of the sporophylls of the new cones are exceptionally well preserved, and good radial sections have been obtained of them.

These show that the sporophyll consists of a narrow pedicel, from about 2·5 to 3 mm. long, carrying an upturned lamina with a broad fleshy base. The base is prolonged into a thick dorsal lobe below and a larger ventral lobe above, the latter bearing the sporangium. In all previously known sections the lamina has disappeared, leaving only the base with its two lobes, which gave the appearance of a peltate sporophyll-head. The true form of the sporophyll is represented somewhat diagrammatically in Fig. 3; the two examples photographed (Phots. 3 and 4) contributing the main features to the diagram.

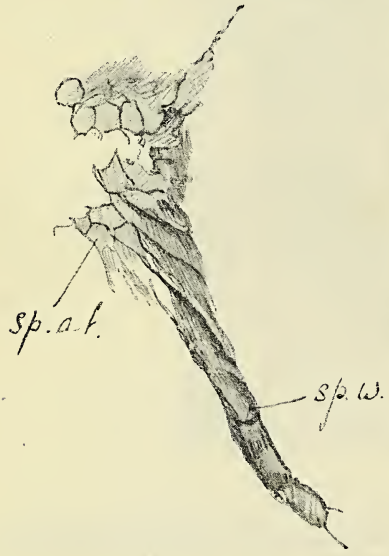


FIG. 4. Portion of sporangium-wall (*sp. w.*) in continuity with some cells of the sporangial attachment (*sp. at.*).

The structure of the pedicel, which has been fully described in Dr. Scott's paper, is clearly shown in the cross-sections of it which appear in photograph 7.

The ventral lobe is usually the best preserved portion of the sporophyll-head. It consists of elongated, somewhat thick-walled cells, and bears a cushion of small delicate cells to which the sporangium was attached. This elliptical disc of small cells forms a conspicuous object in several of the slides, especially in cases such as that represented in photograph 8, where it is cut by a section passing more or less parallel to the adaxial surface of the ventral lobe. This section shows that its horizontal width is about ·5 mm. and its vertical breadth about ·3 mm.

In the two cones the sporangium has not been found attached to the lamina, but the sporangium-wall is sometimes continuous with a few small isodiametric cells similar to those of the cushion on the ventral lobe (Fig. 4); the wall appears frequently to have ruptured at the place of attachment.

The distal limb of the sporophyll is best shown in photograph 4; in

this example the dorsal lobe is considerably decayed. The vascular bundle, which is clearly shown in photograph 3, runs from the pedicel into the lamina, and can, in several cases, be traced passing up it for a considerable distance. It is noteworthy that no branch has been observed passing to the ventral lobe and sporangium.

The surface of the cone was probably completely covered by the leaf-like laminae of the sporophylls, as those of each whorl extended at least to the level of the third whorl above. The broad base of the distal limb, where it joins the ventral lobe, appears to have been the widest part of the sporophyll. Unfortunately this width cannot be measured directly, owing to the lack of good cross-sections of the sporophyll-head. From determinations of the circumference of the new cones, and the distance between adjacent whorls, it is evident that each sporophyll-head, exclusive of the lamina, occupied a rhomboidal area about 3·7 mm. in width by 2·3 mm. in breadth. It may therefore, I think, be assumed that the ratio of the tangential width of the head to its vertical height, measured through the ventral and dorsal lobes, was about 3 : 2. The same measurements were applied to some of the examples in the Williamson Collection; these were not very definite, as the sporophylls are always considerably decayed, but they seemed to indicate that in these cases the tangential width was more nearly equal to the vertical height of the sporophyll head, the ratio being about 4 : 3.

Spores. The characteristic spores are well preserved, particularly in one of the cones.

In no case has any reticulum been found similar to that apparent in some of the spores in the slides of the Williamson Collection.

A few sporules occur within the sporangia similar to those described by Dr. Scott, but only two have been observed within the spore.

Summary.

The relationship of this family to other genera among the Palaeozoic Lycopods, as outlined by Dr. Scott in his paper, is practically unaffected by the facts brought to light by the examination of the new specimens. The presence of a sporophyll with a leaf-like lamina emphasizes the relationship to the *Lepidostrobi* and to M. Zeiller's *Sigillariostrobus Crepini*, but the attachment of the sporangium to the sporophyll-head above its junction with the pedicel is a character which separates it definitely from the former genus. Besides other points of difference, such as the structure of the sporangial wall and of the spores, and also the arrangement of the leaf-traces, *Spencerites* appears to differ from the *Lepidostrobi* in having no ligule, no trace of it having been found, although well-preserved

radial sections through the ventral lobe and sporangial attachment are frequent.

The distal attachment of the sporangium to the sporophyll has suggested a relationship with *Sphenophyllum*; in that case, the well-marked ventral lobe would represent the sporangiophore of that type. The absence of any trace of a vascular bundle running to the lobe, however, makes this suggestion as to its nature a very doubtful one.

It is evident from the foregoing comparison that the new specimens vary considerably in several points from the examples of *Spencerites insignis* already known, particularly in the absence of Dictyoxylon structure in the cortex, the more ovoid form of the sporangia (corresponding to a greater length of the sporophyll-pedicel), and the greater tangential width of the sporophyll-head as compared with its vertical height. In these characters the new cones show a closer resemblance to *Spencerites majusculus*, and it is questionable whether they do not belong to a species intermediate between *S. insignis* and *S. majusculus*, though certainly much more closely allied to the former. It seems, however, inadvisable to multiply species, and the differences are hardly greater than such as might possibly occur between a young and old cone, or between the top and bottom portions of a long strobilus.

The diagnosis given by Dr. Scott in his paper 'On *Spencerites*' must, however, be considerably modified in order to include the new cones as well as those in the Williamson Collection at the Natural History Museum.

With his permission, therefore, I have introduced the following diagnosis, and take this opportunity of gratefully acknowledging the help I have received from him in dealing with this new material.

Spencerites.

Cone, consisting of a cylindrical axis, bearing numerous simple sporophylls, arranged spirally or in crowded alternating verticils.

Sporophylls, formed of a sub-cylindrical pedicel expanding into a large lamina.

Sporangia, solitary on each sporophyll, inserted by a narrow base on the upper surface of the lamina, but free from the pedicel.

Sporangial wall, formed of a single layer of prosenchymatous cells.

Spores, winged.

Spencerites insignis (Will.).

Cone, pedunculate, peduncle bractigerous.

Whole cone, 8 to 10 mm. in diameter.

Axis, 3·5 to 5 mm. in diameter.

Sporophylls. Pedicel from 2 to 3 mm. long, expanding into a distal limb bearing ventral and dorsal lobes.

Sporangia, spherical or ovoid, seated on the ventral lobe.

Spores, tetrahedral, becoming spheroidal when free, with a hollow equatorial wing. Maximum diameter of spore without wing, about 0·14 mm.; with wing, about 0·28 mm.

Wood of axis, 20-arch, without prominent angles; with or without pith.

Locality, near Halifax and Huddersfield, and at Dulesgate.

Horizon, Lower Coal-Measures.

EXPLANATION OF PLATES XI AND XII.

Illustrating Miss Berridge's paper on *Spencerites*.

Phot. 1. Radial section of strobilus. *s.*, stele; *i. c.*, inner cortex; *o. c.*, outer cortex; *sp.*, best preserved sporophyll. × 7.

Phot. 2. Tangential section through cortex showing the verticillate arrangement of the leaf-traces, *l. t.* × 7.

Phot. 3. Sporophyll-head seen in radial section (*sp.* Phot. 1), showing the sporangial attachment and the vascular bundle passing out to the distal limb, which has perished. × 27.

Phot. 4. Sporophyll-head with distal limb attached, seen in slightly tangential section. The dorsal lobe is much decayed. × 32.

Phot. 5. Transverse section of inner part of axis. *m.*, pith with central strand of thick-walled cells; *x.*, xylem; *i. c.*, inner cortex; *l. t.*, leaf-trace. × 35.

Phot. 6. Transverse section of sporophyll, with distal limb (*a*) attached. × 32.

Phot. 7. Tangential section of strobilus. *sp. ped.*, cross-section of pedicel of sporophyll. × 20.

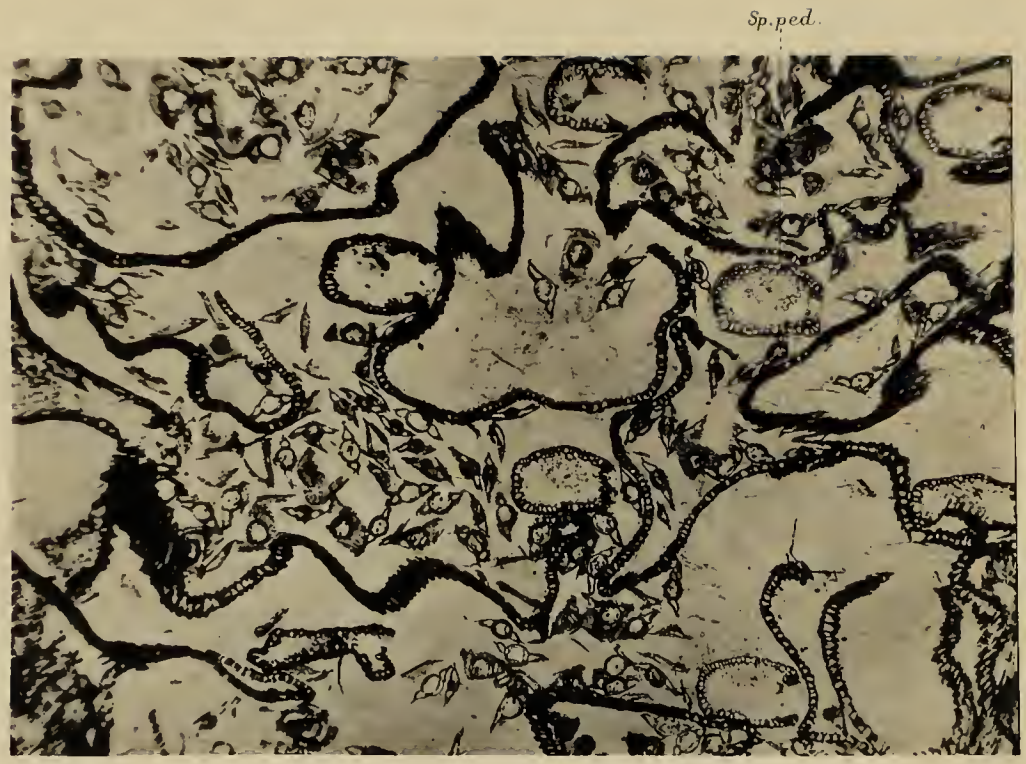
Phot. 8. Section through the ventral lobe and sporangial attachment, *sp. at.* × 40.

Phot. 9. Portion of radial section of the axis, showing the arched leaf-trace, *l. t.*, passing through the outer cortex, *o. c.*, into the base of the sporophyll-pedicel, *pd.*; *x.*, xylem; *i. c.*, inner cortex. × 40.

Phot. 10. Portion of transverse section of axis, showing two leaf-traces. Each leaf-trace appears twice in the section owing to its arched course.

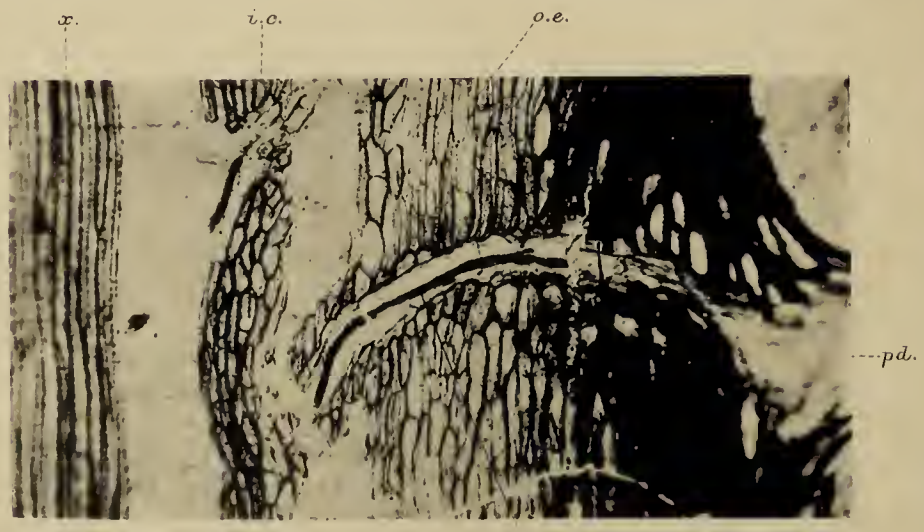
E.M. Berridge, Photo

MIS



Phot. 7.

Phot. 8.

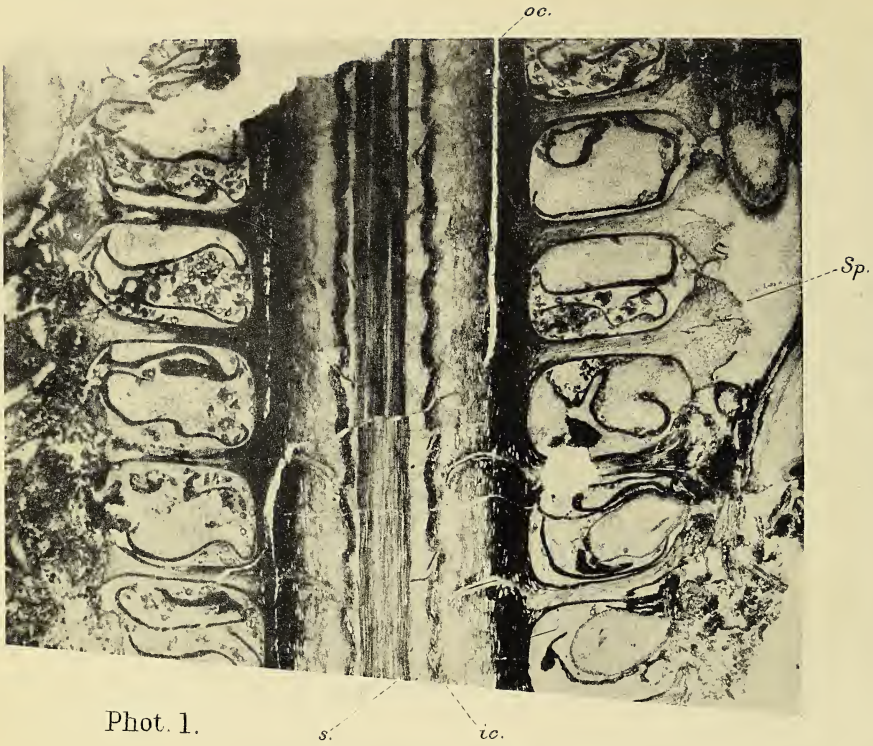


Phot. 9.

Phot. 10.

E. M. Berridge, Photo. Huth, coll.

MISS BERRIDGE — SPENCERITES.



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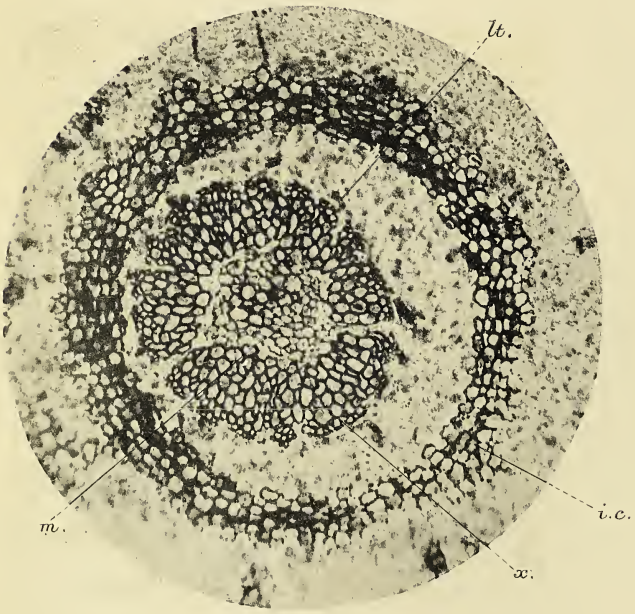
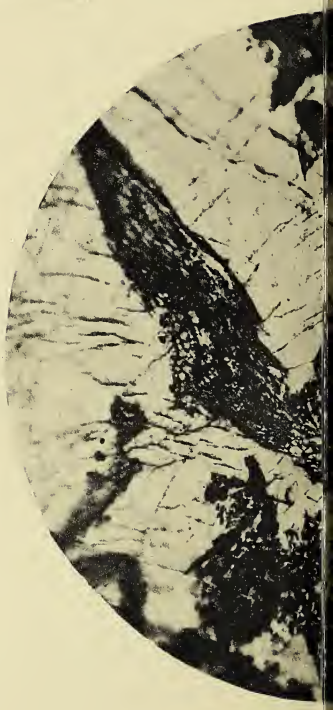


Phot. 5.



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MISS BERRIDGE — SPENCERITES.

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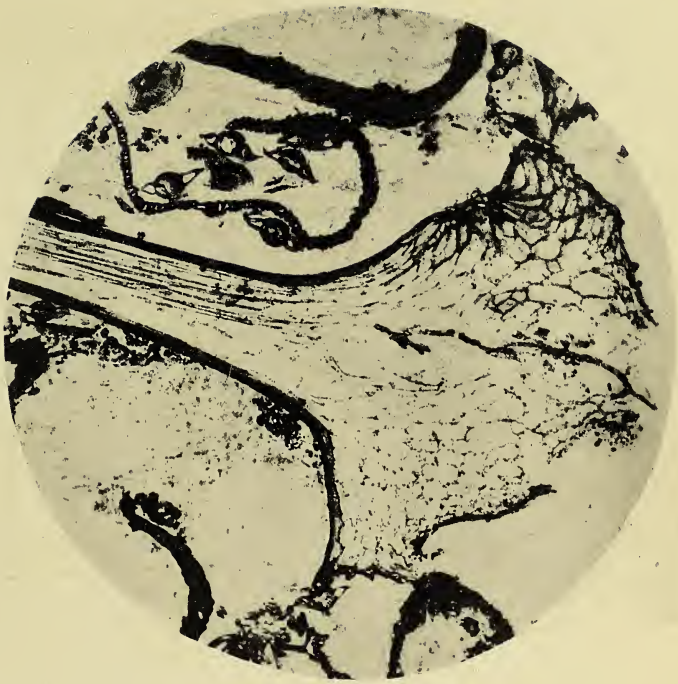

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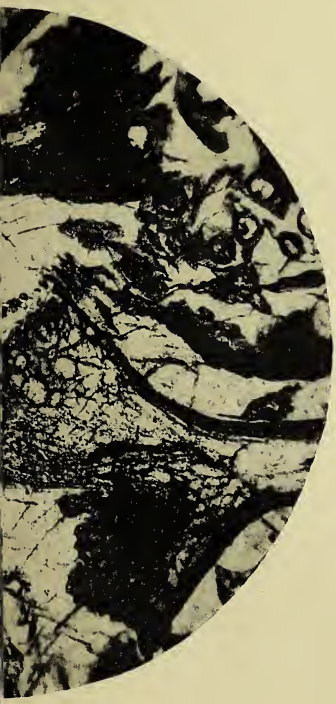

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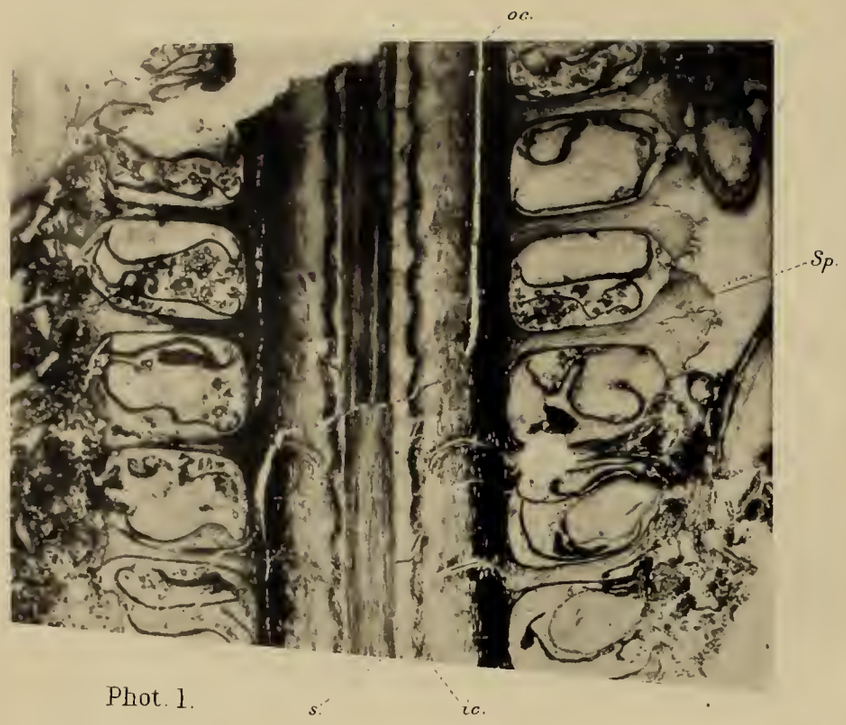

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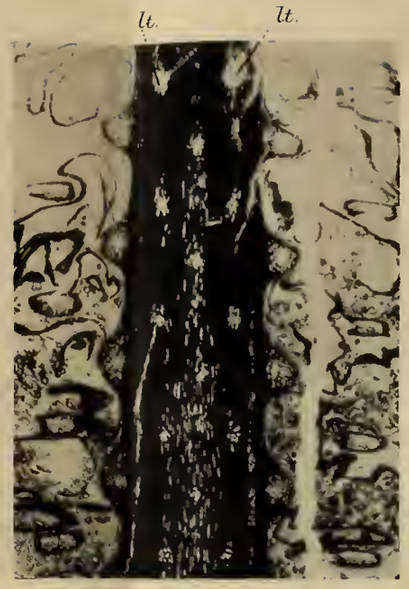

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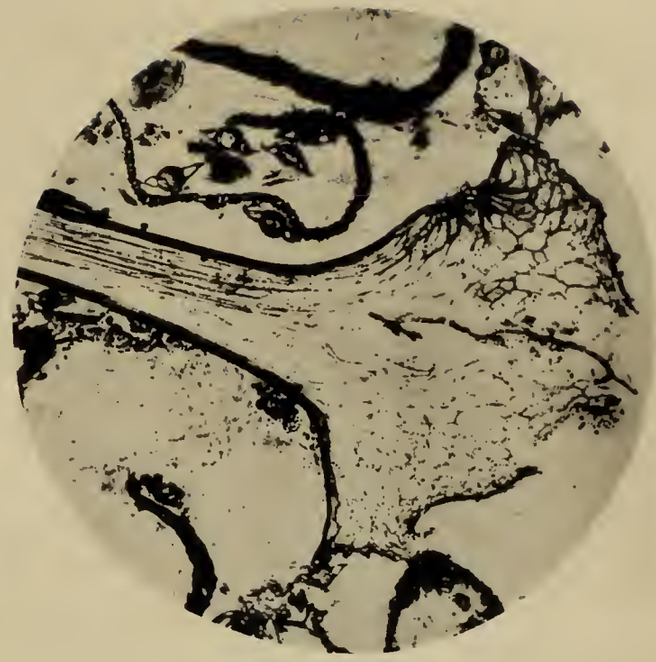

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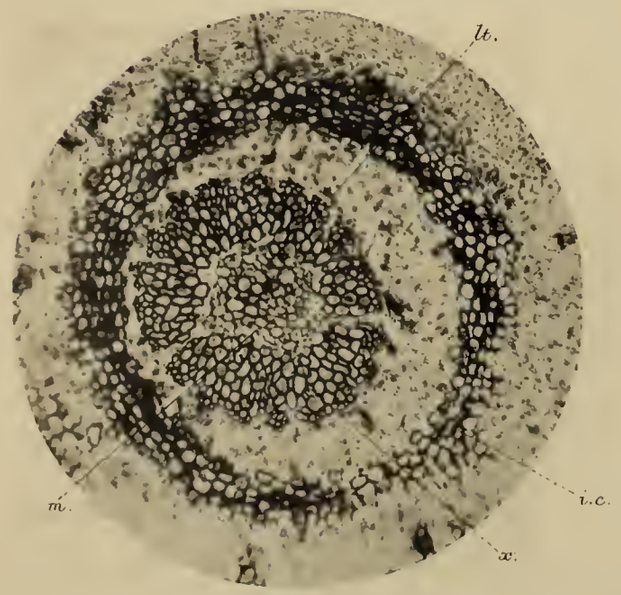

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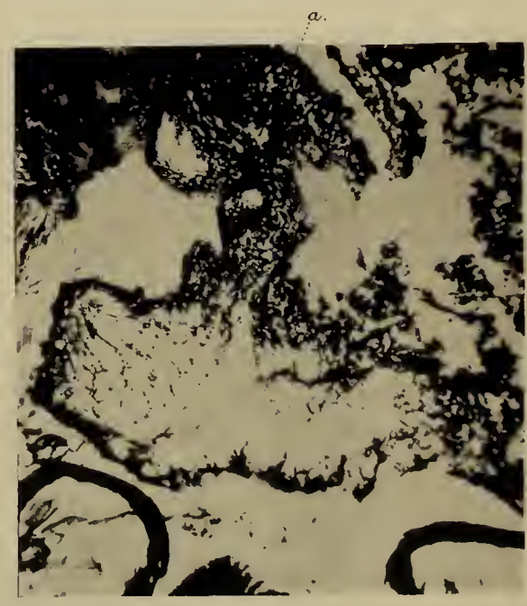

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MISS BERRIDGE — SPENCERITES.

Optima and Limiting Factors.

BY

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With two Diagrams in the Text.

IN this article it is proposed to subject to critical consideration the conception of the 'optimum' as a primary general relation between physiological processes and the external or internal conditions which affect them.

In treating physiological phenomena, assimilation, respiration, growth, and the like, which have a varying magnitude under varying external conditions of temperature, light, supply of materials, &c., it is customary to speak of three cardinal points, the *minimal* condition below which the phenomenon ceases altogether, the *optimal* condition at which it is exhibited to its highest observed degree, and the *maximal* condition above which it ceases again.

As the maximum temperature for most metabolic processes is very near to the death point, exact location of it is attended with considerable experimental uncertainty and precise data are generally wanting. In practice, attention is usually concentrated upon the optimum of the condition and upon the general form of the middle part of the simple curve, which is usually accepted as a satisfactory graphic expression of the relation between the function and the condition.

In the treatment of the assimilation of carbon dioxide in all text-books we find mention of optima of temperature, of light, and of carbon dioxide-supply for this process. After some years of experimental study of the effect of external conditions upon carbon-assimilation the writer has demonstrated that much of this treatment is quite incorrect, and from this position has passed to the general conviction that there is much that is misleading in that treatment of the effect of an external condition which involves giving definite values to its cardinal points.

[Annals of Botany, Vol. XIX. No. LXXIV. April, 1905.]

I.

We will at present confine our attention to the condition of temperature, and will begin with certain *a priori* considerations derived from chemical dynamics.

The rate at which all normal chemical change takes place is increased by a heightened temperature condition.

Most reactions *in vitro* take place so quickly that it is impossible to measure their rate, but with all that go slowly in aqueous solution and resemble the processes of the organism, such as the saponification of esters, the inversion of sugar by acids, and others, it has been found that the acceleration produced by increased temperature is about the same. This has been generalized by van't Hoff[1] into the rule that for every rise of 10° C. the rate of reaction is about doubled or trebled.

If this rule of chemical dynamics does not hold good for chemical reactions within the organism it is the duty of the physiologist to attempt, at any rate, to explain the aberration. Now it is interesting to note that this relation has actually been found to hold, as regards medium temperatures, say from 10° C. to 27° C., for quite a number of cases in animal and vegetable organisms so diverse in nature that the law clearly is *primarily* applicable to chemical change in the cell as well as the test-tube. Thus the respiration numbers of Clausen[2], for lupine seedlings and for *Syringa* flowers, show between 0° and 20° C., an increase of two and a half times for a rise of 10° C., the assimilation numbers obtained by Miss Matthaei and the writer for cherry-laurel leaves a coefficient of 2·1, and for sunflower leaves 2·3, while to come to more complex metabolic changes, the times required for spore-formation in *Saccharomyces pastorianus* (Herzog[3]), and for the development of frogs' eggs (as calculated by Cohen from Hertwig's data, cf. Hober[4]), at different medium temperatures both proceed within the limits of this rule.

As regards the rate of metabolic chemical change in the organism at high temperatures, this law clearly does not express the whole truth. If it did we should expect, with increasing temperature, all vital processes to proceed with ever-increasing velocity till the fatal temperature was reached at which some essential proteid coagulated or some other connexion was dislocated, and the whole metabolic machinery came suddenly to a standstill.

What then does happen as we approach the upper temperature-limit of the working of the organism? An important new factor, the *time-factor*, comes into play.

[1] Vorlesungen ü. theoretische Chemie, 1898, Pt. i, English translation, p. 227.

[2] Landwirtschaftliche Jahrbücher, 1890, Bd. 19, p. 893.

[3] Zeitschrift f. physiologische Chemie, 1903, Bd. 37, p. 396.

[4] Physikalische Chemie der Zelle u. der Gewebe. Leipzig, 1902.

In later years this factor hardly receives the attention that it deserves. Sachs[1], however, clearly pointed out that the higher the temperature the more quickly the fatal effect ensues, and that short exposure to a very high temperature may not kill, when a prolonged exposure to a slightly lower temperature is fatal ; see also Pfeffer, Physiology, Section 65.

Miss Matthaei's experiments on Carbon-assimilation[2] show, for a given case, precisely how important this same time-factor is in the relation of a single function to temperatures which are high but not fatal. The facts observed indicate the following three laws :—

(1) At high temperatures (30° C. and above for cherry-laurel) the initial rate of assimilation cannot be maintained, but falls off regularly.

(2) The higher the temperature the more rapid is the rate of falling off.

(3) The falling off at any given temperature is fastest at first and subsequently becomes less rapid.

This falling off makes it experimentally impracticable to attain the highest possible assimilation value at any given temperature. The theoretical initial value can, however, be arrived at indirectly by continuing back the curve of falling values actually attained, taking of course due account of the time that elapsed between the initial heating and the earliest actual estimation. Besides this first method there is also a second method available for arriving at these initial theoretical values, which is based on the law of uniform acceleration of reaction mentioned above. At low temperatures, below 25° C., the assimilation-rate does not fall off appreciably with successive estimations—no time-function is involved—therefore at these temperatures the first actual estimations made give an exact measure of the initial values of the function, and by the relation between them we determine the coefficient of increase of rate for a rise of 10° C. In the case of the cherry-laurel leaf this coefficient is 2·1. We can then obtain the theoretical values for higher temperatures by calculation.

We have now to apply these two methods to the case of assimilation, as to which function alone we have adequate numerical data, and to see how far they indicate similar theoretical initial values.

This is carried out graphically in Diagram I. By the van't Hoff rule, starting from the ascertained values of the assimilation at 9° C., and at 19° C., which were 3·8 mg. CO_2 (*A*), and 8·0 mg. CO_2 (*B*) respectively, giving the coefficient of 2·1, we continue the series, and by interpolation on the calculated curve obtain the initial value for any desired temperature. The values for 30·5° C. (*C*), 37·5° C. (*D*), 40·5° C. (*E*), 45° C. (*F*), 50° C. (*G*), are represented by dots in the interrupted line which forms the calculated hypothetical curve of the initial relation of assimilation to temperature.

[1] Handbuch der Experimental-Physiologie, 1865, p. 52 ff.

[2] Phil. Trans. Roy. Soc., vol. 197 B, p. 85, 1904.

We have now to apply the other method. Prolonged assimilation-estimations were made at high temperatures, the rate of falling off being determined at 30·5° C., at 37·5° C., and at 40·5° C.[1]. In each case the

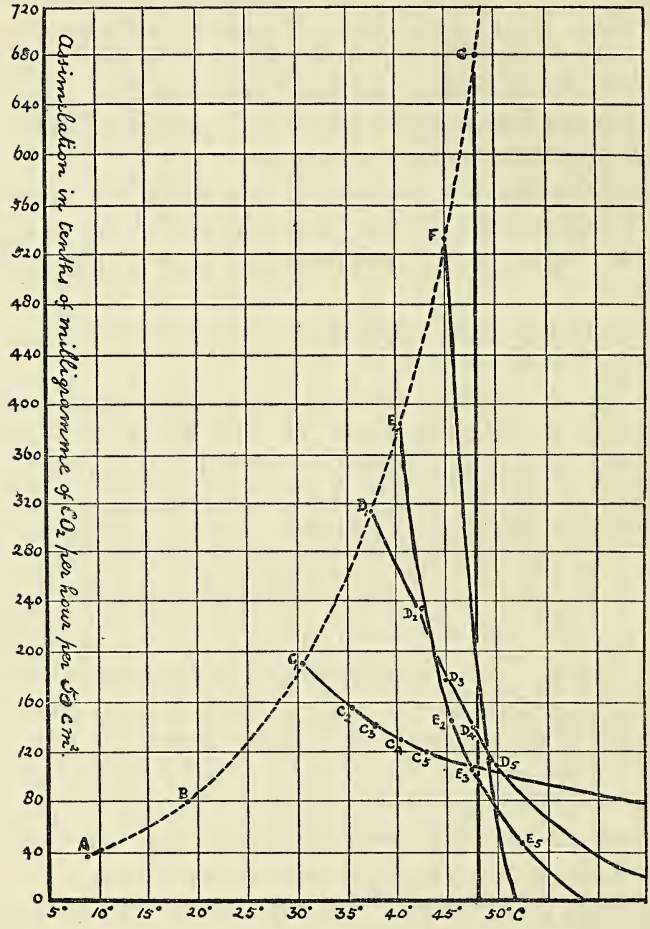

FIG. 5. DIAGRAM I.

estimations were four in number, of one hour's duration each[2], preceded by one and a half hours' preliminary between the initial heating and the beginning of the first estimation. To plot these on the diagram we regard the base line as having only a time significance, each division

[1] See the experiments 58, 59, and 60 in Miss Matthaei's paper.

[2] The numbers obtained for the 'real' assimilation (assimilation corrected for contemporaneous respiration) were as follows, in tenths milligramme CO_2 per hour per 50 cm^2. of leaf-area.

$C_2 = 157$	$D_2 = 237$	$E_2 = 147$
$C_3 = 140$	$D_3 = 176$	$E_3 = 108$
$C_4 = 129$	$D_4 = 139$	$E_4 = 98$
$C_5 = 120$	$D_5 = 109$	$E_5 = 48$

The value for E_4 is clearly out of place, and the curve has not been made to pass through it.

representing two hours, and plot out the falling series of readings obtained at 30·5, 37·5, and 40·5° C. in curves starting from the initial values indicated by method I.

It then becomes at once obvious that the calculated initial value and the observed subsequent values fall into one fairly harmonious curve for each temperature. We thus attain a graphic demonstration that both methods indicate practically identical initial values, and this affords, it seems to me, satisfactory evidence for a preliminary acceptance of the theory that such values actually occur, though it is not possible to measure them.

At low temperatures—up to 25° C.—the assimilation-values keep up to the initial value for a long period, but at these higher temperatures the fall continues and in time brings the assimilation down to zero, and that the more rapidly the higher the temperature. The points at which the respective curves reach zero on the base line are partly outside the limits of the diagram, but the various curves have been continued by freehand beyond the actual estimations in the way that is indicated by other experiments.

Extending the schema that we have arrived at to higher temperatures than 40·5° C., we find at 45° C. a still more rapid fall of the assimilation, for which, however, we have no suitable numerical data, and this declines to zero in a comparatively short time. This is represented in the diagram by the curve starting from *F*.

Finally, to conclude the series we ought to find a temperature at which the earliest estimation that could be actually made would give no measurable assimilation. The lowest temperature to give this result might be called the 'extinction temperature,' and here we should hypothecate that, for the first few seconds after attaining it, each chloroplast would give a higher assimilation rate than at any lower temperature, but that the rate would immediately fall, and that so rapidly that it would become *nil* almost at once (say in 100 seconds, for the accepted specific extinction temperature would of course have to be arbitrarily defined in time-units).

In estimating carbon-assimilation in the presence of over-balancing respiration, it is almost impossible that this temperature should be determined directly, but we are accumulating evidence as to the location of it. I have placed it at 48° C. in the diagram. We thus arrive at a complete theoretical schema of the primary relation between the rate of the photosynthetic process, different temperatures, and time.

Now, without going into details again, it may be stated that for cherry-laurel leaves the process of respiration, although it contrasts powerfully with assimilation as to its metabolic significance, also shows quite the same kind of relation to temperature.

I therefore venture to suggest that, making suitable changes in the

coefficients of temperature and of time, this schema may possibly exhibit the *hypothetical primary relation of all metabolic processes whatever to temperature.*

It has been mentioned that before taking the assimilation estimations at high temperatures a 'preliminary' of one and a half hours was allowed after the initial heating up to the experimental temperature. Hardly any investigators have allowed a shorter preliminary time, so that it is clear that published values, for physiological processes generally, at high temperatures are too low, and special experiments designed to get trustworthy estimations as near the initial effect as possible should give higher values.

Now it is most important to note that not only would the value at each high temperature be thus increased, but that these values would be increased *in different ratios.*

Such special experiments would give values—to take the case of assimilation—that were no higher for temperatures up to 25° C., slightly higher values for 30·5° C., and very much higher for 40·5° C. The important consequence follows that the observed 'optimum' temperature will be raised by such special procedure. Compare, for instance, the two extreme cases on the diagram, in one of which the investigator may be supposed to have taken our set of actual first readings, i. e. those at one and a half hours after heating up, B, C_2, D_2, E_2, and in the other to have allowed four and a half hours' preliminary and so to have obtained our set of fourth readings, B, C_5, D_5, E_5.

The first set[1] will give the 'optimum' at 37·5° C. with a steep falling off to 40·5° C., while the other set will give an optimum at 30·5° C. with a gentle falling off to 37·5° C. A more adventurous investigator whose method would work with only a quarter of an hour preliminary should get an experimental optimum over 40° C., and possibly so close to the extinction point that he would decide that a real optimum was absent. Now it is in this contradictory state that knowledge really stands as regards the relation of *respiration* to temperature. Clausen[2] has recorded a well-marked optimum for a variety of plants at 40° C., while Kreusler[3] finds for *Rubus* no optimum, certainly up to 46° C.

It is not to be supposed that these differences in regard to respiration optima depend on different lengths of *actual* preliminaries, rather no doubt they are to a greater extent dependent on different degrees of high-temperature-impermanence of the function with different plants.

Physico-chemical finality is not to be attained in this matter, but special research might at least show how far the recorded optima for assimilation and respiration are real metabolic truths and how far they are illusions of experimentation.

[1] See numbers in footnote, p. 284. [2] loc. cit. [3] Landwirthschaftliche Jahrbücher, 1887.

A further experimental complication lies in the rate of heating up, which must depend partly on the method and partly on the conductivity of the organ investigated. If heating up is slow, then the falling off produced by passing slowly through the temperatures 40°–44° C. would lower the value obtained for 45° C., to take a concrete example.

The optimum has by some investigators been regarded as the highest temperature which can be permanently sustained without depression of function, but more usually a real optimum is held to be characterized by this, that the retardation produced by exposure to super-optimal temperature must not be of the nature of permanent injury, and that therefore on cooling again to the optimum temperature there must be a return of the function to its highest value.

There has been little attempt to apply this principle experimentally, and it looks as if everything would depend on the *time* of exposure to the super-optimal temperature. Rather than by direct experiment, it is probable that the high transient values will in future have to be estimated by the convergence of the lines of evidence that we have already indicated.

Only respiration and assimilation have been yet mentioned, and they are conditioned by comparatively simple factors, or rather by factors which can be kept under control so that these two processes might be expected to show the primary relation to temperature not greatly masked.

In the case of such a complex process as growth one cannot start analysis with any such expectation.

The available published data as to growth are of very little use for our inquiry. Those that deal with a wide range of temperatures mostly consist of single readings, and these after a long preliminary—in one classical set as long as forty-eight hours (Köppen)[1]; the more detailed studies by Askenasy[2] and Godlewski[3] are concerned only with medium temperatures: True[4] has a couple of not very significant experiments at a 'super-optimal' temperature. It is, however, the universally held opinion that growth exhibits a well-marked optimum in its temperature relation. This optimum is placed by Sachs[5] at 34° C. for seedlings of flowering plants, and in many other cases it is lower, so that the optimum is so far removed from the fatal temperatures as to make it impossible that the phenomenon should be wholly an illusion of experimentation. Were it possible to make critical sets of growth-readings fairly close to the hypothetical initial values, the position of the optimum should be found to be higher than after long preliminaries, but it could hardly be pushed upwards to such an extent as to become uncertain by reason of its nearness to fatal temperatures.

[1] Cf. Pfeffer, Physiology of Plants, vol. ii, p. 77. [2] Ber. d. deut. Bot. Ges., 1890.
[3] Bot. Centralb., Bd. 47. [4] Annals of Botany, vol. ix, 1895. [5] Pfeffer, l. c.

Admitting, then, the existence of a 'real optimum,' not to be broken down by the most ideal experimentation, we have to seek a clue to its causation.

In vitro, a few chemical processes are known which take place *more slowly* as temperature rises, but it is agreed that this abnormality is not a primary effect of the temperature, but that secondary causes are at work.

The rate of hydrolytic action of isolated enzymes, however, always shows a marked optimum temperature effect. Thus Kjeldahl[1] showed that malt-diastase hydrolysed increasing quantities of starch up to about 63° C., after which the action fell off quickly, becoming *nil* at 86° C. The interpretation of this is quite simple, and the diminished effect at super-optimal temperatures is due to an actual destruction of the enzyme by the heat. It has been clearly proved that the destruction is the faster the higher the temperature, and that the apparent optimum is the effect of the two opposed processes at work. If the supply of enzyme could be kept up constantly to counterbalance its destruction, then the true rate of its hydrolytic action at high temperatures could be determined. This result could be also indirectly arrived at by the method adopted for calculating the initial values of assimilation, but to my knowledge neither of these experiments has yet been carried out.

Should not the optimum for growth be interpreted in some similar way? I think we may regard it as certain that it is due to some *secondary* cause working against the causes that have made for increasing growth as the temperature has risen from 0° C., and not due to failure of these causes as a direct effect of the increased temperature.

It is possible that the destruction of an enzyme, or the action of some anti-enzyme, plays a part in causing the 'real optimum' which growth exhibits, but in the absence of any analytical study of the behaviour of growth at high temperatures it is idle to put forward any explanatory hypothesis.

Growth is indeed the finished product of the metabolic loom, and, in order that the weaving of the specific pattern of the individual plant shall continue for a measurable time at the racing speed of metabolism which high temperature enforces, extraordinary co-ordination is required.

It may well be that, soon, co-ordination will fail in some question of supply of material, and it is significant that high temperature does not distort the specific pattern of the plant as do negative or positive extremes of light or of moisture; the machinery slackens and the output is less, but the pattern is the same.

It is therefore easy to conceive that falling off of growth-rate may be due to a variety of causes, and what is really required is a careful investiga-

[1] Compt. rend. Carlsberg Lab., 1879; cf. Czapek, Biochemie der Pflanzen, Bd. 1, p. 345.

tion of the separate factors that are involved in growth to see whether or not in some simple case it is merely the inadequate working of a single one of them that is checking growth. Peradventure food reserves cannot be translocated fast enough, or oxygen cannot quickly penetrate to the deep-lying growing cells.

This analytical attitude brings us naturally to the second part of this study, to the consideration of those influences which I propose to call 'limiting factors.'

II.

We start this section with the following axiom.

When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor.

I think one may fairly express surprise at the extent to which this principle has been overlooked by those who have proposed to work out the relation between a function and some *single* one of the various factors that control it.

This desirable end often cannot be really accomplished without taking deliberate thought to the other factors, lest surreptitiously one of *them*, and not the factor under investigation, becomes the real limiting factor to an increase of functional activity.

We will consider in some detail the application of this axiom to assimilation, and briefly its application to respiration and growth.

Carbon assimilation furnishes the most instructive case for the consideration of the inter-relation of conditioning factors, because these factors are largely external ones, whereas in growth they are internal and less under control.

Let us then consider first the case of assimilation. We can recognize five obvious controlling factors in the case of a given chloroplast engaged in photosynthesis.

(1) The amount of CO_2 available,
(2) the amount of H_2O available,
(3) the intensity of available radiant energy,
(4) the amount of chlorophyll present,
(5) the temperature in the chloroplast.

In theory any one of these five might be the limiting factor in the total effect, and it is comparatively easy to experiment with (1), (3), or (5) successively as limiting factors.

Many experimenters have indeed done this without premeditation. The experiments of Reinke [1], in which with increasing light the rate of assimilation (as measured by the bubbling of *Elodea*) suddenly ceased its proportional increase and remained stationary while the light increased yet

[1] Bot. Zeit., 1883.

another tenfold, I interpret as probably a case in which the supply of carbon dioxide was the limiting factor: its limit of arrival by osmosis being once reached no further increase of assimilation was possible.

The experiments of Kreusler [1] on the effect of temperature on the assimilation of a shoot of *Rubus* gave, as higher and higher temperatures were used, at first a steady rise of assimilation up to 15° C., but after this the assimilation practically never rose further. This state of things has been shown by Miss Matthaei [2] to be a case in which inadequate illumination limited the assimilation to that obtained at 15° C., and so further heating produced no increase. There are also contemporary examples of such misinterpretation which will be discussed elsewhere.

When the rate of a function exhibits, in experiment, a sudden transition from rapid increase to a stationary value, it becomes at once probable that a 'limiting factor' has come into play. The form of curve obtained is then like the curve *A B C* in diagram II, where the limiting factor has soon come into play. If the factor in question only becomes 'limiting' when the function is near its high values, then the curve *A B F G* represents the result attained. If the factor only 'limits' when the function is close to its highest values we may get a curve recalling the conventional optimum curve with the top cut off.

The relation of assimilation to intensity of illumination is shortly to be treated elsewhere, but something may be said here with advantage about the relation of assimilation to the supply of carbon dioxide. The willingness to believe in an 'optimum amount of CO_2 for assimilation' is almost universal, and the belief is quite general that Godlewski [3] showed it to be about eight per cent. In my opinion there is no justification whatever for speaking of an optimum at all in this connexion.

Suppose a leaf in a glass chamber to have enough light falling upon it to give energy equal to decomposing 5 c.c. of carbon dioxide per hour. Then, as one gradually increases the carbon dioxide in the air current through the chamber from the amount (or pressure) that causes 1 c.c. to diffuse into the leaf through its stomata up to five times that pressure, so steadily the assimilation will increase from 1 c.c. to fivefold. After that, further increase of the carbon dioxide will produce no augmentation of the assimilation, but will give continually an effect of 5 c.c. of carbon dioxide assimilated—the light being now the limiting factor. The curve obtained will be of the form *A B C*. Ultimately, if the supply of carbon dioxide in the air current be increased up to 30, 50, or 70 per cent., the carbon dioxide will have a general depressing effect on the whole vitality, and before suspension of all function a diminution of assimilation undoubtedly occurs; this is, however, quite a separate process. Now, secondly,

[1] l. c.; see also Pfeffer, Physiology, vol. i, sect. 58.
[2] l. c., p. 69.
[3] Arb. bot. Inst. zu Würzburg, Bd. 1, 1873.

suppose the light falling on the leaf to be sufficient for the decomposition of 10 c.c. of carbon dioxide per hour, then twice the external pressure of carbon dioxide will be required to reach the limit and the angle of the curve, which will now be *A B D E*. With still stronger light we should get *A B D F G*. Those who would be prepared to admit that a curve like *A B C* shows an optimum, only with a very long drawn-out top, would have to further admit that for *each intensity of light falling on a leaf there is a different optimum amount of carbon dioxide*. This is not to be entertained.

The light-energy available fixes an upper limit to the carbon dioxide that can be decomposed, and when that amount is attained, which even for direct sunlight could be provided with a current of air containing less than 1 per cent. if the current were sufficiently fast, the limit of effect of carbon

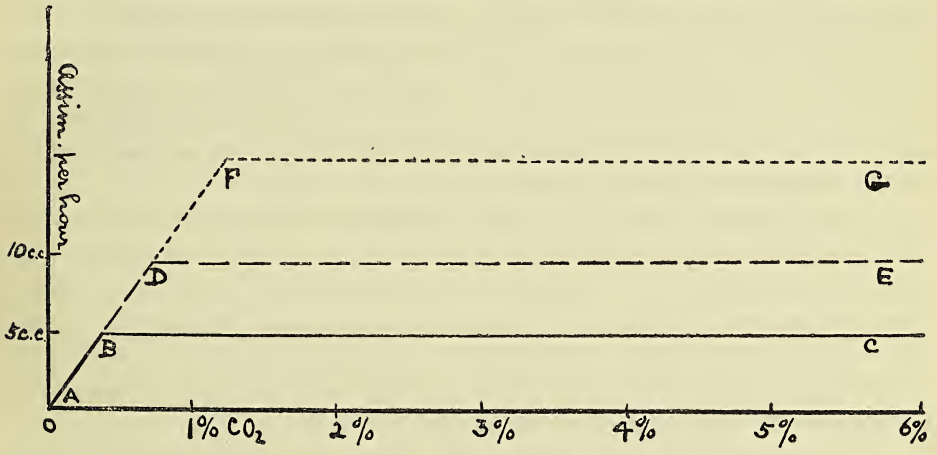

Fig. 6. Diagram II.

dioxide is reached: any more provided is wasted, and has no further effect till many times that concentration is reached and a general depressing effect comes in. Just as little can one speak of an optimum amount of carbon dioxide required to use up a fixed amount of radiant energy (i.e. a given intensity of light) as one can speak seriously of the 'optimum amount' of water required to fill a litre flask, while to attempt to speak of an optimal amount of carbon dioxide for assimilation in general is like speaking of 550 c.c. as the optimal amount of water *to fill flasks*, when the two flasks in question happen to be the one a litre flask and the other a 100 c.c. flask.

With regard to the function of respiration, a factor that sometimes comes markedly into play as a limiting factor is the amount of plastic material available for oxidation, and it is by no means easy to separate analytically the effects of this factor and those of the temperature factor.

Further consideration of this question would, however, involve the quotation of definite examples, and must be postponed.

In the case of growth, besides the more subtle factors, there are two fairly obvious factors which must, each in its turn, and under different conditions, play the part of a limiting factor. These are the temperature and the water supply. To variations in either of these growth is very susceptible, and indeed a vigorous transpiration in dry air has been observed by Frank Darwin[1] to cause actual temporary *loss* of weight in a growing *Cucurbita* fruit, water being drawn off to supply the leaves.

In a quite recent paper by R. H. Lock[2] we meet with what I take to be a most interesting example of the action of limiting factors in growth.

The author made series of measurements of the daily, and sometimes of the hourly, rate of growth of Bamboos growing in the open at Peradeniya, and at the same time noted the temperature and the relative humidity of the air, and the rainfall. The conclusion at which he arrives is that the daily rate of growth is almost entirely a question of the water supply, and that the hourly growth during the day follows very closely the curve of humidity of the air. It is striking that temperature does not appear to come into the causation of the fluctuations at all, and indeed in the fifteen conclusions given temperature is not mentioned. Now during the months of June, July, and August, when the measurements were taken, the temperature was always high, and it is recorded that the extreme range of temperature for these months was between 19° C. and 30° C. Here, then, temperature is favourable to growth, and would presumably permit more growth than actually takes place, so the amount of water attainable is the factor which limits the growth of the bamboo stems. Hence the close correlation between observed growth and water supply.

Now suppose the temperature had been much lower and the water supply not diminished in proportion. Then the temperature would have kept the growth-rate down and would have been the limiting factor, and we should have expected the daily growth to correspond to the fluctuations of temperature, and not to those of water supply, which is now in excess.

I think it abundantly justifies this way of looking at the phenomena when we find that Shibata[3] in 1900, measuring the daily growth-rate of Bamboos growing in the open in Japan, obtained numbers which showed the closest agreement with the fluctuations of temperature and very little relation with the humidity. The temperatures in Japan fluctuated between 11·6° C. and 20·7° C. during the measurements, so that the whole range is quite below the Peradeniya range of temperature and the case entirely fits our hypothesis.

Shibata's measurements are analysed by Lock in his paper, but

[1] Annals of Botany, vol. vii. [2] Annals of R. Botanic Garden, Peradeniya, vol. ii, August, 1904.
[3] Journal Coll. Sci., Tokyo, 1900; cf. Lock, l. c., p. 215.

he has not our clue to harmonize them with his own results. The interpretation here provided harmonizes both sets of observations as examples of the effects of contrasted limiting factors.

Internal factors, such as rate of translocation of plastic material, may no doubt play the part of limiting factors, but clear examples of this correlation are not to hand. One cannot, however, help suspecting that insufficiency of plastic material may play some part in the falling off of growth at high temperatures. As the temperature rises above the 'optimum' for growth, respiration goes on still increasing enormously, and large quantities of carbon are compulsorily lost to the growing plant in this way. Presumably in some cases, possibly quite generally, the insufficient residue of available plastic material in the vigorously respiring part would limit growth.

To take the hypothetical case that translocation could just bring in, per unit-time, enough carbonaceous material for the growth at the optimal temperature *plus* the respiration at the same temperature. Then, as the temperature rose further and the respiration increased *faster and faster*, so necessarily there would be *less and less* carbon material available for growth. The falling curve of growth would become the complement of the rising curve of respiration, and it is of interest that to a large extent, whatever be its significance, the two curves actually have this appearance.

To conclude this section on limiting factors, it seems to me instructive to point out that in the equality of all conditions except one—which is the essence of the 'control-experiment' method of investigation—may lurk a dangerous pitfall if either of the equalized conditions becomes a limiting factor in the result.

Suppose that it were proposed to test the effect upon assimilation of some specific factor that should have an augmenting effect. What more natural than to place two similar leaves side by side under similar medium conditions for assimilation, one subject to this factor and the other not? This would be the typical 'control experiment.' Yet the assimilation of the two leaves might be equally limited by the small amount of carbon dioxide in the air, or, if this were augmented, by the moderate light or by the low temperature; thus equal assimilations might be obtained, and a negative result announced though the specific factor might really show an augmenting effect were another factor not limiting the assimilation.

III.

In conclusion, it is desirable to make some reference to a particular class of agents that complicate the problems of the magnitude of metabolic phenomena which we have been seeking to simplify by analysis.

So far we have spoken of conditions that affect the rapidity of vital chemical processes, whether these be (1) conditions of supply of material or of energy, or (2) tonic conditions that affect only the rate of metabolism. The former act in proportion to the quantity of matter or energy available, but the latter, of which temperature is the type, act by altering the velocity of chemical change. The further class of agents to be just mentioned here is that sometimes known as 'chemical stimuli'—substances of which small traces may produce large alterations of the rate of metabolism, noticeably of the rate of respiration.

This susceptibility to the presence of small traces of unessential substances is so easily manifested that respiration is often justifiably spoken of as exhibiting stimulation effects, and as being controlled as to its magnitude by stimuli as well as by tonic conditions.

Variations of this order would naturally be most disturbing in investigating the effect of tonic conditions, and it is therefore important to have as exact an idea as possible before one of their causation, significance, and possibilities.

It is only within the last two or three years that our conception of the chemical organization of the cell has acquired sufficient solidarity to allow the investigator to face such facts without flinching.

Regarding the cell, as we now may [1], from the metabolic point of view, as a congeries of enzymes, a colloidal honeycomb of katalytic agents, as many in number as there are cell-functions, and each capable of being isolated and made to do its particular work alone *in vitro*, we look for light on the action of chemical stimuli in the cell to their effect on the action of isolated enzymes *in vitro*. Here, too, law and order is now known to reign, and while enzymes only 'accelerate' reactions without being incorporated in their end products, yet the acceleration produced is proportional to the mass of the enzyme present, minute as it is, and the effects of 'activators' and 'paralyzators' of this action are also in proportion to their masses.

Thus all these effects belong to the province of chemical dynamics, and the accelerating effects of ferments and activators upon respiration fall into the same category as the accelerating effect of increase of temperature.

The analytical treatment of metabolic phenomena which is outlined here is then not made any the less certain in its procedure, though it is made more complex by the interaction of those metabolic effects which have been described by their investigators as stimulatory.

These phenomena need not be considered further at present, and the essential quantitative laws of metabolic 'velocity of reaction' may no doubt

[1] For recent views on these points see Fr. Hofmeister, Die chemische Organisation der Zelle, Braunschweig, 1901; G. Bredig, Anorganische Fermente, Leipzig, 1901; F. Czapek, Biochemie der Pflanzen, Zweites Kapitel, 1904; E. F. Armstrong, Proc. Roy. Soc., vol. lxxiii, 1904.

be arrived at without the disturbing effect of introduced 'chemical stimuli.'

On the conclusion of this survey it will be generally conceded, I think, that the way of those who set out to evaluate exactly the effects of changes in a single factor upon a multi-conditioned metabolic process is hard, and especially so when the process is being pushed towards the upper limits of its activity. In this latter department of investigation, I think it may be fairly said that at present our science entirely lacks data that will stand critical analysis from the point of view indicated in this article.

Several preliminary analytical investigations in this field are now in progress here.

CAMBRIDGE, *March*, 1905.

The Localization of the Indigo-producing Substance in Indigo-yielding Plants.

BY

H. M. LEAKE, M.A., F.L.S.

With Plate XIII.

THAT there is present in numerous plants scattered through the vegetable kingdom a body which, under certain conditions, is capable of yielding indigo has been a matter of general knowledge from very remote ages. This knowledge, however, was chiefly acquired as the result of the utilitarian purposes to which indigo is put; thus the woad, *Isatis tinctoria*, L., was largely cultivated in Europe until the dye so obtained was replaced by indigo imported from the East; *Polygonum tinctorium*, Ait., was, and is still, cultivated in China; *Indigofera argentea*, L., is the ancient 'nil' known to, and cultivated by, the Egyptians; and there are cultivated, chiefly in India and Java, other species of *Indigofera* too numerous to be mentioned individually.

Literature.

Schunck in 1855 (1) attempted to obtain some knowledge of the body which existed in the woad and from which indigo could be obtained. To this body he gave the name 'Indican.'

During the last twelve years a considerable amount of work has been published, the greater portion of which comes from the pens of Molisch and Beijerinck. These two investigators, however, are chiefly concerned with the industrial processes and questions concerning the fermentative and bacterial action.

Of work dealing more particularly with the localization of the indigo-forming substance within the plant there is comparatively little. The earliest suggestion of a method for indicating the situation of this substance within the plant occurs in the Botanische Zeitung for 1871 (3). Here Göppert, in dealing with the effect of frost on plants, notes that, when a flower of *Phajus grandifolius*, Lour., is killed by frost, part of the flower becomes blue. The fact is, however, only noted incidentally, and no effort is made to localize the indigo-forming substance. Attention is again drawn by Müller-Thurgau (4) to this precipitation of indigo in the flower of *Phajus grandifolius*, when it is killed by freezing.

[Annals of Botany, Vol. XIX. No. LXXIV. April, 1905.]

The first experiments to be undertaken with the definite object of localizing the indigo-forming substance within the tissues of the plant are those of Molisch, published in 1893 (5 and 6). In these experiments he uses two methods for ascertaining whether a particular tissue contains this substance. The first, or macro-chemical, method consists in boiling the tissue with dilute ammonia (2 c.c. commercial in 98 c.c. water); indigo is thus formed and will become evident on filtering, when the masking colour of the filtrate is removed. Chloroform may be added to the unfiltered ammoniacal extract, when the chloroform will separate out as a blue layer, for indigo is slightly soluble in chloroform. In the second, or micro-chemical, method the tissue is exposed in a confined atmosphere to the action of alcohol vapour for a period of twenty-four hours, after which the chlorophyll may be removed by immersion in absolute alcohol. The presence of indigo, and hence of the indigo-forming substance, will be indicated by the blue coloration of the tissue. In a later paper in 1899 (7) he suggests chloroform as an alternative for alcohol, and later again, in the same year (8), strong ammonia. Direct immersion in 40% alcohol also results in a formation of blue within the tissues. Sections can now be cut, or the material teased and examined under the microscope.

Beijerinck, in 1899 (9), in applying the 'alcohol test' of Molisch to *Indigofera arrecta*, Hochst. (termed by him *I. leptostachya*), found that indigo-formation occurred in only the youngest leaves. In the 'woad,' again, only a slight blue deposition occurs. As an alternative and more satisfactory method, he immersed the tissue used for experiment in mercury, or exposed it in a vacuum, for a few hours, after which he exposed to ammonia vapour as before, finally extracting the chlorophyll with alcohol.

In 1900 (10) he uses as a macro-chemical method the addition of hydrochloric acid and ferric chloride to a decoction of the tissue to be examined, and adapts this reaction for micro-chemical examination by boiling a section of the living leaf or tissue in a mixture of strong hydrochloric acid and ferric chloride. As an alternative he uses in the same manner a solution of isatin in hydrochloric acid, by which reagent the indigo-forming substance is supposed to yield indigo-red, which is deposited *in situ* within the tissue. The former method of indicating the presence of an indigo-forming substance by the formation of indigo as the result of the action of an acid and ferric chloride is also used by Hazewinkel (11).

Methods.

The above methods are in no case of such a character as to give to the tissue a condition at all comparable to that of material treated by the ordinary fixing and hardening reagents, and the microscopic examination

must consequently be somewhat imperfect; moreover, with the exception of one plate (Ber. der D.B. Gesell., Bd. xviii, 1899, Taf. 18), in which the distribution in the cells of *Isatis* and *Calanthe*, as indicated by the deposition of blue induced by exposure to alcohol vapour, is figured, the results are not illustrated. It seemed, therefore, that if a suitable method could be obtained for precipitating the indigo within the cells of the tissue and at the same time producing all the results of a fixing reagent, thus leaving the material in a suitable condition for hardening and staining in the ordinary manner, there would be room for further examination. This especially applies to examples of the genus *Indigofera*. By far the greater portion of the previous work has been carried out in Europe, and hence this genus, which is chiefly tropical in its habit, has received comparatively little attention. Such a method was eventually found and will be briefly described. The tissue to be examined is cut into small pieces and rapidly immersed in a solution of the following constitution:—

Acetic Acid, Glacial	2 c.c.
Sulphuric Acid, Concentrated . .	1 c.c.
Ammonium Persulphate . . .	0·5 g.
Water to	100 c.c.

In compact tissues, such as those of *Indigofera*, where the intercellular spaces are small, penetration is slow and the pieces must be relatively small—$\frac{1}{8}$ in. square at most; in those tissues having relatively large intercellular spaces considerably larger pieces may be employed.

As penetration takes place a deposition of indigo within the cell is effected, and at the same time the protoplasmic contents are fixed in the normal manner. After penetration is completely effected—the tissue should be cut in pieces of such a size that penetration is complete in 4–6 hours, or after twelve hours' immersion at most—the solution is poured off, and the material, after 3–4 washings of twenty-four hours each in 50% alcohol, is in a condition to be taken up through the alcohols to absolute alcohol in the usual manner.

As far as I am aware the only reference for the use of a persulphate in the formation of indigo in plant extracts or tissues occurs in a paper on the Fermentation of the Indigo Plant by Bergtheil (12). Here the author says: 'The best method for precipitating indigotin from an extract of the plant is that devised by Rawson in 1901 for the analysis of indigo-yielding plants. The extract is made strongly acid with hydrochloric acid, and a solution of ammonium persulphate is gradually added, the indigotin being precipitated in a finely crystalline form.' The method here stated to be devised by Rawson has not, as far as I can ascertain, been published by him. Its employment as here indicated, without modification, is not to be recommended, since with this combination a liberation of chlorine is

effected which gives rise to a loss of indigo by oxidation. This loss is avoided by the use of sulphuric acid. Even now the proportion of persulphate to sulphuric acid is of considerable importance, since an excess of the former produces a further oxidation of indigo to isatin. Experiment has shown that in the above proportions a loss does not occur [1].

The hardened material is now embedded in paraffin wax and ribbon sections cut in the normal manner. The thickness of the section depends, to a certain extent, upon the nature of the material. Thus, for *Indigofera sp.* a thickness of 4–5 μ was found to be most suitable on account of the small size and dense arrangement of the cells. For *Polygonum tinctorium* the most suitable thickness is 8 μ; while for *Isatis*, *Strobilanthes*, *Phajus*, and *Calanthe* a thickness of 10–12 μ is advisable.

Staining. For this purpose a combination of Haematoxylin and Eosin was found most suitable. The sections, after successive immersion in xylol and absolute alcohol, are placed in a solution of Haematoxylin consisting of:—

Delafield's Haematoxylin . . .	50 c.c.
Water	300 c.c.

in which they are left for at least twelve hours. They are then transferred to acid alcohol (1 % HCl in 50 % alcohol) until sections are almost colourless to the naked eye. After the removal of all traces of the acid and alcohol by washing in water, the sections are transferred to eosin—1 % solution of Grübler's water-soluble eosin—for at least an hour, after which they are rapidly dehydrated by absolute alcohol, passed into xylol and mounted in balsam. By this method a delicate differential coloration of cell-walls and cell contents is obtained in which the grains of indigo-blue stand out prominently and are hence readily localized.

Examination of Material.

The material employed in the preliminary experiments was obtained from two species of *Indigofera*, *I. sumatrana*, Gaert., and *I. arrecta*, Hochst. These two species were made the subject of the most minute investigations, for the two reasons that they are the two indigo-yielding plants cultivated in Behar, where the work was commenced [2], and that the indigo-yielding species of the genus *Indigofera*, being inhabitants of

[1] In the 'Report to the Government of Bengal on the Research Work carried out at the Dalsing Sarai Research Station,' the above-described reagent is dealt with more fully from a macrochemical standpoint by W. P. Bloxam. This Report is, at the time of writing (Sept. 1904), still in the press.

[2] At the Dalsing Sarai Research Station. Here the plant was grown and the material prepared. Examination was deferred and finally carried out at the Botanical Laboratory, Cambridge.

tropical climates, have received less attention than the corresponding species of various other genera which are either cultivated in temperate climates (*Isatis* and *Polygonum*) or are commonly met with under artificial conditions (*Phajus* and *Calanthe*). The various genera which have been subjected to investigation in the above-described manner will be considered separately.

I. Indigofera.

Several species of this genus have been examined (loc. cit.) by Molisch, but the examination was—in the case of *I. leptostachya* (=*I. arrecta*) (13) and *I. anil* the only two species of those which he examined which yield indigo in any quantity—confined to dried material, and specimens of *I. arrecta* grown in the gardens at Prague.

According to his results (5), *I. Dosua*, *I. argentea*, *I. chinensis*, *I. decora*, *I. hirsuta*, and *I. galegoides* yield no indigo. The absence of an indigo-yielding power in *I. Dosua* is also noted by Bréaudat (14).

In *I. anil* indigo can, according to Molisch, be precipitated in the leaves, especially in the mesophyll and epidermis, but not in the roots, stems, fruits, or seeds.

In *I. arrecta* he finds indigo precipitated as in *I. anil*, but on further examination of fresh material (7) this statement is amplified. He finds, by employing the 'alcohol-test' above described, the greatest amount of precipitation to have taken place in and on the chloroplasts of the chlorophyll bearing parenchyma of leaf-lamina. Very little occurs in the epidermal cells, with the exception of the epidermal hairs, in which larger amounts occur.

The following species have been examined recently after treatment with the Sulphuric-Acetic-Persulphate reagent:—

I. Dosua, Wall. Cat.	*I. anil*, L.
I. galegoides, D.C.	*I. sumatrana*, Gaert.
I. oligosperma, D.C.	*I. arrecta*, Hochst.

I. Dosua, Wall. Cat., growing in the Botanical Gardens, Cambridge, showed no trace of indigo.

I. galegoides, D.C. Material obtained from this plant growing in the Royal Botanic Garden, Calcutta, showed no trace of indigo after treatment.

I. oligosperma, D.C. Material from this plant was also obtained in the Royal Botanic Garden, Calcutta. The leaf only has been examined, and, when the chlorophyll had been removed by alcohol, appeared a distinct, though faint, blue colour. Microscopic examination of sections showed the distribution of indigo to be essentially the same as in *I. arrecta* described below.

I. anil, L. This plant was also growing in the Royal Botanic Garden, Calcutta, where the material was obtained. Indigo was precipitated abundantly in the mesophyll of the lamina. In the remaining tissues of the leaflet only traces occurred; the distribution, however, is essentially the same as in *I. arrecta*, the difference is merely one of the degree of precipitation in the various tissues. The leaflets alone have been examined.

I. sumatrana, Gaert., and *I. arrecta*, Hochst. These two plants have been examined in greatest detail, and, since the distribution of the precipitated indigo is practically the same in both plants, they may be considered together.

Leaf-lamina. In the lamina of the leaflet of both species there is a marked palisade-parenchyma beneath the epidermis of the upper surface and a spongy parenchyma of small cells, compactly set, with small intercellular spaces. Between these two normal kinds of parenchymatous tissue, or isolating on the side of the palisade-parenchyma a few cells of the spongy parenchymatous type, occurs a layer, one cell in thickness, of large, irregular cells with a small amount of protoplasmic content and relatively few chloroplasts.

In all these varieties of parenchyma of the leaf-lamina indigo is precipitated, but it is especially abundant in the palisade-parenchyma, where it occurs as stellate masses either outlining a chloroplast, on the surface of which the indigo may be deposited, or suspended freely in the protoplasmic network of the cell (Pl. XIII, Figs. 1 and 2). In the epidermis it also occurs abundantly, not only in the normal but in the two specialized types of epidermal cell—the guard-cells of the stomata and the epidermal hairs (Figs. 2–5). In the vascular bundles indigo is also abundant, and its distribution is best studied in the case of the midrib of the leaflet. Here the structure in no way departs from that of a normal leaf. The parenchymatous tissue, especially on the dorsal surface of the leaf, is composed of thick-walled cells with little or no chlorophyll. In the vascular bundles fibres are present to a very limited extent, the vessels being separated by thin-walled parenchymatous cells rich in protoplasmic contents.

In the xylem-parenchyma an abundant deposition of indigo is effected by the above-mentioned reagent. In the xylem-vessels alone is there no trace of deposition. Owing to the minute size of the elements of the phloem it is difficult to ascertain definitely where deposition actually takes place. A careful examination, however, shows that indigo may be present in all elements; but the material is most unsuited for a clear demonstration (Fig. 6).

In the extra-vascular tissue of the midrib indigo is again present in abundance, more especially in the thick-walled dorsal parenchyma (Fig. 6).

The Rachis. The structure of the rachis differs from that of the mid-

rib and approaches more nearly to that of the stem, in that the vascular strand has lost its dorso-ventral character and become completely circular, enclosing a central pith. There are two minor vascular strands situated in the two dorso-lateral ridges. Both the xylem and the cortex show a marked development of fibrous tissue which, in the latter case, forms a complete ring enclosing the central vascular strand. There are also numerous cells, situated both in the cortex and pith, which form an incomplete laticiferous system.

Considering the vascular cylinder first, we find indigo deposited in some quantity in the pith, except in the apparent laticiferous system where no trace occurs; it is also deposited in the cells forming the medullary rays. In the xylem it is completely absent from the vessels; traces alone occur in the fibres; while there is an abundant deposition in the xylem-parenchyma. Indigo is present in all elements of the phloem. In all the cortical elements indigo is abundantly deposited, the above-mentioned cells of the laticiferous system alone forming an exception. Here no indigo is found. In the fibres there is a considerable deposition (Figs. 7, 8).

Stem. Indigo is only present in the young stem; as the distance from the growing point increases, the amount of indigo becomes progressively less and finally disappears. Deposition occurs in all the elements except, as before, in the xylem-vessels and laticiferous cells. For the first time a permanent cambium is found, in the cells of which indigo is deposited (Fig. 9). The phloem is bounded externally by a very definite and strongly developed fibrous layer, and outside this lies a well-defined endodermis. In the cells of both of these indigo occurs. Cortical fibres are not present, but the stem is strengthened by a considerable development of sclerenchyma at the angles opposite the primary bundles. In this sclerenchymatous tissue indigo is found deposited (Fig. 10). The epidermis, as before, contains blue deposited in the normal cells, guard-cells, and epidermal hairs.

Throughout the stem, however, the amount of blue deposited is relatively small. It is apparent to the naked eye, after the chlorophyll has been extracted with alcohol, as a faint though distinct blue colour which is very different to the deep blue of the rachis and leaflet. The abundant deposition in these latter ends abruptly at the swollen and somewhat prominent pulvinus.

Roots. There is no indication that the indigo-forming substance occurs in these.

Organs of Reproduction. The flowers of both species are small and only rendered conspicuous by the red colour of the two alae. The distribution of the indigo-forming substance varies slightly in the two species. In the calyx of *I. sumatrana* a considerable amount of deposition takes place, while, in all cases examined, that of *I. arrecta* showed a complete

absence of blue. Of the corolla, the carina and vexillum show only the merest traces of blue deposition. No deposition could be traced in the alae of *I. sumatrana*; but in *I. arrecta* these contained a sufficient quantity to give a faint coloration, barely perceptible with the naked eye. In neither case did the filaments of the stamens show any trace of blue. The walls of the ovary up to, and shortly after, fertilization show a considerable deposition in the parenchymatous cells, as do the highly meristematic cells of the ovule of both species at the time of, and shortly after, fertilization. This applies to each of the component parts of the ovule, to the integuments, the nucellus, and even to the embryo-sac (Fig. 11).

Shortly after fertilization all trace of blue vanishes from all parts of the ovary, nor does it appear in the ripe seed.

II. Isatis tinctoria, L.

Owing to the fact that a temperate climate is best suited to the growth of this plant, it has supplied the material for some of the most detailed work which has so far been carried out, both on the subject at present under consideration and on the biological and fermentation problems connected with indigo and its formation from plant tissues.

Molisch in 1893 (5) used for a macrochemical investigation dilute ammonia. In localizing the blue-forming substance by alcohol vapour he finds traces in the roots, except those of flowering plants and of plants under fourteen days old, and in the cambium and epidermis of the stem. As regards the leaves he says: 'Schon die beiden im Lichte ergrünten Keimblätter und die jungen, die Plumula umhüllenden Primordialblätter enthalten sammt dem Vegetationspunkte des Stengels reichlich Indican.' He further locates the precipitated indigo in the epidermis, mesophyll, and those elements of the vascular bundles which have protoplasmic contents, especially the latter, so 'dass die ganze Nervatur als blaues Netz in Erscheinung tritt.' In the cotyledons it is present, while the flower-buds, but not the opened flower, contain a deposit of indigo.

In repeating the investigation in 1899 (8) he uses ammonia instead of alcohol-vapour. The position of the blue, as here described, differs somewhat from that previously allotted to it. The chlorophyll granules of the mesophyll stand out a deep blue. In the epidermal hairs and the epidermis, with the exception of the guard-cells and a few of the cells bordering on the guard-cells, only traces are present. The vascular bundles also show only traces.

Beijerinck, in 1900 (10), localizes indigo 'besides in the young leaves and buds, also in the young root-peridermis, in the root-buds and in the growing root-ends.' It is absent in the thick stems and all the thicker

roots, the root-stock, the cambium and secondary tissues of woad-roots, flower-buds, embryo, seeds, and fruits.

Again, in 1900 (15), by the action of boiling hydrochloric acid and ferric chloride on a thin section, indigo will be found deposited in the epidermal cells, and especially the hairs, of young leaves. It is also precipitated in the mesophyll and other parenchymatous tissues.

The sulphuric-acetic-persulphate reagent does not produce any large development of blue in *Isatis*, and it is consequently somewhat difficult to trace the locality of this deposition. In the epidermis precipitation occurs, especially in the guard-cells of the stomata. The main deposition occurs in the mesophyll and veins of the lamina, in which latter indigo occurs in all elements except the xylem-vessels. In the midrib there is only sufficient in the epidermis and extra-vascular parenchyma to give the faintest blue coloration. The merest traces occur in the parenchyma of the vascular strands of the midrib. The root-stock roots, flowering stalk, and organs of reproduction show no trace of blue after the above treatment.

III. Polygonum tinctorium, Ait.

As in the case of *Isatis tinctoria*, the present knowledge chiefly depends on the work of Molisch. According to him (5) the indigo-forming substance can first be identified in the earliest foliage leaves, but not in the cotyledons; in these latter none is present, nor is there any in the root or hypocotyl of the young plant. In the fully-developed plant all parts, except the leaf-lamina, are, after treatment, colourless and devoid of indigo blue. The petioles and principal veins of the leaf, the stipules, stem, root, flower, and fruit are therefore devoid of the indigo-forming substance. In the epidermal cells of the lamina there is little, nor is there any abundance of indigo deposited in the parenchyma of the vascular bundles. In the mesophyll alone is it deposited in any quantity.

Beyond this little or no work has been undertaken on the localization in this plant. The examination made by Beijerinck in 1900 (10) is only of a cursory nature, and his results, as far as they are stated, agree with those of Molisch as given above.

The present examination indicates that, as Molisch has shown, the leaf-lamina—especially of the young leaves—is the only locality where the dye-producing substance occurs. Beyond this, however, there is little agreement between the present results and those obtained by Molisch. The greatest deposition of indigo occurs in the epidermis, including both the guard-cells and the epidermal hairs. In the unspecialized epidermal cells deposition is so great that the plasma and nucleus are rarely distinguishable.

The mesophyll contains a considerable amount of indigo, and so

do the vascular bundles, with the exception of the midrib. In the vascular bundles indigo is deposited in all elements except the xylem-vessels. In the older and fully-developed leaves the larger veins, in addition to the midrib, remain colourless on treatment.

IV. Phajus grandifolius, Lour.

As early as 1830 this plant was recognized by Clamor Marquart to yield indigo (16). Reference has already been made to the work of Göppert and Müller-Thurgau (3 and 4), whose methods need not be recapitulated here. According to the former the localization in the flower is the same as in *Calanthe*: 'das Labellum der Blüthe und Operculum am dunkelsten, während die Pollenmassen, aber diese nur allein, ihre natürliche gelbliche Farbe . . . behalten'; the flower, stalks, and bracts become blue, as do also the leaves. Müller-Thurgau only deals with the petals, the labellum in particular, in all of which he finds indigo deposited.

The distribution was next investigated by Molisch (5), precipitation of indigo being obtained by exposure to alcohol vapour. Briefly, he localizes indigo as follows:—

'Wurzel. Die relativ grösste Menge von Indican findet sich in den Meristemzellen der Spitze und in 1–3 Zelllagen knapp unterhalb der Wurzelhülle (Velamen). In den Wurzelhaaren, dem Velamen und dem übrigen Wurzelparenchym sehr wenig, nur Spuren in einzelnen Zellen des centralen Gefässbündelcylinders.

'Der Stengel und die knollenförmigen Verdickungen (Scheinknollen) desselben führen reichlich Indican.

'Blatt. In der Epidermis wenig, im grünen Parenchym viel, im Gefässbündel nur Spuren des Glykosids.

'Blüthe. Alle Theile, und zwar nahezu alle Zellen mit Ausnahme der Pollinarien indicanhaltig.'

Repeating his experiments in 1899 (8) he localizes the blue more particularly in the mesophyll, and especially the chloroplasts, of this tissue. The vascular bundles and epidermis, with the exception of the guard-cells, remain colourless to the eye, while the hairs, sieve-tubes, and raphide-cells contain no indigo.

Beijerinck, on the other hand, obtains blue depositions in the mesophyll and epidermis by boiling a section in hydrochloric acid and ferric chloride (10).

Only the aerial vegetative organs—the leaves and pseudo-bulbs—have been examined by the sulphuric-acetic-persulphate reagent. By this means the blue is deposited in the cell-plasm as very minute granules, the smaller of which are difficult to identify as such, even under the high powers of the microscope.

In both the young and old leaf an abundant deposition takes place in the parenchyma of the mesophyll, the xylem-parenchyma, and in the guard-cells of the stomata (Fig. 12). In both the dorsal and ventral bundles of fibrous tissue embracing the vascular bundles less occurs, and the same may be said of the elements of the phloem; the epidermis, with the exception of the guard-cells already mentioned, contains only traces, while the xylem-vessels contain none (Fig. 13).

The pseudo-bulb contains a few vascular strands scattered throughout a parenchyma of unspecialized cells containing numerous starch-grains. Isolated in this parenchyma and scattered throughout it occur raphide-cells. Indigo is deposited throughout the tissues of the pseudo-bulb excepting in the xylem-vessels and raphide-cells.

The amount of blue deposited in the vascular strands both of the leaf and pseudo-bulb is small compared with that in the mesophyll. As a result of this the vascular bundles appear to the naked eye as white strands embedded in a blue matrix.

V. Phajus Wallichii, Lindl., and Phajus maculatus, Lindl.

So far as I am aware, the only reference to these two species occurs in the article by Göppert on the freezing of plants (3). The aerial vegetative organs alone have been examined, and in all respects blue deposition is here similar to that in *P. grandifolius*.

VI. Calanthe.

Calanthe was also first investigated by Göppert (3), and, as far as his investigation goes, the species of this genus investigated by him agrees in all respects with *Phajus grandifolius*.

Molisch (5), after an examination of both plants, states for *Calanthe veratrifolia*, Br.: 'Im Wesentlichen Alles so wie bei *Phajus*.'

This is the case, and it is therefore unnecessary to deal with the genus at length. Only two species have been examined—*C. vestita*, Lindl., and *C. Veitchii*, a cross between *C. veratrifolia*, Br., and *C. rosea*, Benth., in both of which species only the aerial vegetative organs have been subjected to the test.

Among other indigo-yielding plants which have been previously examined, but of which material has not been forthcoming for the present examination, are, *Marsdenia tinctoria*, R. Br. (5), *Echites religiosa*, T. and B. (17), *Wrightia antidysenterica*, R. Br. (17), and *Crotalaria sp.* (17).

VII. Strobilanthes flaccidifolius, Nees.

This member of the *Acanthaceae* is largely cultivated in Assam for its indigo-yielding properties. A general account of it occurs in Watt's

Dictionary of Economic Products (18). I can find no account of the localization of indigo in this plant, and on the present occasion opportunity has only offered for examining the stems and leaves of plants cultivated in Bengal. To the naked eye the deposition of indigo-blue produced by the sulphuric-acetic-persulphate reagent appears greater in the tissues of this plant than in those of any of the species previously considered.

Microscopic examination, after sectioning and staining, indicates that the blue is deposited in practically all tissues. The xylem-vessels alone are free from indigo. Traces indeed occur even here, but it is probable that, in a moderately succulent plant like the present, the mere cutting of the fresh tissue across the vascular bundles will transfer from the injured mesophyll-cells to the xylem-vessels a certain amount of cell-sap which, though small, will be sufficient to cause a deposition of blue in the latter. With this exception indigo may be said to be deposited in abundance in all tissues. The large, elongated cystolith-cells are especially prominent (Fig. 14) owing to the dense mass of blue deposited in them and around the cystolith. In the leaf the epidermal cells, including the guard-cells and the two forms of epidermal hairs (Fig. 15), the mesophyll, the collenchyma of midrib and lateral veins, the parenchyma of the vascular bundles and the sieve-tubes all contain indigo. In the stem the cortical collenchyma and the thin-walled chlorophyll containing parenchyma (Fig. 16), the xylem-parenchyma, the phloem-parenchyma, the few scattered fibres of the phloem and sieve-tubes and the pith similarly contain a deposit of indigo within the cell-plasm. The xylem-fibres, which form the greater bulk of the older stem, contain only traces, and the same may be said of the cambium (Fig. 17), which is not, however, very prominent. Here again, therefore, indigo is only absent from the xylem-vessels. No opportunity has yet occurred of examining the flowering stem and organs of reproduction.

The Supposed Relation between the Chloroplast and the Indigo-producing Substance of the Plant.

Molisch, after an examination of *Phajus grandifolius*, *Calanthe veratrifolia*, *Isatis tinctoria*, and *Indigofera sp.* concludes (8):—

'Erstens, dass die Chlorophyllkörner der Indicanpflanzen, wenn auch nicht den ausschliesslichen, so doch den Hauptsitz des Indicans darstellen, und zweitens, dass hiermit die Anwesenheit eines stickstoffhaltigen Glykosids im Chlorophyllkorn der genannten Pflanzen zum ersten Male nachgewiesen erscheint.'

The method already described for precipitating indigo within the tissue is used for the examination. An examination of the figures given by him are not convincing; moreover, Molisch himself shows that indigo

occurs in certain cells where chloroplasts do not occur, e.g. the epidermal cells and vascular strands of *Isatis* (5); the blossoms of *Echites religiosa* (17); laticiferous vessels of *Echites* (17), &c.

It is clear, therefore, that the indigo-forming substance is not located *only* in those cells in which chloroplasts occur; a fact which the foregoing account has made, if possible, more clear. The presence in tissues, other than those containing chloroplasts, does not of necessity preclude these bodies from taking an active part in the formation of the substance. The indigo-forming substance is undoubtedly a soluble body, and retained within the cell in a condition of solution. It may, therefore, pass from the chloroplast, where it is supposed to be formed, to the various tissues of the plant. It becomes necessary, therefore, before any part in the formation of the indigo-forming substance can be claimed for the chloroplast, to prove some definite relation between this and the blue precipitated. This can only be done by a minute examination of the distribution of indigo within a cell containing chloroplasts. For this purpose the cortical cells of a young stem of *Strobilanthes* is most suitable. Examination under a high power (Fig. 16) shows the blue distributed throughout the plasm of the cell. Here and there a granule may be found deposited in contact with a chloroplast, but in no case has it been possible to locate the blue *within* the latter body, as Molisch claims to have done.

Further, in no case where the deposition of indigo within the cells of a chlorophyll-bearing parenchyma has been examined has it been possible to trace deposition within the chloroplast. The indigo is, moreover, frequently found deposited in contact with the nucleus, and it seems impossible to lay greater stress on this contact in the one case than in the other. Until further proof, obtained from work along different lines, is obtained it is impossible to accredit to the chloroplasts any direct function in the production of the indigo-forming substance.

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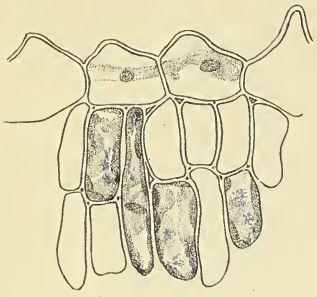

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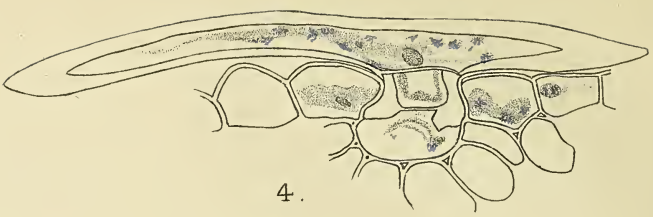

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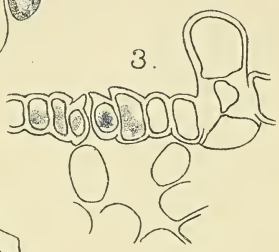

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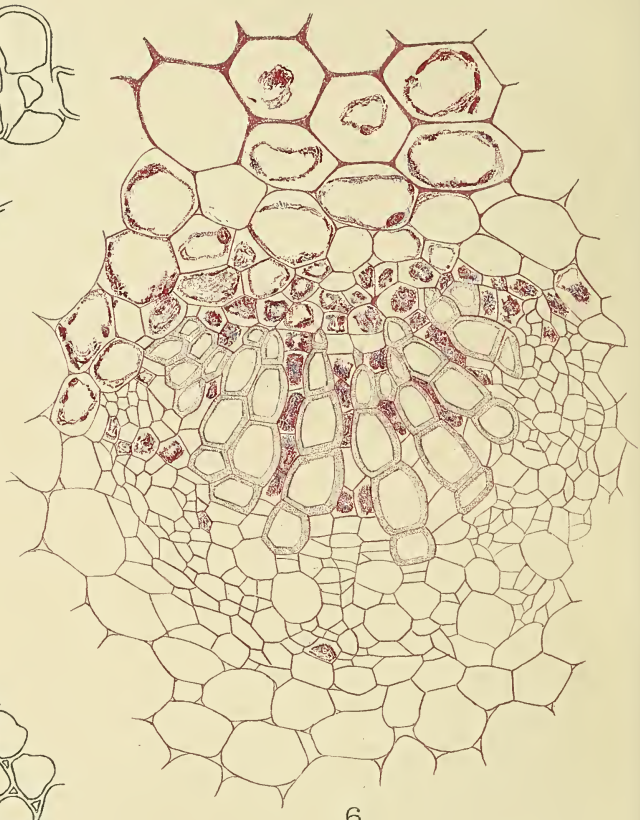

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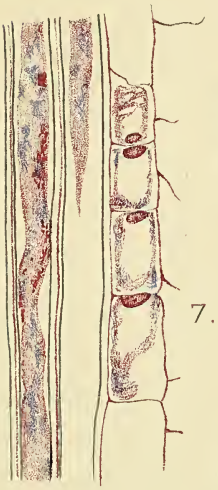

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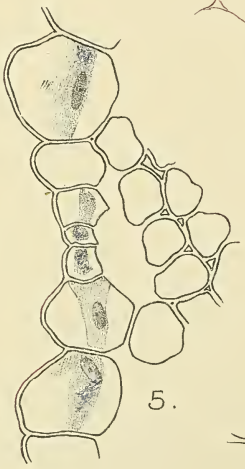

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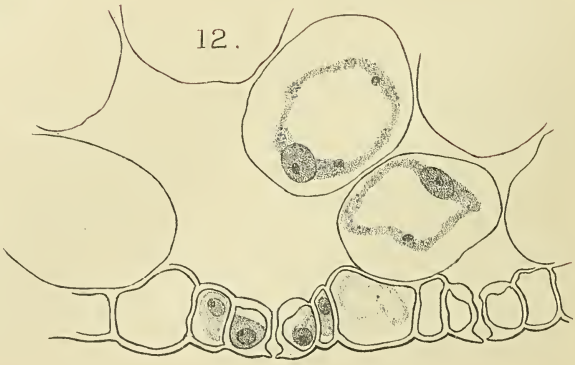

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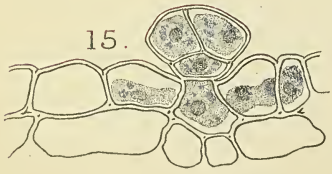

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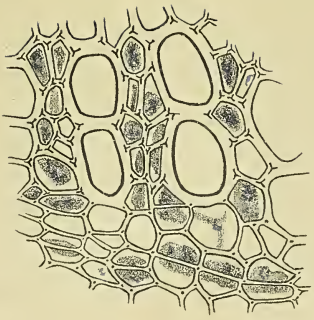

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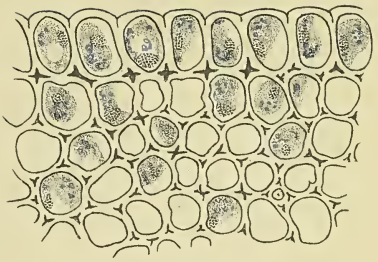

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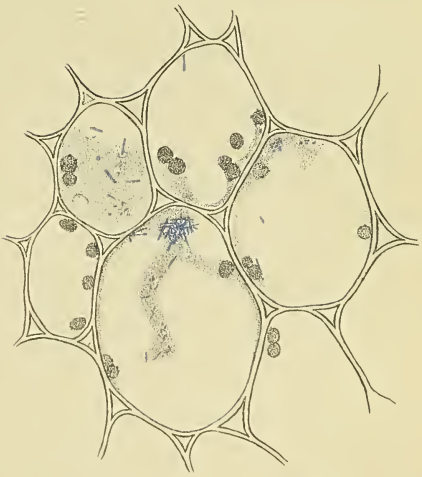

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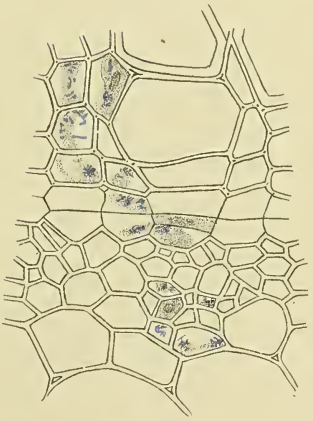

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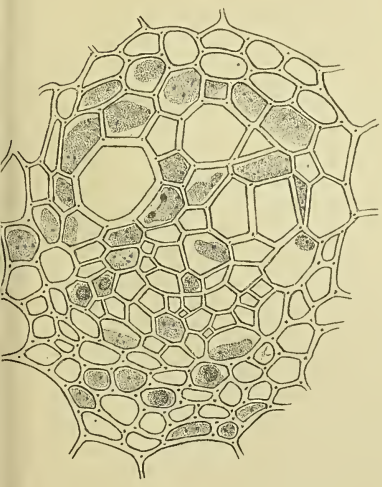

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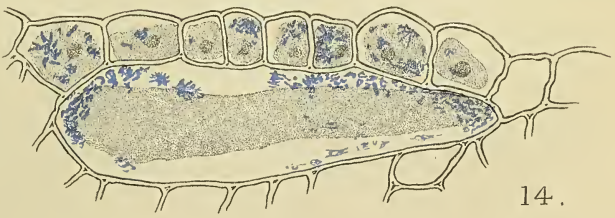

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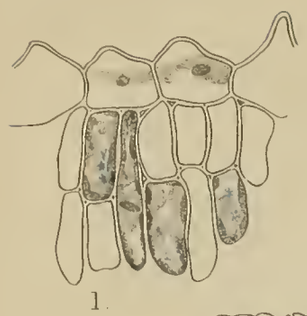

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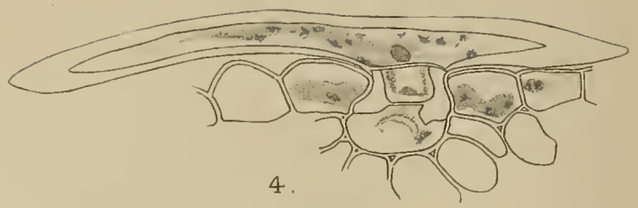

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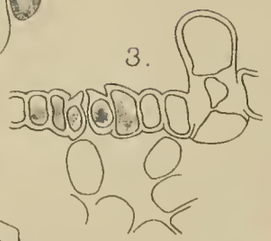

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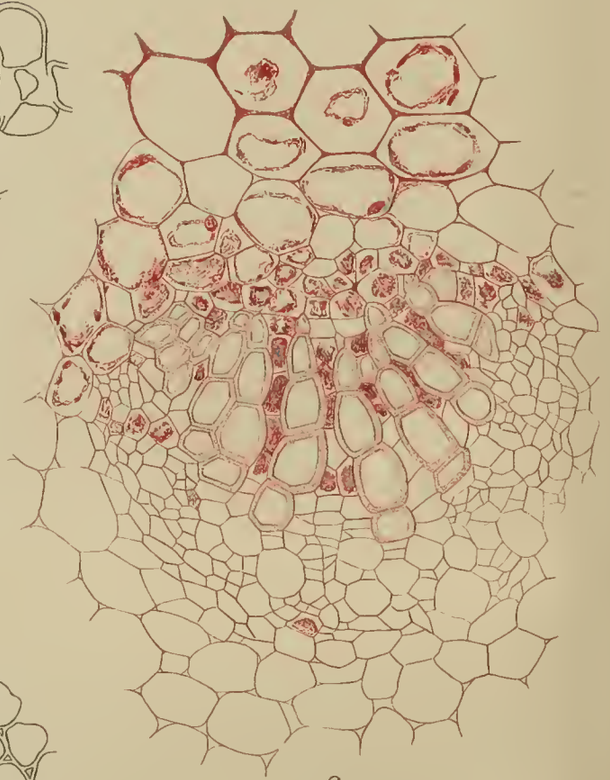

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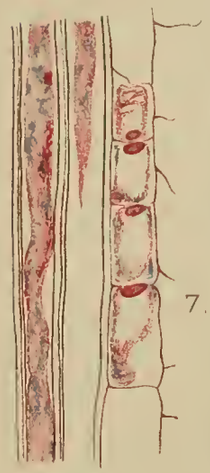

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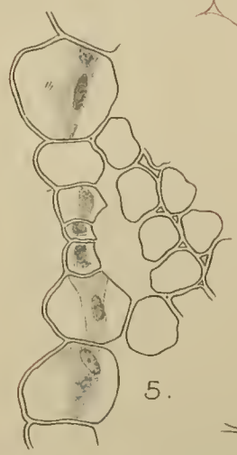

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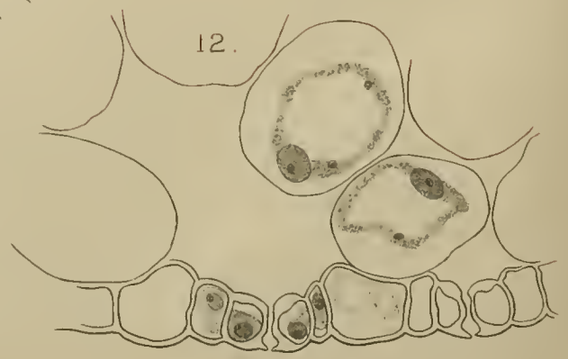

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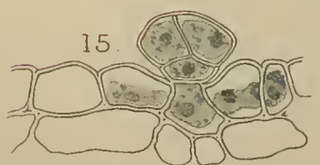

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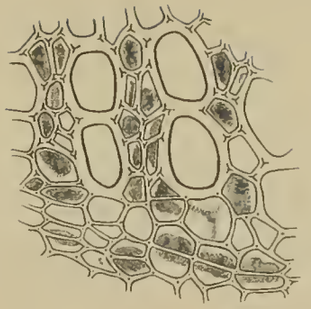

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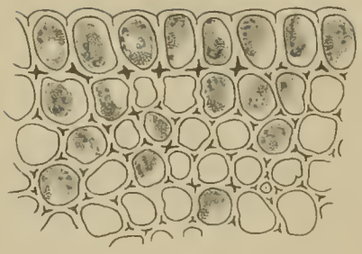

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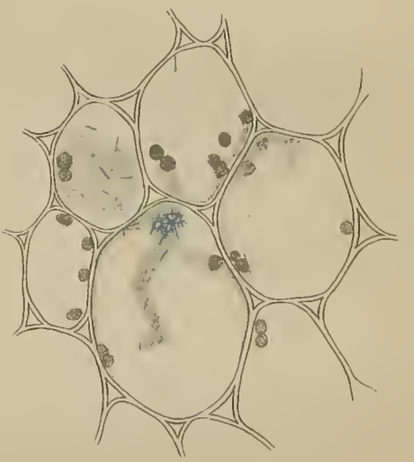

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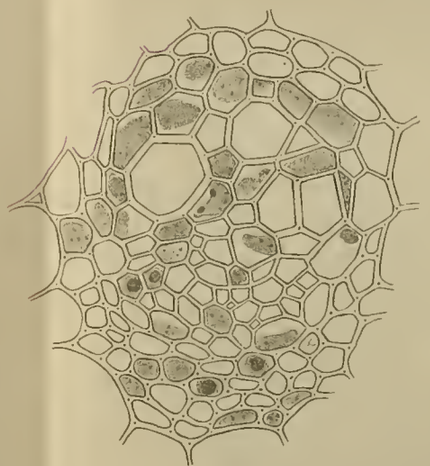

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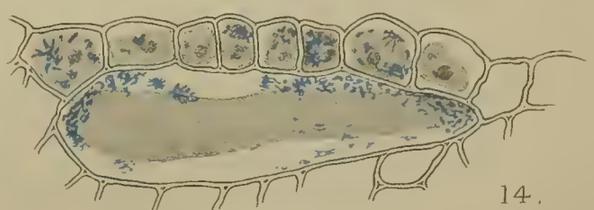

14.

Geotropic Response at Various Angles of Inclination.

BY

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A. Angle for Maximum Response of Primary Roots and Stems.

I. Introduction.

In his fundamental work on the growth of roots, Sachs[1] gives as the second of his three laws for the curvature of roots, that zones of equal development show various angles of curvature in the same time if the roots are placed at various angles with the vertical, and the curvature becomes the stronger the nearer the axis of the root is placed to the horizontal. Sachs does not give a very firm basis for this law, since he does not cite specific experiments from which the reader may judge the evidence. One finds, however, here and there a statement from which it is learned that Sachs laid seedlings at various angles of inclination, noting the latent period of curvature and the angles attained in given periods of time.

Bateson and F. Darwin[2] tested the detached inflorescence-stalks of *Plantago lanceolata* and of *Brassica oleracea* by fastening them at various angles of inclination, allowing them so to remain for two hours, then releasing them and laying them horizontally under water so that their former right and left sides became the upper and lower. The angle of curvature after one hour was taken as an index of the strength of geotropic stimulation. The results agreed wholly with those of Sachs on roots, in finding the strongest curves in those stems which had been kept horizontal.

Czapek[3] next took up the problem with both roots and stems. His method consisted in tying the plant members to rods of wood or glass, or encasing them in closely-fitting glass tubes, and thus exposing them to gravitation stimulation for periods of three to six hours at temperatures of 17° to 19°. The objects were then released and rotated on the klinostat for twenty-four hours, when the angles caused by the gravitation stimulation were measured and used as an index of relative intensity of response. In this way Czapek tested the roots of *Lupinus*, *Vicia Faba*, *Phaseolus*, *Pisum* and *Zea*, the hypocotyl of *Helianthus*, and the sporangiophores of *Phycomyces*. As a general result the author states that the greatest angles were formed

[1] Ueber das Wachsthum der Haupt- und Nebenwurzeln. Arbeit. bot. Inst. Würzb., i, 1873, p. 454.

[2] 'A Method of Studying Geotropism.' Ann. of Bot., ii, 1888, p. 65.

[3] Untersuchung über Geotropismus. Jahrb. f. wiss. Bot., xxvii, 1895, p. 283.

[Annals of Botany, Vol. XIX. No. LXXIV. April, 1905.]

when the plant member was exposed to the gravitation stimulus at 135° from the position of normal equilibrium. Czapek attempted to use Sachs' latent period method also for measuring relative geotropic response, but found the curves beginning in approximately the same time at all angles of inclination between 20° and 160° from the position of stable equilibrium.

Stone[1], in a very brief report of work with the roots of *Vicia Faba* and stems of grass, found the horizontal position to be the one of greatest response. He used dynamometers to measure the force of bending, measured the angles attained at different inclinations of plant axis, and compared the after-effect curvatures.

A moment's reflection over the results recorded in the foregoing abstracts of investigations will show one that the subject treated is left in a very unsatisfactory condition. In the first place, if it is conceded that a representative number of roots has been tested, the number of species of stems used is far too small. The hypocotyl of *Helianthus*, the stem of some grass, and the scapes of the cabbage and plantain alone have been tested. In the second place, the method employed to secure Czapek's results—the most extensive work of all—seems to be open to several serious objections. The forcible retention of roots in glass tubes may, for ought we know, bring in traumatic phenomena. More serious still seems to me to be the consideration, the possibility of which must be admitted, that a weaker stimulus may accomplish as much as a stronger one, provided the period of operation is long enough. That is to say, Czapek forced his roots to lie at their various angles of inclination for three to six hours, a period far exceeding their latent period. It is easily conceivable that the gravitation-stimulus might in such a period effect the same result in roots stimulated at 90° and at 135° from their position of stable equilibrium, no matter which might be the angle of stronger stimulation, or stronger response under fairer conditions. One may question, too, whether the arbitrary selection of twenty-four hours as the period for the measurement of angles is likely to lead to reliable conclusions.

With these thoughts in mind, but principally because stems had been insufficiently tested, Miss Haynes, in this laboratory, was assigned the subject of the angle of inclination of stems showing the strongest geotropic curvature, the test to be made by the method of alternate stimulation for a period less than the latent period, at angles of 90° and 135° from the position of stable equilibrium.

This method of alternate or rhythmic stimulation on opposite sides of a plant member was used by F. Darwin and Pertz[2] in 1892. It has since been used in a modified way by various authors, among them Czapek[3],

[1] 'Geotropic Experiments.' Bot. Gazette, xxix, 1900, p. 136. Abstract of paper read before Soc. Plant Morph. and Physiol., Dec., 1899.

[2] 'On the Artificial Production of Rhythm in Plants.' Annals of Bot., vi, 1892, p. 245.

[3] Ueber die Richtungsursachen der Seitenwurzeln und einiger anderer plagiotroper Pflanzentheile. Sitzungsber. d. k. Akad. d. Wiss., Math.-Naturw. Cl., civ, 1. Abt., 1895, p. 1197.

who employed it to determine the angle of inclination for maximum geotropic response of lateral roots. This method seems to me wholly free from objection. The plants are subjected for equal periods of time to gravitation stimulation, first on one side, then on the other, till by a summation of stimulations they curve. The direction of the curve, provided the great majority of plants behave alike, can be nothing less than an index of stronger geotropic stimulation or response.

By this method, Miss Haynes, whose work is soon to be published, tested the stems, young and old, of ten to twelve species, and found all of them to curve in response to the horizontal position. It occurred to me that primary roots ought to be tested in the same way also, inasmuch as this method had not been used with them. The following pages contain a record of this and other tests with a discussion of the results.

II. Experimentation and Discussion.

1. *Determination of the angle for maximum response by the method of perception-period.*

This method is not quite the same as that of the latent period discarded by Czapek [1], because he found the curves in roots beginning in about the same time when the angle of inclination was anywhere between 20° and 160° from the position of stable equilibrium. I have varied the method somewhat by exposing in each experiment two pots of seedlings of the same age for the same time, but less than the latent period, one at 90° and the other at 135° from the position of stable equilibrium ; revolving them subsequently so as to neutralize gravitation, and observing the number of curvatures and the relative amplitude of the angles. In this series only the hypocotyls were used. The experiments were conducted in a dark room, at a temperature of 22° C.

The first seedlings used were *Brassica alba*, L. Their latent period at 22° was found to be 20 min. for the earliest curve observed, while most of the stems showed no curves after 33 min. A pot with eight seedlings was turned horizontally, and another with fifteen seedlings was turned with the stems pointing 45° below the horizontal, both remaining in these positions for 33 min. The pot at 90° then showed three stems curved, while the one with stems 135° from the position of stable equilibrium showed but one curved. After 15 minutes' revolution on the klinostat in the vertical plane of curvature, six of the eight stems stimulated at 90°, and ten of the fifteen stimulated at 135° showed geotropic curves. In each set three stems had attained an angle of 45°.

In all the other experiments with the hypocotyls of *Brassica alba* the period of stimulation was less than the latent period, being in some cases 17½ min. and in others 20 min. Records were made at several times during the revolution, but the period of 30 min. seemed to be the best one

[1] Untersuchungen über Geotropismus. Jahrb. f. wiss. Bot., xxvii, 1895, p. 283.

and the fairest one, and that is given here. The total number of hypocotyls stimulated at 90° was forty-one, and the same number was stimulated at 135° from the position of stable equilibrium. After the stimulation for 17½ min., or for 20 min., and the subsequent rotation on the klinostat for 30 min., twenty-four geotropic curves were observed in those exposed at 90°, and thirteen curves in those exposed at 135°. In only one of the five experiments did the number of curves in the seedlings stimulated at 135° equal those in the seedlings stimulated at 90°; and that was in the last experiment, where each pot of seven seedlings showed only one curve. In amplitude of angle the curves formed in response to the stimulation at 90° were certainly the larger on the average; but there was a great deal of individual variation, and the evidence in this direction was not very strong, though more careful measurements might lead to stronger evidence.

Two pots of seedlings of *Raphanus sativus*, L., were used in the same way as the foregoing *Brassica* seedlings. The latent period at 22° C. was found to be 25 min. for the first stems to curve. In one pot, eighteen hypocotyls were stimulated in the horizontal position; while in the other, seventeen were placed 135° from their position of stable equilibrium. After lying 20 min. in these positions, and then revolving for 40 min. on the klinostat, each pot showed eleven hypocotyls curved geotropically. After revolving on the klinostat for 60 min., eleven of the eighteen stimulated at 90° were curved, while seven of those stimulated at 135° were curved.

The same seedlings just described were allowed to stand for 1 hour and 30 min. and were used again as before. Stimulation was for 15 min. only; the seedlings that were laid at 90° before now being placed at 135°, and those before at 135° now being at 90°. The largest number of curves in each pot came after 45 min. of revolution on the klinostat; then those seedlings stimulated at 90° showed four curves, those at 135° one curve. Subsequently all the hypocotyls began to straighten.

The foregoing results cannot be said to furnish conclusive evidence for either the position at 90° or that at 135° as giving the stronger geotropic response. The indications, however, point to the horizontal position as the more effective, since the larger number of curves and the larger angles came in response to that position.

2. *Determination of the angle for maximum response by the method of alternate stimulation.*

Alternation at 90° *and* 135°.—To facilitate this alternate stimulation there was constructed a device consisting of a heavy board base, rising about a foot, to the top of which was hinged a board capable of a rotation of 135° about a horizontal axis.. The base and hinged board were 5 feet in length, thus giving room for several plant pots or damp chambers at one time. The hinged board was pierced by holes to receive the small plant crocks, and was provided with hooks and wires to allow ready means of securing

the pots or damp chambers in place. The base itself had two faces at right angles to one another, upon either one of which it could lie on the table. The plants could thus stand in their normally erect position while they were being secured to the hinged board, and then, when all the containers were secured, the whole apparatus could be turned through 90°, and all the plants put at the same inclination at the same instant.

All the experiments were made in the dark room the temperature of which ranged from 20° to 24°C. The alternating process continued till several to many curves had appeared, but not always long enough to bring curves in all the plants. The period varied from 4 hours for *Brassica*, *Raphanus*, and *Lupinus*, to 10 hours for *Vicia Faba* and *Zea Mays*. The results were so generally uniform and decisive that details of individual species and experiments may be omitted, and the whole given in tabular form. The stimulation was usually begun with the plants inclined 135° from their position of stable equilibrium. This would give this position a slight advantage over the 90° position. The plants remained in each position 15 min. It required about 1 second to change from one position to the other. In changing from one position to the other, the plants always passed across the vertical plane, thus bringing alternately the opposite sides of the plants toward the earth.

TABLE I.

Showing results of stimulation alternately at 135° *and* 90° *from the position of equilibrium.*

Plants.	*Total number.*	*Curves caused by* 90°.	*No curves.*	*Curves caused by* 135°.
Brassica alba, hypocotyl	76	50	26	0
Raphanus sativus, „	69	47	19	3
Helianthus annuus, „	27	18	8	1
Vicia Faba „	5	4	0	1
Zea Mays, coleoptile (White dent)	14	14	0	0
Total stems	191	133	53	5
Brassica alba, primary root	19	13	4	2
Raphanus sativus, „ „	19	10	8	1
Helianthus annuus, „ „	5	5	0	0
Lupinus albus, „ „	12	12	0	0
Vicia Faba, „ „	8	4	3	1
Zea Mays, „ „ (White dent)	14	9	4	1
Total roots	77	53	19	5

The results are capable of easy demonstration. Let any one who wishes make the test with *Helianthus annuus* for stems and roots, or with *Lupinus albus* for roots. *Vicia Faba* and *Zea Mays* (except for the

coleoptile) are among the most insensitive and aberrant of plants commonly worked with. These two species have played leading rôles in plant physiology far too long. With them one must continue the foregoing experiment twice as long as for the other species used.

The results shown in the preceding table are decisive. They show the need of a critical examination of the method and results of Czapek by which he inferred a stronger response at 135° than at 90°. We have the alternative on the one hand of regarding Czapek's method or the one employed here as faulty, or on the other of supposing that different tests, all fair, will lead to different conclusions as to the angle of inclination bringing the strongest geotropic response. On a later page of this paper evidence will be offered which is believed to indicate that the discrepancy in results by the two methods is due to the unreliability of one. It is difficult to see how the method used in this paper can be called unreliable. It is the method used by Czapek[1] himself to determine the inclination at which lateral roots give their strongest geotropic response. The number of stems and roots shown in the tables as remaining straight are of importance only in indicating that the experiments were not continued long enough to bring curves in all cases, and that there is considerable variation.

Alternation at 90° *and* 112·5°.—If we accept the foregoing experimental proof of a stronger geotropic response at an inclination of 90° than at 135°, we have still to determine whether the horizontal position is the one giving the strongest response. The following table shows the results obtained with a few seedlings alternated from 90° to 112·5°, conditions being the same as for the preceding series of experiments. The curves did not show till 8 to 10 hours after beginning the experiment.

TABLE II.

Showing results of stimulation alternately at 112·5° *and* 90° *from the position of equilibrium.*

Plants.	*Total number.*	*Curves caused by* 90°.	*No curves.*	*Curves caused by* 112·5°.
Brassica alba, hypocotyl	5	5	0	0
Helianthus annuus, ,,	7	6	1	0
Zea Mays, coleoptile (White dent)	6	6	0	0
Total stems	18	17	1	0
Lupinus albus, primary root	10	9	1	0
Helianthus annuus, ,, ,,	2	2	0	0
Total roots	12	11	1	0

[1] Ueber die Richtungsursachen der Seitenwurzeln etc. Sitzungsber. d. k. Akad. d. Wiss., Math.-Naturw. Cl., civ, 1. Abt., 1895, p. 1216.

Alternation at 90° *and* 100°.—In this test the curves in the roots of *Lupinus albus* came more slowly than in those of the preceding experiment, though the temperature here was 24° and there 22·5°. This would indicate that the stimulations on the opposite sides of the roots were more nearly equal. The result shows unmistakably that the position at 90° gives a stronger effect than at 100°. The plants were placed first in the position with their axes inclined 100° from the position of stable equilibrium, thus giving probably a slight advantage to the stimulation in that position. The alternation was made every 15 min. for 12 hours. Ten seedlings of *Lupinus albus*, about 6 cm. long, in damp chambers showed six root-tips bent slightly in response to stimulation at 90°, one apparently in response to 100°, and the other three roots straight. In a pot of twelve seedlings of *Brassica alba*, seven hypocotyls ben in response to the position at 90°, one apparently in response to 100°, and the other four grew straight. In a pot of eleven seedlings of *Raphanus sativus*, seven hypocotyls bent in response to the position at 90°, and the other four grew straight.

Alternation at 90° *and* 67·5°.—For this test seedlings of *Lupinus albus* and of *Helianthus annuus* were used on the alternating rack. The former were in damp chambers, and were observed for the behaviour of roots; the latter grew in pots of sawdust, and only their hypocotyls were studied. After the alternating process had been continued for eight hours at a temperature of 23°, all of the twelve *Lupinus* roots were curved in response to the position at 90°; while of the fifteen hypocotyls of *Helianthus*, twelve had bent in response to the position at 90°, and the other three remained straight.

Alternation at 90° *and* 45°.—Only one set of seedlings was tested at these angles—the primary roots of *Lupinus albus*. After the alternation had been continued for 5 hours in temperature 22·5°, eight of the twelve roots showed curves in response to the position of 90°; the other four roots remained straight. In this set of seedlings the hypocotyls were too short for good results and were not observed.

The position at 90° stimulates to bending more strongly than at 45°, 67·5°, 100°, 112·5°, or 135°. From this we may infer that the position of strongest geotropic stimulation or response is 90° from the position of normal equilibrium.

3. *Determination of the angle for maximum response by the method of the after-effect.*

Under the foregoing heading I have placed several experiments conducted after the manner of those of Czapek. Good seedlings were selected for the test, the glass tubes were shaped to fit the root-tips closely, and the revolution on the klinostat began immediately on the removal of the glass tubes. The tubes were washed thoroughly before using, were

about one centimetre in length, and were open at both ends, the tapering end having but a small pore, and the growing root pushing the glass tube forward. The glass tubes were placed over the root-tips, and the roots, always in two sets, laid the one set at 90° the other at 135° from the position of stable equilibrium. The glass tubes lay on a plane support below, so that their weight did not rest on the roots. The seedlings were so placed that during revolution when the curves came the curves were parallel with the vertical plate of the klinostat. The following table is so constructed as to show changes in the angles of roots as the experiment progressed, and to show variation among the members of the same set of seedlings. The third column shows the period of stimulation while the roots were covered with glass tubes. The fourth column gives the time of observation after the glass tubes were removed. The fifth and seventh columns show the position of individual roots at two or three periods during the experiment; all of the numbers in the same vertical row in any one experiment give the angles for the same root. The sixth and eighth columns give the average angles at the observation nearest 24 hours. The four numbers marked '*' indicate original angles reversed. The angles as given are all for the place of the original curvature. The subsequent curves nearer the apex of the roots are not indicated.

An inspection of the following table shows such an individual variation on the part of the roots that the whole method must appear unsatisfactory. It is true that three of the four experiments give average results favouring the position of 135°; but the fourth experiment reverses this result. Moreover, in each experiment of five roots there are at least two roots from the position of 90° that, after many hours' rotation, exceed in their angles the two smallest from 135°. As worthy of note in the behaviour not shown in the table it may be said that roots kept in the forced positions in glass tubes usually tend to reduce their original angles after a few hours' stay on the klinostat, and even continue to reduce the angles after the apex of the angle has passed beyond the region of the so-called elongating zone. In the last experiment, for example, given in the table, several of the root-tips after 20 hours were 15 mm. beyond the angles; yet the angles were reduced during the ensuing 16 hours.

TABLE III.

Showing angles attained on the klinostat after prolonged forced stimulation.

Species.	*Temperature.*	*Period of stimulation.*	*Time of observation.*	*Angles attained.*			
				From 90°.	*Av.*	*From* 135°.	*Av.*
Vicia Faba	22°	5 hrs.	30 min.	90, 90, 30, 90, 90, 60		90, 45, 70, 90, 90	
			25½ hrs.	45, 30, 45, 12, 50, 15	33	40, 50, 35, 80, 95	60
Vicia Faba	26°	5 hrs.	5 min.	40, 30, 30, 0, 30		45, 20, 45, 15, 50	
			19 hrs.	15, 0, 15, −15*, 15		30, 30, 30, 0, −25*	
			24 hrs.	15, 0, 0, −15*, 10	5	30, 30, 30, 0, −10*	18
Lupinus albus	23·5°	4 hrs.	10 min.	0, 70, 70, 80, 60		45, 30, 45, 0, 45	
			12 hrs.	0, 30, 30, 15, 20		55, 40, 30, 15, 55	
			24 hrs.	0, 30, 30, 0, 0	12	45, 45, 30, 0, 45	33
Lupinus albus	23°	4½ hrs.	25 min.	30, 30, 45, 60, 60		60, 60, 10, 10, 45	
			20 hrs.	30, 30, 0, 60, 40		75, 10, 0, 0, 45	
			36 hrs.	15, 10, 0, 10, 10	34	45, 0, 0, 0, 15	26

In the experiments made by Czapek there are no details given from which we can judge of the similarity of behaviour of his primary roots. From the few like tests recorded in the present paper we may, however, infer that Czapek's results were correspondingly irregular.

B. Relative Intensity of Response at Equal Angles above and below the Horizontal.

When Sachs[1] and Stone[2] state that the geotropic response of primary roots and stems is approximately proportional to the cosine of the angle of inclination during stimulation, the deduction is that equal angles of inclination above and below the horizontal will cause equal geotropic reactions. Czapek[3] contests this conclusion, stating that for roots all angles of inclination above the horizontal cause stronger curvatures than equal angles below; while for stems all inclinations below cause stronger curves than equal angles above the horizontal. The method employed to obtain these results was, however, the objectionable one used to find the angle for greatest geotropic effect. The experiments giving the best evidence for Czapek's conclusion are to my mind those of Miss Pertz[4] on

[1] Ueber das Wachsthum der Haupt- und Nebenwurzeln. Arbeit. bot. Inst. Würzb., i, 1873, p. 454.

[2] 'Geotropic Experiments.' Bot. Gazette, xxix, 1900, p. 136.

[3] Untersuchung über Geotropismus. Jahrb. f. wiss. Bot., xxvii, 1895, p. 283.

[4] 'On the Gravitation Stimulus in Relation to Position.' Annals of Bot., xiii, 1899, p. 620.

grass stems. In these experiments the intermittent klinostat was used, exposing grass stems alternately for the same interval to various equal angles above and below the horizontal. In twenty-seven cases in the thirty-four the stems bent toward the horizontal axis of revolution, thus indicating that the positions below the horizontal caused a stronger response.

A year ago I carried out a limited number of experiments from which the inference was drawn that the conclusions of Sachs and of Stone were wrong, and that those of Czapek and Miss Pertz were right. A report of my results was made incidentally in a paper[1] dealing with the limitations of the klinostat. At the same time Fitting[2] published a paper merely mentioning results which induced him to support Sachs' view, that stimuli at equal angles above and below the horizontal are equal. Fitting's method seemed adequate, and on other points his results were the same as my own. I could not doubt the results shown by my plants, but I resolved to subject the apparatus I had had made to a critical examination. This examination showed faulty construction, as the result of which the two positions of alternate stimulation, instead of being always 180° apart, were but 177° apart, all of the shortening being on one side of the vertical plane. This regrettable error was corrected, and another series of experiments carried through as detailed in the following lines.

On the alternating rack.—This device was the same as that described in the first part of this paper. The plants were fastened to the hinged board, and the latter was alternated from one side of the vertical plane to the other, and stopped in positions such as to give the longitudinal axes of the plants in the two positions equal angles with the horizontal plane, in one position above, in the other below the horizontal. In all these experiments the plants were so placed as to serve as checks on any irregularities in the apparatus, some of the pots or damp chambers being reversed with respect to the others. Thus, for example, while, in one position of the alternating stimulation, some of the roots were 45° above the horizontal, the neighbouring reversed container would have its roots at the same time 45° below the horizontal.

Seedlings of *Pisum sativum*, to the number of thirty-eight, were employed for the behaviour of their primary roots when alternated at intervals of 10 minutes from 45° above to 45° below the horizontal. In the two experiments the duration was in one case 7 hours and in the other 9 hours, at a temperature of 23°. Eleven roots bent slightly as though responding to the position above the horizontal, three as though responding to the position below the horizontal, and the other twenty-four remained straight. Twelve seedling roots of *Lupinus albus* in a similar experiment

[1] 'Limitations of the Klinostat as an Instrument for Scientific Research.' Science, New Ser. xx, 1904, p. 376.

[2] Geotropische Untersuchungen. Vorl. Mittheil. Ber. d. d. bot. Gesellsch., xxii, 1904, p. 361.

showed, after 8 hours' alternation, four individuals curving as though responding to the position above the horizontal, two curving as though responding to the position below the horizontal, and the other six straight.

Three small potted plants of *Fuchsia* and three of *Verbena* were subjected to alternate stimulation, the stems of the former being respectively 45°, 65°, and 70° from the horizontal, while those of the *Verbena* were 35°, 45°, and 60° from the horizontal. After 8 hours' alternate stimulation only one stem, a *Verbena*, curved, and that was in the direction as though responding to the position above the horizontal. At the end of the alternation the plants were laid upon their sides at rest to see whether they were in a sensitive condition. After an hour and a half four of the six stems had curved geotropically.

On the klinostat.—If stems and roots are not equally stimulated geotropically, at equal angles above and below the horizontal position, revolution on the klinostat with horizontal axis will, by summation of stimulation, cause curves. In making experiments to determine the question suggested, it is of the very first importance that the axis of the klinostat should be strictly horizontal, and the revolution should be uniform in every part of the course, since a very slight deviation in either direction will lead to erroneous conclusions.

The klinostats used in my experiments revolved within the limits of once in 4 minutes, and once in 12 minutes. The temperature ranged between 17° and 25°, and the periods varied from 28 to 48 hours. A curve of a root toward the horizontal would indicate a stronger response from the position above the horizontal, while a curve away from the horizontal would indicate a stronger response from the position of the root inclined below the horizontal. Primary roots of *Pisum sativum*, to the number of nine, when adjusted 45° from the horizontal, showed three bending toward the horizontal, one away, and five straight. Twelve hypocotyls of *Raphanus sativus* showed one bending toward the horizontal, two away, and nine straight. Four hypocotyls of *Brassica alba* all grew without curving. Eleven coleoptiles of *Zea Mays* bent variously, but without any orientation. Two stems of *Fuchsia* showed no curves.

These results certainly indicate that orthotropic roots and stems receive equal stimuli at equal angles above and below the horizontal. The curves which were formed in the foregoing experiments are regarded as autotropic, the plant having no external directive influence. The experimenter knows that in the absence of directive influences such devious curves are not rare.

C. Summary.

The present paper deals with two questions: (1) the angle of inclination of primary roots and stems at which the strongest geotropic reaction is effected; (2) the relative geotropic effect at equal angles of inclination above and below the horizontal plane.

It has been shown that evidence on these questions has been heretofore too meagre, principally on account of the insufficiency of method employed. One of the methods employed by Sachs to settle the first question—that of the determination of the latent period—has been found by several experimenters to be difficult to use because the latent period seems to be almost the same for all angles of inclination, except for very small angles. The method of the comparison of angles attained in a given time, when roots and stems are placed at various angles of inclination, is not satisfactory, because the angle of inclination begins to change as soon as the curve begins. The method of measuring the angles attained after releasing plant members forced to retain fixed angles of inclination during prolonged stimulation is objectionable theoretically; and, in practice, roots so treated are found to behave very irregularly.

In the present paper the method of alternate, intermittent stimulation has been most employed, and, in addition, the method of determining the perception period at 90° and 135° from the position of stable equilibrium, by stimulating for a time less than the latent period and then rotating the plant on the klinostat.

The tests made by determining the perception period of the hypocotyls of *Brassica alba* and *Raphanus sativus* were not very decisive, since there is considerable irregularity of results; but the weight of evidence indicated the position at 90° as having a shorter perception period than at 135°.

The experiments by alternate stimulation on opposite sides of the same plant member for equal periods of time, but at different angles, leave no ambiguity. All of the five species of plants used showed their seedling stems responding to the position at 90° rather than to that at 135°, or any other angle from the position of stable equilibrium; and all of the six species of plants used showed the same reaction with their primary roots.

The experiments made to determine whether the geotropic effect is the same on plant members at equal distance above and below the horizontal were less uniform. By alternating plants every 15 minutes from a position above the horizontal plane on one side of the vertical plane to a position an equal distance below the horizontal on the other side of the vertical plane, the ensuing curvature after some hours would indicate which position was most influenced by the gravitation stimulus. It was found that for both roots and stems there were irregular curves, but that the large majority grew straight.

The whole matter of the inclination at which roots and stems show their strongest geotropic response is brought to this condition, as shown principally by the method of alternate stimulation :—

The orthotropic stems of plants receive their maximum gravitation stimulation at an angle of 90° from their position of stable equilibrium. This is shown by every one of the five species of seedlings used in the present paper, and by some ten species of plants with older stems, as will soon be shown by the publication of other work from this laboratory.

The orthotropic roots of plants receive their maximum gravitation stimulation at an angle of 90° from their position of stable equilibrium, as shown by every one of the six species of seedlings alternately stimulated, as recorded in this paper.

The lateral roots of plants Czapek found by the method of alternate stimulation to receive their maximum gravitation stimulation at 60° to 90° above their position of stable equilibrium.

Orthotropic roots and stems of plants, as shown in the present paper, receive equal gravitation stimulation at equal angles above and below the horizontal.

Lateral roots, as found by Czapek, curve more readily when displaced above their position of stable equilibrium than at equal distances below.

NOTES.

ON THE PRESENCE OF BINUCLEATE CELLS IN THE ASCOMYCETES.

Some time ago I indicated, from a morphological standpoint, the origin of the Protobasidiomycetes and the Basidiomycetes from conidial forms of the Ascomycetes [1].

In a criticism on this paper Harper [2] remarks as follows :—

'The widespread occurrence of regularly binucleate cells in the Basidiomycetes, with the additional evidence that these cells reproduce by conjugate division and constitute the reproductive series (*Keimbahn*) in each individual through at least a considerable part of its life-history, leading up to the formation of basidia, while no such cells are found in the Ascomycetes either in vegetative or ascogenous hyphae, shows that the two groups are widely separated phylogenetically. In the face of such differences, resemblances of outer form and method of spore-formation between conidiophores and basidia must be regarded as superficial and of uncertain value, and as wholly inadequate evidence for the conclusion Massee wishes to draw.'

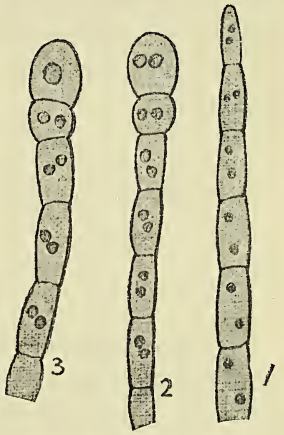

FIG. 7. Binucleate cells in conidial form (*Mycogone*) of *Hypomyces perniciosum*. 1. Vegetative hypha. 2. Conidiophore with terminal conidium containing two nuclei. 3. Conidiophore showing the terminal conidium after the fusion of its two nuclei.

Through lack of appreciation of the value of nuclei in indicating affinity or descent no special search was made for binucleate cells in the Ascomycetes. Quite recently, however, during an investigation of a disease of the cultivated mushroom (*Agaricus campestris*) caused by *Hypomyces perniciosum*, a fungus belonging to the Ascomycetes, it was observed that the conidial form of the *Hypomyces* known as *Mycogone* had the cells of the hyphae and the conidia constantly binucleate. It was also observed that the two nuclei present in the conidium fused at an early stage of development, and that on germination the germ-tube of the conidium consisted of uninucleate cells.

The cells of *Exoascus deformans*, one of the Ascomycetes, are binucleate.

In *Sclerotinia fructigena*, also one of the Ascomycetes, the conidial condition long known as *Monilia fructigena*, has both hyphal cells and conidia multinucleate, whereas in *Sclerotinia aucupariae*, an allied species, both hyphal cells and conidia of the conidial form are uninucleate.

[1] Journ. Linn. Soc., 34, p. 438 (1900).

[2] Bot. Gaz., 33, p. 1 (1902).

In the genus *Cystopus* fertilization is effected in *C. candidus* by the fusion of two nuclei, whereas in an allied species, *C. bliti*, about one hundred nuclei fuse in pairs to effect the same object.

Finally, in the Uredineae binucleate cells are usual during one phase of the life-cycle, but in *Entyloma glauci*, one of an allied group, the cells during this same phase are multinucleate.

The above examples illustrate only some of the variants as to number of nuclei in the cells of closely allied species, after which much faith is necessary to admit the value of the number of nuclei present in cells as indicating phylogenetic affinities.

Returning to the conidial form of *Hypomyces perniciosum*, where the binucleate cells are reproduced by conjugate division, or, in other words, where the two nuclei in a cell divide simultaneously, viewed from Dangeard's standpoint the fusing of the two nuclei in the conidium is a sexual act, and the conidium becomes an oogonium; hence what is considered by common consent to be an asexual conidial form is, according to Dangeard, the sexual form, and the ascophore stage emanating from the germinating conidium and having uninucleate cells, which is considered as representing the sexual phase, becomes according to Dangeard a conidial or asexual phase.

GEO. MASSEE.

Jodrell Laboratory, Kew.

ON SOME NEW SPECIES OF LAGENOSTOMA[1]: A TYPE OF PTERIDOSPERMOUS SEED FROM THE COAL MEASURES (ABSTRACT).—The recent discoveries of the seeds of two genera of the Cycadofilices, *Lyginodendron* and *Medullosa*, mark an important epoch in the history of our knowledge of Palaeozoic plants. As a corollary of this work, attention has been called afresh to the impressions or casts of seed-like bodies which occur, here and there, in the sandstones and shales of the Coal Measures, with the result that two new species, described here, have been identified as members of the genus *Lagenostoma*. Although the anatomical structure is not preserved in either case, these seeds in their external morphology agree so closely with the three species of *Lagenostoma* previously recorded from petrified material that there can be no hesitation in referring them to the same genus. In view of the recent attribution of the seed *Lagenostoma Lomaxi* to *Lyginodendron* by Professor Oliver and Dr. Scott, it is highly probable that these new species belonged either to that genus or to some closely related member of the Lyginodendreae. These specimens also throw light on the habit of these plants, especially with regard to the manner in which the female organs were borne, a character which is, with rare exceptions, extremely difficult to ascertain in the case of fossil plants owing to the fragmentary nature of the evidence.

[1] Abstract of a paper read before the Royal Society on February 23, 1905.

Lagenostoma Kidstoni sp. nov.

The first species, for which the name *Lagenostoma Kidstoni* is proposed in honour of Mr. R. Kidston, F.R.S., to whom I am indebted for information respecting the specimens, was obtained many years ago from the Lower Coal Measures at Swinhill Colliery, Stonehouse, Lanark. There are two specimens showing these seeds, one of which is in the British Museum (Nat. Hist.), and the other in the Hunterian Museum, Glasgow.

The seed, *L. Kidstoni*, is of the radiospermic type, and measures on an average about 6 mm. in length, and 2·5 to 3 mm. at its greatest width. The integument, at the apex of the seed, is divided into several short, blunt lobes, which appear to be usually six in number. The seed is slightly ridged longitudinally, the number of ridges probably corresponding to the number of apical lobes. In point of size and in its general morphology, *L. Kidstoni* agrees fairly closely with *L. physoides*, Will.

A large number of these seeds have been examined, and in every case they have proved to be naked. In only one instance has any organ been observed which could be regarded as of the nature of a 'cupular' investment, similar to that of *L. Lomaxi*, and here it does not obviously subtend the seed.

The seeds are in nearly every instance detached. Associated with them are several long, naked rachis-like structures, which correspond somewhat closely with portions of certain highly-compound fronds of the *Sphenopteris* type. In one particular case several seeds may conceivably be still attached to what is probably the termination of one of the finer branches of these axes. If this specimen is rightly interpreted, there would appear to be some evidence, though not as conclusive as one could wish, for the provisional view that these seeds were borne sessile on the terminations of the finer branches of a foliar organ probably of the *Sphenopteris* type.

Lagenostoma Sinclairi, Kidston MS.

For the loan of these specimens I am indebted to Mr. Kidston, who has recorded them, and has since proposed in MS. the specific name *L. Sinclairi*. They were obtained from the Lower Coal Measures at Grange Colliery, Kilmarnock, Ayrshire.

These specimens are particularly interesting, since many of the seeds are enclosed in a 'cupule-like' investment, and are still attached to the axes on which they were borne in the living state.

The seeds are radiospermic, and vary from 4 to 5·5 mm. in length, and from 1·5 to 3 mm. in breadth at their widest part. The integument is slightly notched or fluted at the apex, and in this respect recalls *L. Lomaxi*. The 'cupules' vary from 8 to 9·5 mm. in length, and are attached to the axis slightly below the seed. They enclose the seed somewhat loosely, and are divided at the apex into several, apparently erect, lanceolate lobes.

It seems probable that the axes on which the seeds are borne are the segments of a highly compound frond with reduced lamina, in all probability of the *Sphenopteris* type.

General Conclusions.

The chief conclusion arrived at from a study of these new seeds relates to the light which they throw on the habit of members of the Pteridospermeae. At present we are only acquainted with one genus, *Medullosa*, in regard to the manner in which the seeds were borne.

In neither *L. Kidstoni* nor *L. Sinclairi* is there any direct evidence as to the type of sterile frond with which they were associated, but the general morphology of the branched axes bearing the seeds affords a valuable clue to the habit of the sterile fronds. These axes are regarded as portions of a compound frond with reduced lamina. In the case of *L. Kidstoni* the long rachis-like structures present many points of morphological similarity to the fronds of the *Sphenopteris* type. In *L. Sinclairi* the frond, had it possessed a lamina, would in all probability be placed in the same genus. Thus there is every reason to suppose that the sterile foliage associated with these seeds was of the *Sphenopteris* type.

This conclusion is supported by the recent attribution of the seed *L. Lomaxi* to *Lyginodendron*, a stem known beyond doubt to have possessed fronds of this nature. There is thus strong evidence that these new species, which in the morphology of their seed-bearing axes approach so closely to the foliar organs of *Lyginodendron*, and, in their seeds, agree so well with *L. Lomaxi*, were borne by stems either of *Lyginodendron* itself, or of some closely related member of the same family possessing the *Sphenopteris* form of sterile foliage.

There is, therefore, to be found in these specimens the first definite clue to the habit of the Lyginodendreae with regard to the manner in which the female fructification was borne. If this conclusion is correct, we may picture these plants as bearing, in addition to numerous highly-compound fronds of the *Sphenopteris* type, others in which the lamina was wholly or partially reduced, and in which the ultimate branches terminated in seeds, with or without a 'cupular' investment.

In the lax arrangement of the fructification, the Pteridospermeae must have presented a striking contrast in habit to the members of most of the other great Palaeozoic groups, in which compact strobili were for the most part conspicuous and dominant types of sporangial aggregation. Among living plants, almost the only analogue is to be found in the female sporophyll of *Cycas*.

E. A. NEWELL ARBER.

Trinity College, Cambridge.

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ANNALS OF BOTANY

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ASSISTED BY OTHER BOTANISTS

London
HENRY FROWDE, AMEN CORNER, E.C.

Oxford
CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1905

Printed by HORACE HART, *at the* CLARENDON PRESS, *Oxford.*

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Studies on the Araceae, III.

BY

DOUGLAS HOUGHTON CAMPBELL,

Professor of Botany in the Stanford University, California, U.S.A.

With Plates XIV, XV, XVI, and XVII.

DURING the summer of 1899 the writer obtained good series of several species of Araceae from the very large collection cultivated at Kew. Some of the results obtained from the study of these collections have already been published in previous papers [1]. The present contribution to the study of these plants is concerned mainly with a study of the embryo-sac and embryo of two species—*Anthurium violaceum*, Schott, var. *leucocarpum*, and *Nephthytis Liberica*, Schott. These investigations have yielded several somewhat important additional facts bearing upon the development of these interesting plants.

Anthurium violaceum, Schott, var. leucocarpum.

The genus *Anthurium* is a large one, comprising some 200 or more species distributed through the American tropics. The showy foliage of certain species, and the brilliantly coloured spathes of others, have made them favourite ornaments for the greenhouse and conservatory. In the damp forests of the West Indies and in Central and South America species of *Anthurium* are among the most conspicuous plants that one encounters.

In a previous paper [2] some notes upon the endosperm of *Anthurium* based upon material collected in Jamaica were published, but owing to the material being imperfectly preserved it was impossible to trace out its development. Among the numerous species grown at Kew, several were collected, among them a small species, *A. violaceum*, var. *leucocarpum*, hort., which was flowering and fruiting freely, so that a very complete series of stages could be collected. These were prepared as carefully as possible, and yielded very satisfactory results.

[1] Campbell, Studies on the Araceae, I: Annals of Botany, xiv, March, 1900; II: Annals of Botany, xvii, September, 1903.

[2] Loc. cit., I.

[Annals of Botany, Vol. XIX. No. LXXV. July, 1905.]

Many small ants were noticed crawling over the plants at Kew, and it is highly probable, although this was not actually demonstrated, that they were the agents in carrying the pollen.

The Flower.

The spadix in *Anthurium* is completely covered with flowers which are all alike. There is a perianth composed of several scale-like leaves which do not, however, completely cover the stamens and pistil. There are four stamens regularly placed about the top-shaped pistil. The latter is composed of two carpels completely coherent, and in cross-section is conspicuously four-sided (Pl. XIV, Fig. 2). The stamens consist of a short filament, and the anther has four loculi [1].

Engler considers the type of flowers found in the Pothoideae, to which *Anthurium* belongs, to be the primitive one for the Araceae, and thinks that the diclinous type found in most genera is the result of a reduction from the hermaphrodite flowers found in *Anthurium* and other similar genera. It seems to the writer more probable that this is not the case, and that the type of flower found in *Anthurium* is less primitive than the flower occurring in some genera.

In *A. violaceum*, the young flower, aside from the perianth, consists of a short top-shaped pistil (Fig. 1) composed of two completely coherent carpels, which in transverse section shows the ovary to be strongly quadrangular, with two chambers, each containing two ovules. The placenta is axial, formed by the coherent faces of two carpels.

The four stamens are in pairs corresponding to the flattened sides of the ovary and in all respects seem to correspond to the stamen of the typical angiospermous flower, so that no special study was made of the development. The divisions of the pollen mother-cells were not followed in detail. In the early prophase of nuclear division the chromosomes were very distinct, and, usually at least, sixteen in number. The divisions are completed while the ovules are still very young. The pollen-grains are small, nearly globular in form, and with a moderately thick wall. It was not determined whether or not the generative nucleus divides before the pollen germinates. The division of the pollen is complete while the ovules are still very small.

The Ovule.

When the ovules are first clearly recognizable, the pistil is a top-shaped body which in longitudinal section shows a central canal extending to the top of the placenta (Fig. 1). The young ovules are in pairs, and

[1] Engler and Prantl, Die natürlichen Pflanzenfamilien, II, 3. Abt., pp. 107, 111.

are nearly hemispherical masses of tissue whose upper cells are larger and indicate the beginning of the papillate hairs which later are so conspicuous at the base of the funiculus. As the ovule enlarges, these hairs become much more evident (Figs. 4, 5), and the ovule itself assumes an oblong form. The apex, which develops later into the nucellus, is somewhat attenuated, and there is visible a slight enlargement below the young nucellus, which marks the beginning of the first integument.

At this stage there could be seen a single rather large sub-epidermal cell, from which is later developed the single embryo-sac. During its further growth the ovule becomes strongly bent. The second integument develops and the first (inner) integument rapidly grows up above the nucellus, forming the micropyle. Later, the outer integument grows much faster and extends beyond the inner one.

The nucellus, as in most Araceae, remains relatively small. The cells at its apex become elongated, and periclinal walls arise in them, so that the young embryo-sac (Fig. 5) is sunk rather deeply in the tissue of the nucellus.

From a study of the younger stages of the embryo-sac, although the actual division was not seen, it seems likely that the primary archesporial cell usually divides once by a transverse wall, the outer and smaller cell (*t*) representing the tapetum, which undergoes no further divisions and later is destroyed. The inner and larger cell develops directly into the embryo-sac. It is possible, however, that the outer cell represents a second embryo-sac and not a tapetum, as in one case (Fig. 11) there was found above the embryo-sac a cell containing several nuclei, which recalls the development of the secondary sacs met with in some other Araceae.

The development of the embryo-sac up to the time of fertilization shows nothing peculiar. The polarity is strongly marked, the two primary nuclei lying at opposite ends of the sac. These nuclei are not very large and are of the usual type (Fig. 9).

The subsequent divisions proceed as usual, and the eight resulting nuclei are distributed in the ordinary fashion. The egg-apparatus, antipodals, and polar nuclei do not differ in any way from the ordinary angiospermous type (Figs. 10–14). The eight nuclei are much alike in size and structure. They stain readily and each is provided with a conspicuous nucleolus.

The egg-apparatus becomes clearly evident at the micropylar end of the sac, while at the chalazal end are the three antipodal cells. The upper polar nucleus moves to the lower end of the sac where it fuses with the lower polar nucleus to form the endosperm-nucleus (Figs. 13, 16).

Previous to the union of the two nuclei they are surrounded by a pretty well defined mass of granular cytoplasm (Fig. 12), which becomes less conspicuous after the fusion of the two nuclei. The details of the

fusion were not followed, but there was nothing to indicate that the process differed in any respect from the usual one. The endosperm-nucleus resulting from this fusion is, at first, not markedly different, except for its greater size, from the polar nuclei; but it subsequently enlarges a good deal and the chromosomes become more evident, preparatory to its entering upon the first prophases of division.

At the time the embryo-sac is ready for fertilization (Fig. 14) the egg-apparatus shows the two similar synergidae at the apex, with the egg-cell somewhat lower down. The egg has less granular cytoplasm and its nucleus is somewhat larger and does not stain so readily as do the synergidal nuclei. The arrangement of the antipodal cells, as is so often the case, is very much like that of the cells of the egg-apparatus, but the three cells are entirely similar as to their contents.

As the embryo-sac approaches maturity, the lateral tissue of the nucellus is almost completely destroyed, and the sides of the embryo-sac are separated from the inner integument of the ovule only by the remains of the nucellar tissue. The apex of the nucellus, as in most Araceae examined, persists as a conspicuous cap covering the apex of the embryo-sac (Fig. 15).

Fertilization.

While a few preparations showed the entrance of the pollen-tube, the details of fertilization could not be treated as fully as could have been wished.

It is possible that the conspicuous papillate glandular hairs at the base of the funiculus are concerned in directing the growth of the pollen-tube to the micropyle. The pollen-tube pushes down between the cells of the nucellar cap, and apparently penetrates one of the synergidae. In the specimen shown in Fig. 15, the synergid into which the pollen-tube seemed to have penetrated was decidedly more granular in appearance than the other one, and was probably being destroyed by the growth of the pollen-tube through it. The most satisfactory preparation that was found is shown in Fig. 17. In this case the pollen-tube had crowded between the synergidae, one of which remained intact while the other was much contracted and the nucleus had lost its regular contour. It was not certain whether or not the pollen-tube had really penetrated the synergid, or merely was appressed to it. Apparently within the tube was an irregular deeply stained body (*t.n.*), which it was concluded was the tube-nucleus. Two much smaller nuclei, δ^1, δ^2, outside the tube and presumably discharged from it, were interpreted as the two generative nuclei. One of these, with clearly defined contour and a conspicuous nucleolus, seemed to be within the egg, while the other, which stained more

strongly, lay within the cavity of the embryo-sac, close to the wall. No preparations were found showing the fusion of the sexual nuclei, but there is no reason to suppose it differs from other forms that have been studied.

Whether or not the second generative nucleus fuses with the polar nuclei must be left undecided for the present. If there is such a fusion it must occur subsequent to the union of the polar nuclei, as this takes place some time before the egg is ready for fertilization.

The Endosperm.

As in other Araceae there is no formation of free endosperm-nuclei in *Anthurium*, but each nuclear division is accompanied by the formation of a division-wall between the daughter-nuclei. The primary endosperm-nucleus lies at the base of the embryo-sac, and its first division occurs while it occupies this position, the formation of the endosperm thus proceeding from the base to the apex of the sac, as has already been described for *Spathicarpa* and *Aglaonema* [1].

Shortly after fertilization has been effected, the antipodal cells, which are at no time especially conspicuous, begin to disintegrate, and before the endosperm formation has proceeded far, are no longer recognizable.

Before the first division of the primary endosperm-nucleus occurs, it increases a good deal in size (Fig. 16). It lies close to the small antipodal cells and soon undergoes its first division. The earliest division stage encountered (Fig. 18) showed the two daughter-nuclei already completely separated, but the nuclear spindle was clearly evident. The chromosomes were still recognizable, and the nuclear membranes had not yet formed. No sign of a division-wall could be made out, but in a later stage (Fig. 19) in which the daughter-nuclei were complete, they were separated by a very distinct cell-wall, which cut off a small flattened cell from the base of the embryo-sac, just above the antipodal cells. In this case, as well as in a somewhat more advanced stage which was seen, the upper nucleus was decidedly larger than that of the lower and smaller endosperm-cell.

The next division takes place in the upper cell and divides it transversely into two cells of nearly equal size; in each of these cells the process is repeated, and a row of four large cells lying above the basal cell is formed. This repeated transverse division is accompanied by a considerable elongation of the embryo-sac, with but little growth in breadth (Pl. XV, Fig. 21).

In the lower of the two primary endosperm-cells the divisions are all vertical, and result in usually four cells which are decidedly different in appearance from the larger cells derived from the upper of the primary endosperm-cells, which give rise to the major part of the fully developed

[1] Campbell, loc. cit., II.

endosperm. The basal cells may divide somewhat further, but the divisions are apparently all vertical ones, and the small group of basal endosperm-cells thus formed looks very much like a mass of antipodal cells, and might very readily be mistaken for them, had not the study of the development of this group of cells shown unmistakably that they were the derivatives of the lower of the two primary endosperm-cells. The true antipodal cells are clearly recognizable up to the time of the first division of the endosperm-nucleus, but soon become so crowded upon by the growing endosperm as to be no longer distinguishable.

In one case (Fig. 11), above the normal embryo-sac containing the egg-apparatus, antipodals, and endosperm-nucleus, there was another cell containing four free nuclei. From a comparison with *Nephthytis* and *Aglaonema* where secondary embryo-sacs frequently occur, it is probable that this cell is a second embryo-sac in which the early divisions of the nucleus had taken place, but which would probably not reach maturity, and would be finally destroyed by the growth of the principal embryo-sac.

The embryo-sac remains decidedly elongated and of nearly equal diameter throughout. The basal endosperm-cells, as we have already indicated, divide only by vertical walls and form a mass of tissue sharply separated from the much larger cylindrical mass derived from the upper cell. While in the latter the earlier divisions are all transverse, later there are also formed vertical walls, but these are less numerous than the transverse divisions, and the sac retains its narrow form until the embryo is far advanced.

With the growth of the endosperm the lateral tissues of the nucellus are quite destroyed, and the apical cap of cells is all that is distinguishable of the upper part of the nucellus. While this cap increases somewhat in size with the growth of the embryo-sac, it does not keep pace with the latter, which finally exceeds it considerably in width.

In its later stages, therefore, the embryo-sac shows a growth of basal endosperm-cells extended vertically, and a much larger mass of thin-walled cells with transparent contents and not very large nuclei, this mass of cells being derived exclusively from the divisions of the upper of the primary endosperm-cells.

The Embryo.

The development of the embryo does not begin until the endosperm is well advanced in its growth. Traces of the synergidae remain visible for a short time, but soon become unrecognizable. The egg enlarges but little, and after the membrane is formed about it is nearly hemispherical or slightly elongated in outline, and is attached by a broad base to the apex of the sac (Fig. 20). The first division-wall is transverse and is

usually, perhaps always, followed by a second transverse wall before any vertical walls are formed.

The next divisions probably vary somewhat, to judge from the varying appearance of the later stages which were found. In most of these there was evidence of intersecting vertical walls in the second and third of the three primary segments of the embryo, so that a longitudinal section shows the second and third segments divided by a median wall into two equal parts. This quadrant division may also occur in the small basal segment, which forms a very rudimentary suspensor, but this does not seem to be always the case.

In most instances the embryo assumes a nearly globular form, less commonly (Fig. 25) it is somewhat elongated, with an absence of the usual quadrant formation. The rudimentary suspensor is apparently of little or no importance as an organ of absorption, but remains very much reduced as in other Araceae that have been studied. This absence of a functional suspensor is accounted for by the early investment of the young embryo by the endosperm-cells with which it is in intimate contact from the first.

As the embryo enlarges, its original globular form is somewhat altered, the basal part becoming a little flattened and the whole embryo assuming a slightly conical form (Fig. 27); but as yet there is no evidence of the differentiation of the organs of the young plant, nor of its permanent tissues. The evidence of the quadrant divisions in the young segments of the embryo is still more or less apparent, but the tissues are still entirely similar, and except for a superficial layer of cells constituting the young epidermis, the tissues are quite undifferentiated.

Owing to the great uniformity of the tissues, it is not possible to decide with absolute certainty the relation of the organs to the primary divisions of the embryo. The latter becomes elongated, assuming a slenderly conical form, and there is formed at one side a depression which marks the position of the stem-apex (Fig. 29, *a*), whose origin is thus seen to be of the characteristic monocotyledonous type. It is quite impossible, however, to say whether its position corresponds in any way to the early divisions of the embryo.

About the same time that the stem-apex is first visible the differentiation in the root-region begins. A clearly defined line, marking the boundary of the root-cap, can be seen (Fig. 29, *a*), this line probably corresponding to the second transverse division in the young embryo, although this point could not be determined with absolute certainty. If this is correct it means that everything except the rudimentary suspensor and the root-cap is derived from the terminal segment of the young embryo.

A section of the embryo at this stage shows the root-cap (*r.c.*), which is lenticular in form, separated by a sharply marked boundary from the other

root-tissues, which are not very clearly defined. A distinct suspensor cannot be recognized.

In the young embryo the central cylinder or plerome of the root can be distinguished, but its limits are not very clear, and it is quite impossible to refer its origin to any definite divisions in the younger embryo. The stem-apex is a flat area lying in a deep cleft between the base of the large pointed cotyledon, and the short thick root, but the limits of the stem are quite impossible to distinguish ; nor at this time is it possible to make out any strictly cauline vascular bundle. In the axis of the cotyledon is a slender bundle of procambium cells much like that in the root. The base of the cotyledon extends around the cleft containing the stem-apex, which later becomes completely hidden by the sheath-like base of the cotyledon (Fig. 36).

The embryo grows rapidly, until it finally reaches a length of about 2 mm. Like various other low Monocotyledons, the embryo is far advanced at the time the seed is ripe, but part of the endosperm also persists. The second leaf is well developed, and this together with the stem-apex is entirely concealed in the cavity formed by the overlapping margins of the sheathing base of the cotyledon. The ripe embryo is in shape very much like that of *Naias*[1], but it does not entirely fill the embryo-sac.

In the mature embryo the young vascular bundles are very evident, but no tracheary tissue has developed. Each of the primary organs (except the stem) has an axial bundle which unites with the bundles of the other organs at their junction. The tissues of the young root (Fig. 35) are arranged like those of certain other Monocotyledons, e. g. *Sparganium.*

The root-cap is clearly separated from the tissues beneath, and has its own initial cells, which are quite independent of those of the other primary tissues of the root. There is an evident plerome-cylinder, between which and the root-cap is a single layer of cells serving as the initial for both the epidermis and plerome. The outer cells derived from this common initial layer divided by periclinal walls, the outer cells forming the epidermis of the root, the inner ones, which also undergo further periclinal divisions, contributing to the growth of the periblem or cortical tissues.

The presence of acicular crystals of calcium oxalate is a marked characteristic of most Araceae, and these are found to be present in considerable numbers in the embryo of *Anthurium.* These crystals are formed in compact bundles in special cells distinguished by more granular contents than those of the neighbouring cells (Fig. 37). They also have larger nuclei, which sometimes show indications of degeneration.

As the seed ripens there is the usual accumulation of reserve-food,

[1] Campbell, A Morphological Study of *Naias* and *Zannichellia.* Proc. Calif. Acad. Sciences. Third Ser. Bot. I, No. 1, 1897.

including a good deal of starch, in the cells of the endosperm and embryo, which, although of large size, does not fill the whole embryo-sac. In the endosperm-cells of the half-grown seed, the characteristic raphides were found, a rather unusual occurrence in endosperm-cells. The bulk of the seed is derived from the embryo-sac. The integuments formed the well-developed testa, but the great development of the chalazal region, so conspicuous in *Aglaonema* and *Lysichiton*, for example, is wanting in *Anthurium*.

The papillae upon the funiculus secrete mucilage very freely, and the presence of this interferes often with fixing the material for study, and it is necessary to avoid aqueous fixing agents. The inner tissue of the ovary wall also becomes mucilaginous, and the ripe seeds are thus imbedded in a pulpy, adhesive mass which no doubt facilitates their attachment to the substratum upon which they germinate.

Nephthytis Liberica, Schott.

The genus *Nephthytis* comprises two species of West African aroids, one of which, *N. Liberica*, was growing and fruiting at Kew, and a fairly complete series of specimens was collected.

This species proved to be a very puzzling one, as there was extraordinary variation shown in the development of the embryo-sac, which departed widely from the ordinary angiospermous type. It approached more nearly *Aglaonema commutatum* than any other form which has yet been investigated, and as the structure of the flower is very similar in both of these old world Araceae, it is quite probable that they are more nearly related than would be indicated by the relative positions given them by Engler[1].

As in *Aglaonema*, the flowers of *Nephthytis* are diclinous, the pistillate flowers being at the base of the spadix whose upper part is completely covered with the crowded naked staminate flowers.

Each pistillate flower consists of a single carpel with a solitary basal, anatropous ovule. The stigma is broad and peltate, the whole flower resembling very closely that of *Aglaonema*[2].

Owing to imperfect development, or to defects in the fixing methods employed, the pollen-grains were very badly shrunken and distorted, and no satisfactory study of their development and structure could be made, so that a comparison with the corresponding stages of the other Araceae was not possible, and the work in *Nephthytis* was confined to a study of the embryo-sac and embryo.

[1] According to Engler (loc. cit., p. 128), the ovule of *Nephthytis* is at the apex of the loculus of the ovary. In the plants labelled *N. Liberica* at Kew, from which my material was taken, the ovule was invariably at the base of the loculus, exactly resembling *Aglaonema* in this respect.

[2] Campbell, loc. cit., II, pp. 668–9.

The Ovule.

In the youngest available ovules the integuments were already completely formed and the archesporium could be recognized. At this stage the ovule closely resembles that of *Aglaonema*, and its further history recalls in many ways that of *A. commutatum*.

The young ovule is very massive, but this is mainly due to the great development of the base of the ovule and the integuments, as the nucellus is relatively small and slender. The apex of the nucellus is flattened and is on a level with the top of the two integuments which are at this time of equal length (Pl. XVI, Fig. 38).

Occupying the axis of the nucellus in the youngest ovules found, were two superimposed cells with large and conspicuous nuclei. These were probably sister-cells, but the actual division of a primary archesporial cell was not seen.

In the other ovules of about the same age the two sporogenous cells were side by side, and in some others there was a more or less irregular group of what might be interpreted as sporogenous cells (Fig. 40). The position of these was sometimes such as to make it probable that they were not derived from the division of a common mother-cell, but may have been the product of two or three independent hypodermal cells—in this respect showing a resemblance to *Arisaema*[1] where the archesporium-cells show, in some cases at least, a similar independent origin.

The cells of the nucellus-apex generally divide by periclinal walls, but some of them may remain undivided. Evidences of the formation of a tapetal cell cut off from the archesporium were seen, but whether this is regularly the case is doubtful, and it seems quite as likely that no tapetum is developed in most cases.

The further history of the sporogenous cells shows extraordinary variation, and in most cases examined the number of embryo-sacs which begin to develop is more than one. In some instances several sacs develop about equally for some time. So variable, indeed, does *Nephthytis* show itself to be, that it is quite impossible to determine what may be called the normal method of development.

As development proceeds the mass of sporogenous tissue becomes very conspicuous and gradually encroaches upon the lateral nucellar tissue. The number of sporogenous cells is variable, and it is impossible to determine just how many are to be looked upon as primary embryo-sacs.

The ovule shown in Fig. 41 was probably abnormal, but it illustrates the remarkable development of sporogenous tissue which may take place before a definite embryo-sac is recognizable. In this case the division-walls

[1] Campbell, loc. cit., II, p. 667. Mottier, Bot. Gazette, 1892.

were largely transverse, but in the specimen shown in Fig. 42, which seemed to be perfectly normal, there were several embryo-sacs lying side by side. The exact number is difficult to determine, and it is not always possible to decide whether the division-walls are not in some cases of secondary origin, and formed within the embryo-sacs themselves.

Each young embryo-sac begins to develop—that is, divisions of the nucleus, and perhaps, sometimes, cell-divisions as well, occur. This makes it extremely difficult to decide how much of the cell-complex found in the centre of the nucellus is the product of a single embryo-sac (see Figs. 42, 43). It seems probable that one sac finally crowds out the others, but, on the other hand, it looked sometimes as if the structures present at the time of fertilization were the combined products of two or more of the primary embryo-sacs.

In no case observed did the mature embryo-sac show the typical structure found in most Angiosperms. Sometimes a nearly normal egg-apparatus was found, but in such cases the other structures of the sac were not of the usual type, either there were no antipodal cells, or else no polar nuclei were developed. Indeed, so great was the variation, that it was quite impossible to make out any prevailing type, and all that can be done will be to describe the most striking forms met with, without attempting to decide what may be considered as the normal type.

The simplest case observed (Figs. 48–50) had a single well-defined embryo-sac with two nuclei, one at each end. The upper nucleus was in the early prophase of division, it was large and conspicuous, and was surrounded by a clearly marked mass of cytoplasm, looking like the egg-cell of a typical embryo-sac. At the antipodal end of the sac was the second nucleus, surrounded by a similar but larger mass of granular cytoplasm like that at the upper end of the sac. The antipodal nucleus was in process of division. The nuclear plate, with about fifty-six chromosomes, was extremely conspicuous, and the nuclear spindle was very clear.

What would have been the future history of this embryo-sac can only be conjectured, but it may very well represent an earlier stage of such a sac as that shown in Pl. XVII, Fig. 52, where there was at the apex an egg-apparatus consisting of the usual two synergidae and the egg-cell, while at the opposite end of the sac there was a single large nucleus embedded in a mass of granular cytoplasm, suggesting a single large antipodal cell. No other nuclei were present, and it looks as if the polar nuclei were quite absent. A condition similar to this has been found in *Aglaonema*[1].

In the sac shown at Fig. 44 there was a single large nucleus at the apex, and at the antipodal end there were apparently three nuclei. The latter were not noticeably different from the nuclei of the adjacent nucellar cells, and it is possible that they may have belonged to these and not to the embryo-sac itself.

[1] Campbell, loc. cit., II, Fig. 23.

In Fig. 45 is shown a sac in which there were two large nuclei surrounded by small starch granules such as are usually found after the fusion of the polar nuclei. These nuclei were separated by a delicate cell-membrane, but whether this was a real cellulose wall was not determined. There was in this specimen a second young embryo-sac with a single nucleus.

Of somewhat similar character is the case shown in Fig. 43. In this case the embryo-sac showed a transverse division into three parts. It is possible that here each division is really a potential embryo-sac, but if this is true, the definitive embryo-sac is formed by the fusion of three primary ones. Of the three divisions, the upper and lower ones each contained two nuclei, while the middle one had but a single one. There was in this case also a single sac (*sp*^{2}.), much smaller, but containing two nuclei. From its small size and position it is pretty certain that it would eventually be destroyed by the growth of the principal sac.

The embryo-sac shown in Figs. 54, 55 is of somewhat the same character as that figured in 43. In the upper compartment a hemispherical mass of cytoplasm, probably the egg-cell, could be seen: there were also in this compartment two other nuclei (*cd.*), which perhaps may be interpreted as belonging to synergidae. In the large central compartment there were four free nuclei, and in the lower compartment was a group of apparently five cells looking like antipodal cells. If the whole structure represents a single embryo-sac, it contains twelve nuclei, instead of the eight characteristic of the typical Angiosperms.

A decidedly different type is shown at Figs. 57–59. The embryo-sac in this instance was not divided by walls, but above it was a small sac with five nuclei. It is possible that these five nuclei were not all contained in a single sac, and there may have been two of the secondary sacs very close together. In the upper part of the principal sac (Fig. 57) was a group of apparently three nuclei in process of fusion, below these being two other free nuclei. Near the base of the sac, but at one side, was an irregular group of either four or five cells (Fig. 58), each with a single nucleus. At the extreme base were three other cells (Fig. 59) closely resembling normal antipodal cells. In this instance it was impossible to say from which of the two lower groups of cells the egg-cell would be derived, but it is clear that it would not be formed in the upper part of the sac, as is usually the case.

The fusion of three nuclei presumably antecedent to the endosperm formation, together with the presence of three extra free endosperm nuclei, have their nearest analogy, perhaps, in *Peperomia*, and are to a certain degree between the condition found in the latter genus and that which obtains in the ordinary Angiosperms.

Another different type is shown in Fig. 53. In this specimen there

was a single apical aggregation of cytoplasm with a nucleus, the whole probably being the egg. Nothing which could be interpreted as synergidae could be seen. In the upper part of the undivided cavity of the sac was a large nucleus in process of division, and nearer the base a second free nucleus (*e. n.*) in a resting condition, was seen. There was no sign of nuclear fusion, but it is possible of course that the division of the upper free nucleus may have been preceded by a fusion of two or more nuclei. A little at one side of the base of the sac was a nearly hemispherical cell mass (*an.*) containing, as nearly as could be determined, twelve cells. These perhaps represent the antipodal cells. The twelve nuclei belonging to this cell mass, together with the three in the upper part of the sac, make a total of at least fifteen nuclei for the unfertilized sac, even if there had been no nuclear fusions. The absence of the synergidae, and the very slight polarity in the arrangement of the nuclei, suggest *Peperomia* in this case also.

The sac shown in Fig. 47 exhibited at the apex a large cell containing what seemed to be two large nuclei in process of fusion. The rest of the sac was divided obliquely by a delicate membrane, the upper division containing a single nucleus, the lower three nuclei, two of which were fusing. No antipodal cells could be seen.

The sac shown in Fig. 46 approached the normal angiospermous type. At the apex were three cells resembling closely a normal egg-apparatus. At the chalazal end of the sac were two large antipodal cells with rather small, but strongly staining nuclei. In the middle of the sac were three large free nuclei. The lowest of the three was smaller than the others, and may have represented a third antipodal. The two larger free nuclei were widely separated and the upper one was evidently preparing to divide. If these two nuclei represent the polar nuclei of the ordinary embryo-sac, it is pretty clear that there is no fusion of these preliminary to the first division of the endosperm. Although in this case there were the typical eight nuclei present, the indicated division of one of the polar (?) nuclei showed that the number would be increased before fertilization took place.

Another case met with was difficult to interpret. It is not impossible that there was here an aggregation of several sacs, and not a single one. At the apex were two cells, apparently with cellulose membranes, one with dense contents and somewhat contracted nucleus, the latter perhaps owing its appearance to the action of reagents; the other with much less granular cytoplasm and perfectly normal nucleus. The space below was incompletely divided, and at the base was a group of three cells, one larger and with more conspicuous nucleus, the whole looking like a normal egg-apparatus. Near this egg-apparatus (?) was a second group of five cells with large nuclei, and probably the antipodal cells. Nothing comparable to polar nuclei was visible. It is possible the two basal groups

of cells may have belonged to two closely appressed sacs, but this does not seem to have been the case.

It is evident from the foregoing statements that there is remarkable diversity in the embryo-sac structures in *Nephthytis*. How far these are normal cannot be certainly determined until material grown under natural conditions can be examined. As the variations find a pretty close counterpart in those of *Aglaonema commutatum*, it is highly probable that some of them, at least, are normal deviations from the type found in most Angiosperms. The most significant variations are the greatly increased number of nuclei, the occasional multiple nuclear fusions, and the less marked polarity of the embryo-sac.

Fertiiization.

Although numerous attempts were made to find fertilization stages, these met with indifferent success, and even where what seemed to be the pollen-tube could be demonstrated, the real condition of affairs could not be satisfactorily made out.

Two cases are shown in Figs. 63, 64, and 70. In 63 there were no antipodal cells, but in the cavity of the sac there were three large nuclei, two of them (see Fig. 62) in a resting condition, and surrounded by starch granules, the upper one somewhat smaller, and evidently in the early prophase of division. At the apex of the sac were two large cells (without cellulose membranes), presumably the egg-cell and a single synergid. The latter was much more granular, lay at the apex of the sac, and its nucleus was smaller than that of the clearer egg, which was placed below it.

Closely applied to the synergid (perhaps passing through it), and extending to the egg, was an irregular body (*p.t.*), staining strongly with gentian violet and looking like a pollen-tube. Within it was an irregular darkly staining body, which may have been one of the generative nuclei. Apparently within the egg, but quite free from the egg-nucleus, was a smaller nucleus which probably was the second generative nucleus.

A quite different case is shown in Fig. 70. At the apex of the embryo-sac was seen an irregular sac-like structure which seemed to be connected with a tubular body (pollen-tube) pushing between the cells at the apex of the embryo-sac. In this were two conspicuous nuclei which may possibly have been generative nuclei, but the whole structure differs much in appearance from the ordinary pollen-tubes. A second less definite but similar pollen-tube was present. The sac contained an egg-cell at the apex, and six (possibly seven) other nuclei. Of these, two showed some indications of fusion.

Beyond these very unsatisfactory observations no light could be thrown upon the processes of fertilization.

The Embryo.

While the egg-cell is most commonly near the upper end of the embryo-sac, it not infrequently is at the side, or even at the base, and the position of the embryo varies accordingly. In this respect there is a resemblance to *Aglaonema*.

When the egg is at the apex it has a broad base of attachment, and the first division-wall is nearly parallel to this. No suspensor is developed, and the subsequent divisions, which do not appear to follow with absolute regularity, transform the embryo into a nearly globular mass of uniform cells. Perhaps more frequently the egg-cell is not attached to the wall of the sac, and the embryo is then surrounded on all sides by the endosperm. The older stages of the embryo were not present in the material at my disposal, so that the development could not be followed. In the ripe seed the embryo constitutes much the greater part. The testa is very thin, and the embryo reaches a length of about a centimetre. The greater part of the embryo consists of the very massive cotyledon. The root and stem are relatively poorly developed.

The Endosperm.

The development of the endosperm varies, of course, with the structure of the sac at the time of fertilization. Where only one nucleus is present besides the egg-apparatus, the endosperm formation begins presumably with the division of the sac into two cells, a small basal one and a large upper one. Such a case is probably that shown in Fig. 51, in which there was, somewhat at one side of the sac, a two-celled embryo, and the sac was divided into two cells, of which the lower one was much smaller than the other, whose large nucleus was especially conspicuous.

Where the embryo-sac contains several free nuclei before fertilization, it is likely that the endosperm-formation begins by the development of membranes between the free nuclei, thus dividing the cavity of the sac into several large cells (Figs. 60, 65). In all cases, however, the sac very early becomes filled with a continuous large-celled tissue, with the small embryo embedded in it. In the older stages the embryo-sac usually shows a decided bend near the middle, resembling in this respect, as well as the others referred to, the sac of *Aglaonema*. The lower endosperm-cells (Fig. 68) have much denser cytoplasm than the upper ones, and their large nuclei show signs of degeneration. It is doubtful whether these basal cells are properly to be considered as antipodals, as their exact relation to the group of cells sometimes found at the base of the unfertilized sac could not be determined. Where there are but two primary endosperm-cells and no

antipodals, as in Fig. 51, the denser basal cells of the endosperm probably are derived entirely from the divisions of the lower of the two primary cells.

Comparing *Nephthytis* with the other Araceae that have been studied, it is evident that it most nearly resembles *Aglaonema*, especially *A. commutatum*[1]. The inflorescence and individual flowers are very similar in the two, and the form of the ovule almost identical. There is the same great variability in the development of the embryo-sac, and the variations are of much the same nature, including the variable number of young embryo-sacs, the position and general structure of the embryo, multiple nuclear fusions, imperfectly marked polarity, and the variable character of egg-apparatus, antipodal cells, and endosperm nuclei.

General Conclusions.

The Araceae, although evidently all more or less intimately related, show a good deal of difference in the floral structures. The simplest type of flower is that where the flowers are unisexual, the pistillate flower having a single carpel, with a solitary basal ovule. *Spathicarpa*, *Aglaonema*, and *Nephthytis* are examples of this type. In the more specialized forms there are two or more carpels which may each develop several ovules, as in *Philodendron* and *Anthurium*. While in some of these, e. g. *Arisaema*, the basal origin of the ovules is apparent, in others, like *Anthurium*, this is not the case. The latter genus represents perhaps the most specialized type of flower, as there is a truly compound pistil and the flowers are hermaphrodite. Moreover there is a rudimentary perianth.

Engler[2] considers the type of flower found in the Pothoideae, to which *Anthurium* belongs, as the more primitive, the simpler flowers of the genera being reductions from this type. In view of the strong probability that the unisexual flowers, in general, are more primitive than hermaphrodite ones, and that in the Araceae most of those (e. g. *Aglaonema*, *Nephthytis*) which seem to have the most primitive form of embryo-sac are unisexual, it seems more likely, in the writer's opinion, that the latter are primitive forms, rather than reduced ones. We incline to the belief that the flower of *Spathicarpa*, for example, a single carpel with the solitary basal upright ovule, comes very near the primitive type from which the others have been derived.

In studying the development of the archesporium it has been shown that although there may be a single embryo-sac mother-cell, there is not infrequently a group of sporogenous cells, and that more than one embryo-sac may begin to develop. Examples of this are *Arisaema*, *Aglaonema*, and *Nephthytis*, and it is probable that the same condition will be found to

[1] Campbell, loc. cit., II. [2] Loc. cit., p. 119.

exist in other genera. While this fact may not be of much importance as indicating an exceptionally primitive position for the Araceae, it must be taken into account in trying to assign these plants to their proper position in the system.

The most striking fact brought out in this series of investigation is the extraordinary variability shown in the structures of the embryo-sac itself. So far as the writer knows no family of angiospermous plants (unless perhaps the Piperaceae) shows so great variation.

While in some species (e. g. *Dieffenbachia Seguine*, *Anthurium violaceum*) the embryo-sac conforms entirely to the usual angiospermous type, in other species (e. g. *Lysichiton*, *Aglaonema commutatum*, *Spathicarpa*, and *Nephthytis*) there are more or less notable deviations from the type. In *Spathicarpa* and *Lysichiton* these differences are secondary, consisting in a remarkable development of the antipodal cells subsequent to fertilization. This marked development of the antipodal cells is, perhaps, most nearly paralleled by that of *Sparganium*[1], where there is a similar growth and division of the antipodal cells after fertilization.

The most puzzling type is that of *Nephthytis* and *Aglaonema commutatum*, where in the same species extraordinary variation is encountered. In neither of these forms was the ordinary angiospermous type found, although approximations to this type were sometimes met with. In some cases, as we have shown, the number of nuclei in the mature embryo-sac is reduced to four, or possibly even two; definite synergidae may be wanting and antipodal cells may be entirely absent.

On the other hand, the number of nuclei may exceed the normal, perhaps in some cases being double the typical number; but it is by no means easy, in all cases, to decide whether the augmented number may not be due to a coalescence of two or more young embryo-sacs. The polarity shown by the typical embryo-sac seems to be often wanting in these forms, and although nuclear fusions may occur, these often seem to include more than two nuclei, and more than one of these fusions may take place in the same sac. In these cases it is not always easy to determine which is destined to become the egg-nucleus, and it not infrequently happens that the embryo is developed at the side, or even at the chalazal end of the embryo-sac.

How far these variations are normal it would be hard to say; but they are hardly paralleled in any other angiospermous plants. Perhaps a study of material of these species collected in their natural habitat, or of other showing the same characters, may make it possible to decide what the normal condition is. As it is they resemble more nearly than any other

[1] Campbell, The Flower and Embryo of Sparganium. Proc. Calif. Acad. Sciences, Botany I, No. 9, 1899.

plants the species of *Peperomia*, where there are usually sixteen nuclei. The multiple nuclear fusions also find a parallel in *Peperomia*.

The great difficulty in finding healthy pollen-grains, and the rarity of fertilization stages, makes it probable that some of the conditions enumerated were pathological, so that it will not be safe to lay too much stress on them. The fact that perfect seed was formed in many cases, however, and the perfectly healthy appearance of the cells, together with the complete absence of the ordinary type of embryo-sac, is a strong indication that the plants really differ normally a good deal from the usual type.

In all the species investigated the endosperm is septate from the first, and soon completely fills the embryo-sac. The basipetal growth as shown in *Anthurium* and *Spathicarpa* is probably characteristic, but there are doubtless some modifications of this. The separation of the endosperm into two marked regions (basal and apical) characterized by different cell contents is not uncommon, and the difficulty of distinguishing the basal endosperm from the antipodals is a strong argument for considering both of these as really gametophytic tissue, the antipodals being merely specialized prothallial cells.

According to Engler[1] the presence or absence of the endosperm in the ripe seed is of great importance systematically, as the natural groups show great constancy in this respect. The great development of the chalazal portion of the ovule, and relatively small embryo-sac in such forms as *Aglaonema* and *Lysichiton*, may be compared to the development of the perisperm in the Piperaceae. The suggestion has already been made by the writer[2] that the resemblances in general habit, as well as the distribution of the vascular bundles in *Peperomia* and the Araceae, might indicate a remote relationship between them; the presence in the latter of nucellar tissue, which might be compared to the perisperm of the Piperaceae, might be cited as further evidence of a possible relationship.

We may conclude, then, that the Araceae are really a primitive family of Monocotyledons, the forms with unisexual flowers being less specialized than those with hermaphrodite flowers. The not infrequent occurrence of a multicellular archesporium and the tendency to great variability in the number of the nuclei of the embryo-sac all point to such a conclusion.

The relationship of the Araceae to other low Monocotyledons has been recognized, but their nearer affinities are not very certain. The possibility of a connexion with some of the lower Dicotyledons through forms like *Peperomia* may fairly be considered worthy of consideration.

[1] Loc. cit., p. 107. [2] The Embryo-sac of *Peperomia*. Ann. of Bot., March, 1901.

EXPLANATION OF FIGURES IN PLATES XIV–XVII.

Illustrating Professor Campbell's Studies on Araceae, III.

PLATE XIV.

All figures refer to *Anthurium violaceum*, var. *leucocarpum*.

Fig. 1. Longitudinal section of a young flower, showing the two-celled ovary, with the young ovules, ♀; the perianth is not shown; ♂, one of the four stamens.

Fig. 2. A transverse section of a flower of about the same age as that shown in Fig. 1.

Fig. 3. Longitudinal section of a very young ovule. × about 250.

Fig. 4. A somewhat older ovule; *p.*, the young papillate hairs of the funiculus; *ar*, primary archesporium.

Fig. 5. An older stage of the ovule; the cell, *t.*, is probably a tapetal cell, but may possibly represent a second archesporial cell; *in*^{1}., first; *in*^{2}., second integument.

Fig. 6. Longitudinal section of an older ovule; the young embryo-sac contains two nuclei situated at its poles.

Fig. 7. The nucellus and embryo-sac from a slightly younger ovule; the single nucleus of the embryo-sac is preparing for division. Leitz, immersion, 1/16, oc. 1.

Fig. 8. A slightly older embryo-sac; the primary nucleus has just divided; *t.*, the tapetal (?) cell. Leitz, immersion, 1/16, oc. 1.

Fig. 9. Embryo-sac with two nuclei.

Fig. 10. Nucellus and embryo-sac, after the nuclear divisions in the embryo-sac are complete; five of the eight nuclei are visible in this section. × about 250.

Fig. 11. Nucellus with a second embryo-sac, *sp*^{2}., containing four free nuclei; the main sac was entirely normal; *a*, the upper part of the sac with the egg, *o*, and one synergid, *sy.*; *b*, the chalazal part of the sac, showing two of the antipodal cells, and the endosperm-nucleus. Leitz, im. 1/16, oc. 1.

Fig. 12. Two sections of the embryo-sac, shown in Fig. 10; *sy.*, the two synergidae; *pn*^{1}., *pn*^{2}., the polar nuclei; *an.*, the antipodal cells.

Fig. 13. One of the antipodal cells, and the endosperm-nucleus, from a somewhat older sac.

Fig. 14. The egg-apparatus, nearly mature.

Fig. 15. Two sections of the egg-apparatus at the time of fertilization; *pt.*, pollen-tube.

Fig. 16. Lower part of the embryo-sac about the time of fertilization; the antipodals are quite inconspicuous, and the large endosperm-nucleus is nearly ready to divide.

Fig. 17. Two sections through the egg-apparatus at the time of fertilization; lying close to the egg, *o*, was one synergid, separated from the second one, *sy*^{2}., by the pollen-tube, *pt.* The second synergid was much shrunken. Within the pollen-tube was a deeply staining body, *t. n.*, probably the tube nucleus, while outside were two small nuclei, ♂^{1}, ♂^{2}, presumably the generative nuclei. Leitz, im. 1/16, oc. 1.

Fig. 18. The first division of the endosperm-nucleus. Leitz, im. 1/16, oc. 1.

Fig. 19. Base of the embryo-sac after the completion of the first endosperm-division. The antipodal cells, *an.*, are still recognizable.

Fig. 20. An older embryo-sac. × about 250. Each of the two primary endosperm-cells has divided into two; the embryo, *em.*, is still undivided.

PLATE XV.

All figures refer to *Anthurium violaceum*, var. *leucocarpum*.

Fig. 21. Embryo-sac with eight endosperm-cells; *en.*, two of the four basal cells; the embryo is still undivided.

Fig. 22. Section of an older ovule; the embryo is two-celled; *in*^{1}., *in*^{2}., the ovular integuments. × about 100.

Fig. 23. Upper part of an older embryo-sac; the embryo is four-celled; *nu.*, the cap of cells formed from the apex of the nucellus.

Figs. 24–26. Longitudinal sections of somewhat older embryos. Leitz, im. 1/16, oc. 1.

Fig. 27. Upper part of embryo-sac and older embryo. × about 250.

Fig. 28. Median transverse section of embryo. Leitz, im. 1/16, oc. 1.

Fig. 29. Two longitudinal sections of an older embryo. × about 100. *st.*, stem-apex; *cot.*, cotyledon; *rc.*, root-cap.

Fig. 30. Nearly median section through the base of the same embryo. × about 250.

Fig. 31. Median section of an older embryo.

Figs. 32, 33. Longitudinal sections of nearly full-grown embryos. × about 45.

Fig. 34. Stem-apex and second leaf of the embryo shown in Fig. 32. × about 250.

Fig. 35. Median section through the root-apex of the full-grown embryo. × 250. *pl.*, plerome-cylinder of the root.

Fig. 36. Transverse section of full-grown embryo.

Fig. 37. Cells from the embryo, containing raphides, *r.*

PLATE XVI.

All figures refer to *Nephthytis Liberica.*

Fig. 38. Longitudinal section of young ovule. × about 100.

Fig. 39. Nucellus of the same ovule, more highly magnified, showing two archesporial cells.

Fig. 40. Nucellus from an ovule of about the same age; several archesporial cells are present, but the exact number is doubtful.

Fig. 41. Two sections from a probably abnormal ovule, with a large number of archesporial cells.

Fig. 42. Two sections from an ovule showing unusual development of the sporogenous tissue. Nuclear divisions have taken place within the cells, and it is not possible to say how many primary archesporial cells are represented. There were twenty nuclei in all.

Fig. 43. Three sections of an older ovule; there is apparently a single embryo-sac divided by partitions into three chambers; a second smaller embryo-sac with two nuclei is also present, sp^2.

Fig. 44. Embryo-sac with a single very large apical nucleus, and three antipodals (?) at the base.

Fig. 45. Embryo-sac with two nuclei separated by a membrane. About the nuclei were numerous starch granules; a second embryo-sac with a single nucleus was present.

Fig. 46. Four sections of a large embryo-sac approximating the ordinary angiospermous type; *a*, shows two synergids (?), and *b*, the egg-cell; two antipodal cells were present, and three free nuclei (polar nuclei?), one of which, *c*, was in an advanced prophase of division.

Fig. 47. Embryo-sac with large fusion-nucleus in the upper part.

Fig. 48. Section of ovule with embryo-sac containing but two nuclei.

Fig. 49. Upper part of sac shown in Fig. 48; the nucleus is preparing for division.

Fig. 50. Lower nucleus from the same sac in process of division; about fifty-six chromosomes could be counted.

Fig. 51. Two sections of an embryo-sac containing a two-celled embryo, *em.* The sac was divided into two cells; no traces of antipodal cells could be found. × about 100.

PLATE XVII.

All figures refer to *Nephthytis Liberica.*

Fig. 52. *a*, upper part, *b*, base of an embryo-sac with a normal egg-apparatus, but only a single nucleus at the base.

Fig. 53. Embryo-sac with a single cell (egg?) at the apex, and a large mass of cells, about fifteen in number, at the antipodal end. Two free nuclei, *e.n.*, one in process of division, were also present.

Fig. 54. Section of ovule and embryo-sac, the details are shown in Fig. 55.

Fig. 55. *a–d*, four sections of the apex, *e*, antipodal region of the sac shown in 54; there were five nuclei in the antipodal region.

Fig. 56. Two fusing nuclei from a sac of about the same age as that shown in 55.

Fig. 57. Upper part of the nucellus showing a secondary embryo-sac, *sp*^{2}., with five nuclei, of which only one shows in this section; the apex of the principal sac contains a large fusion nucleus, apparently made up of three.

Figs. 58, 59. Chalazal end of the same embryo-sac; in 58 is shown the group of antipodal (?) cells, five in number; in 59 the egg-apparatus (?), consisting of three cells; there were two free nuclei in the sac.

Figs. 60, 61. Two sections through the apex of an embryo-sac, with the one-celled embryo preparing for the first division; there were about fourteen nuclei in the embryo-sac, but it was not certain that each one belonged to a distinct endosperm-cell.

Figs. 62–64. Three sections through the apex of a sac, apparently just fertilized; there were two large cells, *o*, *sy*., which probably represent an egg-apparatus with a single synergid; the nucleus, *n*., lay close to the egg, but it may have been derived from one of the cells of the integument, as it had the appearance of an ordinary vegetative nucleus; the two irregular bodies, *s*, *s*', perhaps were the generative nuclei, but this is not certain; two large free nuclei (Fig. 62), one preparing for division, completed the contents of the sac. × about 250.

Fig. 65. Embryo-sac, containing a four-celled embryo.

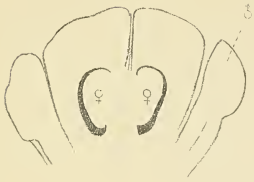
Fig. 66. Embryo-sac with an older embryo. × 50.

Fig. 67. Upper part of a sac and embryo of about the same age. × 250 (about).

Fig. 68. Antipodal region of the same sac.

Fig. 69. Embryo-sac and embryo. × 100 (about). The basal endosperm-cells have denser contents and larger nuclei.

Fig. 70. Upper part of embryo-sac, with large pollen-tube (?) containing two nuclei. A second similar tube was present. Leitz, im. 1/16, oc. 1.



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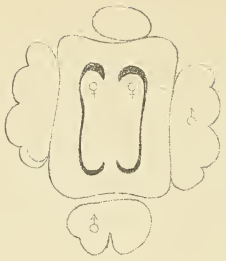

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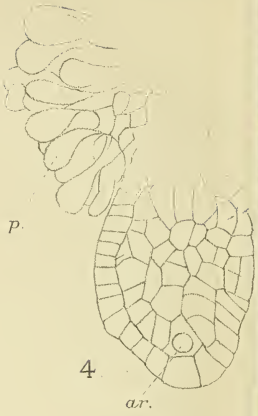

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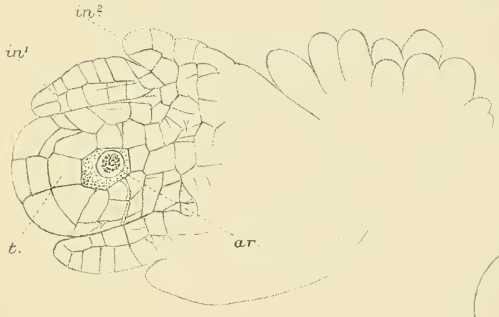

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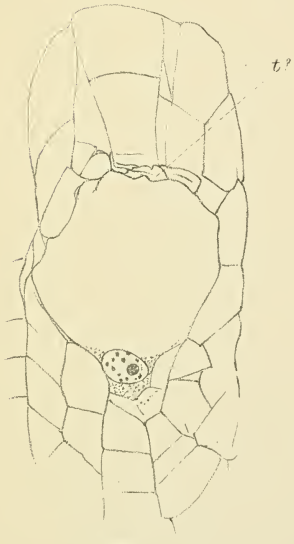

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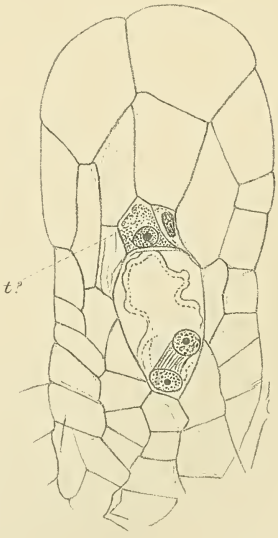

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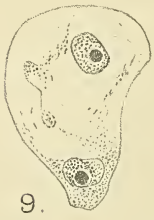

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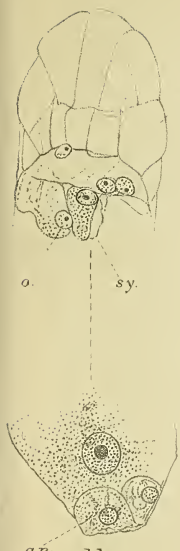

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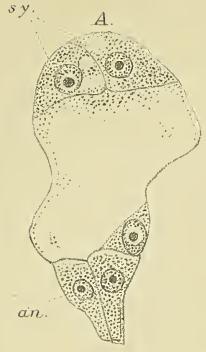

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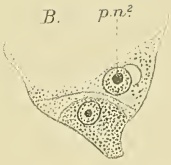

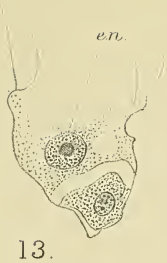

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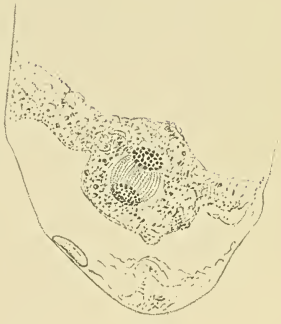

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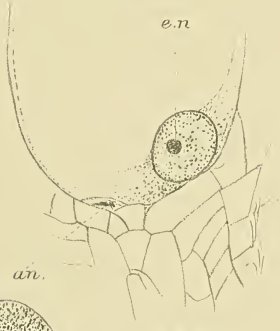

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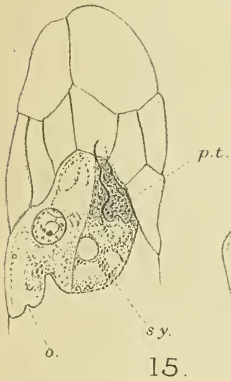

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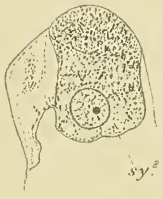

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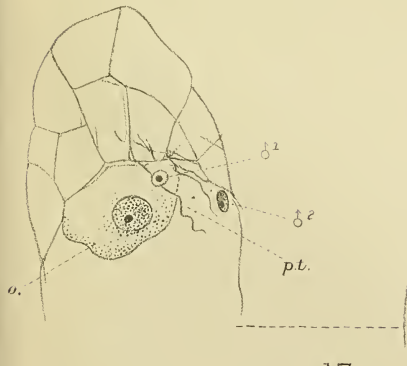

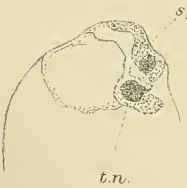

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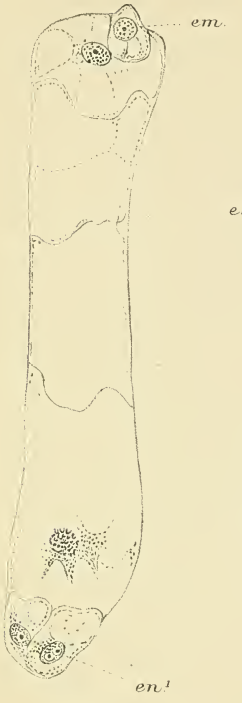

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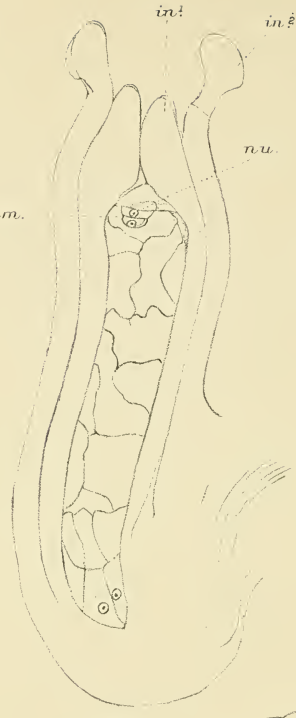

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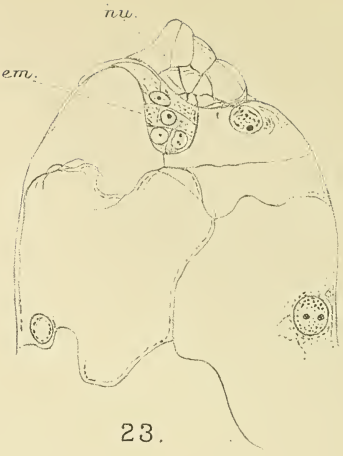

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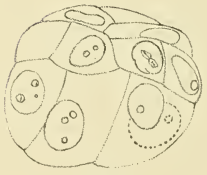

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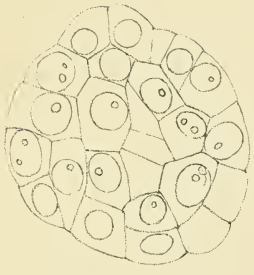

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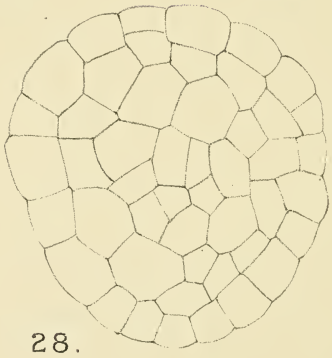

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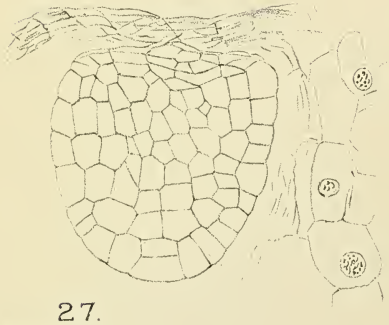

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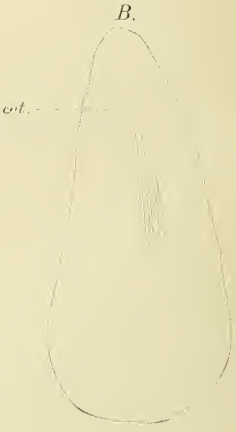

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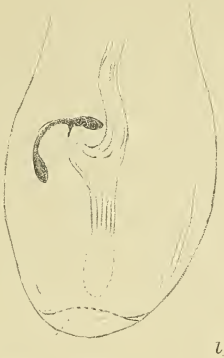

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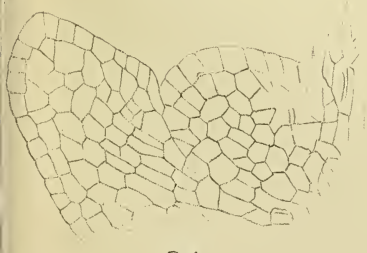

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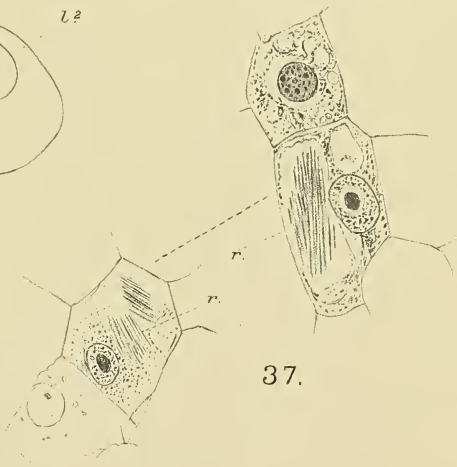

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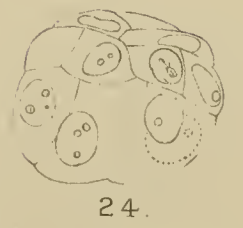

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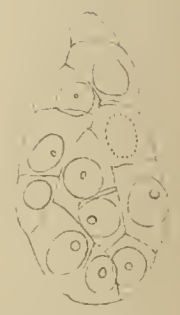

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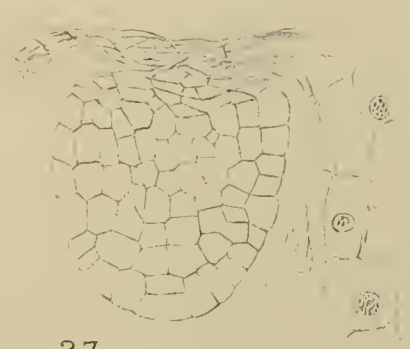

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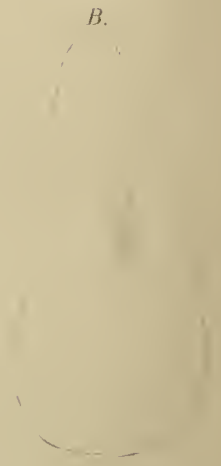

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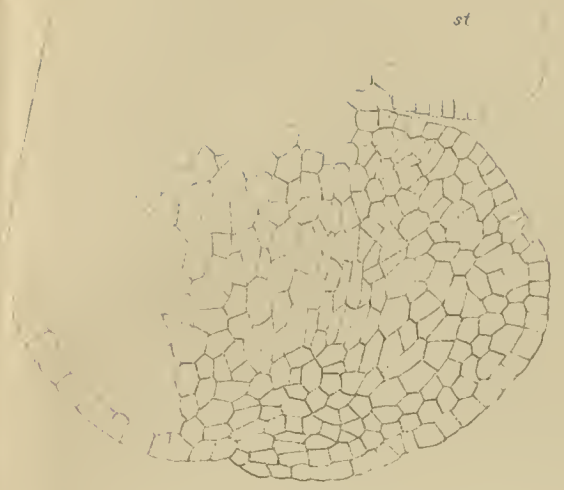

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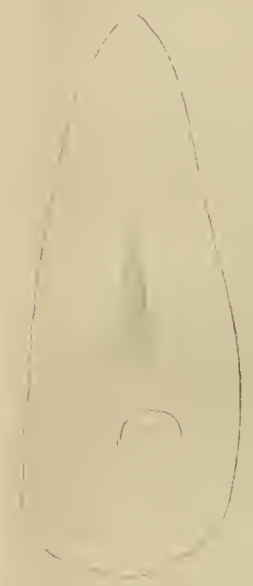

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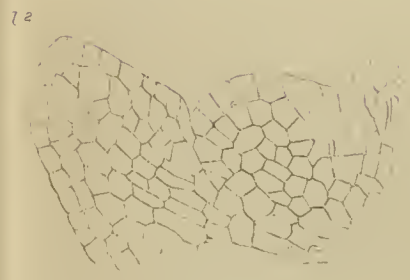

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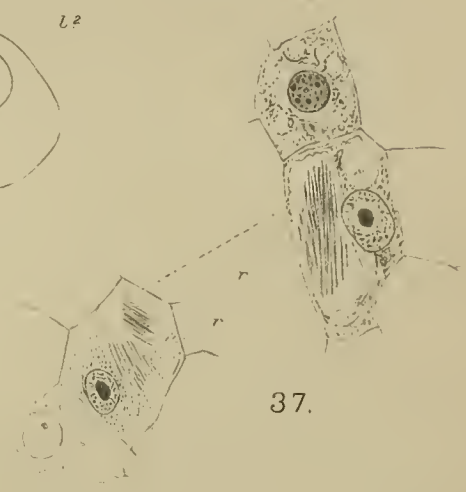

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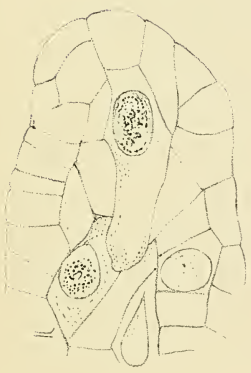

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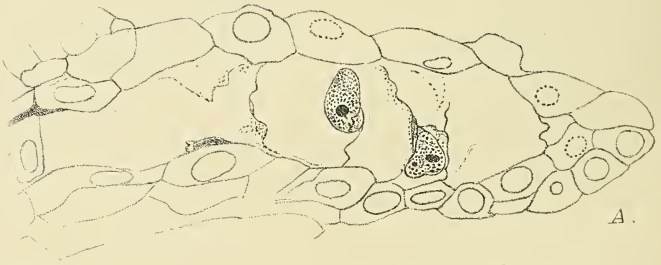

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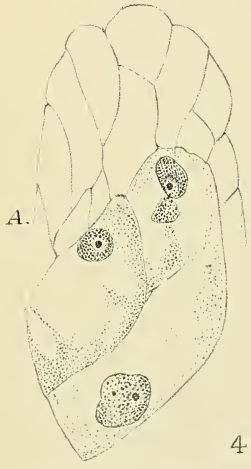

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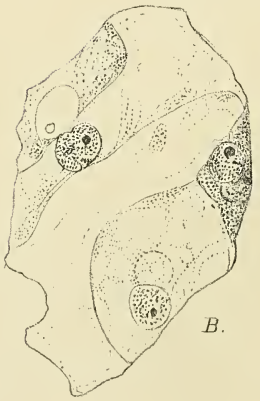

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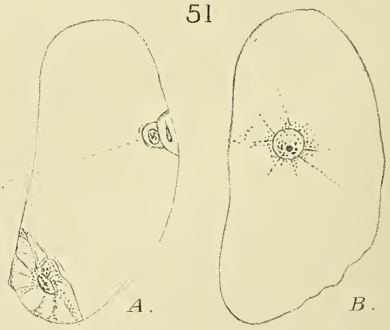
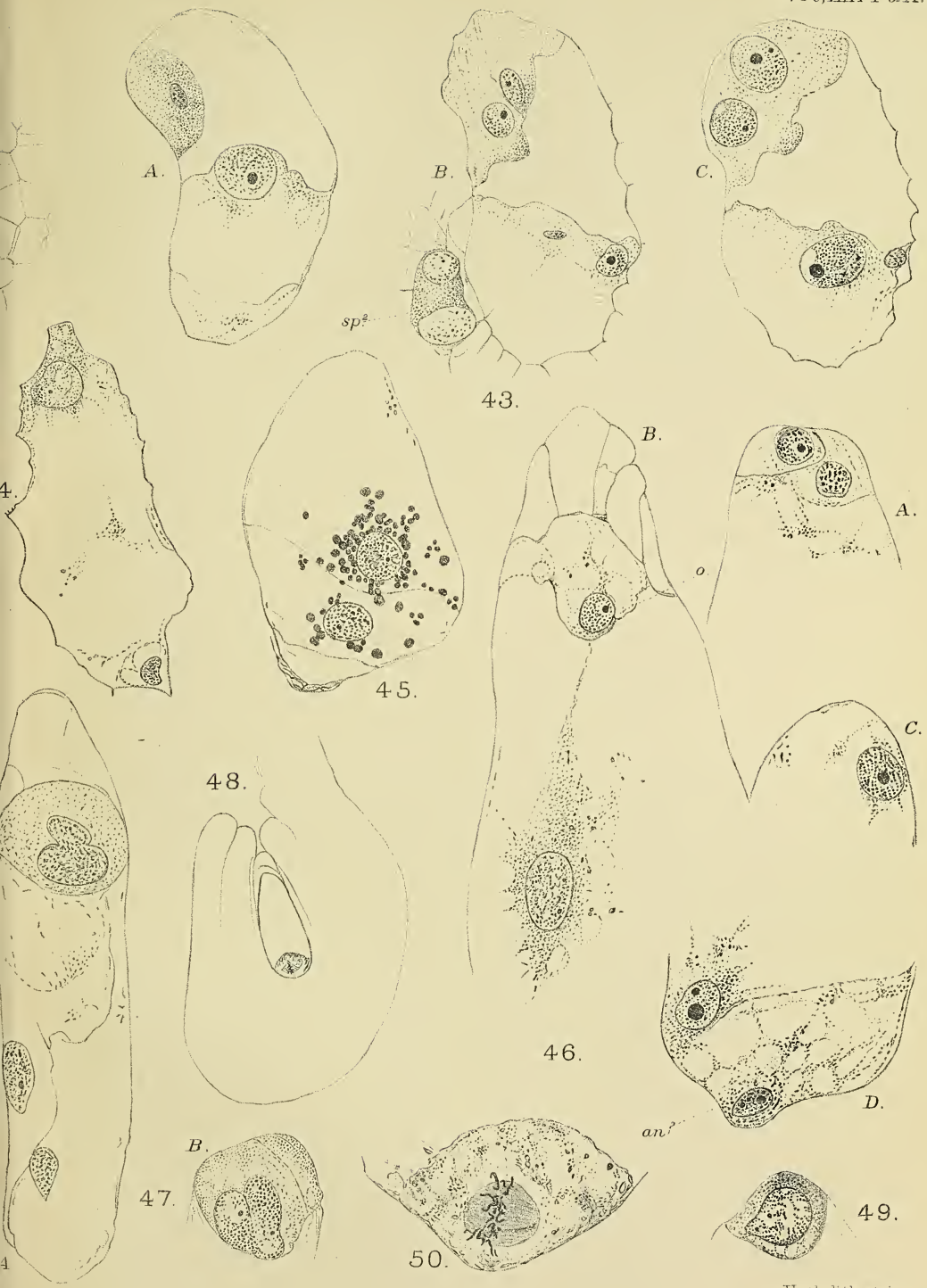


D.H.Campbell, del.

CAMPBELL—ARACEÆ.



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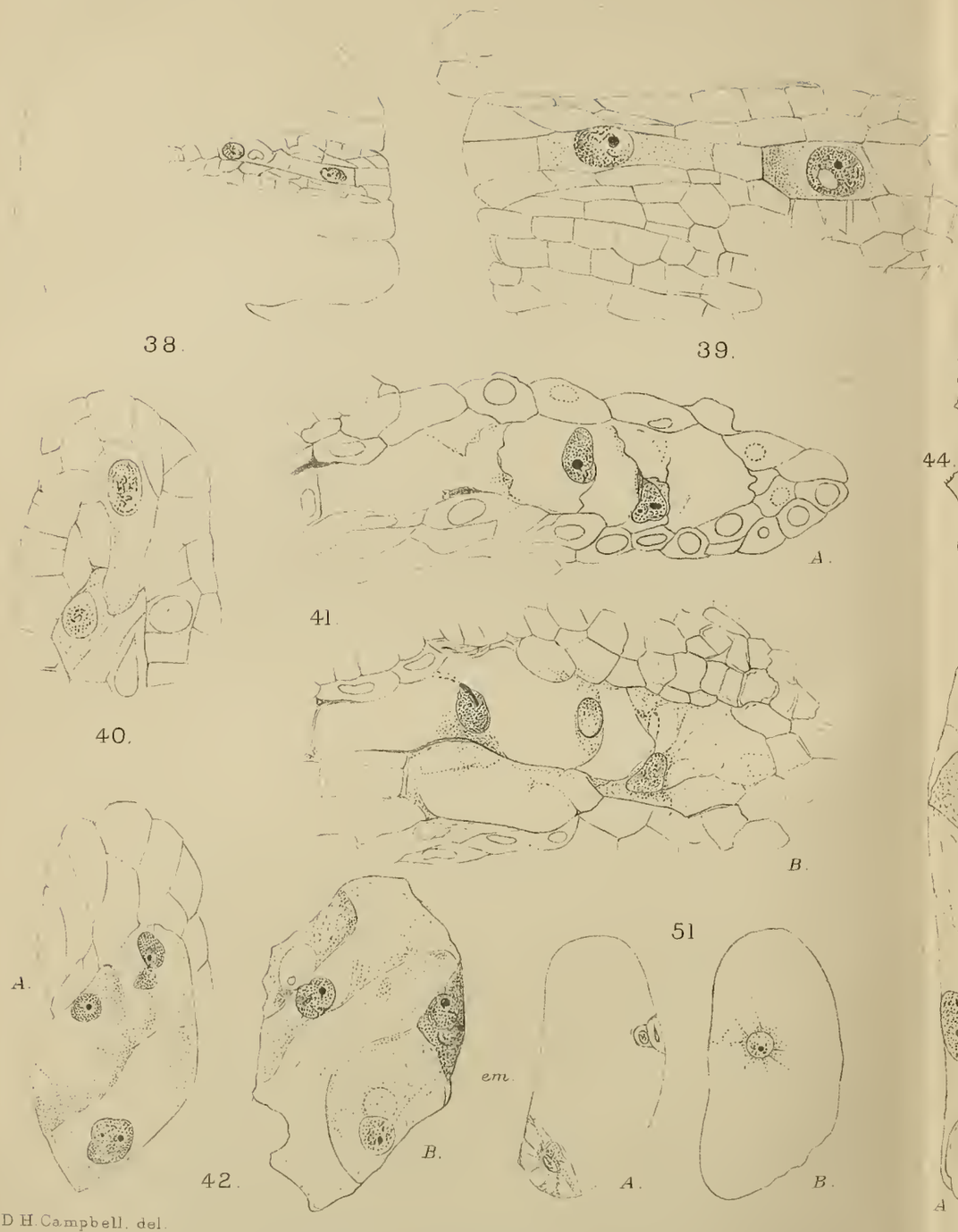

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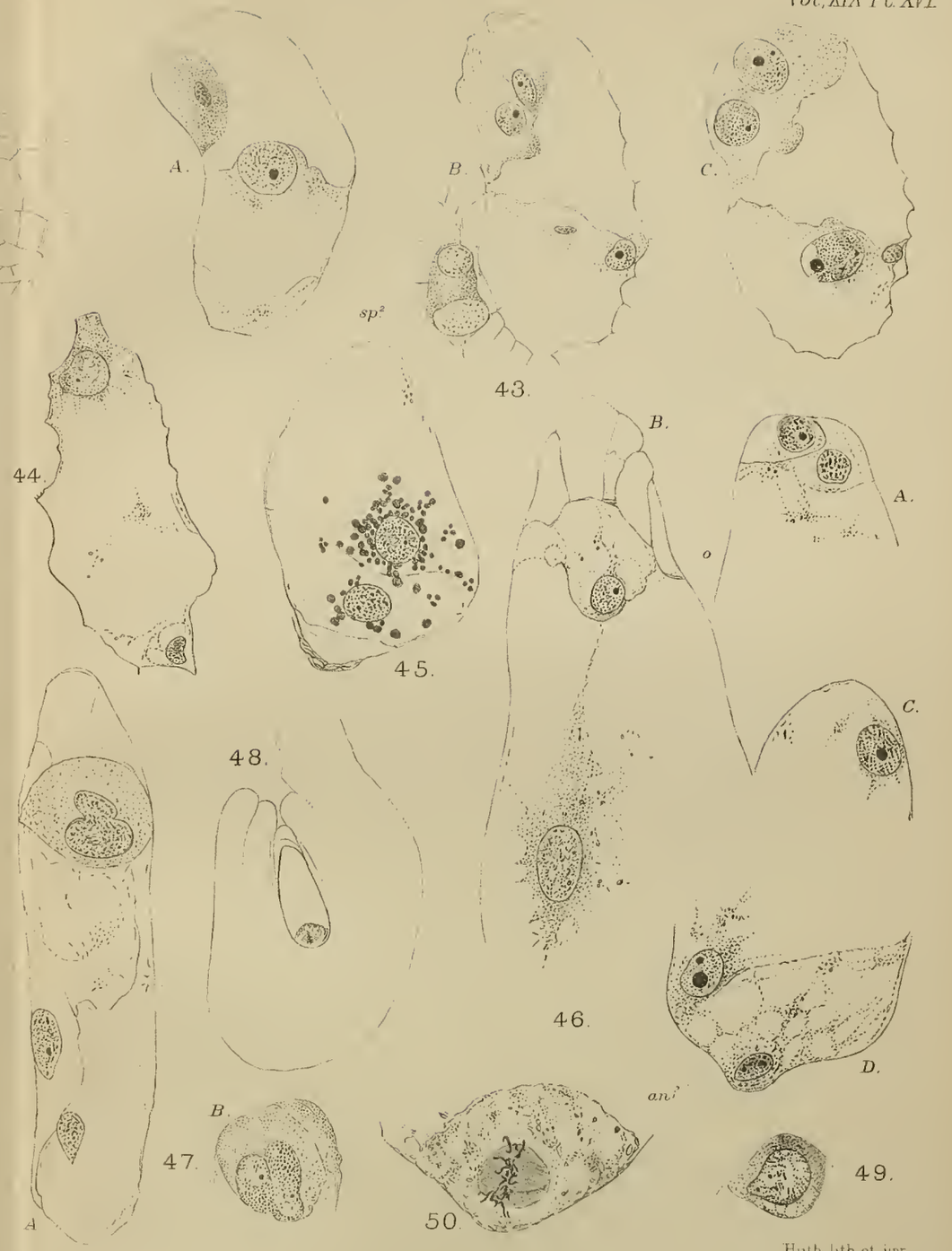

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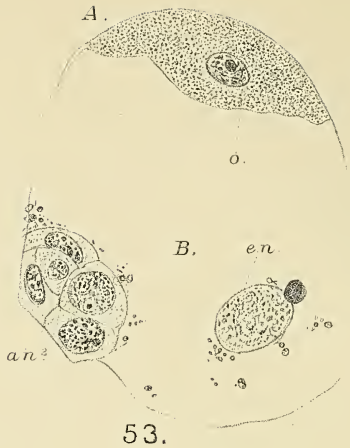

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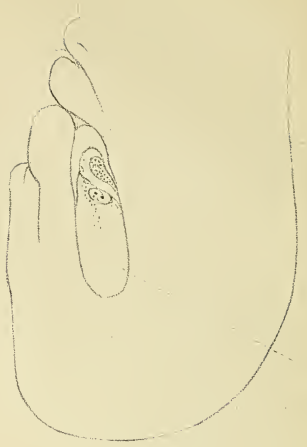

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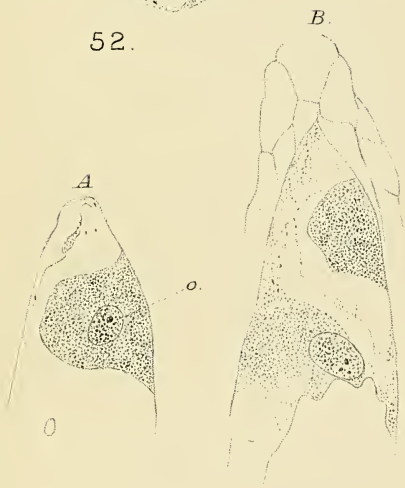

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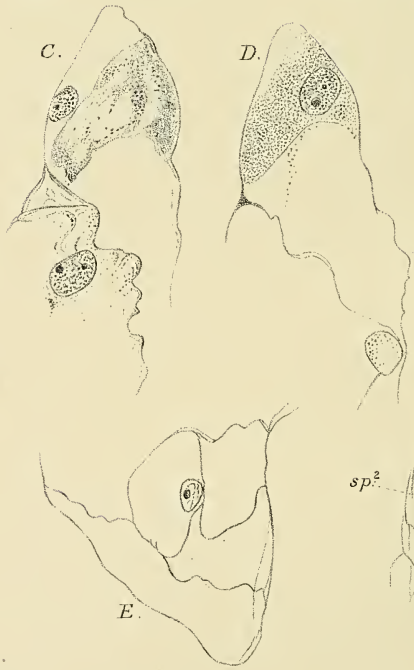

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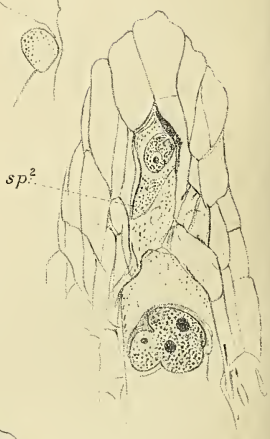

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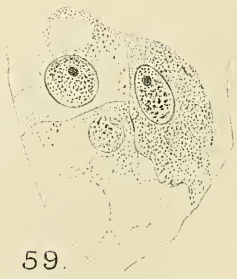

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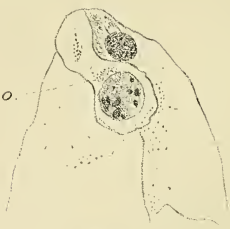

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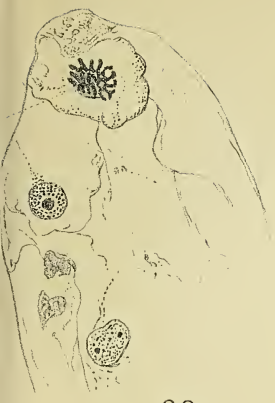
CAMPBELL—ARACEÆ.



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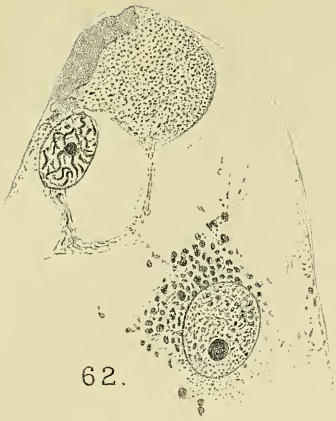

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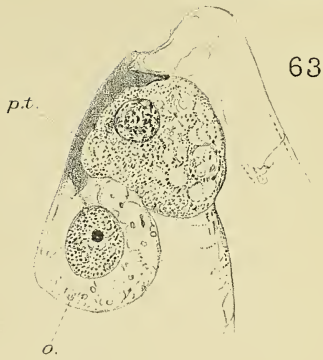

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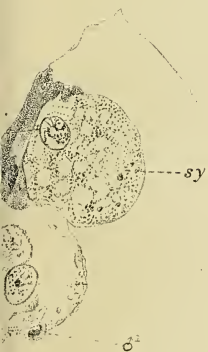

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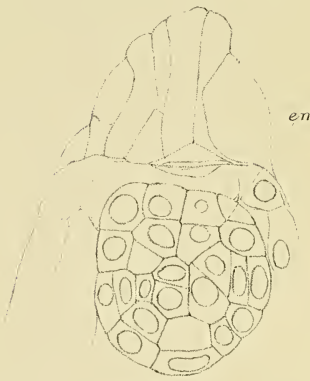

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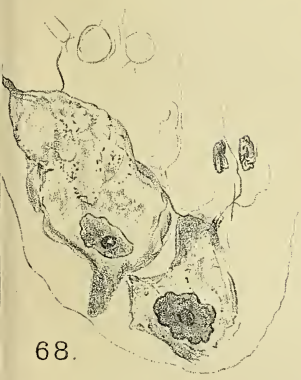

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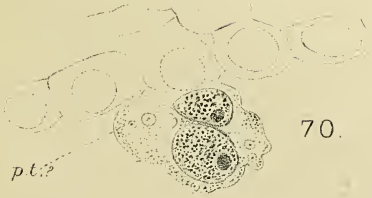

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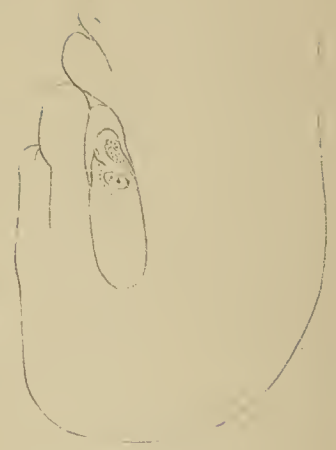

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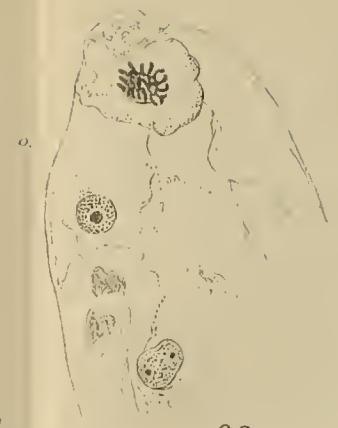

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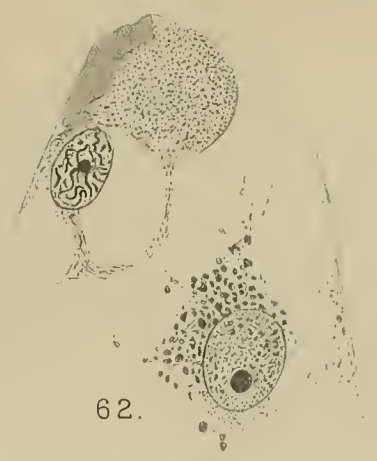

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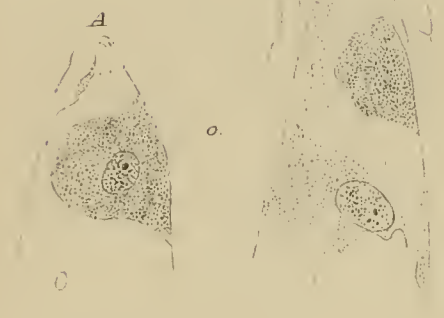

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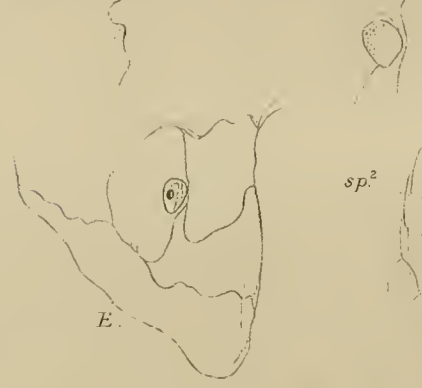

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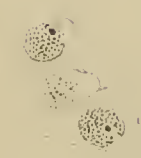

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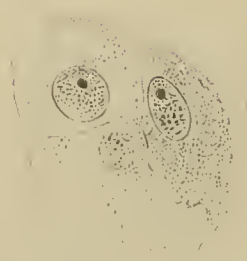

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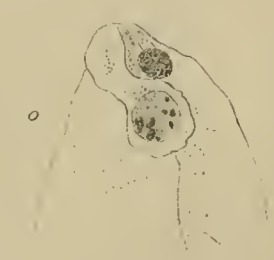

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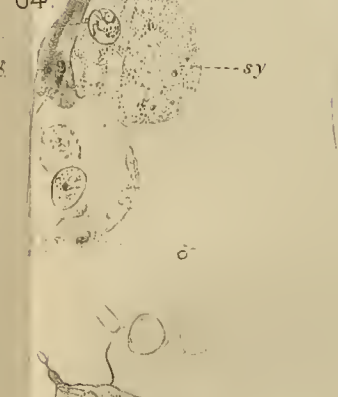

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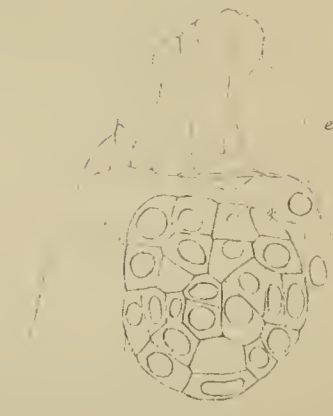

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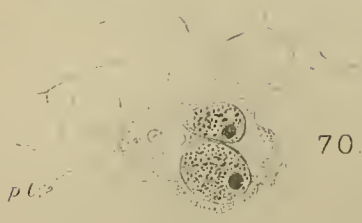

70.

D.H Campbell. del.

CAMPBELL—ARACEÆ.

Huth. Lith. et imp.

On the Dispersal of Seeds by Wind.

BY

HENRY N. RIDLEY, F.L.S.,

Director of the Botanic Gardens, Singapore.

'THE work of the wind as a means of seed-dispersal and of spore-dispersal is one of the most important subjects in Geographical Botany. We cannot say that a conclusive opinion has yet been arrived at regarding it.' Thus writes Schimper in 'Plant Geography' (English Edition). 'A. de Candolle and Kerner estimate the efficiency at a very low figure in the case of seed plants.' This important subject having been always of some interest to me, I would make some suggestions and record some observations I have had opportunities of making from time to time in the tropics, as a contribution to the study of the question; and this the more readily as I have observed that most of the observations on the subject recorded have been made in cold climates, where the country is more open and less afforested than it is on the equator.

Seeds or fruits adopted for dispersal by wind may be put into three groups: (1) Winged fruits or seeds, such as those of most of the Dipterocarpeae and Bignoniaceae; (2) Plumed fruits or seed, e.g. Compositae and Apocynaceae; (3) Powder-seed, fine and dustlike, as in Orchideae or Fern spores.

Wing-seed.

In the Malay Peninsula we have a considerable number of trees of great size and also of lofty climbers which have winged seeds or fruit, and which are disseminated from the parent tree by the aid of these wings. To what distance do these seeds or fruits travel, and what is the rate of progress in this way of the species over a given area?

I will first deal with the wing-fruited Dipterocarpeae, of which a number of species occur in the jungle in the Singapore Botanic Gardens and are fruiting at the present time (September). The weather is squally, with often violent storms of wind and rain of short duration. Prolonged violent wind is very rare in this country, but small cyclonic storms occasionally occur. Typhoons such as are characteristic of the Chinese sea are quite

[Annals of Botany, Vol. XIX. No. LXXV. July, 1905.]

unknown. The wind usually persists in one direction, according to the time of year; but at present, though from the south-east, it veers round occasionally to the west.

Shorea leprosula, Miq., is a very common tree in most parts of the peninsula, occupying a very large area and occurring also in Sumatra and Borneo. It attains a great height, 100 to 150 feet, with a straight tall stem and a big head of foliage. It usually fruits about once in five years, and then produces a vast amount of fruit. As in most of these trees, a very large proportion of the fruit is barren, and this falls first. Then after a time the ripe fruit falls. The fruit is about half an inch long, with three equal wings (modified calyx-lobes) $2\frac{3}{4}$ inches long, half an inch broad, linear, oblong, blunt; when the fruit falls it rotates rapidly, descending with the ovary downwards.

It appears to have no other modification for dispersal, and is apparently not disseminated in any way by animals; but the fruits of these Dipterocarpeae are often eaten apparently by rats, in which case the fallen fruits attacked by them are quite destroyed on the spot.

A big tree in the Botanic Gardens, 100 feet tall, stands in an open spot, but about 20 yards away on one side is the jungle, through which wide paths run, so that the plant has exceptional advantages for dispersing its seeds.

I surmise that the tree is about 100 years old, perhaps more. These Dipterocarpeae are of slow growth, and the Shoreas at least do not fruit till they are fully thirty years old, when they are about 30 feet tall. Fruits from small trees do not fly so far as those from large trees, as the surrounding foliage of the forests being much taller prevents their receiving the full force of the wind. I have been in high forests in a gale, when, though the wind was tearing through the upper branches of the trees 100 feet high and upwards, and throwing down large boughs, there was no wind below, where I was standing.

The furthest limit I have found the fruit from this *Shorea* was a single one 98 yards from the tree-base, on a grass plot. The greatest number fell within 20 yards of the tree, and continued abundant up to 40 yards. I saw none beyond this distance in the jungle; on paths and grass they naturally get drifted a little further after alighting, pushed along by wind, or the gardeners' brushing, or some such accident not possible in the forest. The distance may therefore be for purposes of estimation calculated at 100 yards at the outside, though practically it must be rare for them to go 50 yards.

The furthest young tree descended from this big *Shorea leprosula* is 40 yards away from the parent tree, and though many thousands of seeds must have been blown into the neighbouring jungle, there are only about a dozen young plants in the neighbourhood. Fruits dropped from my

verandah, 17 feet high, where there was no wind, fell vertically for 5 or 6 feet before beginning to rotate, and lit within 6 feet of the place where they would have fallen had they had no wings.

If we assume that a tree flowers and fruits at 30 years of age and the fruits are disseminated to a distance of 100 yards, that the furthest fruits always germinate and so continue in one direction, it will be seen that under such most favourable circumstances the species can only spread 300 yards in 100 years, and would take 58,666 years to migrate 100 miles. This seems an extraordinarily slow rate of migration, but I believe I have much overrated the rapidity. Occasional increases might occur, such as the falling of a seed on a log drifting down a stream, or into the stream itself; but there are very few or no streams in the hilly slopes of the peninsula which are strong enough to carry the seed. Again, excessively violent storms of wind might carry them further than usual, but such storms are nowadays at least very rare. I have seen these Dipterocarpeae in fruit during as violent wind storms as we ever have, and even then the fruits did not fly 100 yards. Allowing for all these possibilities, it will be seen that in these calculations I have estimated the flight at 100 yards, whereas few fruit have been found more than 40 yards from the tree, nor have young trees of any age been found at a much greater distance. Further, in the progress of the species it has been assumed that the very furthest fruit in one direction have developed into trees, whereas it usually happens that bad seed drifts further than the heavier fertile seed, and it is also assumed that the wind always blows violently in the same direction when the tree is in fruit, which is not always the case; again, in the exceptional cases in which these fruits were found at upwards of 100 yards from the tree, the tree was very much taller than the surrounding vegetation, and had a clearer line of flight than it would have had in the primaeval forest, where many trees would be on the same level, and their foliage would check the flight from the adjacent trees; and again, trees of thirty years of age being comparatively short would get much less of the wind than the taller century-old trees, and their fruit would certainly take a shorter flight.

The Malay Peninsula consists to a large extent of hilly country, the hills rising to a height of 7,000 feet, but *Dipterocarpeae* are not met with much above 2,500 feet altitude. The hills, and indeed the whole country where these plants occur, are densely wooded, and it might be suggested that the fruits would fly to greater distances down the hill slopes in heavy gales. This might be so; but it must be remembered that in its migrations a species would have to fly uphill quite as often as it flew down, and its progress uphill would be proportionately slower. Taking all these facts into consideration, it would not seem that I have under-estimated the time taken by a Dipterocarpous tree to migrate a hundred miles, but that on

the contrary it is probably on an average nearly twice as long. If this is so it follows that the family must be of great antiquity, as must also be the forest-region which most of the species now occupy.

As it is impossible that the fruits of these trees can cross the sea in good enough condition to germinate, even if they ever reached the sea or could grow on the sea-shore when stranded, it follows that the whole area occupied by them must have been connected by dry land when they migrated, whether from Cambodia to the Philippines, or Borneo or Sumatra; but at what period this was we cannot even surmise.

Shorea macroptera, Dyer. A tree about 70 feet tall grows in a wood near the house. It is evidently an old tree, as it is partly hollow. It is a smaller species than *S. leprosula*, and fruits rather earlier; a tree planted in 1884 is now fruiting, though only about 30 feet tall. The furthest fallen fruit I can find is just 40 yards from the tree, and the only seedlings not further than 20 yards. This tree well overtops the rest of the surrounding wood, and is in a well-exposed position, so that it gets the full force of the wind.

The wood has grown up on ground evidently cleared at some period, with only a few of the original forest trees left, including the *Shorea*. The whole of the trees, as far as I can see, are those whose seed is regularly distributed by birds, bats and civet cats, together with stray fruit trees, planted, or seedlings from fruit grown or eaten near the house.

Another tree about 30 feet tall in jungle had the greater part of its fruit lying just beneath it, and nearly all destroyed by mice; but I found a few seed about 30 yards away from it in the jungle.

Shorea gratissima, Dyer. A tall tree, about 80 feet high, growing in a wooded part of the gardens, fruits tolerably regularly more or less each year, but often dropping a year or two. The most distant fruits were found about 16 yards from the base of the tree. These fruits are five-winged, but three wings are larger than the other two. It flies less well than *Shorea leprosula*.

Shorea rigida, Brandis. A very large tree in the jungle, with trees as tall surrounding it. The fruits fall about 20 yards at the furthest, and young trees are to be seen about the same distance from the parent. Trees planted in another part of the gardens about twenty years ago are still quite small.

Anisoptera costata, Miq. Tree over 60 feet tall in jungle. It fruits more or less every year, but only in quantity once in five years. It flowered this year, but I could only find one fruit. The fruit is large, with broad wings and heavy; surrounded by jungle the greater part of the fruits fall within 6 yards of the trunk, and the furthest young trees I can find are within 15 yards of the parent tree.

The Dipterocarpeae, at least almost all the species with winged fruits, inhabit only dense forests far from streams by which the migration of the

fruit might be hurried on; *D. oblongifolius* is the only exception I know. It lives on the banks of streams, and the fruits drift down, and I have even seen them in the sea, but dead. Yet several species range from the mouth of the peninsula to Banka and Borneo. *Dryobalanops aromatica*, Gaertn., occurs in three localities in the peninsula only, so far as is known, and is confined to small areas in those localities. They are at Rawang in Selangor, on the west of the peninsula; Endau river, Johor, and Kwantan in Pahang in the east of the peninsula; and it is also abundant in Borneo and the Philippines; so that to reach Rawang from Kwantan (about 110 miles, going in a straight line) under the most favourable circumstances it would take 58,300 years, and from Borneo (300 miles further) 266,710 years. This seems almost incredible, but it is improbable that the tree moved even as fast as this. *Dipterocarpus grandifolius*, Blanco, ranges from the peninsula to the Philippines, and if we assume that at the time of its migration the Philippines were connected by land with the peninsula, the shortest way the plant could go would occupy 1½ million years. The fruit of this tree, however, is much heavier than that of *Shorea*, and it is improbable that it would drift with the wind as far as 100 yards. The whole area of the wing-fruited Dipterocarpeae extends from Burmah to the Philippine Islands. The most widely distributed seem to be—

Dryobalanops aromatica, Gaertn. Rawang, Malay Peninsula, to the Philippines.

Anisoptera glabra, Kurz. Yoma, Cambodia, and Cochin China to Singapore.

Anisoptera costata, Korth. Singapore to South-east Borneo.

A. Curtisii, King. Penang to North-west Borneo.

Dipterocarpus Griffithii, Miq. Mergui, Andamans, to Pahang.

D. Hasseltii, Bl. Malacca to the Philippines.

D. pilosus, Roxb. Assam to Sumatra and Banka.

D. grandiflorus, Blanco. Penang, Banka, Philippines.

Hopea Pierrei, Hance. Cambodia, Singapore, and Borneo.

Shorea furfuracea, Miq. Malay Peninsula, Sumatra, Banka, Philippines.

S. gratissima, Dyer. Tenasserim to Singapore.

S. leprosula, Miq. Perak to Singapore, Sumatra, and Borneo.

The short distance to which these winged fruits or seeds drift is not confined to Dipterocarpeae, as will be illustrated by observations on plants of other orders.

Terminalia subspathulata, King (Combretaceae), is a lofty tree as much as 100 feet tall. It is endemic and rather local. The fruit is flat, thin, oblong rounded, one inch long and two inches across, with a single seed in the centre.

When detached by the wind it drifts along, rotating round its long axis and sometimes rising a little if the gust is strong. I found its limit of

flight in a strong breeze was 38 to 46 yards. These furthest flights were along a path, and on an open grass plot, where they may have drifted a little after reaching the ground, which could not happen and does not when they fall in jungle. Dropped from the verandah with *Shorea leprosula* fruit, they went a little further than that, and took longer to reach the ground. They flew most irregularly, sometimes in one direction, sometimes in another, when there was no wind.

Cumpassia Malaccensis (Leguminosae). Also a very lofty tree, often much over 100 feet. The thin one-seeded pod flies more slowly and rather further than those of *Shorea*. In an open space I found it reached 61 yards with a good wind. It is common all over the peninsula, and occurs in Borneo.

Sterculia scaphigera (Sterculiaceae). A very large tree, fruiting at 60 feet and upwards. The fruit consists of from one to five papery, green, boat-shaped carpels, 6 to 8 inches long, with a single seed at the base. The boat has a gibbous bend towards the base, at which point it is broadest. The boats when ripe become detached singly and drift along, the gibbous portion causing them to rotate briskly as they drift. In thick jungle I found the fruits 50 yards from the main tree, none further.

Dyera costulata, Hook. fil. (Apocynaceae). This gigantic tree has a fruit consisting of a pair of wooden follicles which split along the upper edge, and release a large number of very thin, winged seeds about an inch across and elliptic. These seeds in a strong gale flew 40 yards only, though the tree is over 100 feet tall, and stands away from the forest on an open grass plot, so that it is fully exposed to the whole force of the wind. *Dyera costulata* inhabits the forests all over the peninsula, and occurs in Borneo also, and attains a height of over 200 feet.

Albizzia Moluccana, Bl. (Leguminosae). This is a large tree, 60 to 80 feet tall, introduced as a shade tree into the Malay Peninsula. It has thin, several-seeded pods; when ripe they split, and one half carrying the seeds drifts away in the wind. As it is commonly planted in open ground the half pods drift along the ground very fairly far. It is very prolific, and grows with the greatest rapidity, but it does not move so fast if it is surrounded by dense forest, as I have seen it in long-abandoned estates on Gunong Pantai in Johor.

Spatholobus ferrugineus, Benth. (Leguminosae). A climber, often gigantic, very common in woods and also in the denser forests, where it climbs to a height of 80 or 90 feet or more. It only flowers when it reaches the light, and produces very large numbers of its thin-winged pods. A big plant, climbing to the top of the *Terminalia subspathulata* above mentioned and commonly fruiting there, shed its fruits to a distance of 60 feet. There is, however, a plant of this species in the jungle, 188 yards away from the big plant, which may be derived from

it. The fruits are very light, and seem to drift much further than those of the Dipterocarpeae and *Terminalia*.

Ventilago leiocarpa, Benth. A climber to about 50 or 60 feet on forest trees; has small linear-oblong fruits about 2 inches long and a quarter of an inch wide, with a seed at one end. The fruits are very light and thin, and fly about 40 yards, seldom further.

The following are the plants with winged fruits known to me from the Malay Peninsula:—

I. TREES:

Trigoniastrum. Small tree.

Sterculia, S. scaphigera, S. campanulata. Not very common. Distribution, Cambodia.

Tarrietia. Very large trees, fruits rather heavy.

Pentace. Several species.

Pteleocarpa. Medium tree, local. Fruits like those of *Terminalia subspathulata*. Edges of forests.

Dodonaea. Shrub; open sandy places and sea-shores; widely spread.

Melannorhoea. Fruits with wings, widely spreading; common in dense forests. Those with no or abortive wings rare.

Parishia. Fruits winged like a *Shorea*; local. Big tree.

Dipterocarpeae. All but *Retinodendron*, *Vatica* (most), and *Balanocarpus*.

Kumpassia. Gigantic tree, common and widely spread.

Peltophorum. Medium-sized tree, abundant, open country only, never in forests.

Albizzia. Big tree, open country, introduced.

Terminalia subspathulata, King.

Homalium. Scarce, rarely fruiting.

Englehardtia. Tall tree, scarce.

II. CLIMBERS:

Securidaca. Very rare.

Ancistrocladus. Common, fruits like *Shorea*, abundant in open sandy places by the sea. Low scandent shrub.

Hiptage. Open country, usually rather a low climber.

Aspidopterys. Fruit as in *Terminalia subspathulata*; woods and river-banks.

Cardiopteris. River-banks, rare.

Ventilago. Climber, forests.

Gouania. Low climber, open country.

Spatholobus, *Kunstleria*, *Derris*, *Dalbergia* (several species).

Mezoneurum. All edges of woods, open country, rarely in dense forest.

Combretums, *Calycopteris* and some *Illigera*, *Sphenodesma*. Open wood edges, and thickets.

Linostoma. Usually edges of forest.

Winged-fruit Plants in Insular Floras.

Plants whose seeds or fruits are dispersed by the aid of wings are very rare in oceanic islands, about 2 per cent. of the species; and of these in some cases I am doubtful as to whether the plants reached the islands by the aid of the seed-wings. Notoriously absent are the Dipterocarpeae, wing-seeded Apocynaceae, and Bignoniaceae.

In Christmas Island I found only one, viz. *Gyrocarpus*, a sea-shore tree with fruit resembling that of a *Dipterocarpus*, and *Berrya* with smaller winged fruit, but both may possibly have been sea-drifted. In Fernando de Noronha were none, except possibly a *Bignonia*, the seeds of which were perhaps winged, but were not seen. There are none in Cocos Island nor the Admiralty Islands. *Casuarina equisetifolia*, which possesses thin winged seeds, is certainly sea-borne, as its distribution is that of other sea-borne species, and it is exclusively a sea-sand plant, springing up along the edge of the sea-beaches. It occurs on many of the islands of the archipelago.

Of capsular plants with winged seeds, which drift from the split capsules when ripe, there are a number of trees in the Malay Peninsula and a few climbers. They include *Cratoxylon*, *Gordonia*, *Archytea*, *Pterospermum*, *Ixonanthes*, *Triomma*, *Oroxylon*, *Dolichandrone*, *Duabanga*, *Norrisia*, and *Dyera*; and the climbers *Alsomitra*, *Zanonia*, *Coptosapelta*, *Uncaria*, *Wightia*, *Dioscorea*, *Stemona*, *Nepenthes*, and *Aristolochia*. The trees are usually small ones, except *Dyera*, and none are lofty plants like Dipterocarpeae. The climbers, too, are not usually large. The seeds being usually small, it is difficult to find them when fallen, so as to estimate the distance to which they can fly; but I judge from the appearance of seedlings near the trees that they fly but a few yards. *Dyera* has been described already. *Uncaria* seeds are so fine that they fly like dust-seeds when the pods open, and might be drifted in the wind to a very long distance. I do not know of any plant with winged seeds on oceanic islands.

Plumed Seeds and Fruits.

Fruits and seeds provided with silky plumes fly a good deal further than winged fruit, both in forest and still more so in open country. The flight of this class of fruit or seed is better known from observations in temperate countries, where the ground is more open and the fruit has a clear space to drift along; consequently these are more abundant than they are in a forest region, and indeed are much more easy to observe. In the Malay Peninsula we have very few indigenous terrestrial herbs with plumed seeds or fruits. The only ones inhabiting forests which I know of are *Gynura sarmentosa*, which is usually a low climber, and *Blumea spectabilis*. The former inhabits swampy spots in dense woods, but does often occur on the edges and on banks; the latter, a tall herb, on banks and

open spaces in woods. In cultivated land are a number of introduced weeds, Compositae chiefly, which have plumed fruit; but many of these migrate more rapidly by attaching themselves to clothing (e. g. *Ageratum conyzoides*) than by the action of the wind. This is very marked where new country is being opened up by paths through forests, where these adhesive plumed Composites appear in a new clearing connected by a path with an old one long before the non-adhesive ones do. Indeed, of the Composite weeds common in this region the greater number are plumeless.

On sandy or muddy shores, where the country is treeless or nearly so, we have *Pluchea Indica*, and also *Spinifex*, the heads of which are rolled along the sandy shores. The Lalang grass *Imperata cylindrica* has plumed seeds which become detached by the wind and drift away; but the plant is only two or three feet tall usually, and I found in a fairly strong wind only flew on the level for about 16 yards. From hill-tops and such places it might fly further, but it seems never to migrate over a forest belt of about 30 yards thickness. If a clearing is made in forest Lalang does not appear unless through a wide open path, or when it is carried by man. Quite a narrow band of thick wood will often stop its movements completely.

Of jungle trees with plumed seed or fruit we have very few, and these are usually more abundant in open country and edges of woods: *Vernonia arborea*, a fairly tall tree, abundant in open cleared land but not at all a forest tree, *Vallaris*, and two or three *Alstonias*. The seeds of *Alstonia scolaris*, a very tall tree, I have seen drifting at the distance of a hundred yards and more from the parent tree. They perhaps drift much further even in forest, but it is very difficult to follow them in dense woods.

Clematis and *Naravelia* have plumed fruit. They are climbers on the edges of woods generally, and are not common. Of the Apocynaceae, besides the *Alstonia* and *Vallaris* mentioned above, there are a number of plume-seeded genera and species, of which a few inhabit the forests, climbing to the tops of the trees; such are *Urceola* and *Parameria*. The shrubs *Strophanthus* and small climbers *Parsonsia*, *Wrightia*, &c., only occur in open country or edges of woods.

Parameria polyneura is a big forest climber ascending to 60 or 70 feet on the trees. It fruits heavily. The seeds drift in a good wind for 60 to 100 yards, slowly descending. If they fall on open ground such as a path or grass they drift along it still further. From a climber on a tree 60 feet tall in the Botanic Gardens, I find the furthest seed dropping at forty yards in a fair wind and drifting along the path for another 60 yards. Plants occur in the jungle at a distance of about 108 yards from this big plant, and are doubtless derived from it. As these plants fruit when comparatively young, and frequently occur along the edges of woods and such spots where the fallen seeds can drift along more open

ground, these plants doubtless have a more rapid dispersal than the slow-growing wing-fruited trees.

The most abundant Apocynaceae, however, are those with fleshy fruit, dispersed by birds and mammals, or the sea-borne *Cerbera*.

The Asclepiadeae have all plumed seed except *Sarcolobus*, a tidal river plant, the seed of which is adapted for dissemination by water. They are not very abundant in the peninsula, and fall into two classes—the slender climbers, like *Tylophora*, which occur on the edges of the rivers and over bushes by the sea, &c., and are absent from high jungle and mountains, and the epiphytic species, some *Hoyas* and *Dischidia*. Some of the latter are almost confined to the upper branches of lofty trees; others like *D. Rafflesiana* generally occur in low trees about 30 feet high in the open country, especially near the sea, and again on mountain-tops, but not between. *D. coccinea* occurs on very lofty tree-tops in the low country, and on smaller trees on mountain-tops. I believe the seeds of *D. Rafflesiana* fly to a considerable distance, but it is difficult to be certain. I have seen young plants about 60 yards from a tree full of old plants, but I do not by any means think this is their limit.

The only epiphytic Gesneraceae, *Aeschynanthus* and *Agalmyla*, have small plumed seed. They are much more widely distributed than the terrestrial species, the plumeless seeds of which are distributed by rain-water.

Plumed Seeds and Fruits in Insular Floras.

These are much rarer than one would naturally expect. One could easily conceive of their being carried high up into the air by a gale and drifted away to a distant island, but as a matter of fact plants with this mode of dissemination form only about 1 per cent. of the vegetation. In Christmas Island is one *Hoya*, and *Blumea spectabilis*; in Fernando de Noronha one *Gomphocarpus*; in the Admiralty Islands one *Hoya*. In Juan Fernandez, out of 118 plants there are one *Bromelia* and twenty Compositae which may bear plumed seed; but of these fourteen are endemic species, of two genera, and may of course be all descendants of two species, very early inhabitants of the island; but I have no works here which state whether these fruits are plumed or no.

Powder-Seed.

The very fine powder-like seed of many flowering plants and spores of Ferns, Lycopodiums, and the cellular Cryptogams are well adapted for wind-dispersal, and are often very widely disseminated. It is, however, impossible to follow them in their flight, so that one has to judge of the distance they can go by the appearance of young plants at a distance from

the parent, and from their occurrence in remote islands. Among the flowering plants we have the Orchids, Apostasia, and several species of *Neuwiedia* and *Balanophora*, all of which have very light seed which can be drifted away by the wind.

The distance of the flight of Orchid seeds is probably, occasionally at least, very considerable, but I have but little direct information on this. Some epiphytic species were put in a *Ficus Benjamina* in a very open position—*Cymbidium*, a new species, and *C. Finlaysonianum*, and an *Agrostophyllum*. They fruited, and seeds of *C. Finlaysonianum* flew southwards and grew on *Arenga* trees at a distance of 30 yards, *Agrostophyllum* 90 yards; the other *Cymbidium* 60 yards, northwards. *Dendrobium pandaneti*, which I planted on a Sago palm at one end of the garden, was found on some other Sagos nearly a mile away; but I am not quite certain that the plants descended from the ones I first brought to the garden. The plant only grows on stems of *Sagus*, *Pandanus*, and *Cocos*. One curious thing about these epiphytic Orchids is, that the seed flying from a tree does not usually rest on the side of the tree facing the original plant, but on the further side, as if round the tree there was an eddy of wind which carried it there. I have seen the same thing in *Psilotum complanatum*, the spores of which must have flown from a plant in the plant-house, upwards and south-westwards, and lit on the trunk of a Date-palm 100 yards away. Very few of the numerous Orchids cultivated in the Botanic Gardens have, however, reproduced themselves on the garden trees.

Orchids are not at all common as a rule in small islands. There were none in Fernando de Noronha. In Christmas Island, ten species are recorded; three are endemic, two species of Phreatia, a genus Indo-Malayan and Pacific, and a *Saccolabium*; of the others, *Sarcochilus carinatifolia* occurs also on Pulau Aur, a small island off the east coast of the Malay Peninsula. *Dendrobium crumenatum* ranges very widely over the Malay archipelago, and I have seen quite fresh, green-looking plants of it floating in the sea on the way to Christmas Island. The other *Dendrobium* is allied to a Javanese species. I have also found there a Javanese species of *Thelasis*. Of terrestrial Orchids there is a Corymbis allied to, though distinct from, *C. veratrifolia*, a plant of which hardly distinct forms occur from Africa to Java; a *Zeuxine* and *Didymoplexis pallens*, a saprophytic plant ranging from the Himalayas to Java. This plant has a rather special modification for wind-dispersal, after fertilization. It is self-fertilized and the pedicels of the fruit elongate so as to be actually longer than the stem, and elevate the capsule well above the rotten leaves and the low ferns among which it grows. *Carysanthes*, also a widely distributed Orchid, has the same modification.

As a rule terrestrial Orchids are more widely distributed than epiphytic ones, and saprophytic species are remarkably widely distributed. Con-

sidering how lightly the wind blows in the dense forest at the level at which these plants fruit, it is curious to what a distance they seem to drift. They must often float upwards above the foliage of the lofty trees beneath which they grow. One noticeable fact is that while all terrestrial Orchids have their ripe capsules erect, those of epiphytic species are always pendulous, so that the seed of the former have a tendency to rise in the wind and the latter to descend. Cryptogams, especially epiphytic ones, are certainly very widely and quickly disseminated by their spores. Dr. Treub's well-known paper on the Flora of Krakatau illustrates this. He shows there that the first plants to reach an island after the whole flora has been destroyed are Algae, and these are followed by ferns, and later by phanerogams.

The same phenomenon is seen in the case of epiphytes, as well as on bare rocks, or barren clay banks, or any such places where at first there is no vegetation.

On trees in the Botanic Gardens, Singapore, I note that the first epiphytes to appear are Algae, which settle wherever the trickling of rain-water makes it possible for them to grow. On quite smooth-barked trees such as Betel-nuts, *Areca*, or *Cocos*, nothing further except lichens usually appears, unless there happens to be a notch in the bark, or a small portion of the base of a leaf-stalk remains, which forms a nidus for spores of mosses. The next plants which appear when the moss has grown into a small patch are usually ferns, and later Orchids; occasionally Orchids come before ferns. On smooth cylindric trunks the first lichens that appear are those of thin texture, e.g. *Graphidea*, *Opegrapha*, &c.; later occasionally Parmelias and Collemas develop, and from these thicker lichens start mosses or hepatics.

The same order of appearance takes place on brickwork and bare clay banks, except that Orchids rarely appear, and often mosses do not come; phanerogams, however, always come later than the Algae. On trees after the Algae, mosses, ferns and Orchids (if any are in the neighbourhood); plants whose seeds are disseminated by birds come next.

Powder-seed Plants in Oceanic Islands.

The proportion of powder-seed plants in oceanic islands is usually very large, even if we exclude the cellular plants; but oceanic islands are apt to be dry in the early stages of their afforestation, so that many of this group cannot thrive. Fernando de Noronha possessed no Orchid, only one fern, and very few mosses or hepatics. These plants, however, were not very abundant on the adjacent mainland, which was somewhat dry, and the island itself was in most parts unsuited for the growth of ferns and mosses.

Coral atolls, like Cocos Island, are also deficient, but with other islands it is different.

In Krakatau, after the eruption in 1886, Dr. Treub found that out of 26 plants 11 belonged to this group; Penzig, revisiting it in 1896, found 62 plants, of which 16 are powder-seed plants. In Christmas Islands out of 170 I found 33. South Trinidad out of 11 has 4; the Admiralty Islands out of 118 have 41; Kerguelen out of 27 has 6 ferns and lycopodiums, but it is also accredited with 154 mosses and 26 hepatics; St. Paul and Amsterdam Islands have 19 powder-seed plants (exclusive of cellular cryptogams) out of 33 plants.

These facts are, I think, sufficient to settle to a considerable extent the part played by the wind in seed-dispersal; of the three classes of fruit and seed modified for wind-dispersal, that of *winged* seed and fruit is the slowest. The species migrate very slowly and are, usually at least, unable to cross any large tract of sea by this means alone. *Plumed* seeds and fruits, though easily and probably quickly disseminated over open country, for which they are most suited, are liable to be stopped in their migrations by dense forests. They can, at least occasionally, cross successfully large areas of sea.

Powder-seed, on the other hand, has the most rapid transit probably of any form of seed, and is most widely diffused.

HENRY N. RIDLEY.

On the Arrangement of the Vascular Strands in the 'Seedlings' of Certain Leptosporangiate Ferns[1].

BY

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With Plates XVIII, XIX, and XX.

BEFORE the publication of Jeffrey's[2] account of the development of the vascular system of *Pteris aquilina*, very little attention had been paid to the ontogeny of the 'polystelic' type of vascular anatomy. Gerard[3], Van Tieghem[4], and Leclerc du Sablon[5] had described the very earliest transitional stages of several ferns, but in the majority of cases the descriptions were of the shortest character, and merely concerned with the anatomical changes involved in the transition of the diarch root-structure to the solid protostele occurring at the base of the young stem. These researches, although in themselves of considerable interest, threw very little light upon the processes involved in the vascular elaboration, and it was not until Jeffrey published his account of *Pteris* that definite and accurate information upon this important point was available.

Since the appearance of Jeffrey's paper, much work has been done along exactly similar lines for other ferns, both by that author himself, and also by Boodle, Gwynne-Vaughan, Farmer and Hill, and others. As a result, botanists at the present time have a good general idea of the development of a typical dictyostelic vascular organization, though the interpretation of the facts observed varies to no small degree.

In spite, however, of the considerable amount of work which has been done in this direction, *definite* information with regard to *particular species*

[1] Accepted by the Senate of the University of London as a Thesis for the Degree of Doctor of Science.

[2] Morphology of the Central Cylinder of Angiosperms. Trans. Canad. Inst., 6. 1900.

[3] Recherches sur le passage de la racine à la tige. Ann. Sc. Nat., 6e sér., t. xi.

[4] Sur la Polystélie. Ann. Sc. Nat., 7e sér., t. iii.

[5] Recherches sur la formation de la tige des Fougères. Ann. Sc. Nat., 7e sér., t. xi.

[Annals of Botany, Vol. XIX. No. LXXV. July, 1905.]

is surprisingly scanty, and there can be no doubt that it is just such knowledge which is required at the present time, if the different views which still exist upon the 'stelar' question are to be successfully harmonized.

It was in the hope of making a contribution—necessarily a small one—to such a knowledge that the present work was undertaken. Nearly twenty ferns have been examined, mostly belonging to Polypodiaceae, a family which will doubtless prove of considerable interest in connexion with vascular questions, not only on account of its numerical richness in species, but also on account of the great variety of forms occurring within its boundaries.

The majority of the plants examined were obtained, through the kindness of Prof. Farmer, from Messrs. James Hill and Son, of Edmonton, who afforded the writer every facility for gathering plants of the different ages required.

The methods employed in the investigation were generally of a very simple character. Whenever possible, the plants were embedded in paraffin and serial sections obtained in the usual way. In some cases, however, owing to the presence of sclerenchyma, the celloidin method was resorted to, and occasionally, when even this was impossible, the ferns were cut by hand.

The sections were always double-stained with saffranin and haematoxylene. The well-known method of lightly staining with haematoxylene, and subsequently 'blueing' with ammonia, proved of great value for the differentiation of sieve-tubes, especially in very young plants.

Doodia aspera, R. Br[1].

The primary root of this plant is of the diarch type so general among the ferns. In the transitional region the xylem plate, by the development of tracheides upon its sides, becomes oval and finally more or less circular in section, and, at the same time, the phloem is gradually differentiated as a continuous sheath surrounding the solid xylem rod (Fig. 197). At a slightly higher level, two or three parenchyma cells appear in the centre of the xylem (Fig. 198), quickly followed by one or two well-marked sieve-tubes (Fig. 199). The early appearance of internal sieve-tubes is a point of some interest, since it indicates that almost from its very commencement the primary pith is, in reality, phloem; in other words, it is vascular, as opposed to non-vascular tissue. This point, however, will be referred to later.

Just before the first leaf-trace is given off, the sieve-tubes increase to about four or five in number, taking up a central position in the internal

[1] The nomenclature adopted throughout is that of Hooker's 'Synopsis Filicum,' except when otherwise stated.

phloem, so that at this level we have a relatively broad band of xylem surrounded externally by phloem, and containing at its centre a core of phloem in which the sieve-tubes appear as a central strand surrounded by two or three layers of phloem parenchyma. The pericycle consists of a single layer of cells whose radial walls are not in regular seriation with those of the somewhat poorly differentiated endodermis. The fundamental tissue is composed of parenchyma containing a considerable amount of starch, and, in young plants at any rate, is characterized by a complete absence of the sclerenchyma which is so striking a feature in the seedlings of some other ferns.

The first leaf-gap is formed in a very simple manner, a sector of the vascular strand becoming detached and passing outwards as the leaf-trace. On the completion of the trace, the external and internal phloem become continuous round the horns of the xylem arc, by the differentiation of sieve-tubes at these regions. The endodermis merely bridges the leaf-gap, there being no indication of any 'dipping in' of this layer (Fig. 200). It is somewhat difficult to decide whether the internal phloem 'takes any part in' the formation of the leaf-trace, but the evidence points to such being the case; expressed more correctly, the leaf-strand is concentric even at its junction with the cauline system, the adaxial moiety of its phloem being continuous with the internal phloem strand, and the abaxial portion with the external phloem sheath of the vascular arc. During the separation of the leaf-trace, a root-strand joins the cauline system at a point almost exactly opposite the leaf, its junction taking place in a perfectly simple way. Very soon after the completion of the leaf-trace, the gap is closed in the well-known manner by the gradual extension of the horns of the gap towards one another, the result being the appearance of a ring of xylem surrounded by phloem, and enclosing a central strand of sieve-tubes and parenchyma (Fig. 201). It should be noticed that there is no internal endodermis or phloeoterma. The sieve-tubes still form a solid core in the internal phloem, but this condition of things is not long maintained, for very soon preparations are made for the exit of the second leaf-trace, and at this point a few parenchyma cells appear in the core of sieve-tubes. It is still noticeable, however, that the latter are by far the most numerous and conspicuous elements of the intraxylary tissue.

In the majority of cases, the second leaf-trace is formed in exactly the same way as the first, although individual variations are frequent. The corresponding root-strand then joins the vascular system. The third and fourth leaf- and root-traces are formed in a perfectly normal manner, but in connexion with the fourth leaf-gap we have, for the first time, a differentiation of a blunt projection of ground-tissue between the edges of the gap, the ground-parenchyma being separated from the vascular tissue by a continuation of the normal endodermis. In other words, the endodermis

'dips into' the leaf-gap. As we pass upwards, the ground-parenchyma encroaches more and more upon the vascular tissue, so that the internal phloem loses its strand-like character, and appears as a layer of sieve-tubes and parenchyma lining the internal concavity of the xylem, the horns of which gradually approach one another as the gap is closed (Figs. 1–3). While these changes are taking place, and before the closing of the gap, the xylem, at a point almost exactly opposite the latter, breaks into two, sieve-tubes and parenchyma appearing between the two portions; the continuity of the endodermis is not affected. As in many similar cases, to be described below (an especially good example occurring in *Lomaria gibba*), this new break in the xylem is in reality the first preparation for the exit of the next leaf-trace, for very soon after its appearance a concentric strand of vascular tissue is nipped off from *each* of the cauline arcs, the changes involved in the process being indicated in Figs. 3–5. The two concentric strands, each of which is surrounded by its own endodermis, pass outwards as the leaf-trace, which is thus double at its junction with the cauline system. Long before the strands enter the petiole, however, union has taken place, so that the leaf-stalk itself possesses a single concentric vascular strand.

In most other cases of ferns possessing multiple leaf-traces it has been found that the latter do not appear suddenly, as in the case just described, but only after the few preceding leaf-traces have shown a very complete series of transitions between the primary single leaf-trace and the more complex system supplying the later leaves. The only hint at such a transitional series occurring in *Doodia aspera* was the occasional slightly bilobed character of the xylem of the previous leaf-strand.

As soon as the exit of the double leaf-trace is complete, the former gap closes and a root enters. As will be seen from Figs. 6 and 7, the cauline system at this region of the stem consists of but a single curved strand. A consideration, however, of the vascular system as a whole up to this level will establish its essentially tubular (siphonostelic) character. This is rendered more obvious by subsequent changes, for very quickly the single strand divides into two parts, one much larger than the other (Figs. 8 and 9). From each a small concentric strand is nipped off, the two forming the next double leaf-trace, which, as before, is single before it reaches the petiole, though in this case the two xylem patches are separated by phloem for a short distance at the base of the leaf-stalk. The larger of the two cauline strands then divides into two (as in Fig. 12), and the next leaf-trace is formed in exactly the same way as its predecessors.

It is unnecessary to follow the further development of the vascular system; its final siphonostelic condition has been reached, and subsequent changes are merely an elaboration of the stage just described.

Nephrodium spinulosum v. dilatatum, Hk[1].

The series of changes occurring in the transitional region of this plant resemble those of *Doodia aspera*, and need be described in no great detail.

The primary root is diarch, and somewhat quickly passes into the protostelic condition. A few parenchyma cells, followed later by one or two well-marked sieve-tubes, appear at the centre of the xylem strand, their appearance being succeeded by the differentiation of the first leaf-trace, which takes place in the simple manner described above for the previous fern. The gap remains open through a considerable distance, and only closes just before the exit of the second leaf-trace. The phloem is continuous round the edges of the gap, and, on the closing of the latter, the cauline system consists of an amphiphloic vascular rod. The pericycle consists of relatively large squarish cells; it is somewhat variable in thickness, not only at any one particular level, but also at different parts of the plant, a double layer of cells often persisting through several consecutive sections. The endodermis is narrow, but very conspicuous.

The first three or four leaf-traces are differentiated in the same simple manner, and involve the formation of no real leaf-gap, the break in the vascular system being merely bridged by the endodermis. After the exit of about the fourth leaf-trace, however, the gaps become more pronounced, and at the level of the sixth leaf the xylem has assumed the shape of a curved band, into the bay of which the endodermis 'dips' sharply. The phloem, especially that portion of it lining the concavity of the xylem, is particularly well differentiated.

At a slightly higher level, the seventh leaf-trace leaves the cauline system as the central portion of the curved vascular band, with the result that, after the exit of the trace, the stem is supplied with two concentric vascular strands, each surrounded by a separate endodermis.

The xylem of the last-mentioned leaf-trace has a distinctly bilobed character—an indication of the double or multiple nature of the later-formed traces.

After the closing of the seventh leaf-gap, the vascular arc again breaks into two portions, and the next leaf-strand is double at its origin, though single before it enters the leaf-stalk. By the splitting of one of the vascular strands before the previous gap has been repaired, we have the appearance of three concentric strands in the cauline system at this particular level, and later elaborations of an exactly similar type quickly result in the adult dictyostelic structure being reached.

[1] Hooker, Species Filicum, iv, p. 127.

LOMARIA GIBBA, Labill.

The diarch xylem plate of the primary root very quickly becomes oval and finally circular in section, sheathed externally by a complete layer of phloem derived from the tangential extension of the two original phloem strands. A characteristic feature of the xylem is the presence of a considerable amount of parenchyma among the tracheides, and even in the earliest stages small parenchyma cells (persisting, however, through only two or three sections) appear scattered in various parts of the small xylem strand. The pericycle consists of a single layer of small cells crowded with contents, and is succeeded by a very definite endodermis. As is so often the case, the stem bends somewhat sharply at the transitional region, and the changes occurring at this level are frequently difficult to follow. Oblique sections, however, clearly show that a few parenchyma cells with densely staining contents are differentiated at the centre of the xylem, the parenchymatous core often being temporarily connected with the external phloem by irregular radial extensions.

On the stem resuming the vertical position, two or three well-marked sieve-tubes appear in the central parenchyma, and immediately the first leaf-trace passes out, its structure and mode of exit being essentially similar to that described for *Doodia aspera*. The resulting gap, however, was generally found to differ from the corresponding gap of nearly all other ferns examined, in being of relatively large size and remaining open through some considerable distance. The usual state of affairs is the formation of a small, quickly closed gap.

The gap is repaired in the usual way, viz., by the gradual extension of the vascular tissue tangentially, and we have a ring of xylem surrounded by phloem and containing a strand of the same tissue, the two phloems having been in continuity through the leaf-gap (Figs. 13, 14).

The second leaf-trace is formed exactly opposite the first and in a perfectly similar manner (Fig. 15). Like its predecessor, the trace is a very simple concentric strand, consisting of a few tracheides surrounded by phloem. The third leaf-trace, however, has a distinctly bilobed strand of xylem, a fact hinting at the occurrence of double leaf-traces to supply the later leaves. The preparations for the third leaf-trace are also of some interest. The xylem of the vascular ring breaks temporarily just before its exit, the break being effected by an 'encroachment' of the internal phloem; the actual gap, however, is occupied merely by parenchyma. As will be seen below, this breaking of the xylem foreshadows the preliminary division of the cauline strands which plays so important a part in connexion with the formation of the later leaf-traces.

At a slightly higher level, and before the repair of the last leaf-gap, the xylem breaks immediately opposite the latter by an encroachment

of the internal phloem exactly as before. In this case, however, the break is larger, and occupied not merely by parenchyma, but by sieve-tubes as well. The external and internal phloem thus become continuous through the break, which is, of course, a preparation for the exit of the next leaf-strand.

The gap in the xylem gradually increases in size, and a root-trace joins one of the xylem arcs in the usual way; its entrance is followed at an immediately higher level by the closing of the third leaf-gap (Fig. 18). In some cases the break in the xylem was temporarily bridged over by a single row of two or three tracheides, but the latter persisted through only a few sections. Very soon preparations are made for the actual exit of the leaf-trace. A strand of tracheides is nipped off from each of the xylem horns, and the two strands, separated by a narrow band of sieve-tubes and surrounded by common phloem and endodermal sheaths, pass out as the leaf-trace (Figs. 19–21). The single nature of the leaf-traces is therefore still maintained.

In a small proportion of the plants examined, a shallow and indefinite 'ground-tissue pocket' was associated with the exit of the leaf-trace just described, an irregular patch of endodermal cells appearing between the cauline strand and the outgoing petiolar bundle, just before the latter was quite free. The pocket was of so simple a character, compared with similar structures fully described elsewhere for other ferns, that no further description is necessary. Its occurrence, however, is of interest, since it emphasizes the essential uniformity between apparently somewhat different types of transitional changes.

The fourth and following leaves occur in more rapid succession than their predecessors, and, during the exit of the fourth leaf-trace, the xylem at a point exactly opposite the latter is encroached upon by the central phloem and broken into two portions, the break, as before, being occupied by phloem (Figs. 20–22). The fourth leaf-gap then closes, and the fifth trace is given off, as its predecessor, from the two horns of the broken xylem ring; it is still of the simple type, though the two xylem patches are separated by a comparatively wide band of phloem and parenchyma. Immediately opposite the leaf-gap, a root-strand joins the cauline system.

The break formed in the initial stages of the exit of the sixth leaf-trace is much wider than in previous cases, though the endodermis merely bridges the gap, as shown in Fig. 24. Higher up, however, the xylem strands become further separated, and the sieve-tubes of the internal phloem, which up to the present have occupied a more or less central position in the 'pith,' are now arranged lining the internal surface of the xylem. At the same time the phloem-parenchyma cells become larger and hence more conspicuous.

As the two portions of the xylem become more separated, ground-

parenchyma is differentiated between them, the endodermis being pushed, as it were, before the advancing ground-tissue (Fig. 25). The two vascular strands (the previous leaf-gap has not yet been closed) do not at this level become completely separated by the ground-tissue, so that we have, in transverse section, two patches of xylem each surrounded by phloem, the whole being ensheathed in a common pericycle and endodermis (Fig. 25).

At the moment when the complete separation of the two vascular strands by the ground-tissue seems almost effected, a concentric strand is gradually nipped off from each of the horns bounding the enlarged sixth break in the vascular tissue ; sometimes the strands were found to be nipped off simultaneously, but in other plants they became free from the cauline system at different levels, a state of affairs found to be very common in connexion with such multiple leaf-traces.

The two strands, each of which is surrounded by its own endodermis, never fuse together, as in previous cases, but remain quite separate and pass out to form the first double leaf-trace. During the complete separation of this trace, the larger of the two cauline strands begins to divide into two (Fig. 28), one portion fusing with the remaining main strand and thus closing, at last, the fifth leaf-gap. Fig. 29 shows that at this level the cauline system consists of two concentric vascular strands each surrounded by a separate endodermis. Subsequently a double leaf-trace is formed in the manner indicated in Figs. 29–31, and we have the final appearance of three cauline strands by the division of one of the original two, the actual changes being represented in Figs. 33–35.

Subsequent developments consist of a gradual elaboration of the vascular system, but the processes involved in the actual formation of the leaf-traces are essentially the same as those described above ; that is to say, we have a splitting of the vascular tissue followed by the separation of the two petiolar strands from the adjacent horns, and the subsequent closing of the previous leaf-gap. The siphonostelic character of the vascular system of this plant is therefore quite evident.

A very considerable amount of variation was met with in the earliest transitional stages, necessitating the examination of a comparatively large number of specimens before the varying appearances presented could be reduced to some common plan. As mentioned above, the xylem of *Lomaria gibba* possesses a considerable amount of parenchyma among the tracheides, and it seems almost certain that the variations met with can be attributed to this fact, for the parenchyma cells appear in the xylem of the very youngest plants. The following exceptional case is interesting. A single parenchyma cell, quickly followed by three or four others, appeared in the normal diarch plate of the root. At a higher level the ordinary protostelic structure occurred, but the parenchyma cells now appeared as a band stretching across the xylem, and continuous at either end with

the external phloem. The xylem was thus divided into two portions, and the appearance of another parenchymatous band, almost at right angles to the first, divided the xylem into three portions, the whole strand for several successive sections almost exactly simulating the appearance of a triarch root. At a slightly higher level the two bands had taken up a more or less central position in the xylem as two parenchymatous islands, one gradually dying out, the other maintaining its size, and, after one or two transitory connexions with the external phloem, appearing with a central strand of a few sieve-tubes. This occurred just before the first leaf-trace, which was separated from the cauline strand as a sector of vascular tissue by the development of parenchyma cells between the external and internal phloem.

In other plants similar variations occurred, all obviously connected with the parenchymatous nature of the xylem. A description of these variations, though interesting in itself, is unnecessary.

Lomaria Spicant, Desv.

The writer is indebted to Mr. T. G. Hill for an abundant supply of material of this plant. The transition from root to stem is somewhat rapid, but takes place in the usual manner. The root is diarch, and the protostele of the stem soon possesses a few parenchymatous cells at its centre. Just before the exit of the first leaf-trace one or two sieve-tubes appear in the parenchyma, so that at this stage we have, as before, a central strand of phloem surrounded by xylem, which, in its turn, is ensheathed in a cylinder of phloem. The most striking feature of the anatomy of the younger plants is the thick-walled character of the cells of the fundamental-tissue. The pitted walls are deep brown in colour, and the cell contents consist largely of densely staining tannin. The fundamental and vascular tissues are thus sharply contrasted.

The first two or three leaf-traces leave the amphiphloic protostele in a perfectly normal way, the external and internal phloem becoming continuous at the gap, which is merely bridged by the somewhat small-celled endodermis.

At the level when the endodermis bridges the third or fourth leaf-gap, and while the latter is still unclosed, one or two cells appear in the central phloem in the neighbourhood of the gap. The cells have thickened, yellowish, glistening walls and are thus readily distinguishable from the phloem. These cells, as we pass upwards, are immediately succeeded by a few others which have all the characters of the fundamental-tissue, viz., hard, thick, pitted walls, and tannin-impregnated contents. The most interesting fact, however, is that this group of fundamental-tissue cells is surrounded by an endodermis exactly resembling the outer endodermis in its small narrow cells, and which would readily escape anything but careful

observation (Fig. 51). At this stage preparations are made for the exit of the next leaf-trace, which is formed opposite the previous one and exactly above the preceding root. The strand of fundamental-tissue gradually assumes a more central position in the phloem, and a few sections higher up has increased in size and taken up a position near the outgoing leaf-trace. The previous leaf-gap is not yet closed. As will be seen from Figs. 54–57, the leaf-trace when complete is a simple concentric strand surrounded by an endodermis, the inner half of which is derived from the outer part of the endodermis surrounding the central strand of fundamental-tissue, and not from the external endodermis by a process of 'nipping in,' as is so generally the case.

As soon as the leaf-trace is completed, we have the inner half of the inner endodermis continuous with the outer endodermis round the horns of the xylem arc, the internal strand of fundamental tissue thus effecting a junction with the main mass of ground-parenchyma (Figs. 55–57).

The previous leaf-gap then gradually closes, and finally we have an arc of xylem including a patch of phloem, which is encroached upon at the leaf-gap by the ground-tissue. The latter is limited at this region by an endodermis having quite a different origin from a mere 'invagination.' As we pass upwards, the encroachment of the ground-tissue upon the central phloem becomes less and less, and finally the endodermis merely bridges the gap.

The sixth leaf-trace is formed in exactly the same way as the previous one, with the exception that the internal strand of fundamental-tissue is, from the first, in close proximity to the leaf-trace with which it is to be finally associated. After the trace is completed, however, the 'invagination' of the ground-tissue is not diminished as before, but increased, the more so as the preparations for the next leaf-trace advance (Figs. 61, 62). This leaf-trace differs from the preceding in being double and not single, and consequently different processes are involved in its exit.

In Fig. 60 we see the xylem arc broken at x in preparation for the trace, the two portions being separated by a band of phloem. This break is in reality the unclosed fifth leaf-gap, which is never fully repaired. In Figs. 61 and 62 the fundamental-tissue has advanced upon the medullary or internal phloem, which finally becomes relegated to a position merely lining the internal surface of the tracheary strand. This advance of the ground-parenchyma is complete, as it were, in Fig. 63, and we have two strands of vascular tissue separated from one another by the conspicuous fundamental-tissue, and each surrounded by phloem, pericycle, and endodermis. At a stage shown in Fig. 64, the two strands which will form the future trace are beginning to be nipped off, and it will be observed that the two horns of the xylem arc have approached one another, and that there is but a single fused endodermal layer between them. As the leaf-

trace becomes more advanced this endodermal layer disappears, and the mature leaf-trace at this stage consists of two vascular strands surrounded by a common endodermis. The further processes involved in the exit of this first double leaf-trace are illustrated in Figs. 65–67.

In older plants the double character of the leaf-trace is maintained, but each strand is surrounded by its own endodermis. This modification entails no additional complications at the actual exit of the trace, as will be seen below. As soon as the first double leaf-trace is completed the previous leaf-gap is repaired, and indications of the next appear in a breaking of the xylem arc into two portions, one considerably larger than the other. From each of these two vascular strands a portion is nipped off, each surrounded by its own endodermis, the structure of the older leaf-traces mentioned above being thus attained. The accompanying changes are indicated in Figs. 68–71. In Figs. 72 and 73 the two portions of the xylem are again joining, the junction being followed by a new split, to the horns of which the new leaf-trace is attached. Subsequent changes are merely a repetition and elaboration of the same process.

The occurrence, in connexion with the third or fourth leaf-trace, of a strand of fundamental-tissue surrounded by an endodermis is of considerable interest. A single transverse section at this particular level exhibits a structure closely approximating to that of a typical 'gamostele' as illustrated by the rhizome of *Marsilia*. Again, support is apparently afforded to Jeffrey's hypothesis of the primitive amphiphloic stele with an internal endodermis. A mental picture of the whole region of the leaf-gap will, however, at once show these ideas to be erroneous. What we really have is the formation of a fundamental-tissue pocket which is ensheathed on all sides by an endodermal layer, and hence closely resembling the 'endodermal pockets' occurring in *Schizaea dichotoma* [1], *S. malaccana* [2], and *Lindsaya* [3]. There can be no doubt that the first two or three small cells with yellowish glistening walls which appear in connexion with the third or fourth leaf-trace are really the endodermal cells sheathing the apex of the cone or elongated dome of ground-tissue laid down from the growing point in the medullary phloem [4].

In the above account, the roots have been practically omitted for the sake of brevity and clearness. It will be sufficient to say that the roots are perfectly normal, and that the leaf-traces are always formed, in young plants at any rate, directly over the position of the last root, and very soon after the latter has joined the stelar system.

[1] Boodle, Comparative anatomy of the Hymenophyllaceae, Schizaeaceae and Gleicheniaceae, iv. Further observations on *Schizaea*. Annals of Botany, xvii.

[2] Tansley and Chick, On the anatomy of *Schizaea malaccana*. Annals of Botany, xvii.

[3] Tansley and Lulham, On a new type of Fern-stele, &c. Annals of Botany, xvi.

[4] The term 'fundamental-tissue pocket' or 'ground-tissue pocket' has been employed since the writer believes that such a term more correctly indicates the essential nature of the phenomenon.

Blechnum brasiliense, Desv.

The ontogeny of the vascular system of this plant so closely resembles that of *Lomaria Spicant* that further description is unnecessary.

The radiciferous strands described by Trécul[1] and Lachmann[2] as occurring in this plant were not noticed, probably owing to the examples examined being comparatively young.

Asplenium bulbiferum, Forst.

In investigating this plant it was intended to compare the transitional changes in the seedling with those in the adventitious buds which are so common on the mature fronds. Unfortunately, the seedlings could not be obtained, and hence the following account is concerned with the adventitious buds alone. It is hoped the seedlings may soon be available and the necessary comparison made.

The buds arise as outgrowths of the leaf-tissue in the immediate neighbourhood of the swollen end of a vein supplying the large sorus. The suitability of such a point of origin is obvious. The young bud possesses a simple concentric vascular strand, connected with the vein of the leaf and surrounded by a well-marked endodermis. The strand is oval in section, and the xylem, consisting of tracheides and parenchyma in about equal proportions, shows no distinct protoxylem elements. The phloem completely ensheathes the xylem, and the sieve-tubes are well marked. The pericycle and endodermis have their cells in seriation, as is the case in so many ferns. The endodermis itself is often two or three cells deep locally, and has conspicuous brown cell-walls.

The ground-parenchyma is quite normal in structure but the more external cells are frequently meristematic, their division resulting in the formation of radial rows of cells, which, however, soon lose their radial arrangement.

In older stages, the parenchyma of the xylem has taken up a central position, and sieve-tubes very quickly appear, frequently as a strand at the centre. We thus have the usual amphiphloic structure so general in the other cases examined, but in the absence of a primary root, the preceding protostelic condition naturally does not occur.

The first leaf-trace is quite simple, a sector of the vascular ring affording the necessary strand. The gap is immediately closed. It is interesting to note, in connexion with the later traces, that the xylem of the trace as it leaves the cauline strand is somewhat bilobed.

[1] Remarques sur la position des trachées dans les Fougères. Ann. Sc. Nat., 5e sér., xii, p. 274.

[2] Contributions, etc., Thésis présentée à la Faculté des sciences de Paris, sér. A, no. 116, 1889.

A root-trace then joins the cauline system approximately opposite the previous leaf, but hardly is its connexion complete when the vascular ring opens, and from each of the two horns thus formed a strand is constricted off to form a double leaf-trace, a condition hinted at in the bilobed appearance of the xylem of the first leaf-trace. The two leaf-bundles pursue a curved course through the cortex, leaving the cauline system almost horizontally. During their exit, a group of endodermal cells appears in the internal phloem between the xylem arc and the outgoing trace, and at a slightly higher level an endodermal ring surrounds a group of large parenchyma cells closely resembling the fundamental-tissue. At the leaf-gap, this inner endodermis joins up with the external sheath, which, owing to the horizontal and band-like leaf bundles, presents at this level the somewhat peculiar appearance represented in Figs. 152–154. We thus have in this plant, as in *Lomaria Spicant*, a pocket of fundamental-tissue laid down in the region of the leaf-gap, and shut off from the vascular system by an endodermis.

After the completion of the trace, the two strands of which quickly unite to form a single petiolar bundle, the cauline strand has the form of a curved band, which receives a root-trace opposite the last leaf. The xylem then immediately breaks into two strands, the previous leaf-gap remaining open. The whole cauline strand, however, is still surrounded by a common endodermis (Figs. 156, 157).

The next leaf-trace is double, and formed from the horns guarding the new break in the xylem by a process of simple constriction. There is no pocket of fundamental-tissue formed in connexion with this leaf-trace, whose extreme simplicity of origin is somewhat masked by the difference in levels at which the two components leave the cauline system, and by their horizontal course through the cortex. As before, the final trace is single, but its double origin is obvious (Figs. 158, 159).

After the exit of the trace, the ground-tissue gradually separates the two vascular arcs, each of which finally possesses its own endodermal sheath, as in Figs. 159 and 160. One of the vascular arcs receives a root-trace, and, before its junction with the latter is really complete, divides into two parts; a fusion of one part with the remaining original cauline vascular strand occurs, and a leaf-trace is formed by the nipping off of two strands exactly as before (Figs. 162–164). The whole process is then repeated again and again, the double leaf-strands remaining separate for increasingly longer periods, fusion finally occurring in the base of the petiole.

In connexion with the later-formed leaf-traces, it was found that the division of the cauline strand commences just before the entry of the corresponding root-trace, and consequently the latter has a double insertion upon the cauline system. The later roots, too, have a somewhat

unusual path in the cortex, an upward curved course being that most commonly adopted.

It is unnecessary to describe in any detail the later processes in the elaboration of the vascular system. The cauline strands increase in number by repeated fraction, and the mature dictyostelic condition is very quickly attained.

In one or two plants examined it was noticed that small strands which ended blindly in the ground-tissue at a higher level were occasionally given off from the cauline system. Similar strands were also found to occur in *Polypodium aureum*.

Aspidium Tsus-Simense, Hk.

The transitional processes of this fern are of a very simple character and will be described merely in outline. Passing upwards in the young plant, we find the root-strand changing into the protostelic condition in the normal way, though the completion of the change is much longer deferred than is generally the case. Internal phloem is differentiated just before the exit of the first leaf-trace, which is formed in the usual simple manner. Fig. 165 represents the cauline system after the completion of the first trace. It will be seen that although the gap is not yet repaired, a pocket of fundamental-tissue has been differentiated in the phloem at the centre of the amphiphloic strand. The vascular tissue later bulges outwards at a point opposite the gap, the protruding portion, in which the xylem is seen to be arranged in two distinct tracts, soon passing outwards as the second leaf-trace. The inner and outer parts of the ground-tissue become continuous after the exit of the leaf-trace. The curved vascular band subsequently becomes more strongly convex, and, as before, the 'back' of the arc separates from the lateral portions as we pass upwards, to supply the third leaf with a single petiolar bundle. The later formation of the first double leaf-trace is illustrated in Figs. 170, 171.

The mature vascular organization is the result of the elaboration, along ordinary lines, of the simple dictyostelic structure obtaining at this level, and need be described at no further length.

Polypodium aureum, L.

The dorsiventral rhizome of this fern creeps along the surface of the ground, bearing upon its upper surface two rows of leaves which are more or less arranged in pairs. The internodes between consecutive pairs are of considerable length. The change from the radial to the dorsiventral habit is well shown in a fairly young rhizome, in which the conical base is seen to bend sharply at right angles as it rapidly increases in diameter. The adult horizontal position of the rhizome is soon reached.

The rhizome is creamy-white in colour and thickly clothed with long narrow brown ramenta, which at the growing point are greenish and so densely packed together that they may be removed *en bloc* as a firm conical cap, the protective value of which is obvious. After the removal of the ramenta, the surface of the rhizome is seen to be minutely pitted, each pit indicating the place of insertion of a ramentum. The ramenta are narrowly triangular in outline and consist of a single layer of cells with yellowish-brown walls. Each is inserted upon the rhizome at the base of the shallow pit by means of a short multicellular stalk, beyond which the base of the ramentum is produced backwards as two auricle-like lobes with densely papillose margins, sharply contrasting with the irregularly toothed margin of the body of the ramentum. The contour of the cell-walls differs in different parts of the scale; in the lamina the cells are irregularly rectangular in outline, contrasting with the cells in the region of the stalk, which are much smaller and hexagonal, with walls of a deep-brown colour. The cells of the auricular regions have sinuous contours.

The primary root of *Polypodium aureum* is, as usual, diarch. The vascular strand is extremely simple, consisting of but a few elements, which are, however, very definite. In the transitional region a striking difference from the usual course of events is exhibited. Instead of the diarch plate gradually becoming rod-like to form the solid protostele of the young stem, the xylem extends laterally and becomes strongly curved. The phloem extends round the horns of the xylem, but the sieve-tubes are often absent from the extremities for a few sections. One or two root-traces join the cauline strand at or near the middle of its convex surface, and then the first leaf-trace is formed in a very simple and quite exceptional manner, by the nipping off of a small part of one of the vascular horns. The first trace is concentric, and in several plants was approximately equal to the remaining cauline strand. Thus, in striking contrast to other ferns examined, there is no central phloem differentiated at the centre of the solid protostele, and the first leaf-trace leaves no corresponding gap. The absence of early leaf-gaps is, of course, by no means exceptional outside the Polypodiaceae, familiar examples being afforded by the Schizaeaceae, Gleicheniaceae (Boodle), Osmundaceae (*Osmunda*, Faull, *Todea*, Seward and Ford), and Marattiaceae (*Angiopteris*, &c., Farmer and Hill).

Polypodium aureum has already received the attention of Leclerc du Sablon [1] and Jeffrey [2]. The former investigator has correctly described the very earliest stages in the transition, but announces and figures the later appearance of a strand of parenchyma at the centre of the xylem rod. This however has been denied by Jeffrey, who was unable to find any such

[1] Loc. cit.

[2] Structure and development of the stem in the Pteridophyta and Gymnosperms. Phil. Trans., ser. B, vol. cxcv (1902).

parenchyma. It will be seen that the writer's investigations confirm the results arrived at by the latter author.

The cauline strand increases somewhat in size, but is still in the form of a curved band, and the second leaf-trace is formed in a manner precisely similar to the first, but from the other extremity of the vascular tissue (Figs. 91–95). Soon after the completion of the leaf-trace, a root-strand joins the vascular band, which quickly divides into two parts, each being in section an arc of xylem and phloem surrounded by a pericycle and endodermis. One of these secondary strands is considerably larger than the other, and from it the third leaf-trace is nipped off just as in previous cases. The two strands then approach one another and fuse, the junction being maintained for a short distance; they soon separate again, however, and from one of them the next leaf-trace is formed as before, but more or less opposite the previous trace (Figs. 96–102). This radial arrangement of the leaf-traces in the young plant is of considerable interest, since the most striking feature of mature specimens is the dorsiventrality of the rhizome, the leaves being arranged in two rows on the upper surface and the roots confined to the lower and lateral surfaces. A root-trace soon joins one of the cauline strands after the exit of the leaf-trace, and then the augmented strand divides into two portions, one of which unites temporarily with the remaining original cauline bundle (Figs. 103–109); as before, the separation is connected with the formation of the next leaf-trace.

The unusual length of the internodes makes it a matter of some little difficulty to clearly realize what is actually taking place in connexion with the formation of the leaf-traces. In spite of the fact that the protostelic and the subsequent amphiphloic conditions of the transitional region do not obtain in this fern, the somewhat simple siphonostelic (phyllosiphonic) nature of its vascular system is quite obvious as soon as two or three leaf-traces are formed, although, as stated above, the length of the internodes, together with the small size of the cauline strands, makes this fact somewhat difficult of realization. Instead of the leaf-trace being formed in the normal way, we have a splitting of the vascular strand followed by the constricting off of a portion to form the leaf-trace. The subsequent fusion (really the closing of the elongated gap), followed by a new splitting, demonstrates the essentially siphonostelic nature of the vascular system of this plant. It is interesting to note that a somewhat similar series of changes occurs, as an exceptional circumstance, in connexion with some of the early leaf-traces of *Aspidium falcatum* (q. v.), although in this case the transitional region presents the normal protostelic and amphiphloic conditions. To return. The fifth leaf-trace is the first to hint at the double traces which are so characteristic of the mature plant. It is derived from the two portions of the divided cauline strand by the nipping off of a small concentric bundle from each; one half of the leaf-trace is formed at

a considerably lower level than the other, as is so often the case (Figs. 109, 110). The two leaf-strands pass gradually outwards through the cortex, and, in doing so, approach one another and finally fuse, leaving the stem as a single bundle. The fifth leaf, therefore, is supplied with a single trace which has an obviously double origin. The sixth leaf-trace is also double in origin, and formed exactly as before, being preceded by the usual closing of the elongated gap and subsequent splitting. Although the mature trace is single, its double origin is hinted at in the bilobed character of its xylem.

After the gap is closed, the subdivision of the vascular strands proceeds apace. Instead of a single strand dividing into two, the strands divide, one into two, the other into three parts, each being surrounded by its own endodermis. The division of the actual strand is nearly always preceded by the constriction of the xylem into the required number of parts, the portions of the xylem being separated by phloem parenchyma and occasionally sieve-tubes. Fig. 118 represents the appearance of the stem in transverse section after the sixth trace has passed out. The next two or three leaf-traces are formed in exactly the same way as before, but retain their double character for successively longer periods. Soon, however, the leaf-traces have an apparently simple origin. At about the eighth or ninth leaf, the stem in transverse section presents six or seven strands embedded in the ground-tissue. The leaf-trace is formed by the nipping off of a vascular strand from each of two neighbouring cauline strands, one being separated from the cauline bundle at a considerably lower level than the other. Both soon leave the stem to enter the petiole, but the exit is gradually delayed in succeeding leaf-traces. It is this fact which explains the apparent anomaly in the formation of the leaf-strands in older plants.

In a moderately sized rhizome there are from twelve to fifteen vascular strands, and at the node two of these are seen to pass out bodily into the petiole. This apparently is quite different from the process obtaining in young plants, but a complete series of sections through two of the long internodes shows that the leaf-trace formations are essentially the same in both cases. In the later internodes, the vascular strands divide and fuse several times, but strands from which the last leaf-trace was derived remain separate for some considerable distance. Finally, however, they subdivide, and the lesser strands fuse among themselves, so that the leaf-gap is closed. The true siphonostelic character of the vascular system is therefore maintained. Two neighbouring strands then divide into two, one much in advance of the other, and the two daughter strands run through the remainder of the internode and apparently belong to the cauline system. At the next node, however, they pass outwards to the petiole and form the leaf-trace as described above. It will thus be evident that the later

traces are formed in essentially the same way as those of the young plants, their true origin being rendered somewhat difficult of realization by the greatly elongated internodes. The series of changes is represented in Figs. 123–128. The petioles of the later leaves are generally supplied with two main bundles and one or two smaller ones, which, however, fuse into two strands soon after their entrance into the petiole. As regards the leaf-trace itself, then, we find that the earliest leaves are provided with a single strand ; later, the trace has a double origin, but a single bundle enters the leaf-stalk ; and, finally, the leaf-strands remain separate for successively longer periods until the mature condition is reached, in which two chief strands enter the petiole, fusing a short distance higher up.

Several variations of the normal early transitional changes described above were noted. In two or three plants the xylem of the diarch plate broke into three parts, which were surrounded by a common phloem sheath. One of the xylem strands, surrounded by phloem and endodermis, passed out as the first leaf-trace, while the two remaining strands quickly fused, or separated from one another as concentric strands which reunited to form a single concentric rod at a slightly higher level.

In plants of all ages it occasionally happened that small concentric strands left the cauline system, and, bending sharply outwards, ended blindly in the ground-tissue, generally just beneath the epidermis. A similar state of affairs was noted for *Asplenium bulbiferum.*

Petiole.

The petiole of a moderately sized leaf is almost circular in section, and possesses that peculiar wiriness so characteristic of many fern leaf-stalks. There is a single vascular strand embedded in a ground-tissue limited externally by an epidermis. The latter consists of a layer of cells square in section, and with extremely thick lignified walls almost completely obliterating the cell cavity.

The ground-tissue is composed of hexagonal parenchyma, but the cells of the outer layers are elongated and strongly sclerosed, affording to the leaf-stalk the stiffness mentioned above. The walls of these mechanical cells are bright red in colour and minutely pitted ; the stratification is particularly well marked. The cells remain living for some considerable time, protoplasm and large nuclei being constantly found to be present. The somewhat small intercellular spaces were carefully examined for any indications of the cellulose pegs or outgrowths described by Boodle and others as occurring in similar positions in certain ferns, but such structures seemed to be quite absent.

The petiolar bundle is small and surrounded by an extremely well-marked endodermis, the outer tangential walls of which are strongly sclerosed and of great thickness. The inner tangential and radial walls

are quite normal. The position of the latter, exactly opposite the radial walls of the pericycle cells, demonstrates the origin of the two layers from common mother-cells. The pericycle is two cell-rows deep, except at the protoxylem groups, where it is only one layer thick. Its cells have crowded contents and large nuclei.

In the lower portion of the petiole there are two (or more, *v. s.*) vascular strands, each possessing a curved xylem plate with generally a single protoxylem group. The large scalariform tracheides are surrounded by a layer of parenchyma cells with deeply-staining contents. The parenchyma is succeeded by the phloem, which is arranged in two chief masses, one on either side of the xylem plate ; the sieve-tubes are of the normal type and very prominent, staining intensely with haematoxylene, especially if subsequently treated with ammonia. Round the ends of the xylem plate, the phloem is reduced to a single row of sieve-tubes intermixed with a few parenchyma cells (Fig. 204). The two bundles gradually approach one another, the first stage of the final fusion being the inclusion of the two strands within a common endodermis. The curved xylem bands are situated back to back, separated by phloem, and with the protoxylems widely diverging. A junction is gradually effected, resulting in a **Y**-shaped strand of xylem with a phloem patch in each of the three bays ; the protoxylems occupy the apices of the arms of the **Y** (Figs. 205–207). The actual junction of the bundles takes place at successively higher levels in the later leaves, and in large petioles there are from three to six bundles which are separate throughout. After the junction of the bundles in the earlier leaves, the endodermis cells become even more conspicuous than before, owing to the further thickening of their outer tangential walls. The thickening and lignification frequently extends also along the radial walls of the next outer layer of cells.

Periderm.

In one or two of the plants examined, frequent patches of periderm were noticed. As the cells outside the periderm were broken and disorganised, there seems to be little doubt that the rhizome had been accidentally injured at these places, and that the injury had been followed by the formation of wound-periderm. As noted by De Bary [1], periderm is of extremely rare occurrence in ferns, and, when present, generally seems to be connected with the presence of wounds. Periderm has been described by Holle [2] as occurring in the root and rhizome of *Botrychium*, and more recently, Brebner [3] finds wound-periderm of somewhat frequent occurrence in Marattiaceae.

[1] Comparative Anatomy, p. 108.

[2] Ueber Bau und Entwicklung der Vegetationsorgane der Ophioglosseen. Bot. Zeit., 1875.

[3] On the anatomy of *Danaea* and other Marattiaceae. Annals of Botany, xvi.

The periderm presented the appearance of a large patch of thin-walled cells in radial rows, the origin of which could be traced to a very narrow phellogen just beneath the crushed cells of the wounded area (Fig. 208). Very little external tissue seems to have been formed by this phellogen, and it was quite evident that the cells of the ground-tissue beneath the phellogen also divided directly. This is exactly the state of affairs found by Holle to exist in *Botrychium*.

The changes undergone by the vascular system in the transitional region, as described above, present some striking points of difference from the usual course of events. Instead of the transformation of the diarch xylem plate of the root into a solid strand, a later appearance of a central patch of phloem, and finally the formation of a leaf-gap, we have what is practically the root-strand nipping off a simple concentric leaf-trace directly, without any preliminary appearance of a central pith of phloem, and without the formation of a leaf-gap.

The absence of gaps in connexion with the formation of early leaf-traces is, as pointed out above, a phenomenon for which it is easy to find a parallel, but the division of the cauline strand itself, after the exit of the first few traces, is so strikingly different from the normal course of events as to warrant, at first sight, the assumption that in *Polypodium aureum* a type of transition obtains which is fundamentally distinct from that occurring in the majority of Polypodiaceae. It has been shown above, however, that in the later-formed regions of the rhizome, an essentially siphonostelic vascular system is present, and in the light of this evidence it is highly probable that the apparent anomaly existing in the young plant can be explained. The earliest transitional changes present no real difficulty; the leaf-traces which do not involve the formation of leaf-gaps are given off directly from a cauline strand, in which, for some reason, the xylem has maintained the plate-like character it possessed in the root. This was found to be by no means without a somewhat similar parallel, even among the relatively small number of ferns examined by the writer. For instance, in some of the seedlings of *Pteris Winsetti* and *Doodia aspera*, the xylem at the level of the first trace had a distinctly oval outline in transverse section, and this occurred occasionally also in other plants. The splitting of the cauline strand is perhaps a point more difficult of explanation. The writer, however, regards the splitting merely as an anticipation of the characteristic double nature of the later leaf-traces. Such a splitting, previous to the actual nipping off of the petiolar bundles, seems to be of very general occurrence in connexion with such double traces (cf. *Doodia aspera*, &c.), and is indeed the most obvious preparation for their formation. If this view of the transitional changes be the correct one, the essentially siphonostelic character of the young vascular system is

established, and *Polypodium aureum* is brought into line with the normal type.

The early stages of *Polypodium aureum* find an almost exact parallel in the young seedlings of *Ceratopteris thalictroides*. Miss Ford[1] has given an account of the transitional changes occurring in this interesting fern, and describes the gradual change of the root-strand into a normal protostele, and the subsequent exit of a few simple leaf-traces. Soon, however, the cauline strand apparently breaks into two, just as in the case described above. Unfortunately, Miss Ford was unable to follow out the later changes, and infers that in *Ceratopteris* we have evidence of a kind which lends support to the essential idea of Van Tieghem's theory, viz., the branching of the original single cauline strand, the protostele. With this inference the present writer is inclined to disagree. It is probable that if older stages had been examined, the breaking of the stem strand would have been found to have been in intimate association with the formation of a leaf-gap, a view supported by the evidence of *Polypodium aureum*, and by the text figure of Miss Ford's paper (p. 106), which clearly shows the bundle system of *Ceratopteris* to be a vascular tube interrupted by elongated and exaggerated leaf-gaps.

The occurrence of similar early transitional changes in the two plants is of some interest, since it supports the conclusion arrived at by Miss Ford with regard to the systematic position of *Ceratopteris*, viz., that the plant finds its nearest relations among Polypodiaceae.

Since the publication of a preliminary note[2] on the present work, the writer has received a communication from Miss Ford confirming the account given above of the early transitional stages of *Polypodium aureum*. Miss Ford further observed a few variations from the normal type, most of which were also noted by the writer.

Nothochlaena sinuata, Kaulf.

The transitional changes occurring in this plant proved to be of a most interesting type. The primary root is diarch, but the vascular elements are somewhat irregularly arranged and by no means well differentiated, the latter being especially the case with the sieve-tubes. Considerable variation occurs in the earliest transitional changes, but generally the xylem plate becomes more or less circular in transverse section, and, as usual, a small strand of parenchyma is differentiated at its centre. It is extremely difficult to say whether sieve-tubes occur among the parenchyma cells; some of the latter are smaller than their neighbours, and

[1] The anatomy of *Ceratopteris thalictroides*, L. Annals of Botany, xvi.

[2] New Phytologist, May 1904.

might possibly be interpreted as sieve-tubes, but, on the whole, it is probable that sieve-tubes are not differentiated in the central 'pith' until after the first leaf-trace. The latter is quite simple, but of relatively large size, leaving a gap which remains open through a considerable distance, a character which is maintained throughout the plant. The corresponding root is quite normal, and, just before the second leaf-trace, definite sieve-tubes appear scattered in the enlarged pith. After the closing of the second gap, the stem presents a characteristic appearance in transverse section. The xylem ring is very narrow (consisting of only two or three rows of tracheides), and encloses a relatively large pith of phloem in which the sieve-tubes are very conspicuous. In these early stages it frequently happens that isolated tracheides, sometimes in small groups, are differentiated in the central phloem, a point of some interest which will be referred to in connexion with *Nephrodium setigerum*. Immediately outside the xylem is a very regular row of parenchyma cells, followed by an almost continuous single layer of sieve-tubes. The most striking feature, however, is the pericycle, which is composed of two or three layers of large squarish cells with conspicuous nuclei and readily-staining contents. The endodermis is well marked, but its cells are not always in exact seriation with those of the pericycle, as so commonly occurs. The parenchyma of the ground-tissue is thin-walled except at the periphery, where the walls are sclerosed and deep red in colour.

The first four root- and leaf-strands are formed in a perfectly normal manner, the xylem gradually dilating and enclosing the enlarged central strand of phloem. In connexion with the exit of the fifth or sixth leaf-trace we have the formation of a typical ground-tissue pocket as described for other forms. The vascular ring becomes strongly oval in outline, and towards one end of the central phloem a group of two or three endodermal cells appears. At a slightly higher level the endodermal cells surround a strand of thick-walled parenchyma very similar in appearance to the sclerotic elements of the ground-tissue (Fig. 174). As in similar cases, the inner and outer endodermis become continuous at the exit of the trace, and the ground-tissue is differentiated to a considerable depth in the central phloem (Figs. 174, 175). The apparent encroachment of the ground-parenchyma upon the vascular tissue increases until the phloem and pericycle merely line the concavity of the xylem, as indicated in Fig. 176. At a slightly higher level the leaf-gap is closed, and we have the very interesting appearance represented in Fig. 177. The resemblance of this isolated section to a similar section of Gwynne-Vaughan's true solenostelic ferns is very striking.

During these changes the xylem ring has become thicker, and exactly opposite the previous leaf-gap begins to bulge outwards in preparation for the next trace. The formation of the latter is indicated in Figs. 177

and 178, in which it is seen that the bulged portion of the vascular tissue goes out as the leaf-strand, resulting in the union of the inner and outer endodermis. The gap remains open for some considerable distance, the vascular ring increasing in diameter, and, before the gap is closed, the next leaf-trace is given off from the back of the xylem arc in the manner indicated in Fig. 179, with the result that the cauline system now consists of two strands. The xylem of the leaf-trace is at first in two distinct strands, a fact hinting at the double origin of the later traces. Soon after the exit of the petiolar bundle, the previous leaf-gap closes as far as the vascular tissue as a whole is concerned, the horns of the xylem, however, not fusing, but remaining separated by two or three parenchyma cells (Fig. 180). This is no doubt to be regarded as a preparation for the next leaf-trace, which is definitely double, as shown in Fig. 181. The closing of the previous gap is immediately followed by the breaking of the cauline vascular strand into two (Figs. 182, 183). The following leaf-trace is double, the two strands being nipped off from the vascular horns resulting from the fractionation of the cauline system (Fig. 184). Two or three rootlets join the stem at this level, and one of the vascular strands then breaks into two portions, a division which is rapidly followed by a fusion of one portion with the undivided strand, and the subsequent formation of another leaf-trace as shown in Figs. 185–187.

The two cauline strands then effect a junction, but quickly separate again. As will be seen from Fig. 187, the diameter of the vascular tube is only slightly less than that of the stem as a whole, the ground-tissue being reduced to a mere band, the outer layers of which are very strongly sclerosed. The xylem has increased in width, but is still comparatively narrow; the phloem is normal, and the pericycle maintains its multiseriate character. The endodermis is extremely well marked, especially that portion of it lining the concavity of the vascular arc, a condition of things no doubt to be correlated with the fact that large numbers of intercellular spaces occur in the loosely arranged central ground-parenchyma. Before the last leaf-gap is closed, the vascular arc again breaks into two, and a new double leaf-trace is formed in the manner already described. The two bundles, which are separated by a strand of sclerenchyma, leave the cauline system very gradually, and their separation from it is not complete until the previous leaf-gap has been repaired. At a level immediately above, the vascular band again breaks, first into two and subsequently into three parts (Fig. 189), and during its division several roots join the vascular system. After the entry of the roots, the two largest of the vascular strands again unite (Fig. 190), and, while the exit of another leaf-trace is being effected, the remaining cauline strand also effects a junction with the curved band thus formed.

Subsequent changes are of a very interesting character, and result from

the branching of the stem at this level. The branching takes place into two such equal parts that one is inclined to infer that true dichotomy must obtain, though this point has not been settled. Fig. 192 represents the stage at which the three cauline strands have completely united; the separation of the leaf-trace is also seen to be taking place. Before the latter is effected, however, the cauline system appears, as we pass upwards, to be 'pinched in' at its centre (Fig. 193). The constriction is completed immediately after the leaf-trace leaves the vascular system, with the result that we have the appearance of two nearly independent solenostelic structures (Fig. 194). In one plant examined, the two rings were completely separate before the stem actually bifurcated (Fig. 196), though the usual state of affairs is for the stem to branch immediately the separation of the vascular rings seems about to occur.

Each of the two branches will therefore be seen to possess at its base a vascular structure, which, in transverse section, presents a typical solenostelic appearance, such as occasionally occurs in the stem before bifurcation takes place. As in the latter case, however, the condition is a very transitory one, the complete rings soon breaking at corresponding points (Fig. 195) in preparation for the insertion of a pair of leaf-strands. From this point onwards, the vascular organization resembles, in main outline, that of the stem before the region of branching, and will be described at no further length.

The method of branching in *Nothochlaena sinuata* closely resembles that occurring in *Schizaea dichotoma* and *Pteris incisa*, as described by Boodle [1], and Tansley and Lulham [2] respectively. In *Pteris* the processes involved in the branching are considerably more complex than those obtaining in *Schizaea* or *Nothochlaena*, but in all three cases we have the occurrence of dichotomy accompanied by the formation of a median leaf-trace.

As shown in Figs. 187, 192, 194, the ground-tissue in all but the youngest plants consists of two sharply contrasting portions. A narrow band immediately outside the outer endodermis is composed of soft-walled cells, as is the whole of the portion surrounded by the almost complete vascular ring. The peripheral part, however, is composed of small cells, the thick walls of which are coloured a deep reddish-brown, contrasting in a striking manner with the colourless thin-walled parenchyma and with the vascular tissue.

[1] Comparative anatomy of the Hymenophyllaceae, Schizaeaceae, and Gleicheniaceae. IV. Further observations on *Schizaea*. Annals of Botany, xvii.

[2] The vascular system of the rhizome and leaf-trace of *Pteris aquilina*, L., &c. New Phytologist, iii, 1904.

NEPHROLEPIS CORDIFOLIA, Baker (N. TUBEROSA, Hk.).

The upright, sometimes horizontal, rhizome of this fern gives rise to numerous thin runners or stolons, which bear at intervals scaly tuberous bodies, and many branching adventitious roots. The stolons themselves, especially when young, are often more or less tuberous, and vary in thickness in different parts of their length.

The root exhibits the usual diarch structure, possessing a well-marked endodermis, and a pericycle of varying width. The cells of the cortex are not sclerosed to any remarkable extent. The early transition to a solid protostele takes place in the usual way, the stem bending somewhat sharply at this region; this is by no means an uncommon occurrence, as pointed out elsewhere. A few parenchyma cells are differentiated in the centre of the protostele, and the first leaf-trace is then given off. The trace consists of but a few elements, being practically a continuation of one of the protoxylem groups of the cauline strand together with a little phloem. The leaf-gap is very small, and, in one or two plants examined, no gap at all was left by the first leaf-trace. The external phloem at this stage is poorly differentiated, the identification of the sieve-tubes being a matter of considerable difficulty. This fact probably explains the apparent absence of sieve-tubes in the central pith until after the third leaf-trace, a state of affairs somewhat at variance with that found in the majority of other ferns examined. It frequently happens, however, that the pith is continuous with the external phloem by means of parenchymatous strands extending through the xylem, and in all probability the pith is really to be regarded as phloem in which the sieve-tubes are by no means well differentiated.

The first three leaf-traces are formed in the simple manner indicated above, and the accompanying root-traces join the cauline strand in the usual way. Soon, however, the latter begins to increase in size, though it is still quite small and presents two protoxylem groups which are in much the same position as the protoxylem groups of the root. The pericycle now becomes more definite, and is usually two rows deep, its outer cells fitting exactly upon those of the endodermis. At this level, the vascular tissue supplying the first stolon is differentiated, and joins the cauline system in a way very similar to that of a root; in fact, in a cursory examination, it is merely its size which distinguishes the stolon-strand from a root-trace. Its structure, however, is very similar to that of the stem at this level, except that a definite central pith is not present, parenchyma cells occurring scattered throughout the xylem. The stolon itself may be regarded as the result of an abrupt branching of the stem.

After the junction of the stolon with the stem, the cells of the pith (which up to this level have frequently had a somewhat scattered arrange-

ment in the central part of xylem) become definitely arranged in a small excentric group, and well-marked sieve-tubes, two or three in number, appear among them. The fourth leaf-trace, quite simple and leaving but a small gap, is then given off exactly opposite the stolon. The gap is merely bridged by the endodermis. Just before the trace is differentiated, an isolated section presents an appearance very similar to that described by Tansley and Miss Lulham for *Lindsaya* [1], the excentric position of the internal phloem being very striking. The explanation of this appearance is, of course, very simple, since the vascular tissue of the stolon, although in perfect union with that of the stem, lies alongside the latter for some distance as a kind of keel (Figs. 133, 134). Gradually, however, the union of the two strands becomes more perfect, and the cauline strand once more assumes its cylindrical condition. The leaf-gap is very quickly closed, and a root, with densely sclerosed cortical tissue, then joins the stem at a point opposite the leaf.

As soon as the stem-strand has regained its normal cylindrical condition, sieve-tubes can no longer be distinguished in the pith, the cells of which resume their scattered arrangement among the central tracheides of the xylem. The somewhat indefinite external sieve-tubes show a tendency to arrange themselves in groups, a feature which is more or less constant in older plants. The next change is the somewhat sudden re-grouping of the parenchyma cells to form a central patch in the xylem, followed by the appearance of a few sieve-tubes. At the same time the pericycle increases in width, the cells of its inner layers not being in radial rows with the cells of the outer layer, which, as before noted, are in seriation with the endodermal cells.

The vascular strand has meanwhile increased considerably in size, and the next leaf-trace, though still small, is much larger than its predecessors. It consists of an arc of xylem surrounded by phloem, and leaves a gap which is very quickly closed. The second stolon then joins the stem, its vascular tissue appearing as an immense root-trace. As before, its junction occurs exactly opposite a new leaf-strand, whose exit leaves a gap into which the ground-tissue is differentiated to a slight depth. A transverse section of the stem at this level shows a rounded mass of tracheides and xylem parenchyma with a shallow gap on one side containing sieve-tubes and parenchyma, which also form a continuous sheath round the xylem as a whole. The ground-tissue, dipping slightly into the gap, is very striking, since its strongly sclerosed polygonal cells contrast sharply with the very definite small-celled endodermis. The phloem in the leaf-gap occupies an approximately central position in the xylem below the level of the stolon, its excentricity, as before, being solely due to the augmentation of the cauline vascular tissue by the stolon bundle (Fig. 135).

[1] Loc. cit.

In connexion with the fifth or sixth leaf-trace there is a formation of a regular ground-tissue pocket very similar to that already described for other ferns. A group of endodermal cells appears between the outgoing leaf-trace and the main strand, and at a slightly higher level we have a patch of strongly sclerosed cells surrounded by an endodermal sheath. At the exit of the leaf-trace the inner endodermis becomes continuous with the outer, just as in previous cases. The leaf-gap does not close, but, if anything, becomes wider as the next stolon joins the stem ; a root-trace enters immediately opposite the gap. After the junction of the stolon and root-strands with the cauline system, the latter in transverse section appears as an arc of xylem (largely parenchymatous), with a broad and shallow gap into which the conspicuous fundamental-tissue dips sharply, but to no great depth (Fig. 136). At a higher level the ground-tissue is differentiated further into the gap and the vascular tissue appears as a reniform strand. The middle portion of the xylem then becomes much attenuated, consisting of only one or two rows of tracheides, and, with its accompanying phloem, bulges out in the manner indicated in the diagrams 137, 138. The fundamental-tissue still further encroaches upon the vascular tissue, but gradually loses its highly sclerotic character, except that portion actually between the horns of the vascular arc, the cells of which are extremely thick-walled. Figs. 140–142 represent the subsequent changes. The attenuated portion of the vascular tissue becomes separated from one of the two main masses, and finally the whole strand divides into three, the arc-like narrow portion passing out as a leaf-trace. The remaining strands then gradually approach and finally fuse, though the vascular portions, surrounded by a common endodermis, remain separated by pericyclic parenchyma for some little distance.

Just before the union of the two cauline strands, one of the latter, viz., that from which the leaf-trace first separated, gives off a portion of its vascular tissue which, as a small strand, joins the leaf-trace at a slightly higher level. This is the first indication of the multiple leaf-traces occurring in mature plants, all previous leaf-strands having been simple. As soon as the union of the cauline strands is complete, another stolon is formed in the usual way, its formation being followed by a redivision of the vascular tissue into two portions. Each of the latter then separates off a small strand, one at a much lower level than the other; a third strand is then nipped off, so that the leaf-trace has three bundles instead of two as before. The three bundles ultimately fuse into a horseshoe-shaped strand.

Subsequent changes consist of a further fractionation of the vascular system, and the gradual elaboration of the leaf-traces.

Structure of the Young Stolons.

The structure of the young stolon is essentially the same as that of the stem at the level at which its junction with the latter is effected. The xylem contains a large amount of parenchyma, and possesses three or four peripheral protoxylem groups. A short distance behind the apex the latter are well marked, the large thin-walled tracheae of the metaxylem occupying the central portion of the strand. The phloem, with fairly conspicuous sieve-tubes, which show a tendency to arrange themselves in groups as in the stem, forms a complete sheath round the xylem. An older stolon shows a more or less sclerotic fundamental-tissue contrasting sharply with the small-celled epidermis. The well-marked endodermis, the cells of which are in seriation with those of the pericycle, is surrounded by two or three layers of extremely thick-walled ground-tissue cells. The xylem is still largely parenchymatous, but the tracheae are in three main groups, each with peripheral protoxylem elements. The phloem and pericycle call for no special description, except that the latter does not vary in thickness to any great extent as in the stem.

NEPHRODIUM SETIGERUM, Baker.

The earlier transitional changes of *Nephrodium* have been described by Leclerc du Sablon[1] in *N. molle*. Only very young plants were examined, however, and the writer has been able to make a more complete study of *N. setigerum*. The root is diarch, and its transition to the solid protostele of the young stem is, on the whole, normal. The phloem, however, takes a considerably longer time than usual to completely surround the xylem rod, and generally the phloem sheath is not complete until one or two parenchyma cells have appeared in the centre of the xylem. Sieve-tubes are very quickly differentiated in the pith, taking up a central position, so that we have internal phloem present before the exit of the first leaf-trace. It is a point of some interest that, in two or three of the plants examined, isolated tracheides were found in the central phloem, thus emphasizing the essentially vascular nature of the cauline strand as a whole at this level (Fig. 202). The first leaf- and root-traces are formed at approximately the same level, separated by about 100°.

The leaf-trace is of the normal concentric type, the xylem being a slightly curved band, and the shallow gap, which is occupied by the phloem of the pith, is merely bridged by the endodermis. The leaf-gap is hardly closed (in one or two cases it was actually not closed) when the next leaf-trace is formed exactly opposite the last, accompanied by two roots. The usual occurrence of a single root for each leaf does not seem to hold in

[1] Loc. cit.

the case of *Nephrodium setigerum*, all the plants examined showing the roots to be more numerous, and somewhat irregularly arranged.

The first few leaf-traces are formed in a manner similar to that described above, the closing of the gaps resulting in the formation of a ring of xylem surrounded by phloem, and containing a phloem pith in which isolated tracheides occasionally occur. After about the fifth or sixth trace, a portion of the xylem ring becomes reduced in thickness to only two or three rows of tracheides, the attenuated arc bulging slightly outwards. At a higher level, this arc of vascular tissue passes out as a leaf-trace, but before the xylem has broken away from the cauline strand we have the formation of so typical a fundamental-tissue pocket that no further description is necessary. A transverse section of the stem through the region of the pocket is shown in Fig. 203. The leaf-trace presents a distinctly bilobed appearance, especially with regard to its xylem, and, as might be expected, this is an indication of the later formation of multiple leaf-strands. Before the gap closes, the cauline strand becomes strongly curved, the middle portion of the arc being thinner than the extremities, and quickly passing out as a leaf-trace which is still more markedly bilobed than its predecessor.

The subsequent closing of the previous gap is followed by a re-splitting of the strand into two unequal parts. The next leaf-trace is joined to the margin of this split, and is double from its origin. The subsequent elaboration of the vascular system results in the formation of the usual dictyostelic structure, and need be described at no further length.

The young plants of *Nephrodium setigerum* would be described as 'soft' plants by the cultivator, sclerenchyma being absent in the ground-tissue, a state of affairs very different from that obtaining in other ferns, e. g. *Pteris palmata*. The endodermis is well marked, but its cells are by no means in perfect seriation with those of the pericycle, which generally consists of but one layer of cells without the very abundant contents which so often characterize this layer. The vascular-tissue elements call for no special description except that the small sieve-tubes are well differentiated, and that the xylem contains a considerable amount of parenchyma, which frequently occurs as plates of cells in connexion with the phloem.

Nephrodium hirtipes, Hk.

The early transitional changes of *Nephrodium hirtipes* proved to be of quite an interesting character. Unfortunately, the writer was unable to verify his results in more than the five plants at his disposal, but the essential uniformity of the few examples investigated affords good reason for supposing that the phenomena observed are those obtaining generally in the plant.

The root is of the usual diarch character, and the passage to the protostele is of the normal type. One or two parenchyma cells appear in the xylem, but do not form a central core as is usually the case. Sieve-tubes are not differentiated. The xylem does not long retain its rod-like character, but quickly becomes irregularly angled as seen in transverse section (Fig. 37); protoxylem elements are not distinguishable at this stage. At a higher level, the irregularities have largely disappeared, and the vascular tissue is in the form of a slightly curved band with somewhat sharply pointed horns (Fig. 38). As soon as the vascular strand has reached this condition, the first leaf-trace is differentiated in a manner remarkably different from that occurring in other ferns. From each of the sharp horns of the arc a concentric strand is constricted off, the two strands gradually passing outwards. The distance between them is maintained until just before the base of the petiole is reached, when they suddenly unite, entering the petiole as a single concentric rod.

The early appearance of such a double leaf-trace is very different from the usual state of affairs, the more so as it appears spontaneously, as it were, without any intermediate stages being passed through. Again, the method of formation of the trace is very unusual, reminding one of the simple process obtaining in *Polypodium aureum* (q. v.). During the exit of the trace, the vascular arc becomes attenuated at its middle, the ground-tissue 'encroaching upon' it at this point to such an extent that, on the completion of the leaf-trace, the cauline system consists of two separate concentric strands (Figs. 39, 40). One of the strands quickly divides into two parts (Fig. 41), but later the three strands thus formed unite into a band of vascular tissue, which at a higher level becomes broad and sharply bent (Figs. 42–44). Fig. 45 shows in ground-plan the differentiation of the next leaf-trace. It is formed in the same way as its predecessor, and like the latter enters the leaf-stalk as a single strand. One of the leaf-strands separates from the cauline system at a slightly lower level than the other (Figs. 44, 45).

Subsequent changes are very interesting in relation to what has gone before, since the third leaf-trace, instead of being double like those preceding it, is single at its origin, although the xylem is slightly bilobed; indeed, the double and, in some cases, multiple character of the later-formed leaf-traces is only arrived at after the preceding traces, from the third onwards, have passed through the usual progressive stages of complexity, starting with a petiolar bundle in which the xylem is merely bilobed, and ending with a leaf-trace composed of two or more separate concentric strands, each surrounded by a pericycle and endodermis. The chief stages in the process are as follows:—After the exit of the second trace, the vascular band enlarges slightly, and its central portion passes out as the third leaf-trace; the xylem of the latter is, as mentioned above, distinctly bilobed. After the differentiation of the third trace, one of the cauline

strands divides into two (Fig. 47) and the subsequent union at a higher level, indicated in Fig. 48, takes place. The fourth leaf-trace is double at its origin (Fig. 49), but before it enters the leaf-stalk the two strands have united. In Fig. 50 we have three cauline strands, and at higher levels in older plants several appear in a transverse section of the stem. The increase in number is, of course, due to the division of the vascular strands before the previous leaf-gap has been repaired, and the repetition of such a process results in the dictyostelic arrangement of the vascular system of adult plants being attained.

The leaf-traces maintain their double character for successively longer periods, with the final result that the petioles of the later leaves possess two or more strands.

Nephrodium hirtipes has previously been examined by Jeffrey, and referred to by that author in his Royal Society memoir [1]. The details of the vascular development, however, were not described.

Asplenium nidus, L.

The vascular strand of the young primary root is extremely small, consisting of but a few sieve-tubes arranged on either side of a minute xylem plate. The first leaf-trace is given off quite early, before the xylem has lost its diarch character, and is quickly followed by a root-trace. The leaf-trace arises merely as a nipping off of one of the protoxylem groups, and is formed in a manner very similar to that obtaining in *Polypodium aureum*. As is so generally the case, the first few leaf-traces are of simple structure, consisting of two or three tracheides surrounded by a single layer of poorly differentiated sieve-tubes. The corresponding root-traces are much more definite, the well-marked endodermis being particularly conspicuous when the rootlets are viewed transversely.

A certain amount of variation was met with in the early transitional region. In a few cases, the first two or three leaf-traces were formed *before* a central pith of phloem had appeared in the xylem strand (cf. *Todea Fraseri* [2], *Lygodium* [3], and *Angiopteris* [4]), but, generally, parenchyma cells and extremely well-marked sieve-tubes were differentiated at the centre of the xylem immediately after the first leaf-trace. Higher up the stem the central phloem increases in quantity, and after the second leaf-gap has closed in the usual manner, a transverse section of the stem presents the appearance found to be so common in the other plants investigated, viz., a ring of xylem surrounded by phloem, and enclosing a strand of phloem

[1] Jeffrey, Structure and development of the stem in the Pteridophyta and Gymnosperms. Phil. Trans., ser. B., cxcv, 1902, p. 131.

[2] q. v. [3] Boodle, Schizaeaceae. Annals of Botany, xv.

[4] Farmer and Hill, On the arrangement of the vascular strands in *Angiopteris evecta*. Annals of Botany, xvi.

in which the sieve-tubes take up a central position. The pericycle is composed of relatively large cells, and is succeeded by an obvious but not particularly well-differentiated endodermis.

The third and fourth leaf-traces are formed in the same manner as the second, the external and internal phloem becoming continuous at the gaps, which are merely bridged by the endodermis. The pericycle gradually becomes two or three layers in thickness. After the exit of about the fourth trace, preparations are made for the change of the leaf-strand from a single to a double character. The change is effected in the usual way, viz., by the breaking of the curved xylem band into two before the closure of the previous gap, followed by a nipping off of a concentric strand from each of the vascular horns so formed. The two leaf-strands, however, unite immediately they leave the cauline system, so that the actual trace is single as before. At the level of the fifth or sixth leaf-trace we have the differentiation of a ground-tissue pocket of the usual type, a single cell, often containing tannin or mucilage, and surrounded by a well-marked endodermis, appearing in the central phloem. A little higher up, this internal endodermis surrounds a small group of ground-parenchyma cells, which become continuous with the main mass of ground-tissue at the next leaf-gap. At other times the endodermis appears immediately after the exit of a leaf-trace, and becomes continuous with the outer layer before the corresponding gap has been closed. In both cases the net result is the same, viz., the separation of the cauline vascular system into two concentric strands each surrounded by an endodermis (Fig. 82).

Later changes are represented in the diagrams. One of the two cauline strands divides into two parts for the insertion of the double leaf-trace, and one part immediately joins with the other main cauline strand, thus closing the previous leaf-gap. The vascular system still consists, therefore, of two concentric strands (Figs. 83, 84). The leaf-trace is then given off; as before, it is double at its origin, but enters the leaf as a single vascular rod. Figs. 86–88 illustrate the closing of the previous gap and the differentiation of the next leaf-trace. At the level of about the ninth leaf, the splitting of the cauline strand and the subsequent formation of the trace takes place *before* the closure of the previous gap, i. e. the gaps 'overlap,' and we consequently have the appearance of three cauline strands (Figs. 89, 90).

Subsequent elaboration need be described in no great detail. By the repeated overlapping of the gaps, the number of cauline strands at succeeding levels is gradually increased, and the dictyostelic structure of the mature plant is soon reached. From about the eighth leaf onwards, the two strands of the leaf-trace remain separate through increasingly longer intervals until they enter the petiole as two, or in the later leaves, more than two, separate strands.

Aspidium falcatum, Sw.

The young plants are characterized by the total absence of sclerenchymatous ground-tissue, even in the rootlets. The transition from the diarch root to the protostele extends over a considerable distance, and, as in *Nephrodium setigerum*, the completion of the phloem sheath is delayed for some time. The xylem of the protostele forms a very small rod of tissue, at the centre of which parenchyma cells are differentiated, quickly succeeded by very obvious sieve-tubes.

The first lateral rootlet generally effects its junction with the stem just before the exit of the first leaf-trace, which is formed in a perfectly normal manner, leaving a small gap bridged by the endodermis. In examining a large number of seedlings, some little variation was observed in connexion with the formation of this leaf-trace, a variation occurring more than once being represented in Figs. 129–132. It will be seen that the xylem ring has opened, and that from one horn a concentric strand of vascular tissue is nipped off which passes outwards as the leaf-trace. In the majority of cases, however, the trace was perfectly normal, and on repair of the gap a ring of xylem enclosing and enclosed by phloem constituted the cauline strand.

The second and third leaf-traces are formed in rapid succession nearly opposite one another, the gaps closing almost immediately. Their exit is followed by a long internode, and then another pair of leaf-strands is differentiated. This regular alternation of long and short internodes held true for all the comparatively young plants examined.

In connexion with the fifth trace we have the appearance of a ground-tissue pocket. The internal sieve-tubes arrange themselves towards the periphery of the internal phloem and therefore lining the xylem, and in the central parenchyma a poorly differentiated patch of endodermal cells appears, which, a little distance above, surrounds a strand of ground-tissue, the latter becoming continuous at the leaf-gap with the main mass of fundamental parenchyma. After the completion of the trace, another leaf-strand is formed exactly opposite the last, and the cauline system then consists of two strands which finally fuse.

Unfortunately, older plants were not available, but the above phenomena are so similar to those described for other types that there can be little doubt that the subsequent fractionation of the vascular tissue takes place in a manner similar to that already described.

Pteris palmata, Willd.

The transitional changes are of the usual type. The root is diarch, the xylem plate rapidly becoming circular in section and surrounded by the phloem. The appearance of phloem in the protostele takes place

before the first leaf-trace. The first two leaf-strands are quite simple, and the resulting gaps are merely bridged by the endodermis. At about the third leaf-trace a fundamental-tissue pocket appears in the central phloem in a way almost identical with that described above for other ferns. A patch of endodermal cells appears, quickly followed at a higher level by an endodermis surrounding a patch of ground-parenchyma cells containing tannin. The inner endodermis becomes continuous with the outer at the succeeding leaf-gap, and the subsequent elaborative changes of the vascular system are essentially the same as in the case of other ferns with similar fundamental-tissue pockets.

The most striking character in the anatomy of this plant is the abnormal width of the pericycle. The excessive width results not only from the treble, and sometimes quadruple nature of the layer, but also from the large size of the cells themselves. The latter are densely crowded with starch, and are not arranged in radial rows. The ground-tissue in young plants, or in the lower parts of older ones, is in striking contrast with the thin-walled pericycle. It is wholly composed of cells with thick, brown, sclerotic walls, even the outer tangential walls of the endodermis being strongly sclerosed; higher up the plant, however, a thin-walled band of ground-tissue separates the vascular strand from the outer sclerotic portion. It will be remembered that an exactly similar state of affairs occurs in *Osmunda*, and also in *Lygodium*, as described by Boodle.

Todea Fraseri, H. and G.

By the kindness of Mr. Boodle the writer had the opportunity of examining seedlings of two species of *Todea*, viz. *T. hymenophylloides* and *T. Fraseri*. Examination of the former fully confirmed the account of the transitional region given by Seward and Ford [1], who found in this fern an essential agreement with the transitional changes occurring in *Osmunda regalis* (Leclerc du Sablon).

The changes occurring in *T. Fraseri* closely resemble those of *T. hymenophylloides*. The root possesses a very simple vascular strand, the xylem being diarch and consisting of but a few tracheides. The sieve-tubes are somewhat ill-defined, as is also the endodermis. The ground-tissue is highly scelerotic. The usual change of the xylem plate to a solid strand occurs, and the first leaf-trace is formed while the xylem of the protostele is still very small in amount, consisting of about six or seven tracheides. No leaf-gap is formed, a few tracheides surrounded by phloem being merely nipped off from the stem strand. After the first trace, the xylem increases in quantity, and the sieve-tubes become much more prominent. The endodermis is very indistinct, and readily torn in the process of cutting; in

[1] The anatomy of *Todea*. Trans. Linn. Soc., 2nd ser., vol. vi, 1903.

favourable sections, however, it was seen that its cells were not in seriation with the pericycle cells, thus confirming a similar statement made by Seward and Ford with regard to *T. hymenophylloides.*

Dicksonia antarctica, Labill.

The development of the vascular system in Cyatheaceae has received but little attention. As far as the writer is aware, the only account of the transitional changes is that occurring in Gwynne-Vaughan's second paper on Solenostelic Ferns [1], in which the author describes the young stages of *Alsophila excelsa.* In addition to the latter plant, the writer has had the opportunity of examining *Dicksonia antarctica.*

The vascular strand of the primary root is of the normal diarch type, resembling that of *Alsophila excelsa* in being extremely small. The vascular elements, however, especially the sieve-tubes, are well differentiated. The usual transition to the solid protostele occurs, and a single parenchyma cell, quickly followed by two or three others, is differentiated in the xylem rod. In the majority of the plants examined this parenchyma strand occupied a markedly excentric position, the appearance presented by this isolated section reminding one of the normal structure of *Lindsaya.* The excentric position in *Dicksonia,* however, is evidently merely connected with the exit of the first leaf-trace, which is concentric in structure, and formed in a perfectly simple manner. It will be noticed that no sieve-tubes appear in the central parenchyma before the exit of the first trace, a state of affairs differing from that obtaining in *Alsophila.* In one case, however, a well-marked sieve-tube was observed in the parenchyma between the cauline strand and the outgoing leaf-trace, and this point, in conjunction with the fact that only four specimens of this fern were available for examination, would seem to indicate that, in all probability, internal phloem is differentiated *before* the first leaf-trace, just as described for *Alsophila.*

At the exit of the trace, phloem appears on the inner surface of the vascular arc, and, after the closing of the gap, we have a ring of xylem enclosing a strand of phloem, largely parenchymatous, and surrounded by a phloem sheath in which the narrow deeply-staining sieve-tubes are very conspicuous. At this level a considerable amount of parenchyma occurs in the xylem, often so arranged as to break the xylem ring into two or three arcs. The ring-like character of the vascular tissue as a whole, however, is perfectly obvious.

The vascular ring then becomes strongly elliptical in outline, receiving a root-trace at one end of the ellipse. The next leaf-trace is formed exactly opposite the root, its gap closing somewhat abruptly. During

[1] Observations on the anatomy of Solenostelic Ferns, II. Annals of Botany, xvii.

these changes the vascular tissue has increased greatly in size, the xylem, as before, being still somewhat parenchymatous. The next leaf-trace is very quickly formed, exactly opposite the last; the corresponding gap soon closes, and the following leaf-strand shows distinct indications of a double character which no doubt obtains in later leaf-traces. The repair of this gap results in the completion of the vascular ring, a condition which is maintained unaltered for some little distance. In connexion with the next leaf-trace, however, we have, just as in *Alsophila*, the formation of a typical ground-tissue pocket, the apex of the pocket in this case being blunt, and occupying a considerable portion of the central phloem. The actual course of events is as follows:—Several cells, some eight or nine in number, and possessing thickened refractive walls, appear at the centre of the phloem and persist through four or five sections. These cells are no doubt endodermal in function, and are succeeded by a patch of parenchymatous cells resembling those of the ground-tissue and surrounded by a definite endodermis, an isolated section presenting a 'gamostelic' appearance. As in similar cases described, the inner and outer endodermis become continuous at the gap, and the pocket-like character of the central ground-parenchyma is demonstrated.

Unfortunately, no older plants were available for examination. The great similarity between the above early transitional changes and those described as occurring in the young plant of *Alsophila*, leaves little doubt that subsequent changes in *Dicksonia* are essentially the same as those given in the excellent account of Gwynne-Vaughan.

Conclusions.

A discussion of the results arrived at in the above research involves a consideration of the stelar question. It would be serving no useful purpose, however, to follow in detail the various changes of thought which have followed upon the enunciation of Van Tieghem's famous hypothesis, since the history of the question is of general knowledge, largely owing to the appearance, from time to time, of excellent *résumés* of the subject, prominent among which are those of Tansley [1], Faull [2], and Schoute [3].

At the present time, botanists are practically unanimous in regarding Van Tieghem's conception of polystely as no longer affording a real explanation of observed facts. His work fails chiefly because the investigation of individual types was not carried far enough, for there can be little doubt that, had Van Tieghem followed out the complete elaboration of the

[1] The Stelar Theory. Science Progress, 1896.
[2] The anatomy of the Osmundaceae. Botanical Gazette, xxxii.
[3] Die Stelär-Theorie. Groningen, 1902.

vascular system of an ordinary 'polystelic' type, the idea of a continuously branching protostele would never have been formulated. Van Tieghem's work was essentially an attempt to reduce to some common plan the various existing arrangements of vascular tissue, and as such deserved, and received, the greatest attention. That it has since been shown to be partially incorrect [1] in no way alters the fact that it has secured the greatest reward which can fall to the work of a pioneer, viz., the stimulation of investigation.

In 1897 Gwynne-Vaughan [2] published his well-known paper on Polystely in the genus *Primula*, in which it was conclusively shown that the idea of polystely, as advanced by Van Tieghem, must be given up for this genus, since the polystelic arrangement does not follow from the branching of an originally single vascular rod, but as a result of repeated perforations of a vascular tube by gaps occurring in connexion with the leaf-traces. This idea was elaborated and extended by Jeffrey in his memoirs of 1897 [3], 1900 [4], and 1902 [5]. According to this writer, there are two types of cauline central cylinder: the first, a solid xylem rod surrounded by phloem (protostele), and the second, the siphonostele, a ring or tube of xylem surrounded externally and internally by phloem and endodermis, the whole vascular tube being sheathed externally by the cortex, and enclosing a central medulla or pith [6]. Of these two types the protostele is regarded as the more primitive. The presence of an internal endodermis separating the vascular tissue from a non-'stelar' pith is an essential point of the theory. As is so well known, the interruption of this vascular tube by the exit of the leaf-traces, followed later in life by an elaborate overlapping of the foliar gaps, results in the polystelic, or preferably dictyostelic [7], type so characteristic of the ferns.

This is the view which meets with very general acceptance at the present day, and the readiness with which its author has extended it to all the great classes of vascular plants makes it all the more convincing. That, however, the theory is not entirely satisfactory is evidenced by the fact that from time to time other views have been put forward, which, although essentially based upon the conception of Jeffrey, nevertheless differ sufficiently from it as to warrant full consideration.

The first of these is that put forward by Boodle [8] in his series of papers on fern anatomy. This view, defined as briefly as possible, appears to be that the whole ring of vascular strands as seen in a transverse section of the stem of a dictyostelic leptosporangiate fern, together with the mass of parenchyma included within the ring, corresponds to, or is homologous with, Van Tieghem's medullated monostele. It follows that the 'pith'

[1] Cf. opinion of Schoute, loc. cit.
[2] Polystely in the genus *Primula*. Annals of Botany, xi.
[3] Trans. Brit. Assn., Toronto, 1897.
[4] Trans. Canad. Inst., 1900.
[5] Phil. Trans., 1902.
[6] Cf. Faull, loc. cit.
[7] Cf. Brebner, loc. cit.
[8] Schizaeaceae. Annals of Botany, xv, p. 404 *et seq.*

parenchyma is wholly distinct from the external or cortical parenchyma. In the light, however, of Gwynne-Vaughan's and Jeffrey's work, this view is, to many, somewhat difficult of acceptance. The weak point in the conception is the implied distinction drawn between the parenchyma outside and the parenchyma inside the ring of vascular strands. There seems to be no reason why any such distinction should be drawn between tissues which are shown by a study of the young plant to be part and parcel of one and the same thing, viz., the general ground-tissue.

The next view is that of Farmer and Hill [1] as put forward in their paper on *Angiopteris evecta*. Acknowledging the importance of Jeffrey's work in bringing the *vascular system as a whole* to the fore in all 'stelar' questions, the authors point out that the weakness of Jeffrey's theory is the retention of the endodermis as an indispensable constituent of the primitive siphonostele. As a result of their work on the Marattiaceae, and from the evidence afforded by *Helminthostachys*, *Botrychium*, and *Osmunda* as to the unreliability of the endodermis as a morphological criterion, the authors maintain that in dealing with all so-called stelar questions, tissues of two categories only need to be considered, viz., vascular and non-vascular.

Schoute, in his memoir already quoted, deals with the question in a very exhaustive manner. Not the least important service rendered by the author in this valuable essay, is his emphasizing a fact which is very apt to be overlooked, viz., that the results of modern research after all only confirm the main contention of Van Tieghem that the monostele is the primary structure in all vascular plants. This author, however, criticizes Van Tieghem's stelar theory from two points of view; firstly, from a study of the development of the plant, both from the embryo and from the growing point of the adult, and, secondly, from the point of view of comparative anatomy. As a final result of his work, Schoute concludes that 'in Stengel und Wurzel der Gefässpflanzen findet sich ein einziger Stelär-Typus, die Monostelie.'

Having thus briefly outlined the present position of the stelar question, the writer may perhaps be allowed to consider the bearing, if any, which the results of his investigations have upon the question.

The simplest type of vascular elaboration above described is (with the exception of *Polypodium aureum*) that of such a fern as *Doodia aspera*. It will be remembered that the primary condition of the vascular system is a solid vascular rod, which later appears with a central strand of tissue which is undoubtedly phloem. The differentiation of this central phloem in no way alters the essential character of the young cauline strand, which is that of a solid rod of vascular, as opposed to non-vascular, tissue. Following upon the appearance of the central phloem we have the formation of several leaf-traces which leave no real gaps in the ordinary sense of the

[1] Loc. cit.

word, the external phloem becoming continuous with the internal at each exit of a trace, and the endodermis merely stretching between the horns of the temporarily broken rod. It will be noticed that there is no question of a primitive xylem rod with a central strand of 'extra-stelar' parenchyma; still less is there any evidence of an internal endodermis. We merely have an amphiphloic vascular rod to which are attached, in the simplest manner, the vascular bundles which supply the leaves. Subsequent processes are merely an elaboration of this primitive strand, the resulting structure no doubt representing the most satisfactory compromise in meeting the requirements of several demands, prominent among which is the necessity for an efficient mechanical distribution of the tissues concerned. The steps by which the mature condition of the vascular tissue is reached are very simple.

At about the fourth or fifth leaf-trace, the gap is no longer very small, but of such a size that ground-tissue is differentiated between the horns of the gap, the ground-parenchyma everywhere being separated from the vascular tissue by the endodermis. Before the gap is closed, the vascular arc breaks at a point opposite it, and, from each of the two horns, a strand is nipped off, the two forming the first double leaf-trace. Soon afterwards, the first gap closes and is followed by a re-splitting for the next trace. In older plants, the overlapping of the gaps is the most striking feature, and a simple type of dictyostely is attained without the previous appearance of Jeffrey's siphonostele, consisting of an amphiphloic vascular ring with an internal and external endodermis.

Although the vascular system of *Doodia aspera* is undoubtedly the simplest type examined, the writer is of opinion that it does not represent the true phylogenetic development of the vascular system of the Polypodiaceae. It is best considered as a simplification of that other type of vascular elaboration which was found to be so general among the plants investigated, viz., that in which an early appearance of a ground-tissue pocket was a characteristic feature. It will be remembered that the appearance of the first ground-tissue pocket usually occurs at that region of the plant at which the leaf-traces show a transition from the single to the double character. This transition is not met with in *Doodia aspera*, and, further, the first double leaf-trace appears relatively early in this plant, supplying as it does the fifth leaf, whereas in the majority of other forms examined it did not appear until the level of the sixth or seventh leaf. That is to say, the stage at which, from the study of other forms, we should expect to meet with a ground-tissue pocket is omitted in this plant. To put the matter briefly, the type of vascular elaboration met with in *Doodia aspera* is probably best regarded as a 'short cut' to the adult dictyostelic arrangement. In the type exhibiting a ground-tissue pocket, the protostele, as usual, soon gives way to a ring of xylem surrounding and surrounded

by phloem ; the early leaf-traces are quite simple, and then suddenly in the central phloem we have the appearance, in surface view, of one or two endodermal cells, followed immediately by definite ground-tissue cells surrounded by a ring of elements possessing all the well-known characters of an endodermis. These phenomena may possibly be interpreted as meeting the demands of the plant for a more peripheral arrangement of its vascular tissues than has obtained hitherto, the demand being met by the differentiation, from the meristem, of a central cone of ground-tissue continuous with the general mass of ground-tissue, and with its apex pointing downwards. Certainly a most striking fact in connexion with this ground-tissue pocket is that the fundamental-parenchyma is at all points shut off from the vascular tissue by a continuous sheath of endodermal cells. It is quite unnecessary to describe the further changes resulting in the perfection of the mature dictyostelic structure, since they have been given in full elsewhere. The interest of these plants lies in the fact that, at a level immediately succeeding the appearance of the internal endodermis, we have a very transitory condition of affairs exactly resembling, in an isolated section, the structure of the primitive siphonostele demanded by Jeffrey's hypothesis (Fig. 201).

In the light of what has been previously said as to the origin of this ground-tissue pocket, its essential structure, and its relationship to the general mass of ground-tissue, the writer believes that the assignment of a morphological significance of fundamental importance to such an arrangement of tissues is, perhaps, unjustifiable. The reason for the appearance of the internal ground-tissue appears to be fairly obvious, if, with Jeffrey, we admit the important part which mechanical considerations must play in the elaboration of the vascular system. The plant, at the time when the pocket is differentiated, apparently requires a more peripheral arrangement of its vascular tissues, and the dilatation of the existing conducting-tissue is probably the least expensive and most efficient means of meeting the requirements of the case. Before the necessity for such dilatation arises, the vascular strand is amphiphloic in structure, and since the separation of vascular from non-vascular tissue is so very generally effected by the presence of an endodermal sheath, the differentiation of internal ground-tissue naturally results in the appearance of an amphiphloic and amphi-endodermic vascular ring to which it is unnecessary, perhaps, to ascribe any profound significance.

The position, therefore, which the writer is inclined to take up with regard to the evolution of the dictyostelic vascular system is that the latter is derived from a certain simple primitive type, as a result of meeting the demands of several requirements, the chief and most obvious of which are, (1) the efficient distribution of the vascular tissue, (2) the increase of the conducting system in the young plant. The primitive type is not regarded

as being a solid rod of xylem surrounded by phloem, and later an amphiphloic tube with an inner endodermis surrounding non-stelar tissue, but as a solid strand of vascular tissue which may be either a solid rod of xylem ensheathed by phloem (protostele), or a ring of xylem surrounded by, and surrounding, phloem (amphiphloic protostele).

It may be objected that at the very base of the young stem a few parenchyma cells are differentiated at the centre of the xylem immediately before the appearance of internal sieve-tubes, and that such parenchyma cannot be regarded as vascular tissue. Definite internal phloem is so quickly differentiated, however, that there can be little doubt that the parenchyma cells, which are in organic continuity with the internal phloem, are best regarded as potential phloem. The wholly vascular nature of the cauline strand at this level is further emphasized by the occasional differentiation of tracheides in the internal phloem, an example occurring in *Nephrodium setigerum* being illustrated in Fig. 202.

The insertion of the leaf-traces upon this primitive strand has resulted in the latter becoming moulded and modified along certain definite lines, the culminating structure being the dictyostelic complex. In all probability this arrangement of the vascular tissues is the most satisfactory for allowing an adequate supply of water and mineral food to reach the relatively large leaves. The portion of the cauline system actually in connexion with the leaf-strands is probably quite insufficient to afford this supply, and the latter is only effected as a result of the ready transference of water from one part of the rhizome to another, which is rendered possible by the complete network of the vascular strands.

It has been stated above that the ontogenetic elaboration of the vascular system proceeds along certain constant and well-defined lines. As has been shown by Boodle and others, not the least interesting point in connexion with this fact is that many of the admittedly more primitive ferns have proceeded for only a relatively short distance along these ontogenetic and, presumably, phylogenetic lines. The elaboration of their vascular system stops short, as it were, at some intermediate stage or condition which is adopted by the plant as the most suitable for its mature habit. Take, for example, a typical dictyostelic fern such as *Blechnum brasiliense*. The first stage is the solid protostele, a structure adopted as the mature organization by *Lygodium*. The appearance of central parenchyma follows, a condition which is exactly matched in *Schizaea*. Later, well-defined sieve-tubes appear in the central parenchyma, and this structure, although rare in mature plants, is found in *Lindsaya*[1]. The region at which we have the differentiation of the ground-tissue pocket is the most interesting in the whole series of elaborative processes. If we imagine this conical pocket continued through successive internodes (cases

[1] Tansley and Lulham, loc. cit.

in which it was continued through two internodes were noted above), then we have a condition of affairs resembling in all essential points the mature structure of ferns described by Gwynne-Vaughan as solenostelic[1]. That such solenostelic vascular systems have passed through the previously described ontogenetic conditions has been shown by Gwynne-Vaughan in *Alsophila*, and by the writer in *Dicksonia*. From the solenostelic to the dictyostelic type the changes are rapid and obvious. They merely depend upon the rates at which the leaf-gaps overlap one another, and the final result is the formation of a vascular network of the type familiar to every one.

A group of plants very interesting in this connexion is the Osmundaceae. De Bary[2] and Van Tieghem both recognized the striking difference of the vascular organization from the ordinary leptosporangiate type, and the latter author, from a study of seedlings, maintains that the plant in question possesses, as in the case of Phanerogams, a medullated monostele. The Osmundaceae have also received the attention of Leclerc du Sablon[3], and later of Zenetti[4], Faull[5], and Seward and Ford[6].

The work of Faull is of especial interest, since a type of vascular system new to the Osmundaceae was discovered in *Osmunda cinnamomea*. This plant, it will be remembered, possesses an internal endodermis; internal phloem, however, is absent, except occasionally at the ramular gaps. The internal endodermis, moreover, is sometimes continuous with the external endodermis through the foliar gaps, and generally so through the ramular gaps. It follows that at such regions the parenchyma surrounded by the internal endodermis, and the external ground-parenchyma, are put into continuity. The author concludes that the internal and external parenchyma are morphologically identical, and from this conclusion there can, perhaps, be little question of dissent; but the contention that the vascular system of *O. cinnamomea* represents a degenerate siphonostelic type is not so convincing. The present writer fully agrees with the criticism of Boodle[7], that the great weakness of Faull's position results from a neglect, no doubt compulsory, of the ontogeny of the vascular system of the plant concerned. In fact, the whole question depends upon *how* and *when* the internal endodermis with its included ground-parenchyma arises, and this can only be settled by a study of young plants. The writer believes that such a study will show that the internal endodermis arises as a necessary accompaniment of a typical ground-tissue pocket, similar to those described above, and differentiated early in the life of the plant. This pocket has, in all probability,

[1] Observations on the anatomy of the Solenostelic Ferns, Annals of Botany, xv.
[2] Loc. cit., p. 319. [3] Loc. cit.
[4] Das Leitungssystem in Stamm von *Osmunda regalis*. Bot. Zeit., 1895.
[5] Loc. cit. [6] Loc. cit.
[7] Further observations on *Schizaea*. Annals of Botany, xvii.

'persisted' through successive internodes, and is only occasionally in continuity with the external ground-tissue at the gaps. That is to say, the structure occurring in *O. cinnamomea* is very closely akin to that of solenostelic ferns, the absence of internal phloem being the chief difference, while the resemblance to *Platyzoma microphyllum*[1] is even more striking.

If the above contention should ultimately prove correct, it would have an important bearing upon the relative primitiveness of the different types of vascular organization occurring in the Osmundaceae. Faull believes that *O. cinnamomea* and *O. Claytoniana* stand at opposite ends of a series, with *O. regalis* as an intermediate form. Strictly speaking, there is no essential difference between the vascular systems of *O. regalis* and *O. Claytoniana*, and hence, for the purpose of comparison, they may be regarded as the same. Of this series Faull regards *O. cinnamomea* as the more primitive, i. e. least modified from the amphiphloic siphonostelic type, and derived from the siphonostele by 'degeneration.' From this it follows that *O. regalis* and *O. Claytoniana* have still further degenerated.

This position has already been subjected to criticism at the hands of Boodle, and, as a result of the present work, the writer is inclined to take a diametrically opposite view of the question. Admitting that a complete knowledge of the young vascular system in the Osmundaceae is urgently needed, he believes that comparison with other and fully-known types points to the conclusion that the type of vascular organization possessed by *O. regalis* and *O. Claytoniana* is that which is more primitive, and that the vascular system of *O. cinnamomea*, instead of being the result of degeneration, is, so to speak, really on the up-grade, and has, indeed, advanced a considerable distance along the phylogenetic road which at the present time leads to dictyostely.

The structure of *O. regalis* and *O. Claytoniana* can be plausibly explained if compared with the young vascular system of a Polypodiaceous fern. The earlier leaf-traces of the latter involve the differentiation of no real leaf-gaps—we have merely the xylem discontinuous at a certain point, and the external and internal phloem more or less continuous between the horns of the xylem. Now, if we consider a plant in which the internal potential vascular tissue does not assume the definite characters of phloem (and this is merely assuming the persistence of the earliest condition of a dictyostelic vascular system)[2], then this first 'leaf-gap' corresponds to a 'medullary ray' of *Osmunda*. If, further, we suppose the first and succeeding leaf-gaps to remain unclosed, we have a structure exactly resembling that found in mature plants of *Osmunda*

[1] Boodle, Comparative anatomy of the Hymenophyllaceae, Schizaeaceae, and Gleicheniaceae. III. Gleicheniaceae. Annals of Botany, xv.

[2] Cf. *Angiopteris evecta.*

regalis, and it follows that the pith of this species must be regarded as belonging to the central cylinder, as maintained by Van Tieghem. On the other hand, Faull's work clearly shows that the pith of *O. cinnamomea* is extra-stelar in nature. Consequently we must regard the pith in these two members of the same genus as of different morphological value, and the vascular arrangements in the two cases as fundamentally distinct. To some, the acceptance of this view would be a matter of difficulty, but it is by no means impossible to find a parallel, an almost equally striking difference in the arrangement of the vascular tissues occurring in the genus *Anemia*, where, in *A. mexicana* we have a solenostelic vascular system, in *A. Phyllitidis* a typical dictyostelic arrangement, and in *A. aurita* an arrangement closely approximating to that of *Lygodium*. If the validity of the above interpretation of the vascular systems of *Osmunda regalis* and *O. cinnamomea* be admitted, then it follows that the vascular organization of *O. regalis* is the more primitive, and that the type occurring in *O. cinnamomea* is less so.

The vascular organization of *O. cinnamomea*, therefore, may be regarded as having been evolved in two alternative ways; either as a result of degeneration from a typical siphonostelic structure, or, on the other hand, it may represent an intermediate halt on the direct line of advance to dictyostely. For reasons already given, the writer is inclined to regard the latter view as the more probable.

To sum up. As a result of the study of certain leptosporangiate ferns, the writer has been led to conclude that:—

(*a*) in dealing with questions of a so-called stelar character, we must confine our attention to tissues of two categories only, viz., vascular and non-vascular;

(*b*) the primitive type of vascular system in the ferns is a solid rod of vascular tissue, which may be a solid xylem strand surrounded by phloem, or an amphiphloic strand;

(*c*) the complex dictyostelic structure results from the moulding and elaboration of this solid vascular strand, the moulding and elaboration being largely due to the necessity for an efficient attachment of the leaf-traces;

(*d*) the differentiation of ground-tissue pockets plays an important part in such elaboration;

(*e*) the development of the vascular system proceeds along certain well-defined lines, and that practically all the intermediate stages have been adopted by different plants as most suited to their individual mature requirements;

(*f*) the ontogeny of the vascular system strikingly resembles what we must suppose to have been its phylogeny.

It would be impossible for the writer to conclude without expressing his grateful thanks to Professor J. B. Farmer, F.R.S., for the continuous interest he has taken in the above investigation. The work was carried out at the suggestion of Professor Farmer, and but for his kindness in allowing the writer the use of a table in his laboratory, the research would have been impossible.

Alphabetical List of Ferns Examined.

Aspidium falcatum, Sw.
Aspidium Tsus-Simense, Hk.
Asplenium bulbiferum, Forst.
Asplenium nidus, L.
Blechnum brasiliense, Desv.
Dicksonia antarctica, Labill.
Doodia aspera, R. Br.
Lomaria gibba, Labill.
Lomaria Spicant, Desv.
Nephrodium hirtipes, Hk.
Nephrodium setigerum, Baker.
Nephrodium spinulosum, var. *dilatatum*, Hk.
Nephrolepis cordifolia, Baker.
Nothochlaena sinuata, Kaulf.
Polypodium aureum, L.
Pteris palmata, Willd.
Todea Fraseri, H. and G.

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EXPLANATION OF FIGURES IN PLATES XVIII, XIX, AND XX.

Illustrating Dr. Chandler's paper on the Vascular Strands in Ferns.

In the diagrammatic figures the following scheme has been adopted :—Endodermis: Represented by a broken line. Phloem : Shaded. Xylem : Unshaded.

Figs. 1–12. *Doodia aspera*, R. Br.

Figs. 13–35. *Lomaria gibba*, Labill. *r.* root.

Figs. 36–50. *Nephrodium hirtipes*, Hk. *r.* root, *lt.* leaf-trace.

Figs. 51–76. *Lomaria Spicant*, Desv. *r.* root, *lt.* leaf-trace.

Figs. 77–90. *Asplenium nidus*, L. *St.* Sieve-tube, *r.* root, *lt.* leaf-trace, *i.e.* internal endodermis.

Figs. 91–128. *Polypodium aureum*, L. *Lt.* leaf-trace, *d. lt.* double leaf-trace, *r.* root, *p. s.* petiolar strand.

Figs. 129–132. *Aspidium falcatum*, Sw. *lt.* leaf-trace.

Figs. 133–151. *Nephrolepis cordifolia*, Hk. *c. s.* cauline strand, *stl.* stolon, *px.* protoxylem, *r.* root, *lt.* leaf-trace.

Figs. 152–164. *Asplenium bulbiferum*, Forst. *E.* Endodermal cells, *lt.* leaf-trace, *r.* root.

Figs. 165–171. *Aspidium Tsus-Simense*, Hk. *lt.* leaf-trace.

Figs. 172–196. *Nothochlaena sinuata*, Kaulf. *Lt.* leaf-trace, *r.* root, *scl.* sclerenchyma.

Fig. 197. *Doodia aspera*, R. Br. Protostele.

Fig. 198. *Doodia aspera*, R. Br. Differentiation of parenchyma at the centre of the solid xylem rod; *p.* parenchyma cells.

Fig. 199. *Doodia aspera*, R. Br. Differentiation of internal phloem; *ph.* phloem.

Fig. 200. *Doodia aspera*, R. Br. Transverse section of cauline vascular system after the exit of the first leaf-trace.

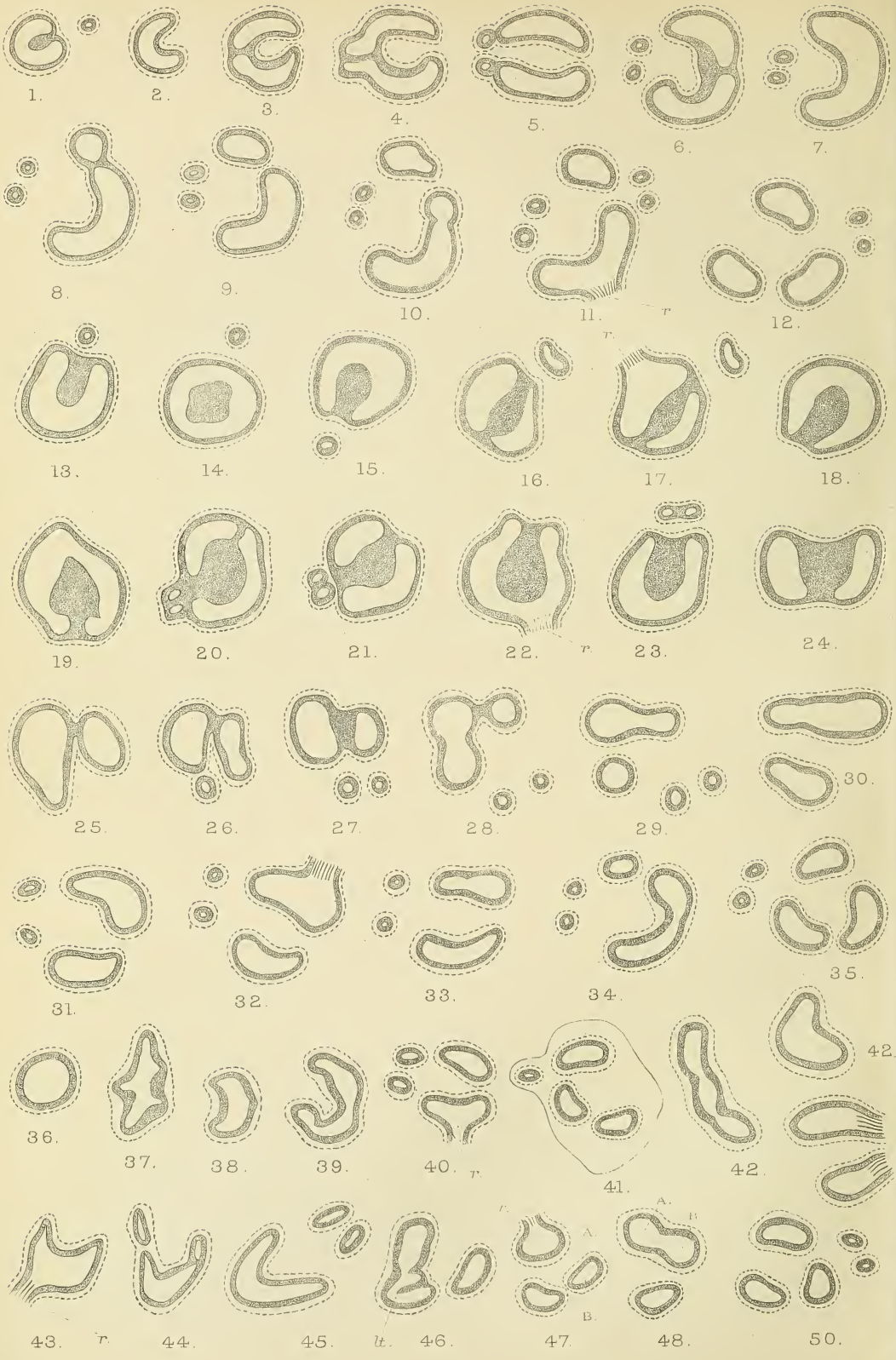
Fig. 201. *Doodia aspera*, R. Br. Transverse section of cauline system after the repair of the first leaf-gap; *st.* sieve-tubes.

Fig. 202. *Nephrodium setigerum*, Baker. Transverse section of the vascular system before the differentiation of the first leaf-trace. Isolated tracheides are shown in the central phloem.

Fig. 203. *Nephrodium setigerum*, Baker. Transverse section through vascular system at the region of a ground-tissue pocket; *i.e.* inner endodermis, *o. e.* outer endodermis, *lt.* leaf-trace.

Figs. 204–207. *Polypodium aureum*, L. Transverse sections of petiole at successively higher levels, showing the gradual fusion of the two petiolar strands.

Fig. 208. *Polypodium aureum*, L. Transverse section of rhizome showing wound-periderm.



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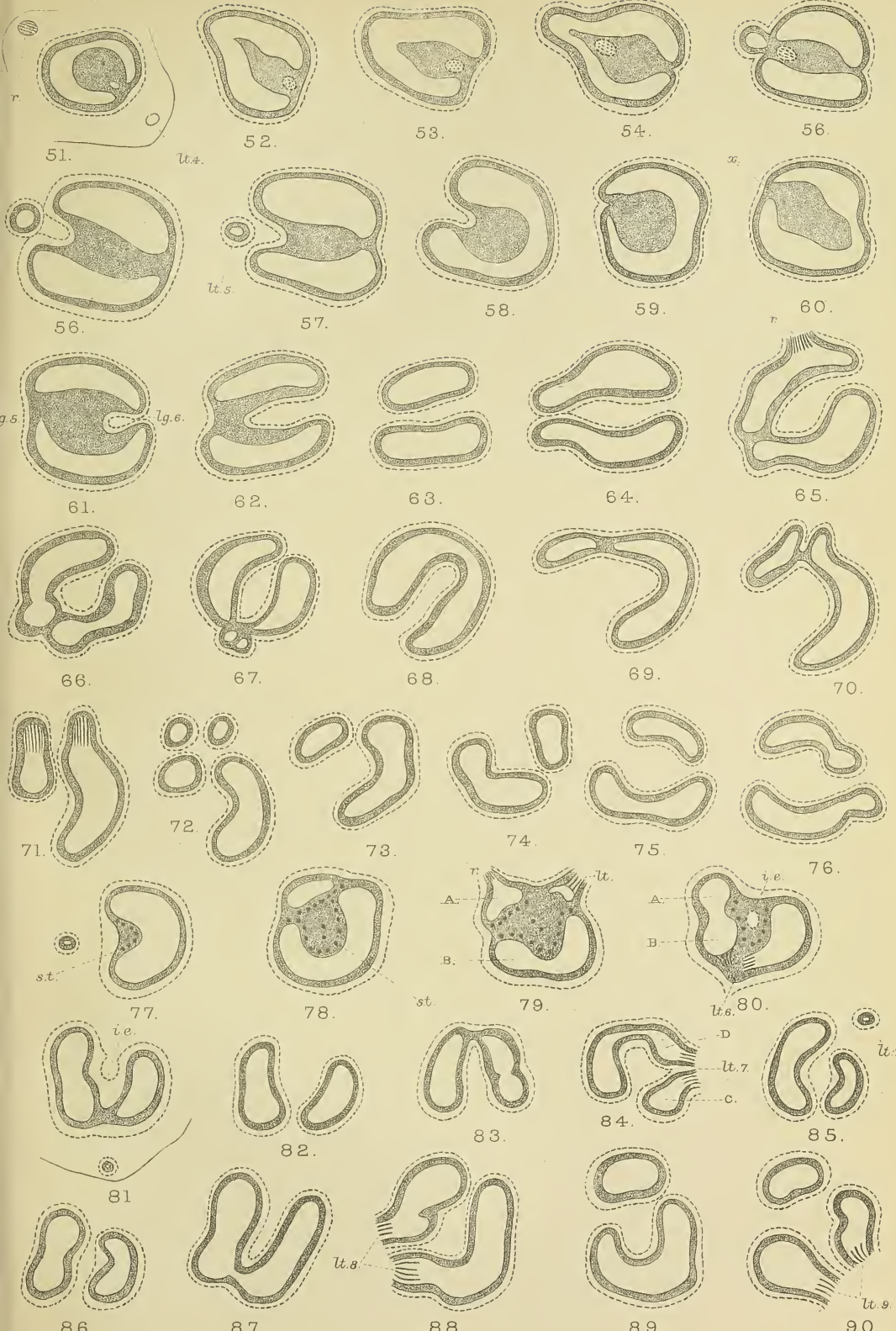
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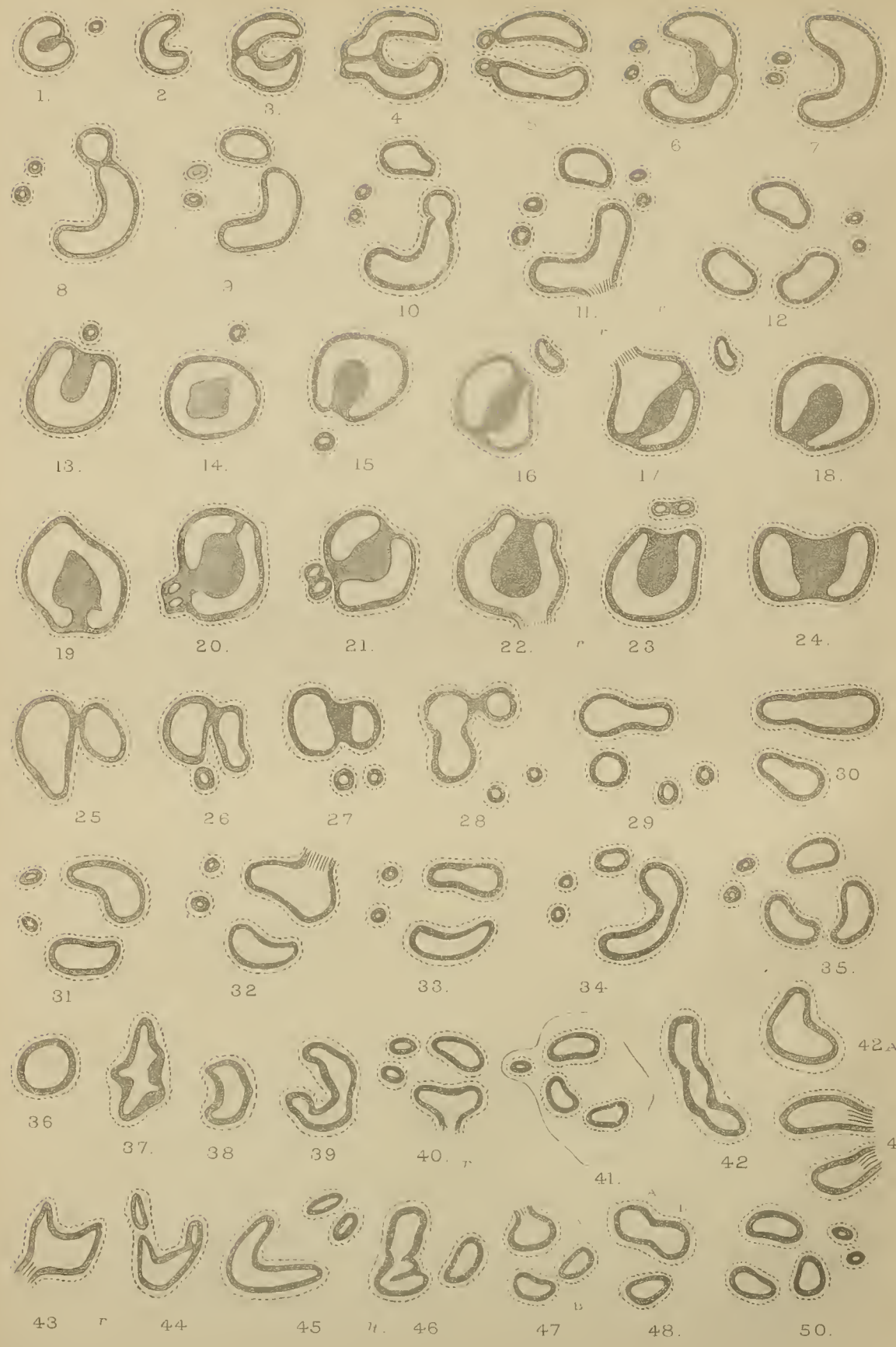

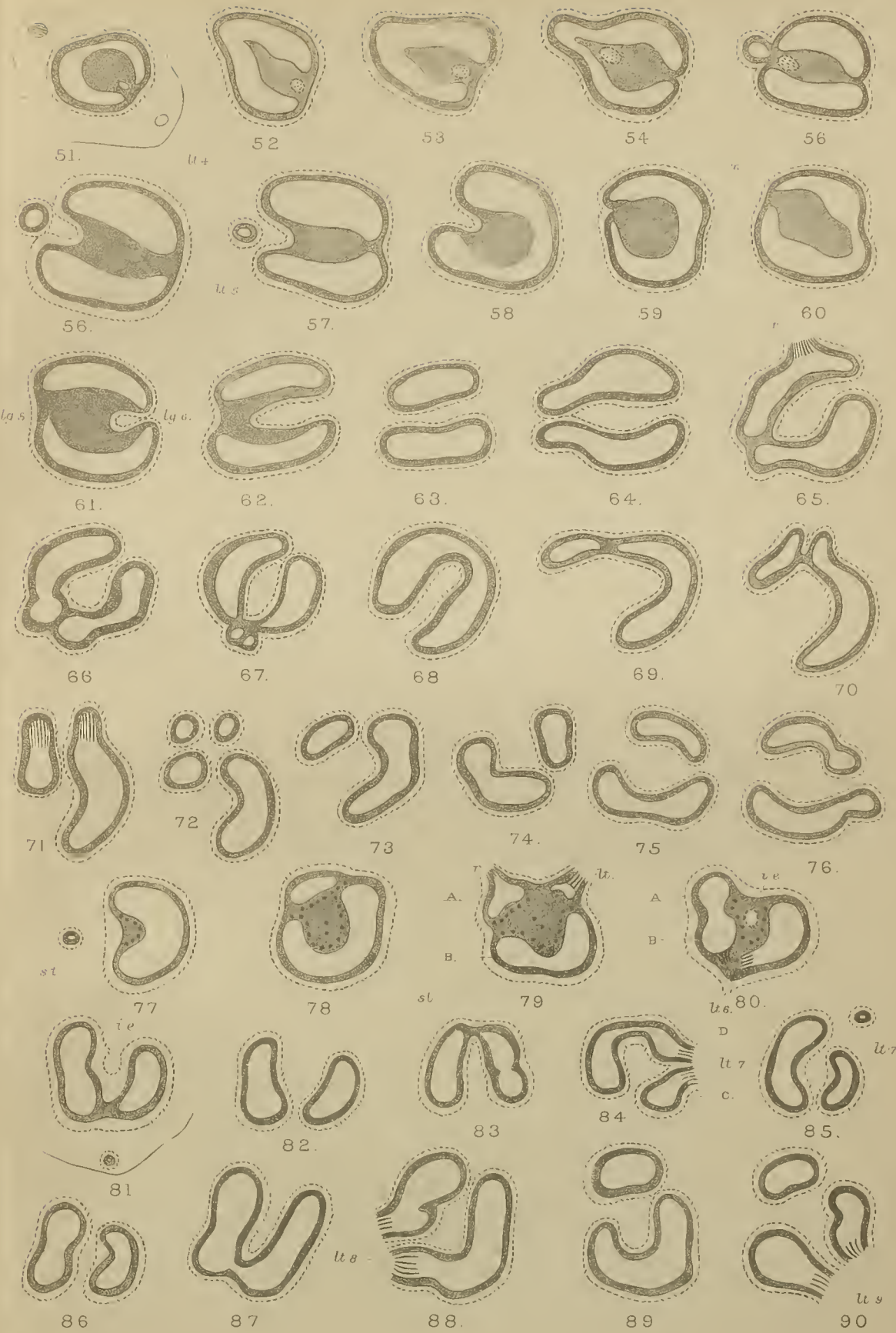

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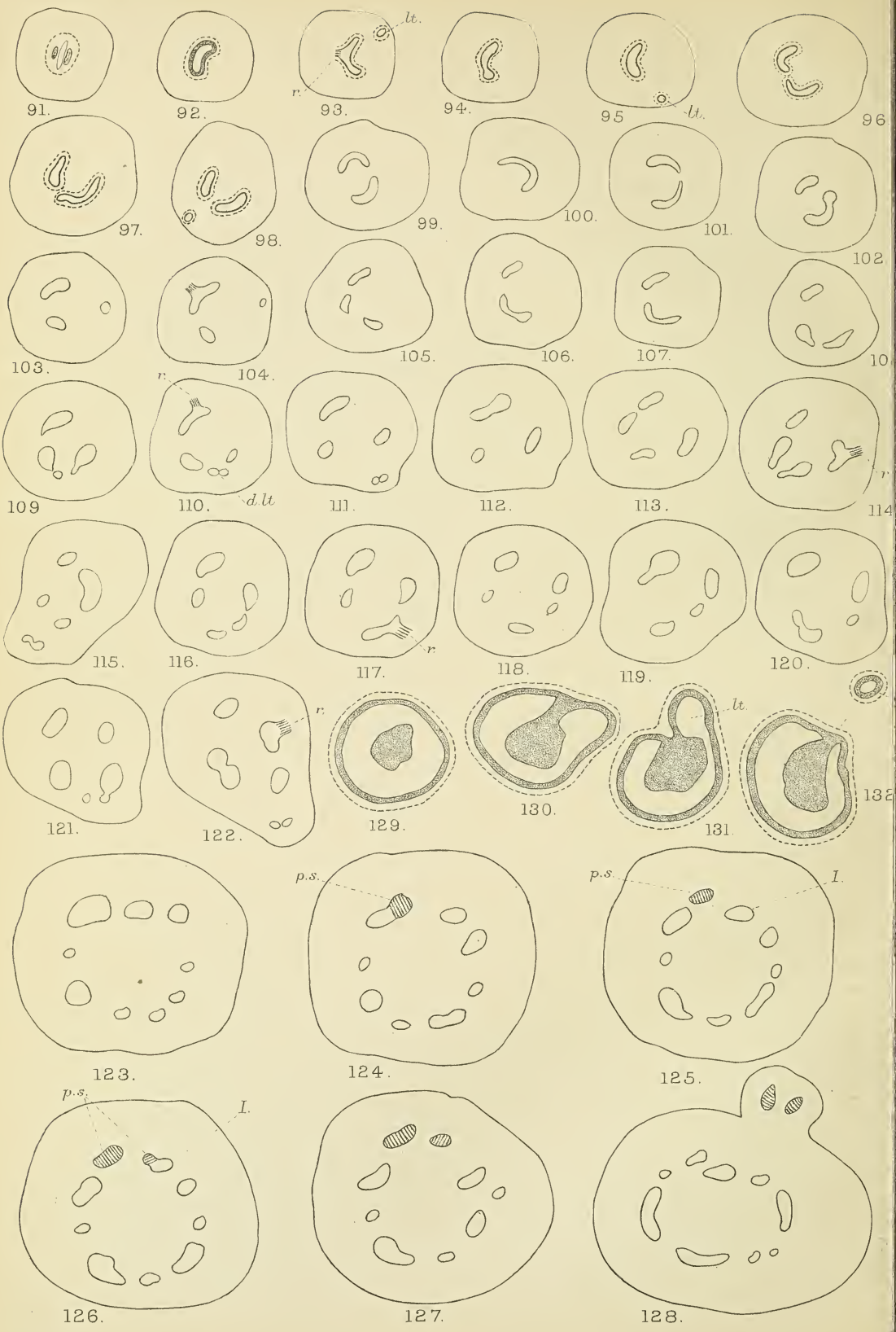

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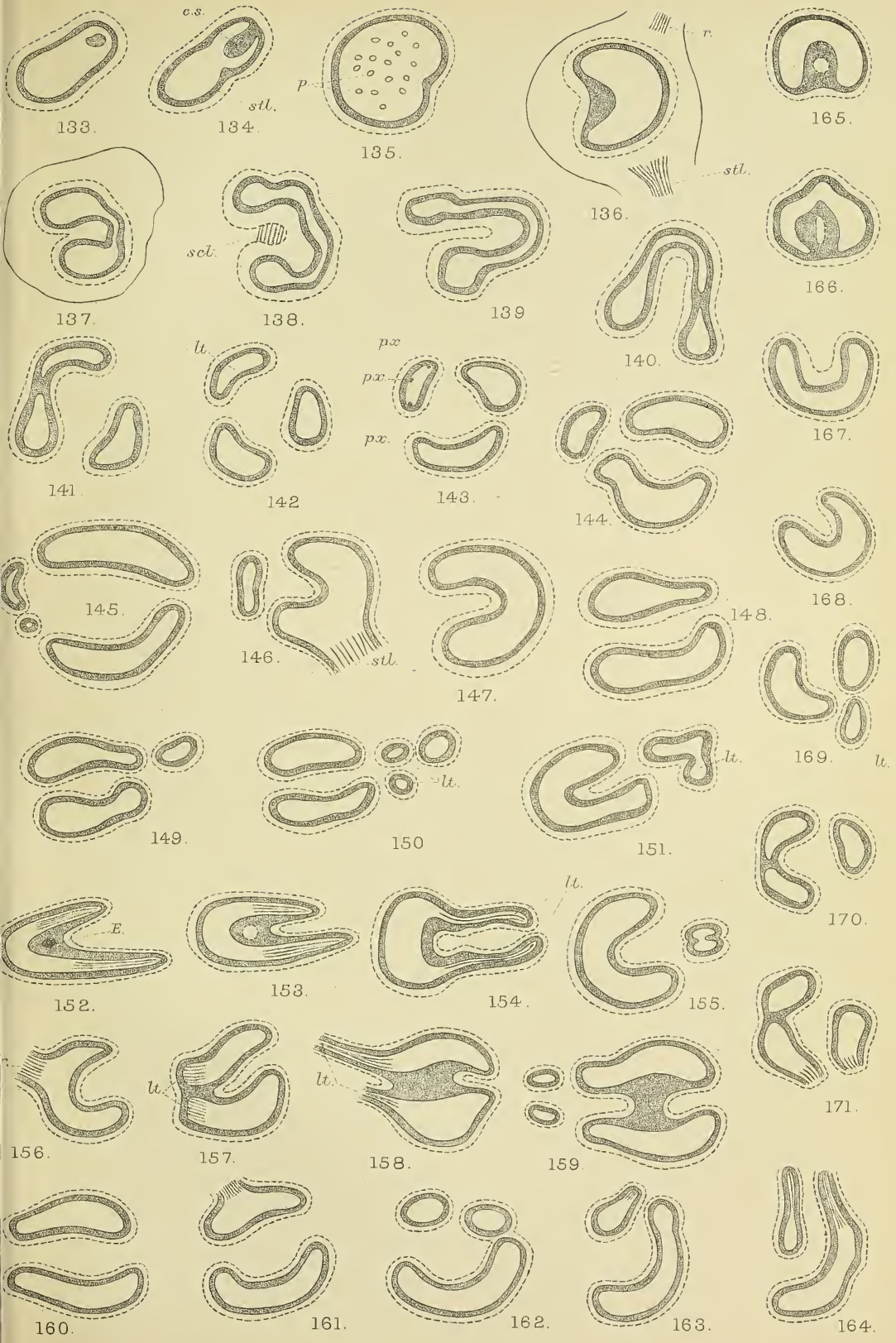
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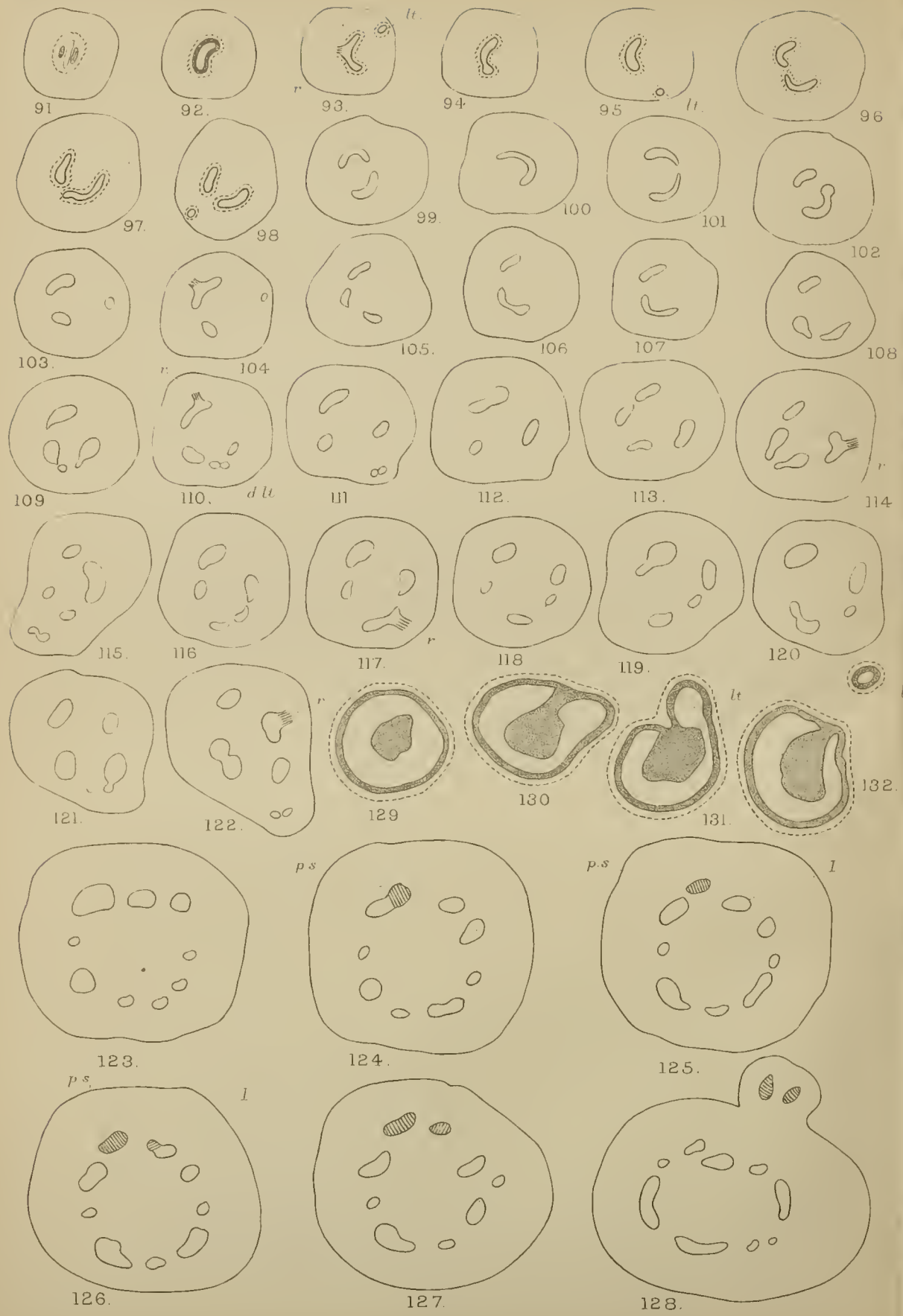

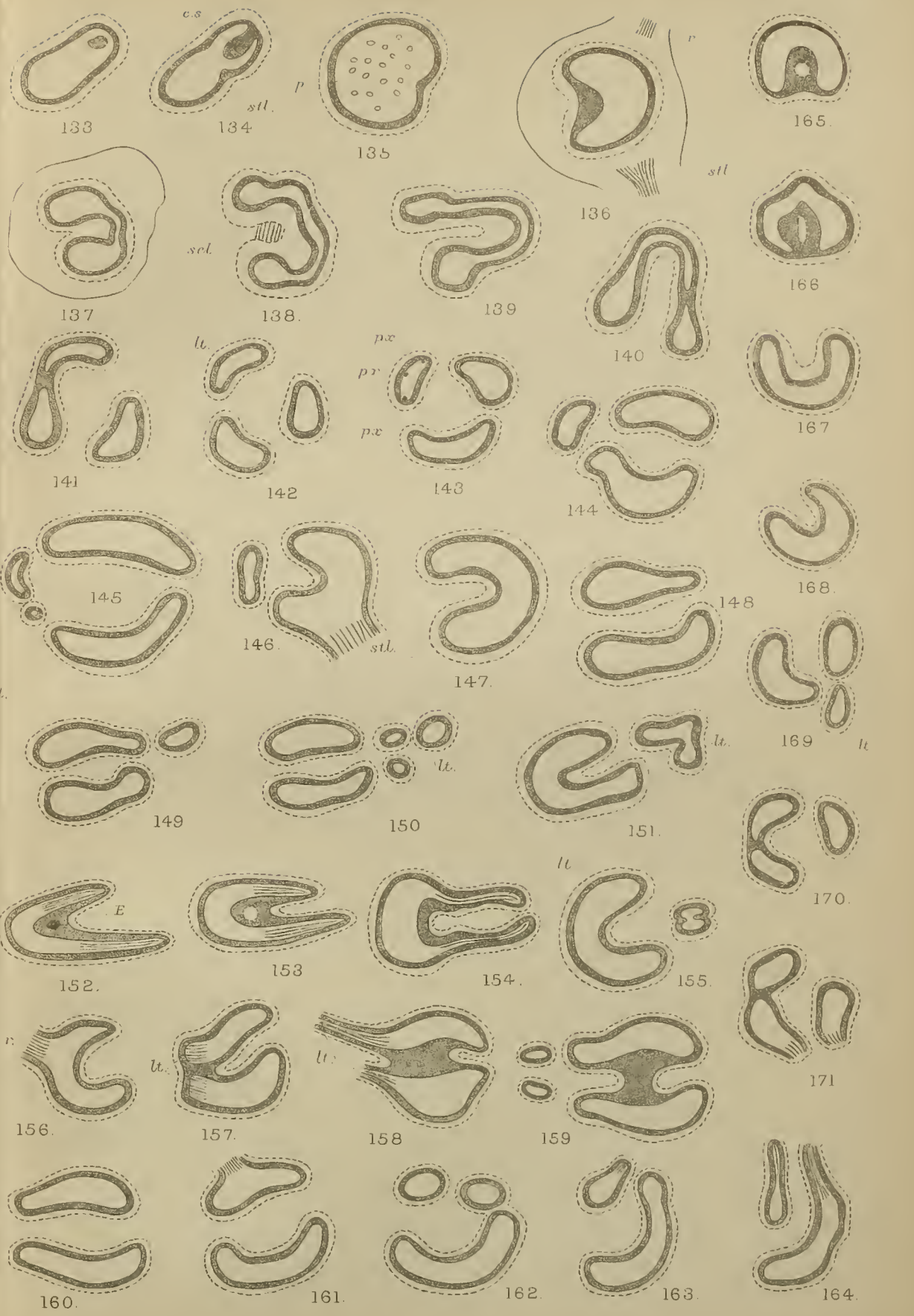

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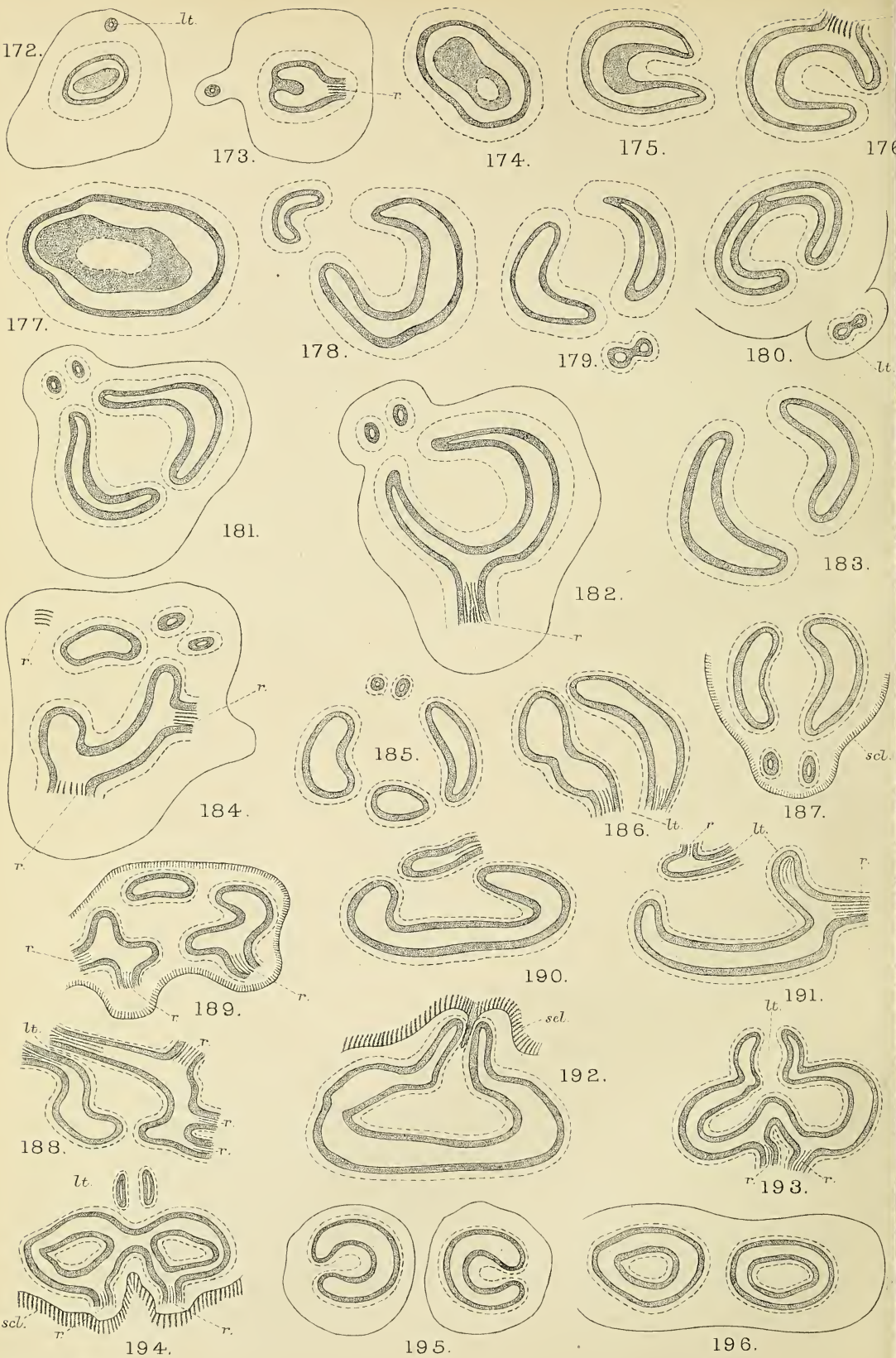
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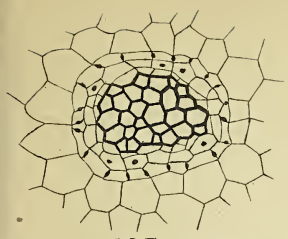

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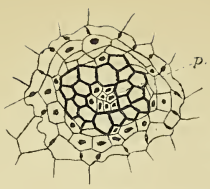

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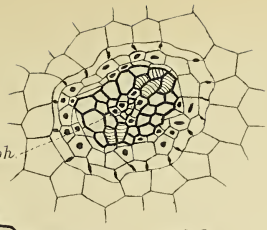

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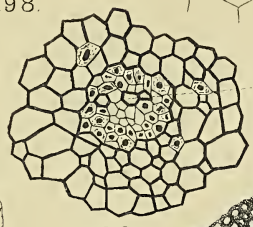

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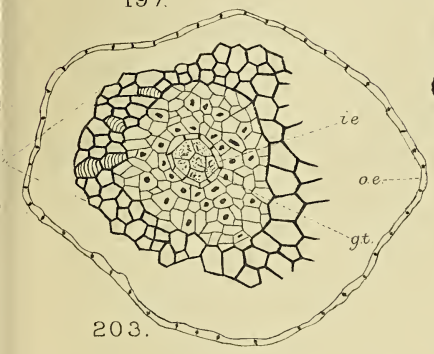

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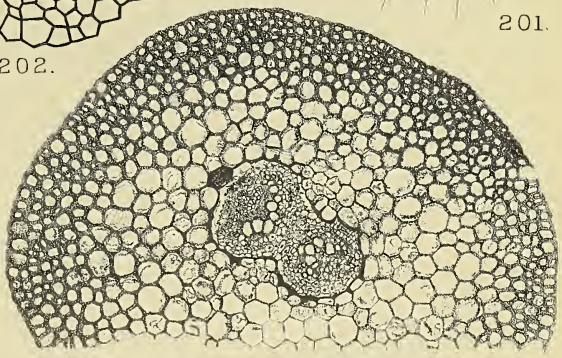

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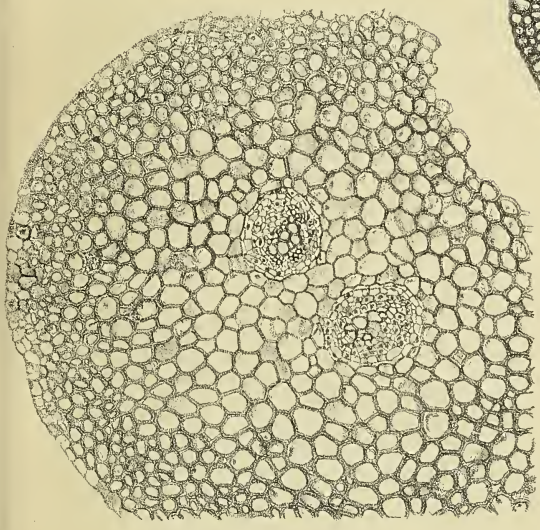

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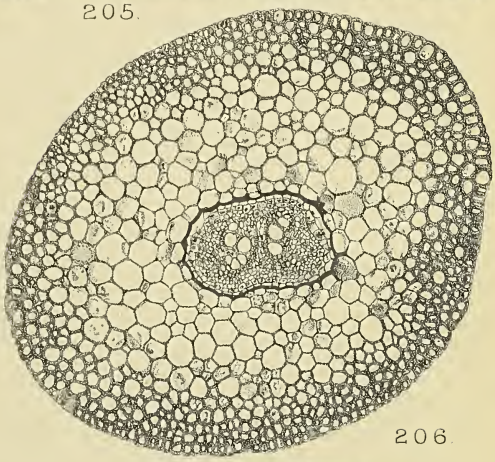

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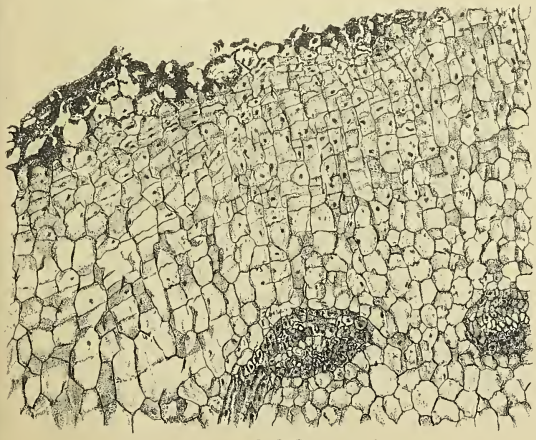

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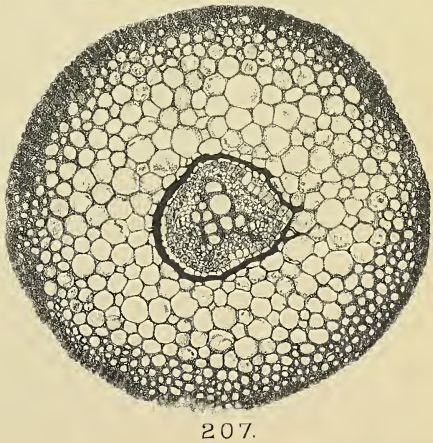

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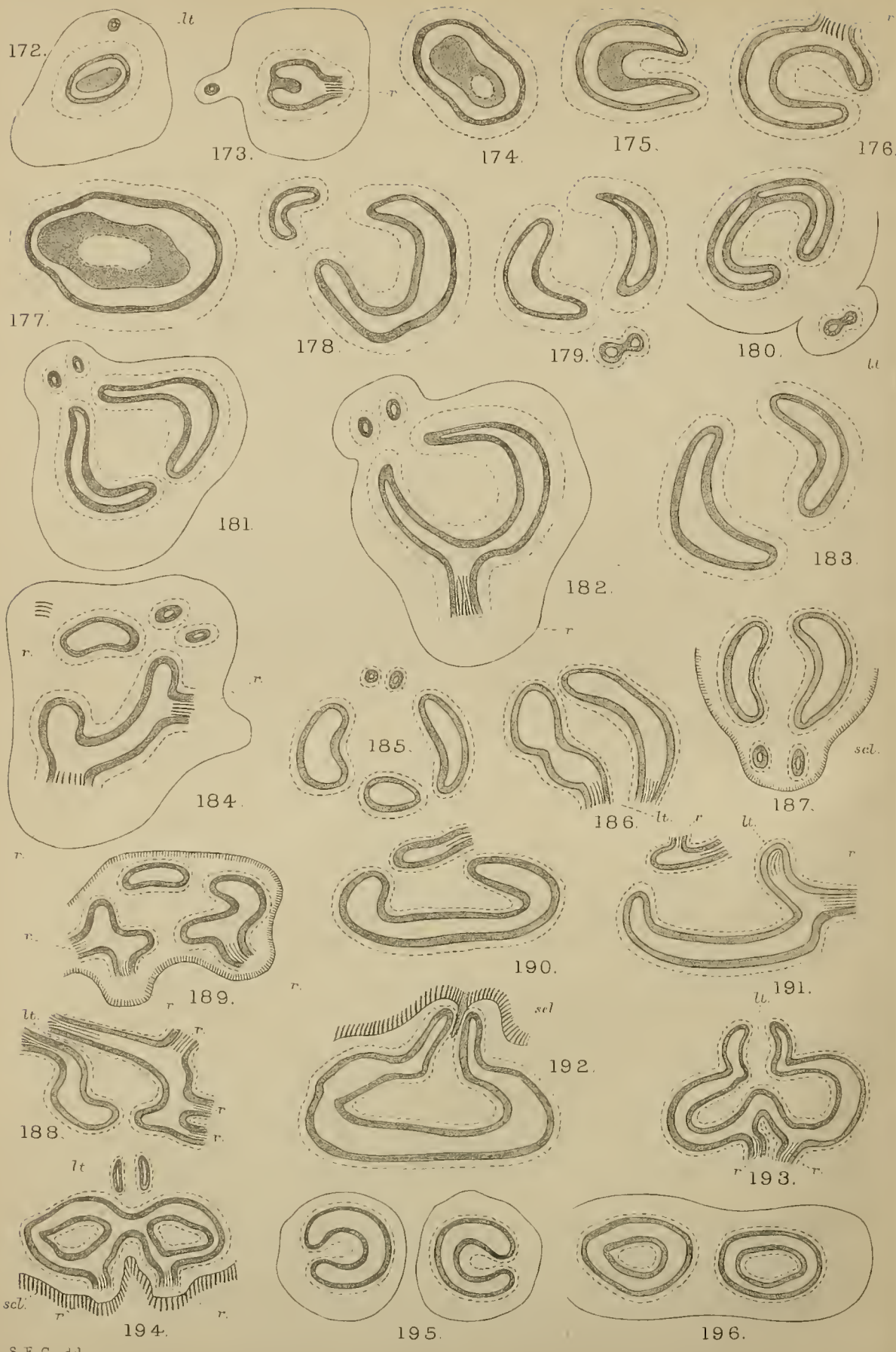

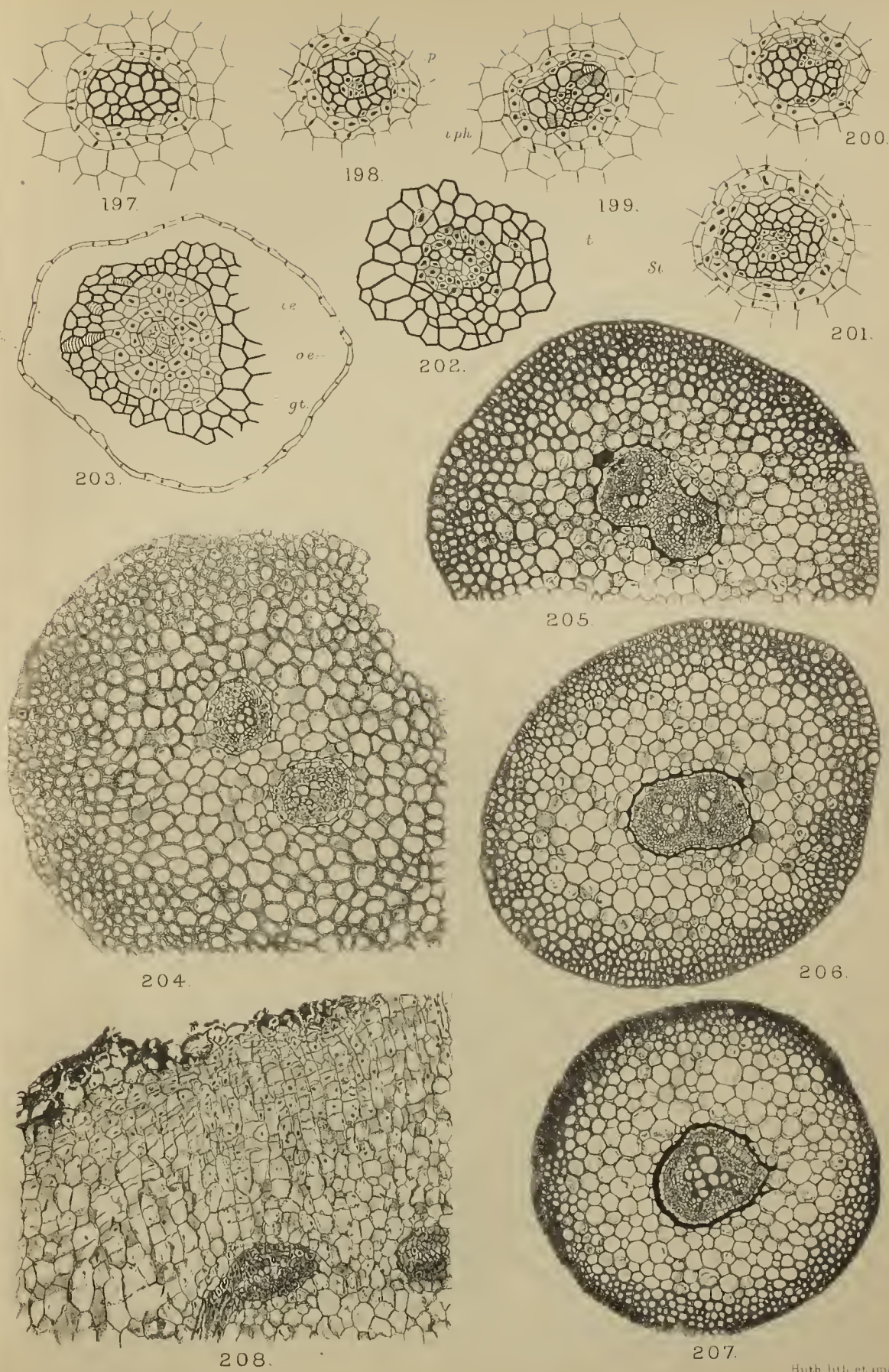

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CHANDLER — VASCULAR STRANDS OF FERNS.

On the Morphology of Cyathodium[1].

BY

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With Plates XXI and XXII.

OUR knowledge of the structure and development of *Cyathodium* is perhaps more obviously deficient than that regarding any other genus of the Marchantiaceae. Apart from systematic descriptions[2], which deal with the structural features of mature plants as displayed in herbarium material, the only considerable detailed description of its morphology is that given by Leitgeb[3], and his observations appear to have been done upon imperfect and probably dry material. Subsequent observers[4] have supplemented this by references to its vegetative structure, based on better preserved material, but there is a complete lack of information as to the development of the sexual organs and of the sporogonium. It has even remained uncertain whether Leitgeb's suggestion, that the antheridium differs from that of all other Archegoniatae in being a stalked unicellular structure, is correct or not.

The interest attaching to the study of this small genus of Liverworts is dependent not merely upon obtaining additional data for phylogenetic comparison, but upon the biological peculiarities of the plants composing it. The structure of the thallus in the majority of the Marchantiaceae may be regarded as adapted to life in exposed situations. The intake of water is limited to special regions of the thallus, and the system of air-chambers, communicating by definite air-pores with the external atmosphere, is such

[1] The expenses of collecting the material for this work were met by a grant of the Royal Society.

[2] Cf. Stephani, Species Hepaticarum, vol. i, p. 62, for a full diagnosis of the genus and species and for references to the original descriptions.

[3] Untersuchungen über die Lebermoose, Heft 6, p. 136, Taf. XI.

[4] Ruge, Beitr. z. Kenntniss d. Vegetationsorgane d. Lebermoose, Flora, 1893, p. 279; Stahl, Ueber bunte Laubblätter, Ann. Jard. Buit., 1896, p. 137, note on p. 201; Kamerling, Zur Biol. und Phys. der Marchantiaceen, Jena, 1897; Andreas, Ueber d. Bau d. Wand u. d. Oeffnungsweise d. Lebermoossporogons, Flora, 1899.

as to limit and control the loss of water vapour. This general view of the significance of the structural peculiarities of the Marchantiaceae is confirmed by the situations in which they are found most abundantly. My own experience, when collecting Hepaticae in Ceylon and the Malay Peninsula, was that the Marchantiaceae were most abundant in open exposed situations, and almost confined to such places ; that some forms also occurred by path-sides in the forest, but that they were almost absent from the depths of the forest itself, where thalloid Jungermanniaceae were abundant.

Among the Marchantiaceae there are, however, one or two genera which not only occur in relatively damp and shaded situations, but exhibit corresponding modifications in the structure of the thallus. In *Dumortiera* (and perhaps in the light of recent investigations *Monoclea* may be added) the air-chambers are rudimentary or absent, and transpiration and assimilation are carried on by the unprotected superficial cells of the thallus, which is thus physiologically comparable to that of *Aneura* or *Pellia*. In *Cyathodium* modifications of a different nature to those in *Dumortiera* appear to stand in relation to a damp and shady habitat. The few species occur in the deep shade of tropical forests, in dark caves, and sometimes in the crevices of walls or on paths in rather more exposed positions. In this genus the layer of air-chambers opening by definite pores is retained, but the basal portion of the thallus is for the most part only a single layer of cells thick. Filaments of assimilating cells are absent from the air-chambers, the epidermis of which has taken over the function of assimilation.

In the present state of our knowledge of the genus it seemed worth while to give the results of my investigation of two forms of *Cyathodium*, which bore sexual organs and sporogonia of various ages, in some detail, although this involves some repetition of known facts. The two forms investigated were collected in the Malay Peninsula and the material was preserved in alcohol. The larger form was found growing on rocks in the deep shade of the forest on Maxwell's Hill in Perak. It grew especially on the vertical faces of damp rocks, and had a distinctly phosphorescent appearance. Comparison with original specimens of *C. foetidissimum*, Schiffn., showed that while differing in some small details, it was referable to this species.

The other form was found growing in extensive patches on the walls and floor of a dimly lit limestone cave near Kuala Lumpur. The small thalli closely overlapped one another, and they also had the same phosphorescent appearance. In a preliminary statement[1] I referred this to *C. aureo-nitens*, (Griff.) Schiffn. Since then, examination of Griffith's original specimens at Kew has shown me that it cannot be referred to that species, but is practically identical with specimens of *C. cavernarum*, Kunze, from Cuba. I do not propose to enter here into the question of the

[1] British Association Report. Cambridge, 1904, p. 782.

systematic arrangement of the forms of *Cyathodium*. A number of them differ from both *C. cavernarum* and from *C. aureo-nitens* as defined by Griffith's original diagnosis and specimens. My specimens from the caves at Kuala Lumpur agree, however, so closely with examples of *C. cavernarum* from Cuba that, with this explanation as to the locality in which they were collected, I shall refer to them below under this name.

It is perhaps of greater morphological interest to make it clear at the outset that while *C. foetidissimum* is the largest and least reduced form in the genus, my specimens of *C. cavernarum* appear to be, if anything, even more reduced than those from Cuba. Thus a comparison can be made between one of the least reduced and the most reduced form in the genus.

External Form and Vegetative Structure.

The thallus of *C. foetidissimum* (Fig. 1) resembles in size and general appearance that of many Marchantiaceae, though its texture is more delicate. My specimens measured about 15 mm. in length by 4 mm. across. As a rule the plants had branched dichotomously, the two branches diverging distinctly from one another. Seen from above, the surface is marked out into well-defined air-chambers. Those over the midrib are of regular outline and have the air-pore in a central position. On passing from the middle line the chambers become more irregular in shape, and the lateral ones are greatly extended over the marginal expansion of the thallus. In these lateral chambers the pore is near to the inner limit of the chamber, which is frequently divided by an imperfect septum running in from the edge of the thallus. Seen from below a narrow distinct midrib is visible, from which numerous rhizoids spring. The cells which are prolonged into rhizoids are conspicuous by reason of their dark brown colour. There are two rows of small ventral scales (cf. Fig. 9). The apical region lies in a depression owing to the greater development of the lateral portions of the thallus. The tips of the ventral scales bend up over the apex, but afford only an imperfect protection to it.

In *C. cavernarum* (Fig. 25) the whole plant is much smaller; my specimens were about 4 mm. in length. The thallus branches dichotomously, and the resulting branches may either be distinctly separated from one another, or repeated dichotomy with little separation of the branches may give a fan-shaped outline to the plant. The air-chambers are more irregular than in *C. foetidissimum*. There is no distinct midrib, its position being only indistinctly indicated by the attachment of the rhizoids and the inconspicuous ventral scales.

Adventitious branches occur in both species (Figs. 2, 3). They originate from the under surface just within the margin of the thallus. Their base is cylindrical and some six cells in thickness, but they soon acquire the ordinary dorsiventral structure.

The rhizoids in *C. foetidissimum* are of two kinds—wider thin-walled ones and others which are narrower and have well-developed peg-thickenings. In *C. cavernarum* none of the rhizoids are thickened, but some are narrower than others and probably correspond to the peg-rhizoids of the larger species.

The ventral scales in *C. foetidissimum* (Fig. 4) are small lanceolate structures, slightly concave towards the thallus and devoid of any trace of purple coloration. There is no distinction of an appendage from a basal portion. The tip is occupied by a small glandular cell, and similar cells occur at intervals round the margin. More rudimentary forms, even to single cells bearing a glandular cell at the tip (Fig. 4,*a*), were found on the bases of adventitious branches, and would doubtless occur on young plants. In *C. cavernarum* the ventral scales were always filamentous structures (Figs. 26, 37). They may simply terminate in a glandular cell, but in the larger ones borne on the involucre similar glandular cells occur at the junctions of the cells of the filament (Fig. 26). A comparison of these with the flat scales of *C. foetidissimum* showed that the latter could be regarded as derived from the filamentous form by a process of branching, the marginal glandular cells indicating the tips of the united branches (cf. Figs. 4 and 26).

The thallus in both species consists for the greater part of its extent of a single basal layer of cells upon which the air-chambers are borne. In *C. foetidissimum*, however, the basal tissue in the region of the midrib is several cells thick (Fig. 5). In the older portions of the thallus these cells were usually inhabited by Fungal hyphae. In *C. cavernarum* there is no indication of a midrib in the vegetative region, but when archegonia are being formed the basal tissue becomes two or three cells thick. The stomata are simple, the large pore being surrounded by five series of narrow cells. In the cells of the basal layer there are only a few small plastids; those in the cells of the septa between the air-chambers are larger, but full-sized ones are only found in the cells of the epidermis roofing over the chambers. This is the true assimilating tissue of the thallus, and, as Stahl [1] first pointed out, its cells are similarly constructed to those of the protonema of *Schistostega*, although the outer wall is not convex. The chloroplasts were sometimes distributed through the protoplasm, but at other times they were grouped around the small nucleus in the convexity of the cell which projects into the air-chamber (Figs. 6, 7). In *C. foetidissimum* there are usually only four or five large chloroplasts in each cell; they are smaller and more numerous in *C. cavernarum*. Between the assimilating cells, and sometimes in the basal layer also, smaller cells, each containing a single oil-body, were present in *C. foetidissimum* (Fig. 7). The oil-body had a protoplasmic limiting membrane, and fine strands passed across the cavity bounded by

[1] Stahl, loc. cit., p. 201.

this. Oil-bodies were absent from *C. cavernarum*. Their abundance in *C. foetidissimum* may perhaps be placed in relation to the aromatic scent of the plant when fresh.

In median longitudinal sections of the vegetative apex in both species there appeared to be a single initial cell cutting off segments parallel to its base (Fig. 8). The details of segmentation as seen in surface view were followed in *C. cavernarum* (Fig. 27). There is a single rectangular initial cell present which cuts off segments parallel to its two sides and to its base. The shape of the initial and its segmentation differs when an apex is forming archegonia, and the basal tissue immediately behind it is more than one cell thick (cf. Figs. 10 and 34).

Sexual Organs and Involucre.

Both species were monoecious, the archegonia being formed in acropetal succession in relation to the apex of a branch of the thallus, while the antheridia are borne on small disk-shaped antheridiophores, developed from the lower surface of the thallus. In *C. foetidissimum* (Figs. 1, 9, 10) the antheridiophore springs from the midrib, and is situated a short distance behind the archegonial group or groups developed from the apex of the branch. In *C. cavernarum* (Figs. 25, *b*, 28) the antheridiophores are borne close to the margin of the thallus. The groups of archegonia are developed at the apices of branches of the thallus, and the antheridiophores very commonly alternate with them (Fig. 28). In other cases an antheridiophore may stand beside an archegonial group in the same apical depression, or it may be developed from the margin in no definite relation to the apex. In one case a young antheridiophore was seen arising from the margin of an older one.

The antheridiophore of *C. foetidissimum* is attached by a short stalk to the midrib (Figs. 9, 10). It is transversely elongated, and the upper surface of the disciform summit is crowded with the projecting openings of the chambers in which the antheridia stand. In a longitudinal section passing through the stalk the latter is seen to expand slightly into the basal region, upon which a number of narrow chambers, each containing a single antheridium, are borne. The outside is formed by a single layer of cells continuous with the basal tissue; this, unlike the septa between the antheridia, persists at maturity. The marginal cells of this peripheral layer project as a ridge circumscribing the upper face of the antheridiophore. The development of the antheridiophore in this species could not be followed in detail. A single specimen showed that it arose as a disk-shaped outgrowth from the lower surface, just behind the anterior margin, and was protected when young by large ventral scales. The two specimens that contained unopened antheridia showed that the youngest were situated at either end of the transversely extended disk. That a succession of

antheridia forms behind these ends, which for a time behave as growing points, is supported by the much greater elongation of these ends in Schiffner's specimens from Java.

The mature antheridium (Fig. 11) is essentially similar to that of other Marchantiaceae. It consists of a short stalk composed of two cells, the wall composed of a single layer of large clear cells, and the mass of spermatocytes. The antheridia throughout their development closely fill the cavities in which they stand, and at maturity have almost obliterated the septa between the chambers. Each of the latter opens by a pore raised above the general surface (Fig. 12).

The antheridiophore in *C. cavernarum* is smaller, and is situated immediately below the margin of the thallus (Figs. 28, 29). Except that no distinct stalk is present, it is similarly constructed to that of *C. foetidissimum*. The projecting rim of cells around the summit of the disk is clearly comparable, in the smaller species, to the clear rim of cells at the margin of the thallus. In the specimen figured in section in Fig. 29 the openings of the chambers in which the antheridia stand can be seen, but the septa between the chambers are obliterated; in younger stages (Fig. 30) the septa are distinct.

The antheridia are similar to those of *C. foetidissimum*. In both species the stalk-cell immediately below the base of the antheridial wall differs in the characters of its cell-wall from the others. The development of the antheridium presents no special peculiarities. The young antheridium is first divided by a number of transverse walls into a row of about six cells. The three lowest form the stalk and the base of the wall; the terminal cell forms the summit of the wall; and the two or three middle cells give rise to the spermatocytes and the other cells of the wall. It is clear from the study of the antheridia in these two species that Leitgeb's surmise that the antheridial wall was not formed of a layer of cells may be definitely put on one side. Even in material preserved in alcohol it is often difficult, in those which have opened, to detect the nature of the antheridial wall.

Comparison with *Targionia* renders it almost certain that the structures, which have been termed antheridiopores above, correspond to the specialized antheridial branches, which in that genus spring from the under side of the midrib. The comparison is closest as regards position in the case of *C. foetidissimum*. The view that the antheridiophores of *Cyathodium* are highly modified adventitious branches is further strengthened by comparison with the sub-marginal vegetative branches of both species. It is, however, difficult to carry the comparison into the details of development, and especially to place the succession of the antheridia in relation to the apical growth of a branch.

The difficulties chiefly appear in the case of the more reduced antheridiophores of *C. cavernarum*, and it is only in this form that I have

been able to study the development to some extent. The young antheridiophore is first recognizable as a projecting disk of tissue just below the margin of the thallus in the apical depression (Figs. 31, 32). Possibly it may in some cases originate from the apical cell itself, but the arrangement of its cells as seen in surface view, taken together with the positions in which the mature antheridiophores were found, makes it clear that this is not always or usually the case. It appears rather to originate from one, two, or several cells of the anterior margin close to the actual apex. In the specimen drawn in Fig. 31 two such cells seem to have been involved, and in that in Fig. 32 three segments can be traced. At this stage the antheridia have not yet appeared, but the septa between the chambers have commenced to grow up (Fig. 33). When the antheridia have developed (Figs. 29, 30) they are found to stand in a regular series, the youngest being next to the margin of the thallus and the oldest being those that stood closest to the posterior border of the young antheridiophore. These facts taken together dispose me to regard the antheridiophore as the equivalent of a much reduced ventral branch, while placing the succession of the antheridia in relation not to the apical growth of the branch, but to the marginal extension of the group of cells forming the rudiment of the antheridiophore. I have, unfortunately, not been able to study the development of the male branch in *Targionia* and *C. foetidissimum*, comparison of which may be expected to throw further light on the question.

In both species of *Cyathodium* the archegonia stand on the morphologically upper surface of the thallus, any branch of which may give rise to a group of archegonia. The actual position in which the archegonia come to stand and the mode of development of the involucre around the group differ to such an extent in the two forms that their separate description is necessary.

In *C. foetidissimum* the state of things is closely comparable to what is found in *Targionia*. By displacement of the apical region the archegonia come to stand on what is apparently the under surface of the branch, though morphologically it is a part of the upper surface. Sometimes there is only one group of archegonia in front of the antheridiophore, the position of which has been described above. Usually, however, the apex of the thallus, after the male branch has been laid down, branches dichotomously, though this dichotomy does not affect the general outline, and is evident simply by the formation of two groups of archegonia instead of a single one. Archegonia may also occur on a branch which does not bear an antheridiophore. The archegonial groups are at first protected by large ventral scales. At this stage (Fig. 9) the displaced region of the upper surface has a **V**-shaped outline, the initial cell being at the point of the **V**, while the arms of the latter correspond to the margin of the thallus, i. e. to the line of junction of dorsal and ventral surfaces (Fig. 13). The archegonia

appear in acropetal succession until the full number, about five, has been formed, so that the youngest archegonium is situated furthest from the anterior margin of the branch. This will be clear from Fig. 10, which represents a longitudinal section through the specimen in Fig. 9. The apical cell is visible some distance from the margin, and the youngest archegonium is close to it, while further forward is an older archegonium. It is also evident from this section that the basal tissue underlying the archegonia is of greater thickness than is usual even in the midrib of this species. After the full number of archegonia have been laid down the involucre commences to form around the group. The sides of the V (Fig. 13) first grow up as plates of cells a single layer thick. Later the growth involves the apex itself (Fig. 14), and this leads to the involucre being composed of two lobes united at the hinder end. The development of the involucre is independent of fertilization. It is throughout one layer of cells thick, and bears neither rhizoids nor ventral scales on its outer surface. Fig. 16 represents two fully developed involucres, the one on the left surrounding a group of unfertilized archegonia, while that on the right encloses two sporogonia. The involucre is roofed in above by a prolongation of the margin of the thallus bearing air-chambers (Figs. 14, 48).

In *C. cavernarum*, on the other hand, the apex does not become displaced, so that the archegonia stand on what is actually as well as morphologically the upper surface of the thallus (Figs. 34–36). The archegonia are in this case roofed in by a single layer of cells, which may be regarded as morphologically equivalent to the epidermal layer of the last formed and incomplete air-chamber (cf. Fig. 34). The basal tissue bearing the archegonia is two layers of cells thick; the further growth of the apical region produces only one layer of cells. Thus the involucre in this species is equivalent to the basal tissue of the thallus itself, and is several layers thick behind, but further forward is a single layer. The lower surface of the involucre bears ventral scales and sometimes rhizoids (Fig. 37).

The structure and development of the archegonium in the two species is like that in other Marchantiaceae. A series of developmental stages of the archegonium of *C. foetidissimum* is shown in Fig. 15, *a–d*, and a young archegonium of *C. cavernarum* in Fig. 34. One point of difference between the two species is of interest as occurring within a genus. In *C. foetidissimum* there is no stalk-cell, while in *C. cavernarum* there is a stalk-cell which from the first projects above the surface. The mature archegonium is borne on a short stalk, the wall of the venter is a single layer of cells thick, and there are six rows of neck-cells. The central series consists of ovum, ventral canal-cell, and eight or nine neck canal-cells which may not all be separated by cell-walls.

Development and Structure of the Sporogonium.

The structure of the mature sporogonium has been described in *C. cavernarum* by Leitgeb [1], and later by Andreas [2], but from the nature of the material used its development was not described. The development of the sporogonium was therefore followed in detail in *C. cavernarum*, and enough of it was seen in *C. foetidissimum* to make comparison possible.

C. foetidissimum. The first division in the fertilized ovum is transverse, and this is followed, as in the other Marchantiaceae, by longitudinal divisions in both upper and lower halves (Fig. 17). Only a few examples of stages intermediate between this and the almost mature sporogonium were seen, but these (Figs. 18, 19) resembled other Marchantiaceae, and made it almost certain that the sporogenous tissue and the greater part of the wall of the capsule were derived from the upper cell of the embryo, while the foot and the base of the capsule-wall were developed from the lower segment. By the stage represented in Fig. 19 the sporogenous tissue was clearly defined from the wall, and was conspicuous by reason of the dense and deeply-staining contents of its cells. With the first divisions of the embryo the venter of the archegonium becomes two-layered, and by this stage it is three or four cells thick. While closely investing the basal region of the sporogonium the young calyptra is separated from the upper portion of the capsule by a space which persists until the spores have been formed (Fig. 20). In the stage represented in this figure the spore-mother-cells had become rounded off and distinct from the young elaters. They are spherical and contain abundant protoplasm and a single nucleus: the elaters were somewhat elongated, but had no thickenings on their walls. The wall of the capsule is composed of a single layer of cells, except at the apex, where a disk of cells similar to that described by Leitgeb for *C. cavernarum* is present. The foot consists, as seen in longitudinal section, of two rows of vacuolated cells. At its base are larger cells densely filled with protoplasm. The calyptra is four to five cells deep around the foot, but thins off above to two layers.

The spore-mother-cells increase in size and the tetrad division takes place without the cell becoming lobed. This stage was indifferently preserved, so that no observations were made on the division. About the same time, however, a remarkable change occurs in the four cells at the base of the foot. These grow out into a number of relatively long tubular processes, each of which may branch once or oftener; these greatly increase the absorbent surface of the foot. Their general appearance will be evident from Figs. 21 and 23, which represent longitudinal and transverse sections of the foot of a sporogonium of this age. A transverse section of the stalk-like portion of the foot is seen in Fig. 22, which shows that this also consists of four rows of cells.

[1] Loc. cit. [2] Loc. cit.

A mature sporogonium is represented in almost median section in Fig. 24. Unfortunately the section just missed the apical disk (cf. Fig. 20). The spores are thick-walled, the exospore bearing rounded tubercles, and between them are the free elaters. The cells of the lower two-thirds of the wall of the capsule have their internal walls slightly thickened and of a brown colour, but have no thickenings on the vertical walls. Those of the upper third bear complete rings, usually thicker to the outside. The apical disk is apparently thrown off entire at dehiscence, for the open sporogonia had eight blunt teeth formed by the splitting of the upper third of the capsule wall. The sporogonium is protected until maturity by the calyptra, which keeps pace with its increase in size and is not ruptured until the time of dehiscence. More than one sporogonium may be developed from the group of archegonia, and thus be enclosed within the common involucre.

C. cavernarum. The material showing younger stages of the sporogonium of this species was more abundant, and enabled the development to be followed nearly continuously. On account of the extremely small size of the sporogonium this could be done with a precision almost impossible in the case of more bulky sporogonia, and the definite delimitation of the several regions may perhaps prove of use for comparison with the latter.

In the mature sporogonium (Fig. 44) we can distinguish a cylindrical foot, consisting of a row of cells and terminating below in a pair of larger cells, which grow out into absorbent processes (Fig. 46). The wall of the capsule, which contains relatively few large spores and some elaters, is composed of a single layer of cells. The cells of the upper third of the wall have ring-shaped thickenings, while those forming the lower two-thirds are thin-walled and of peculiar appearance. They are densely packed with starch grains and the nucleus in each is prominent and had often undergone fragmentation (Fig. 47). The appearance of these cells at once suggests a comparison with tapetal cells, and they have probably a nutritive function. At the summit of the capsule is the apical disk, the position of which is seen in Fig. 44, while its structure in median section is better shown in the adjoining section (Fig. 45). It consists of two series of four cells each, the cells being superposed, and of an inner layer of eight cells. The disk projects into the cavity of the capsule. The mode of opening of the sporogonium is similar to that of *C. foetidissimum*, and has been described and figured by Leitgeb. The spores have a dark brown exosporium, closely covered with spiny projections.

The first division in the embryo is transverse, and, unlike what usually happens in the Marchantiaceae, this is followed by transverse walls in both upper and lower segments (Figs. 38, 39). From the lowest cell of this row of four is derived the basal group of cells (usually two in number) which bear the processes. The upper cell of the lower half of the embryo gives

rise to the row of cells forming the rest of the foot and to the base of the wall of the capsule. The lower of the two cells of the upper half produces the greater part of the capsule and all the sporogenous tissue. After the quadrant divisions in this segment periclinal walls cut off the cells of the capsule wall. The central cells divide further and ultimately separate as the spore-mother-cells. From the uppermost segment of the embryo the upper two tiers of cells in the apical disk are derived. The lowest tier can, however, be traced back to the upper portion of the sporogenous tissue. A single layer of the latter at the upper limit of the group does not separate into spore-mother-cells, but remains as a continuous layer in contact with the uppermost sterile segment (Fig. 43), and forms part of the apical disk. A comparison of Figs. 40 and 41, in which the limits between the original segments are clearly recognizable, with Fig. 39 will enable the course of the segmentation to be followed ; Fig. 42 shows the quadrant and periclinal divisions in the sporogenous segment.

The actual size of the sporogonia and of the spores contained in them presents some features of interest. Figs. 48 and 49 are outlines, drawn to the same scale, of longitudinal sections through the sporogonia of the two forms investigated. They are of practically the same age. In both cases the cells of the upper part of the wall had well-developed brown rings, and the walls of the spores and elaters were also thickened. The calyptra, however, was not ruptured and (as shown by measurements of opened sporogonia) the full size had not been attained. This does not, however, obscure the somewhat surprising fact that, while the sporogonium of *C. cavernarum* is much smaller than that of *C. foetidissimum*, the spores of the former are considerably larger. The sporogonium of *C. foetidissimum* figured (Fig. 48) was 625 μ in length, the capsule being 525 μ ; the length of mature capsules was estimated as 800 μ. The sporogonium of *C. cavernarum* figured was 325 μ in length, the capsule being 250 μ ; the mature capsule was about 400 μ long. Mature spores of *C. foetidissimum* were about 40 μ in diameter, or, including the projecting thickenings of the wall, nearly 50 μ. Those of *C. cavernarum* were 60 μ in diameter, or, including the thickenings, 63 μ. This association of proportionally large spores with a very small capsule, which has probably resulted from a process of reduction, is of interest for comparison with *Archidium*. In this genus of Mosses, as is well known, very large spores are found in a small and possibly reduced sporogonium.

Conclusion.

If we compare *Cyathodium* with the other Marchantiaceae in the light of the additional facts as to its vegetative structure, the structure of the antheridium, and the development of the sporogonium detailed above, the only possible conclusion seems to be that they confirm the close relationship

with *Targionia* expressed by the association of the two genera in the Targionioideae. *C. foetidissimum* stands as an intermediate link between *Targionia* and the smaller species of *Cyathodium*, being less reduced than the latter in its vegetative structure, while the position of both the male branches and of the groups of archegonia is essentially the same as in *Targionia*. The position of the archegonia on the actual upper surface in *C. cavernarum* suggests a comparison with the Corsinioideae, though the facts do not point to this being an indication of direct relationship [1].

It seems clear that *Targionia*, *C. foetidissimum*, and *C. cavernarum* form a series from a thallus of the xerophytic Marchantiaceous type bearing a large sporogonium with a well-developed bulbous foot, to a type with a small and simple thallus bearing a very small sporogonium attached by a very rudimentary foot. There is nothing to suggest that this should be regarded as an ascending series, indicating a progression from forms like *C. cavernarum* to the type of *Targionia*. The facts, on the contrary, appear to point to the series being one of reduction standing in relation to the shady and damp situations in which the species of *Cyathodium* are found, while *Targionia* occurs in open and even exceptionally exposed situations. In specimens of *T. dioica*, Schiffn., that had grown mixed with a form of *Cyathodium* on a bank in a jungle in Ceylon, the thallus was slightly thinner and the purple colour was absent, except from the extreme margin of the thallus and the tips of the ventral scales. I have observed much more extreme modifications in plants of *Fegatella conica* growing in extreme shade. The plants were reduced in size, there was great reduction of the basal tissue, the assimilating filaments were shorter, and the cells of the epidermis (which as in many Marchantiaceae contain chlorophyll) were thin-walled and bulged into the air-chamber much as in *Cyathodium*. The much more profound structural peculiarities exhibited by *Cyathodium* must of course be regarded as the result of adaptation to the changed conditions, and not as directly induced by them.

In reviewing the features presented by *Cyathodium* the persistence of the system of low flat air-chambers may be first noted and contrasted with their loss in such a hygrophytic form as *Dumortiera*. The reduction in thickness of the thallus is at the expense of the basal tissue, which (except at the midrib in *C. foetidissimum*) is only a single layer. Assimilating filaments are completely absent from the chambers. The persistence of the air-chambers and the specialization of the cells of their epidermis for assimilation suggest that *Cyathodium* is to be regarded as primarily a form adapted to shade conditions rather than as a hygrophyte. Were the function of assimilation once transferred to the most superficial layer, as so frequently happens in shade-loving plants, the preservation of the system

[1] The resemblance is increased in those exceptional cases in which the growth continues as a vegetative thallus bearing a sporogonium on its upper surface. Stephani, loc. cit., p. 63.

of air-chambers, although their original protective function had been lost, is readily comprehensible.

C. foetidissimum has been shown to resemble *Targionia* in the position of the archegonia and the development of the involucre. The fact that the archegonia in *C. cavernarum* occur on what is the actual upper face of the thallus can be placed in relation to the greater reduction of the basal tissue, which would make the displacement, as in *Targionia* and *C. foetidissimum*, difficult. The antheridiophores, which appear to correspond to what in *Targionia* are clearly modified branches, are in both species of *Cyathodium* further reduced and specialized. The sexual organs themselves do not appear to be modified.

When the reduction exhibited by the spore-producing generation in *Cyathodium* is considered it is questionable whether any adaptive explanation can be given. It is not obvious that the change of habitat would render the reduction in size of the capsule or foot advantageous, or, to put it in another form, that under the new conditions the larger sporogonium with its greater spore output would have ceased to be of value. A clearer understanding of the facts appears to be reached, if we regard the changes in the sporogonium as imposed upon it by the modifications of the thallus, upon which it is produced and by which it is nourished. The reduction in thickness of the basal tissue renders any considerable accumulation of reserve material, at the expense of which the growth of a large sporogonium could take place, impossible, and the conditions of assimilation of an extreme shade form make it unlikely that the place of such reserves could be taken by the continuous production of the necessary amount of constructive substance. Under these conditions the reduction in size of the sporogonium can be readily understood, and its degree corresponds to that of the vegetative organs. The reduction is evident not only in the capsule, but in the sterile tissue of the foot. A large bulbous foot, such as that found in *Targionia* and all the other Marchantiaceae, would indeed be impossible in connexion with the small mass of underlying tissue in *Cyathodium*. The fact that the terminal cells of the cylindrical foot have their surface greatly increased by the formation of processes after the stimulus of fertilization and of the growth of the embryo have led to the increase in thickness of the base of the calyptra indicates the need for more rapid absorption than at first was possible.

On the grounds of the facts of structure, unfortunately without the assistance of experimental data, I am therefore inclined to regard *Cyathodium* as a truly reduced form, derived from a form not unlike *Targionia* by adaptation of the gametophyte to life in shady and damp situations and by changes in the sporogonium induced by the alterations in the gametophyte upon which it is borne.

Apart from questions of genetic relationship, one point of interest may

be referred to in conclusion. When we look for sporogonia resembling that of *Cyathodium* probably the closest comparison as regards the relation between capsule and foot is presented by *Sphaerocarpus* and *Riella.* The mode of segmentation of the embryo in these genera has much in common with the Marchantiaceae, the comparison between *Sphaerocarpus* and *C. cavernarum* being particularly close. In both *Riella* and *Sphaerocarpus* the mature sporogonium consists of a small capsule connected by a narrow stalk with a small bulbous foot. In some species of *Riella* the stalk consists of a single row of cells, as in *C. cavernarum*, and the bulbous foot, which in *Riella* is composed of more numerous cells, shows a tendency to form absorbent processes. In all the Marchantiaceae and the Jungermanniaceae, with these exceptions, the foot and seta of the mature capsule appear to be relatively bulky. On the other hand, in the Ricciaceae the sporogonium shows no trace of a foot.

As has been shown above, there are reasons for regarding the sporogonium of *Cyathodium* as a reduced structure, and the same may perhaps be assumed for *Riella.* But in the absence of any other forms with a very small sterile portion of the sporogonium these genera are not without interest as indicating the sort of structure which may have characterized the stage intermediate between the Ricciaceous type and sporogonia with a well-developed foot. It is at least possible that the reduction in *Cyathodium* has taken place along the lines of differentiation, and that it may in this respect represent to some extent an intermediate stage between the Ricciaceae and Marchantiaceae.

Such reduced forms must, however, be used with caution in speculations as to the methods of descent, and further information as to the range of variation in the sporogonium under altered conditions must be accumulated before any but tentative conclusions can be drawn from their study. Without attempting further to follow out this line of comparison, the present paper may be left as a description of the main facts regarding the structure of *Cyathodium*, the full explanation of which must depend on further observations and experiments.

EXPLANATION OF FIGURES IN PLATES XXI AND XXII.

Illustrating Dr. Lang's paper on the Morphology of *Cyathodium*.

PLATE XXI.

Cyathodium foetidissimum.

Fig. 1. A plant of *Cyathodium foetidissimum* viewed from below: the thallus has branched dichotomously; an antheridiophore is seen some distance from the anterior margin of each branch, and between it and the margin there are in each case a pair of involucres enclosing sporogonia. × 4½.

Fig. 2. Portion of the thallus bearing two adventitious branches.

Fig. 3. One of the adventitious branches seen from above, showing its relation to the margin of the thallus. × 44.

Fig. 4. Ventral scales. *a*, *b*, reduced forms from the base of an adventitious branch; *c*, the normal form from the well-developed thallus. × 200.

Fig. 5. Transverse section of the thallus in the region of the midrib; the shaded cells were occupied by Fungal hyphae. × 200.

Fig. 6. Two cells of the 'epidermis' in vertical section, showing the position of the chloroplasts in the convexity of the cell. × 353.

Fig. 7. Cells of the 'epidermis' in surface view; the small cell in the centre contains an oil body, the others are assimilating cells. × 353.

Fig. 8. Medium longitudinal section of the apex of a vegetative branch. × 250.

Fig. 9. Fertile branch seen from below, showing a mature antheridiophore and in front of it large ventral scales covering over the groups of archegonia. × 46.

Fig. 10. Longitudinal section of a specimen of the same age as that in Fig. 9, passing through the shortly stalked antheridiophore, and showing the apical cell displaced towards the lower surface and in front of it two young archegonia. × 200.

Fig. 11. Longitudinal section of a mature antheridium. × 530.

Fig. 12. Longitudinal section through the upper part of an antheridium and the opening of the chamber of the antheridiophore that contained it. × 353.

Fig. 13. Sketch showing the position of the developing archegonia and of the V-shaped ridge marking the junction of dorsal and ventral surfaces in the region of the displaced apex.

Fig. 14. Longitudinal section through an apex bearing a fully formed group of archegonia; the apex has commenced to form the hinder portion of the involucre. × 80.

Fig. 15. Series of developmental stages of the archegonium in longitudinal section. × 640.

Fig. 16. View of fertile branch from below, showing the spent antheridiophore and in front of it two fully developed involucres; that on the right is occupied by two sporogonia, while the one on the left contains unfertilized archegonia. × 23.

Figs. 17–19. Three stages in the development of the sporogonium. × 375.

Fig. 20. Median section of a sporogonium with isolated spore-mother-cells; the calyptra enclosing it is shaded. × 130.

Fig. 21. Longitudinal section of the base of the calyptra, showing the absorbent processes developed from the basal cells of the foot of a sporogonium which contained tetrads. × 250.

Fig. 22. Cross section of stalk-like portion of foot. × 375.

Fig. 23. Cross section of basal portion of foot, showing the absorbent processes. × 250.

Fig. 24. Nearly median section of a mature sporogonium; the apical cap of cells is not seen. × 136.

PLATE XXII.

Cyathodium cavernarum.

Fig. 25. Plants of *C. cavernarum*. *a*, sterile plant; *b*, plant bearing three archegonial groups and two antheridiophores. × 7.

Fig. 26. Two of the larger ventral scales. × 200.

Fig. 27. Vegetative apex seen from below, showing the shape and mode of segmentation of the apical cell. × 670.

Fig. 28. Anterior margin of a branch of the thallus seen from below, showing an antheridiophore and two involucres. The involucre to the left encloses a mature sporogonium, while that to the right bears only unfertilized archegonia. × 25.

Fig. 29. Antheridiophore in vertical section; the lower antheridia are mature. × 375.

Fig. 30. Younger antheridiophore, showing the septa between the antheridia. × 375.

Fig. 31. Young antheridiophore in surface view. × 670.

Fig. 32. Young antheridiophore, which can clearly be traced back to three marginal cells, in surface view. × 670.

Fig. 33. Vertical section of a young antheridiophore similar to that in Fig. 32. × 670.

Fig. 34. Median section through an apex which has produced the first archegonium. × 375.

Fig. 35. Median longitudinal section through an older apex, which has formed a group of archegonia, showing the construction of the involucre in this species. This figure is based on two adjoining sections and is slightly diagrammatic. × 200.

Fig. 36. Transverse section across an involucre of the same age as that in Fig. 35. × 200.

Fig. 37. Involucre bearing an advanced sporogonium seen from below to show the presence of rhizoids and ventral scales upon it. × 64.

Fig. 38. Embryo consisting of three cells enclosed in the archegonium. × 375.

Fig. 39. Older embryo, showing the four segments; vertical walls have been formed in the upper two. The venter of the archegonium has become several cells thick. × 375.

Figs. 40, 41. Young sporogonia in which the regions derived from the four first segments of the embryo can be recognized. × 375.

Fig. 42. Transverse section through the lower tier of the upper half of a sporogonium about the age of those in the preceding figure. × 375.

Fig. 43. Longitudinal section through the upper part of the capsule of a somewhat older sporogonium, showing the origin of the lower layer of cells of the apical disk. × 375.

Fig. 44. Longitudinal section of an almost mature sporogonium. × 200.

Fig. 45. The apical disk of the same sporogonium in median section. × 200.

Fig. 46. Foot of a similar sporogonium, showing the processes produced from the large basal cells. × 200.

Fig. 47. Cells of the lower part of the wall of the capsule in surface view. × 530.

Figs. 48, 49. Longitudinal sections through sporogonia of *C. foetidissimum* (Fig. 48) and *C. cavernarum* (Fig. 49) to show their position on the thallus and their relative size. In both cases the sporogonia contained spores and elaters with their walls thickened, but had not quite attained the full size. × 74.

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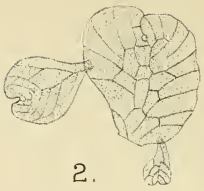

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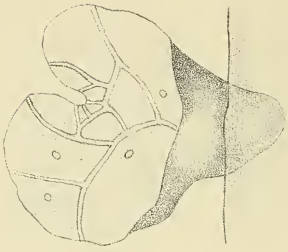

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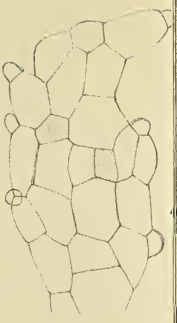

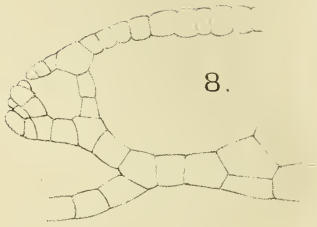

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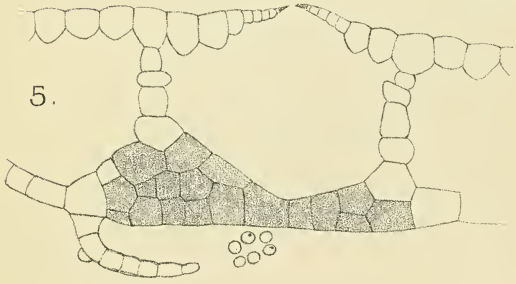

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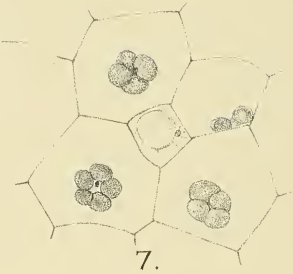

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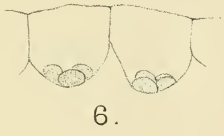

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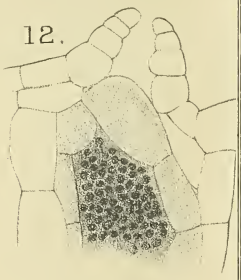

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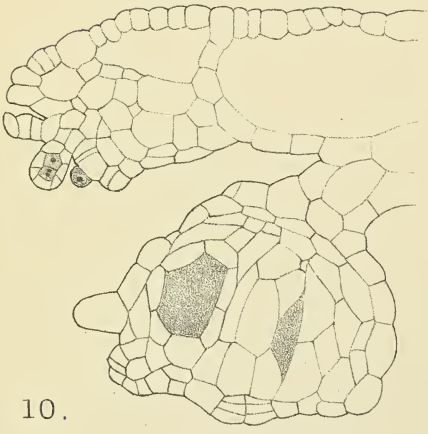

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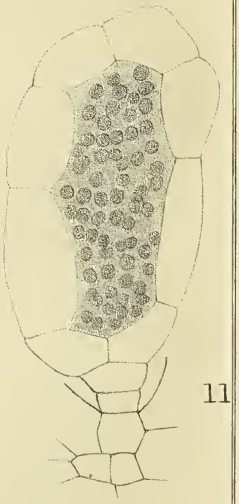

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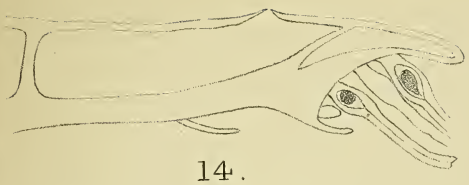

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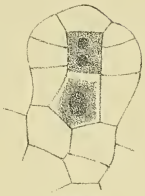

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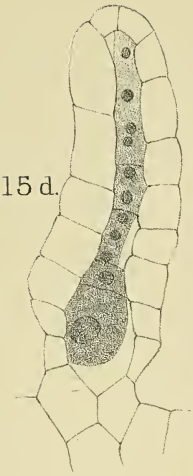

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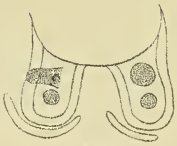

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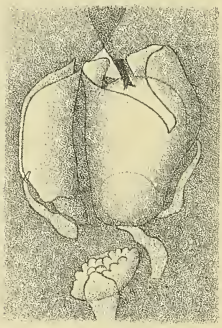

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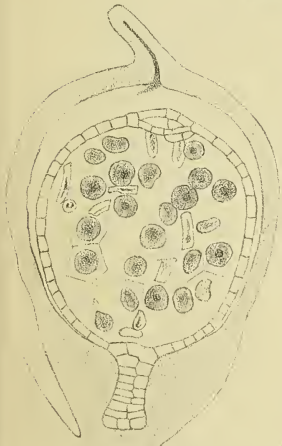

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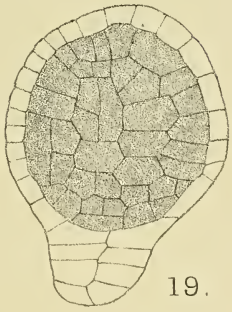

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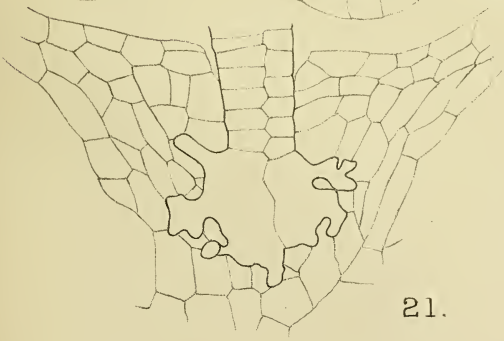

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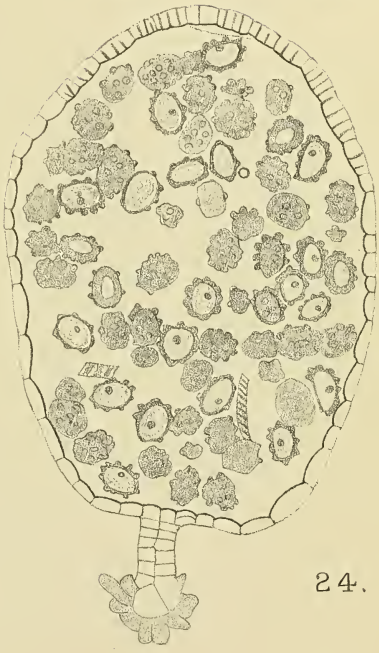

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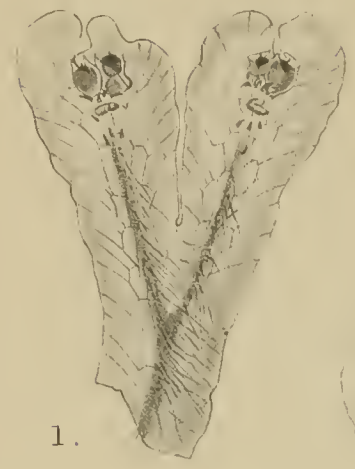
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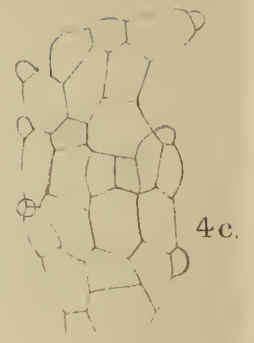
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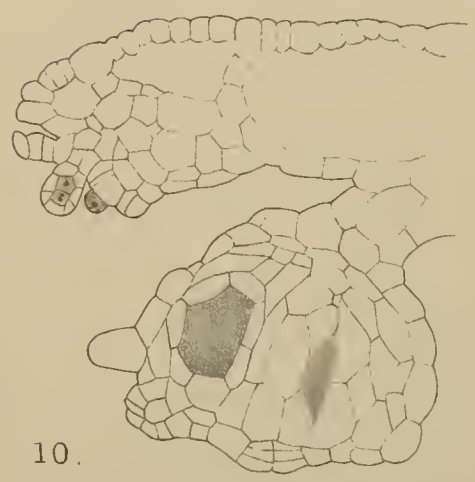
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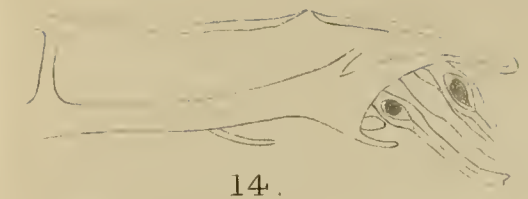
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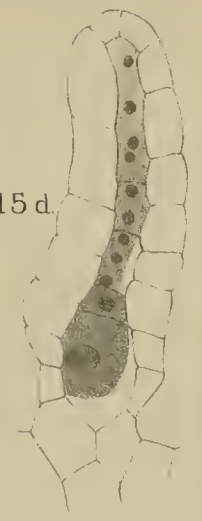
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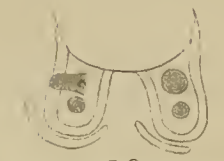
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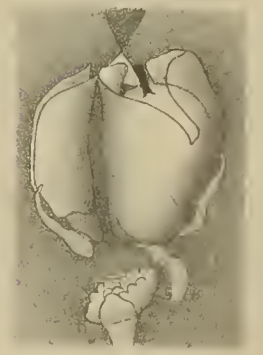
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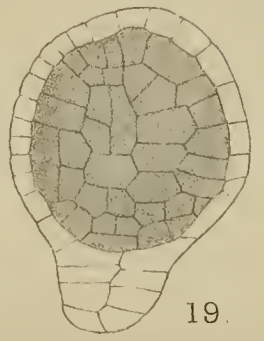
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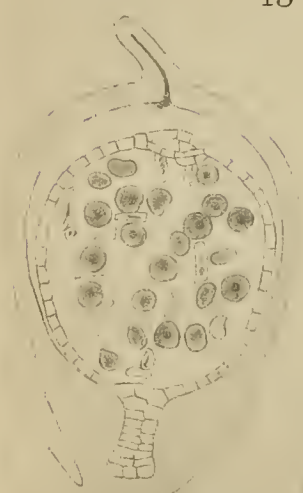
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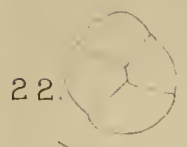
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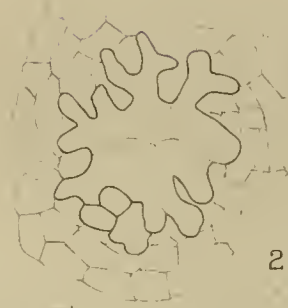
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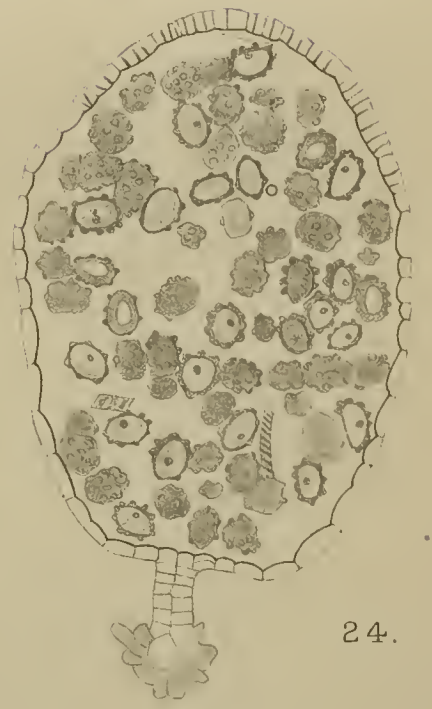
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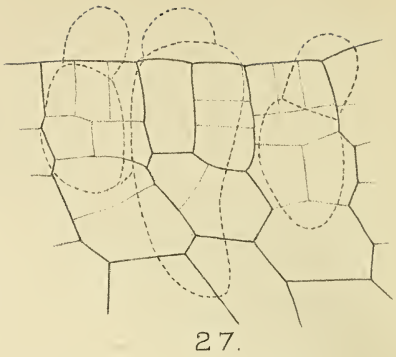

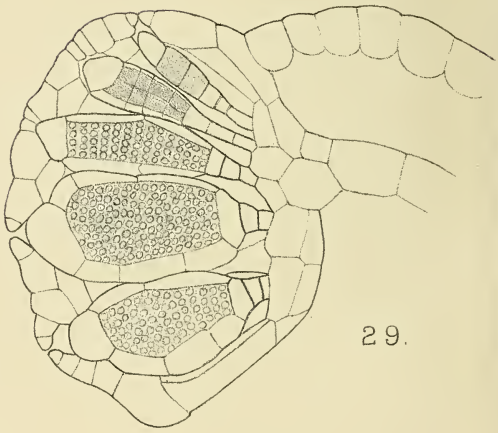

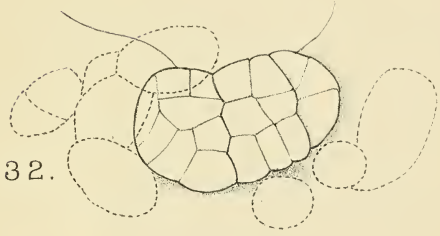

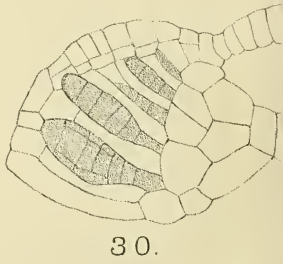

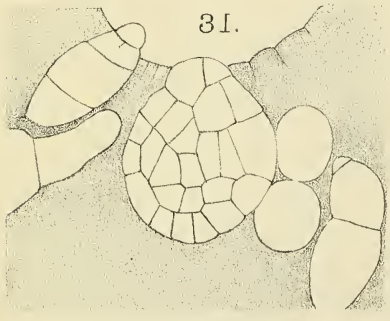

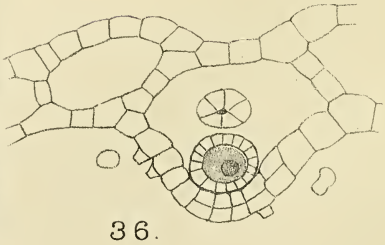

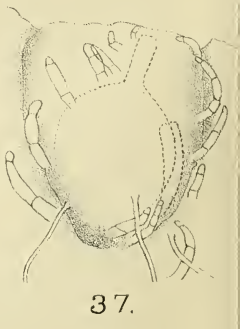

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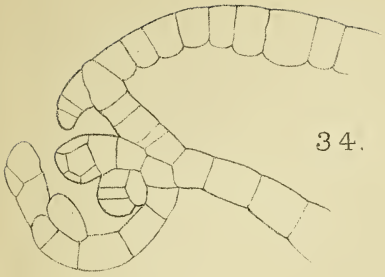

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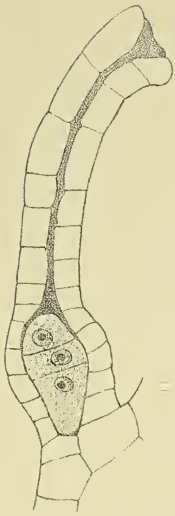

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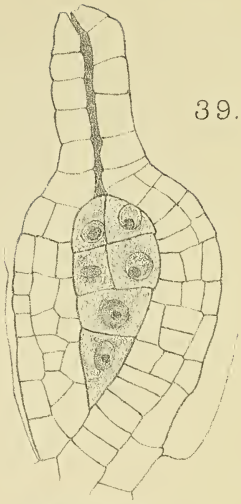

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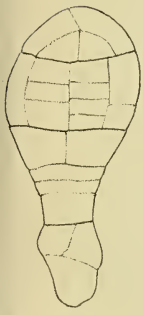

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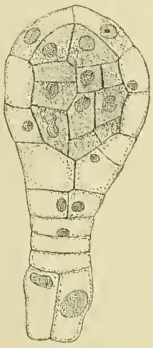

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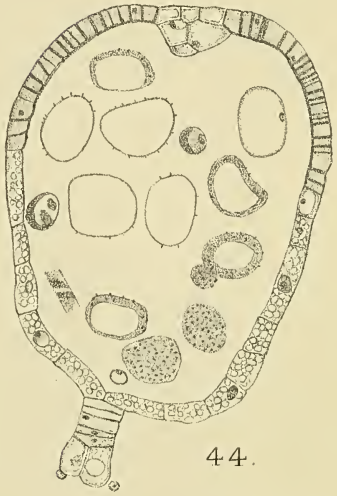

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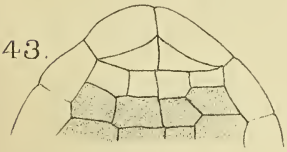

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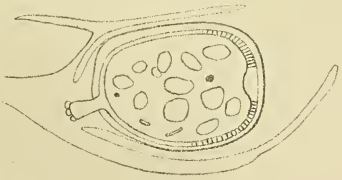

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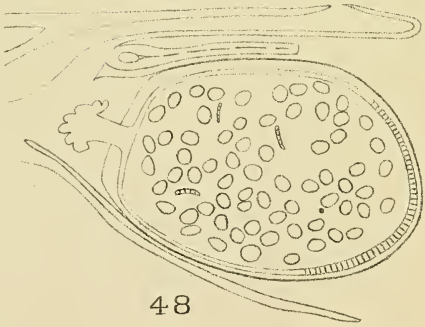

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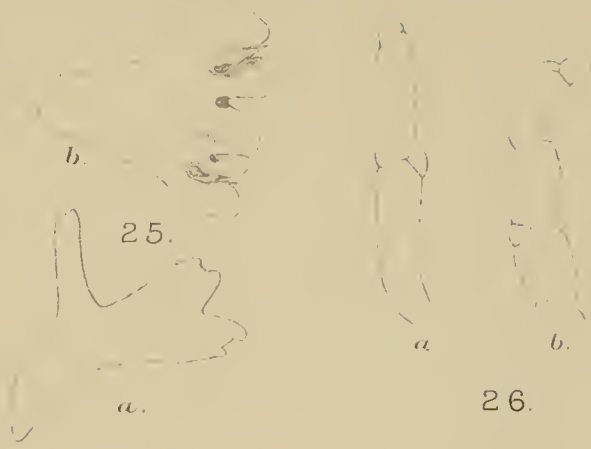

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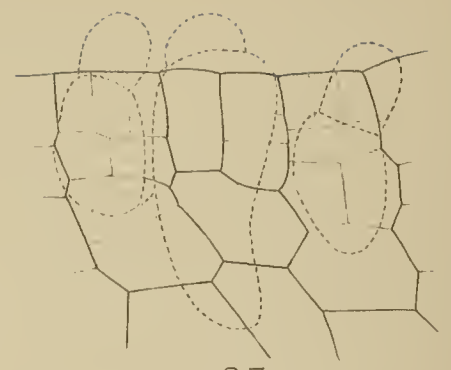

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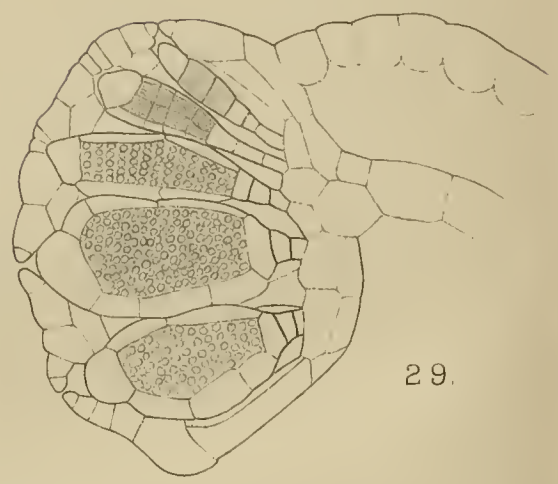

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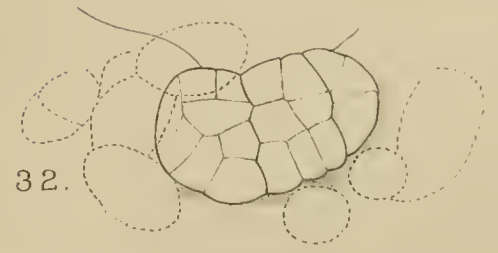

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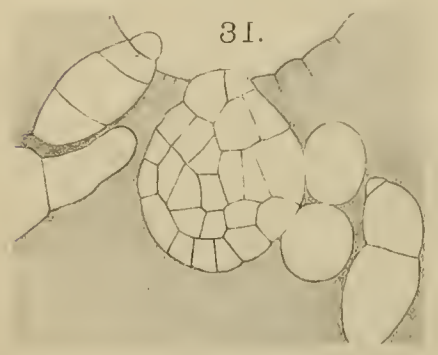

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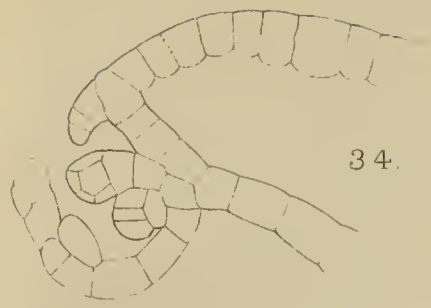

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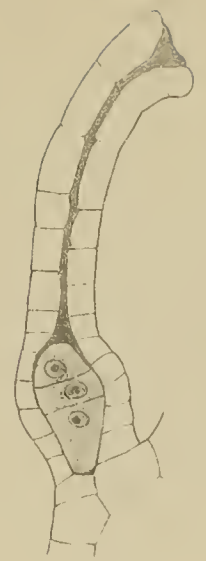

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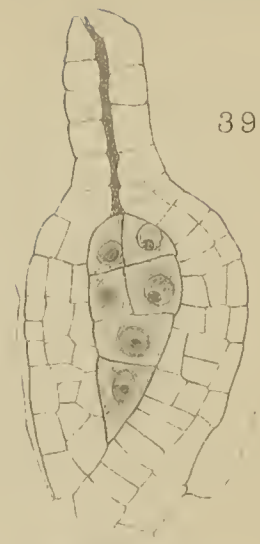

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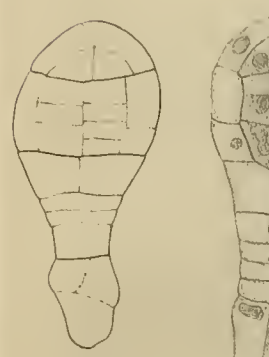

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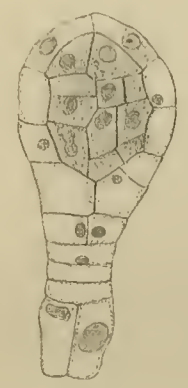

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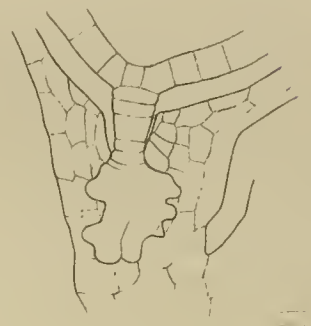

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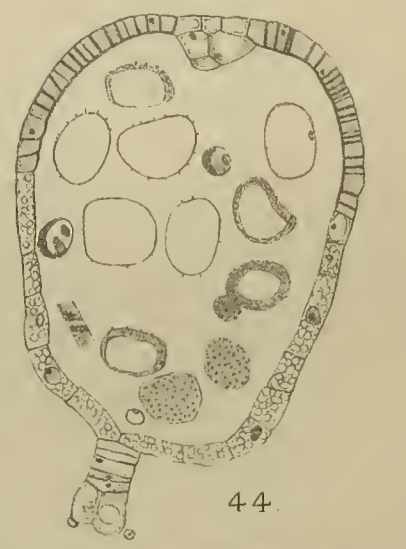

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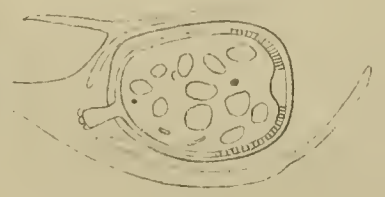

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W.H.L. del.

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LANG — CYATHODIUM CAVERNARUM.

The Reactions of the Fruit-Bodies of Lentinus lepideus, Fr., to External Stimuli.

BY

A. H. REGINALD BULLER, D.Sc., Ph D.,

Professor of Botany at the University of Manitoba.

With Plates XXIII, XXIV and XXV.

THE fruit-bodies of *Lentinus lepideus* were grown upon rotting paving blocks obtained from the streets of Birmingham. The destruction of the pavement by the Fungus and the life-history of the latter will be described in another paper [1].

Lentinus lepideus belongs to the Agaricini. Its fruit-bodies are coriaceous, slow in growth, and live for some weeks. They are very variable in form, and frequently monstrous. Their development on such a convenient substratum as wooden paving blocks afforded me an opportunity of investigating their relations to external stimuli.

Sachs [2] showed that the stipes of a number of Agaricini are negatively geotropic, and that the gills are positively geotropic, while the pilei take up a horizontal position. Brefeld [3] studied the effect of light on species of the genus *Coprinus*. I have endeavoured to give as complete an account as possible of the various reactions to stimuli of a single gilled Fungus, a hitherto uninvestigated species growing upon wood, and to explain the reactions on oecological grounds.

Rotting blocks were obtained from a street pavement, and placed in a large damp-chamber. About fourteen days afterwards a thin snow-white mycelial layer grew out on the surfaces of the blocks, especially on surfaces exposed by the breaking of the wood. After about two days more, small white papillae—the beginnings of the fruit-bodies—began to develop upon the mycelium (Figs. 6, 13, and 19).

The papillae often arise in considerable numbers side by side (Fig. 6). They develop into conical protuberances, the further history of which depends mainly on the condition of light.

[1] In Journ. of Economic Biology, Vol. I, pt. 1, Oct. 1905.

[2] Experimentalphysiologie, 1865, p. 93; also Hofmeister, Ueber die durch Schwerkraft bestimmten Richtungen von Pflanzentheilen, Jahrb. für wiss. Bot., Bd. iii, 1863, pp. 92 and 93.

[3] Botanische Untersuchungen über Schimmelpilze, Heft iii. Basidiomycetes, I. 1877, pp. 87-97.

[Annals of Botany, Vol. XIX. No. LXXV. July, 1905.]

Some of the rotten blocks were placed in a damp-chamber in complete darkness. Under these conditions numerous papillae were formed which developed into protuberances (Figs. 12 and 13). Light is therefore not neccessary for their formation. The direction in which the papillae grow in reference to the substratum was carefully noted. It was found that, whereas the majority grow out more or less perpendicularly to the wood surface (Fig. 13), others grow at angles of 45° and a few quite parallel to it (Fig. 12). It seems that the direction of growth of the protuberances is not determined directly by the substratum, or, in other words, that no somatotropic stimulus is given by the wood. On the other hand, the direction of growth appears to depend on the position of origin of the papillae on the mycelial layer covering the wood. If a papilla arises towards the centre of a mycelial layer which covers a flat wood surface uniformly, it grows out perpendicularly to the mycelial covering and incidentally perpendicularly to the surface of the wood (see the diagram, Fig. 21). If, however, as I frequently observed, the mycelial layer arising on a flat wood surface has rounded edges and papillae arise on such edges, then the conical protuberances (Fig. 21,*o* and *o*[1]) grow perpendicularly to the mycelial layer and consequently obliquely to the wood surface (Figs. 12 and 19). Hence the conclusion seems to be justified that the wood exercises no somatotropic stimulus.

The protuberances develop in the dark into cylindrical rods, which are pointed at the free ends (Figs. 12, 13, and 17). The rods are quite white. Their growth is terminal. They show absolutely no reaction to the stimulus of gravity. In this respect they resemble the hypocotyl of the Mistletoe (*Viscum*), which maintains any direction of growth which it may originally have assumed[1]. They neither curve upwards nor downwards, nor, when growing at an angle of 45° to the vertical (Fig. 12), do they show any tendency to take up a diageotropic position. They simply grow in fairly straight lines and are, therefore, rectipetal.

The fruit-bodies, which develop in the dark, never show the slightest trace of a pileus, and are, therefore, entirely monstrous and abortive (Figs. 12, 13, 17, 19, and 20). They present a very curious appearance, and look more like Clavarias than anything else. Cooke[2] has figured in his Illustrations of the British Fungi some similar monstrosities obtained from a brewery cellar.

Growth of the unpileated rods continues in the dark for an astonishingly long time as compared with normal fruit-bodies growing in light. A number of rods were still growing at the end of two months, and some after three months, whereas under good light conditions normal fruit-bodies were found to come to maturity in less than three weeks. In the course of time the monstrosities become very much elongated. One of the most vigorous

[1] Duhamel. Quoted from Vine's Lectures on the Physiology of Plants, p. 458.
[2] Illustrations of British Fungi, Pl. 1141.

attained a length of nearly seven inches in nine weeks (Figs. 19 and 25). It was still in the form of a stiff tapering rod, the diameter at the base being about three-eighths of an inch. At this stage it was changing from white to brown.

The formation of the pileus depends entirely on the light. This is not the case for all Agaricini. Thus, as is well known, the pileus of the common Mushroom develops in a perfectly normal manner in completely darkened cellars.

A very similar case to *Lentinus lepideus* is that of *Coprinus stercorarius*, investigated by Brefeld [1]. The stipe of this Fungus was observed to grow in the dark to the extraordinary length of between two and three feet. A tiny rudimentary pileus, never producing spores, was, however, always formed at the tip of the stipe. *Lentinus lepideus* in never showing the slightest trace of a pileus in the dark is, therefore, even more dependent on the light for the formation of that structure than the *Coprinus*.

Some rotting blocks were placed in a damp-chamber in weak unilateral light, which was directed obliquely downwards at an angle of 45° with the vertical. Under these conditions the conical protuberances and rods were positively heliotropic. In whatever position the protuberances began their growth upon the blocks they gradually changed their direction of growth so as to bring their axes parallel to the incident rays. Further evidence of the positive reaction to heliotropic stimuli is afforded by the following experiments.

A rod grew out from a block (*Bl* in Fig. 23), and placed its axis in the direction of weak rays of light indicated by the arrow *a*. When it had attained the length *ll*, the block was moved round in a horizontal plane through two right angles so that the direction of the light was changed to that indicated by the arrow *b*. The rod grew in a curve in such a manner as to bring its axis once more parallel to the incident rays. A similar experiment was performed with the rods in Fig. 22. The rods *o*, *p*, *r*, and *s* grew towards the light, the direction of which is shown by the arrow *a*. After the block had been turned round so that the direction of the light came to be that indicated by the arrow *b*, the rods *o* and *p* curved through a right angle towards the light. The rods *r* and *s* had stopped growing and therefore retained their original direction of growth. The rods *t*, *v*, *w*, &c., developed after the light had been changed to the direction indicated by the arrow *b*.

Under well-lighted conditions the rods exhibit positive heliotropism, and usually, when they have attained the length of a few centimetres, proceed to the formation of a pileus. The pileus always arises terminally on the end of the rod which becomes the stipe (Figs. 10 and 14). The end

[1] Loc. cit., p. 90.

of the rod becomes rounded and grows directly into the pileus. The latter is gymnocarpous from the beginning and never forms a gill-chamber.

When rods which have been growing in weak light are placed in strong light they form pilei. The younger and more vigorous the rod, the quicker will the pileus be formed, and the larger it will be. Older and less vigorous rods, when placed in strong light, can only be induced to slowly form very small pilei.

It has been pointed out (p. 428) that, until the pileus begins to develop, the stipe shows no trace of any reaction to the stimulus of gravity, and its direction of growth is completely controlled by the light. When, however, the pileus has attained a breadth of about a centimetre, an important change sets in. The end of the stipe becomes strongly negatively geotropic, so that it takes up a vertical position even in strong oblique light. At this stage the stipe appears to be no longer responsive to heliotropic stimuli.

The change of physiological properties in the stipe may be compared to changes which take place in certain organs of the higher plants during their development. Thus the peduncle of the Poppy (*Papaver*) is positively geotropic whilst the flower is in the bud, but becomes negatively geotropic during flowering and fruiting. The peduncle of *Tussilago Farfara* also shows reversal of its geotropic properties. Whilst flowering it is negatively geotropic. During the development of its fruit the upper part of the peduncle becomes positively geotropic. The final change occurs upon the ripening of the fruit, when the whole peduncle becomes negatively geotropic[1]. Similar instances might be adduced for heliotropism. I am not aware, however, of any case among the higher plants where the direction of development of an organ is at first entirely controlled by the stimulus of light and afterwards by the stimulus of gravity.

The following experiment serves to illustrate the negative geotropism of the end of the stipe. The stipe of a fruit-body had become vertical by bending through an angle of 45° (Fig. 1). The block, upon which the fruit-body grew, was then turned through a right angle so that the top of the stipe was placed in a horizontal position (Fig. 2). In three days the stipe had become vertical once more (Fig. 3). The block was then turned through a further right angle (Fig. 4). In the course of four days the end of the stipe had again executed a movement tending to place the end of the stipe in a vertical position. Owing to cessation of growth, however, the pileus had only been turned through an angle of 45° (Fig. 5). In all, during the two experiments, the stipe had moved round through an angle of 135°.

Another nearly mature fruit-body was placed on its side in the dark. The pileus became partially erected and turned upwards through an angle of 30° in two days (compare Figs. 7 and 8). By this time all growth had ceased. The experiment indicates that the erection of the pileus is not due

[1] Vöchting, Bewegungen der Blüthen und Früchte, Bonn, 1882.

to the stimulus of light, but is a negatively geotropic reaction which can take place in complete darkness. As further evidence that the erection of the pileus is a geotropic and not a heliotropic phenomenon, it may be mentioned that, when grown in the light, the pileus becomes erect however the light may be directed towards the fruit-body.

The variations in shape of the mature pileus now require discussion. It was found that, when a stipe, before the formation of the pileus, was placed in a vertical position and rotated about its axis by means of a klinostat revolving horizontally under good light conditions, a symmetrical pileus with equal gills was formed. On the other hand, it was noticed that, when a fruit-body grew in an ordinary damp-chamber, it always had an oblique stipe (e.g. in Fig. 1), owing to the heliotropic reaction already explained. The pileus was then either symmetrically developed (Figs. 1 and 11) or sometimes its development was very unequal (Figs. 9, 16, and 26). In the latter case the longest gills were always on the lower side of the stipe, whilst the gills on the upper side were considerably shorter, or even undeveloped beyond the most rudimentary stage (Fig. 26).

These variations appear to depend on the manner in which the fruit-bodies respond to the stimulus of gravity during the development of the pileus. The pileus in the youngest stage, as already stated, is symmetrically developed on the end of the stipe (Figs. 10 and 14). Let us now suppose that up to this stage in response to heliotropic stimuli the stipe has grown obliquely towards the light (Figs. 1 and 11). The end of the stipe below the decurrent gills then becomes negatively geotropic and bends upwards, thus bringing the pileus into the vertical position. Then, apparently according to the individual peculiarity of the fruit-bodies, either every side of the pileus elongates equally, and all the gills attain to the same length (Figs. 1 and 11), or the side of the pileus which was on the lower side of the stipe grows much faster than the opposite side. The top of the pileus thereby finally becomes oblique. The gills on the side of the pileus where rapid growth has taken place become very much elongated, whilst those on the opposite side elongate but little or not at all (Figs. 16 and 26).

The fact that when the gills are unequally developed, the longest ones are always on the lower side of the stipe, suggests that the inequality is brought about by the stimulus of gravity. The possible action of light must, however, be taken into account, for, owing to the heliotropic reaction of the young stipe, it so happened that the best developed side of the pileus was not only on the lower side of the stipe, but also received most light. A young, obliquely-growing fruit-body, upon which a pileus was just beginning to form, was, therefore, taken and placed in such a position that the light was directed towards the upper side of the stipe and pileus. The gills developed as before, most vigorously on that side of the pileus which was continuous with the lower side of the stipe. The least lighted side of the

pileus had here developed best. Another fruit-body grew out from near the middle of the vertical side of a paving block in a horizontal direction and almost parallel to the surface of the wood during rotation of the block upon a horizontally revolving klinostat. Under these conditions, whilst the pileus was turning upwards and becoming unequally developed, the side next to the black surface of the block received least light, that turned away from the block received most light, and the sides continuous with the upper and under sides of the stipe were fairly equally illuminated. Nevertheless, the least and best lighted sides of the pileus developed gills of equal length: the side of the pileus continuous with the lower side of the stipe developed the longest gills, and that continuous with the upper side the shortest, although these two sides had been about equally illuminated. Want of sufficient fruit-bodies at this stage of the work unfortunately prevented the carrying out of further experiments of a similar nature to the two described. The evidence obtained, however, is in favour of the view that when a pileus is developed unequally, the stimulus of gravity and not that of light induces the inequality.

The young pileus is convex above (Fig. 1). During further development the margins of the pileus turn upwards so that the top becomes depressed and concave (Fig. 5). During the change the gills undergo considerable elongation. Their outer margins become stretched to such an extent that they finally become very much torn and thus assume a serrated appearance, which is characteristic for the genus *Lentinus*[1] (Figs. 5, 8, and 11).

As the gills develop they become strongly positively geotropic and can alter their direction of growth so as to bring themselves into vertical planes. A fruit-body, which had developed its gills in the ordinary manner, was turned through a right angle and placed in the dark (Fig. 7). In two days the gills had altered their direction of growth so that they were all directed downwards (Figs. 8 and 9). During an experiment on geotropism, already described, the pileus ceased to be moved round at an angle of 45° with the vertical. The gills under these conditions were no longer vertical with respect to the earth, but inclined to it. In about two days the gills altered their position so as to bring themselves into vertical planes as far as circumstances would allow. They all turned downwards towards the earth and thus exhibited positive geotropism (Fig. 5). When grown in unilateral light the gills show no reaction to light in the direction of their growth. They are, therefore, in no way heliotropic.

The reactions of the fruit-bodies to the stimuli of light and gravity can, I think, be explained on oecological grounds without much difficulty. It should be remembered that, so to speak, the main object of a developing fruit-body is to form its pileus in the open air, so that the spores may

[1] G. Massee, British Fungus-Flora, vol. ii, p. 299.

be freely disseminated by the wind. In the case of seedlings of green plants the young shoot, whilst still covered with soil, is almost universally negatively geotropic. This reaction is admirably adapted for bringing the shoot into the light, for the surface of the soil is on the average horizontal. The surface of the wooden substratum on which fruit-bodies of *Lentinus lepideus* develop (i. e. the surface of fallen logs, &c.) is not on the average horizontal. It is in any direction whatsoever. It would be of no advantage, therefore, to the young fruit-bodies to have a specific geotropic reaction. They are, therefore, ageotropic. It is, however, advantageous that the direction of growth should be controlled by light, and, accordingly, the young fruit-bodies are positively heliotropic. Very weak light will cause them to grow outwards from the substratum through cracks in bark, leaves, and other obstacles, so that in the end the growing points will reach the open air. Here stronger light will cause the pileus to develop. The dependence of the pileus for its development on light ensures that the structure shall only be produced in suitable places for the distribution of the spores. As soon as the pileus has begun its development in the open, the end of the stipe ceases to be heliotropic and becomes negatively geotropic. This change is of the highest advantage. Negative geotropism leads to the erection of the pileus, by which operation the best position and spacial arrangement is provided for the spore-bearing gills. Continued positive heliotropism would only interfere with the erection of the pileus. Hence, when the latter is maturing, the reaction becomes eliminated. If the axis of the pileus is erect, the gills, by simply growing outwards radially, are at once in vertical planes, i. e. in the best position for the falling of the spores. If, however, any obstacle should prevent the axis becoming erect, then the gills, owing to their positive geotropism, grow vertically downwards in order to place themselves, so far as the falling of the spores is concerned, in the best possible position under the circumstances.

Summing up this discussion, the conclusion seems to be that the various reactions of the fruit-bodies to the stimuli of light and gravity are adaptations for ensuring that spore-production shall take place in the most economical manner.

A copious excretion of somewhat viscid and often red drops frequently takes place from stipe, top of pileus or gills (Figs. 10 and 15), provided that the air of the damp-chamber is saturated with water-vapour. The excretion of the drops may be started or increased by watering the paving blocks, on which the fruit-bodies are growing. The excretion, therefore, probably serves the purpose of getting rid of excess water from the mycelium.

The colourless spores are shed in vast numbers. Their fall appears, as probably for most Agaricini, to be largely determined by the hygroscopic state of the atmosphere. Half of the pileus was cut away from a fruit-body and placed upon a piece of black paper in a dry situation.

In twenty-four hours a white deposit of spores was formed on the paper. Practically all the spores had fallen during that time. The block bearing the other half of the fruit-body was placed under a bell-jar, the atmosphere of which was saturated with water-vapour. Black paper was laid beneath the fruit-body. No spores were deposited for a week. At the end of that period the air in the bell-jar had become relatively dry. Spores then began to fall, and a white deposit accumulated on the paper. It is evident from the experiment that when the air is moist, the spores do not separate so soon from the basidia as when the air is dry. The oecological advantage of this is obvious. In dry weather there will be no more chance of the spores being scattered than in wet weather. The fall of the spores, therefore, is arranged to take place when the atmosphere is dry and is hindered by its saturation with water-vapour.

It was found that some of the fruit-bodies grown in a weak light became branched. Cooke has figured a large branched monstrosity in his Illustrations of the British Fungi, Pl. 1141. Branching seems to be frequently induced by the formation of abortive pilei. A rod was often observed to grow slowly without branching in weak light and then to form a small pileus, which did not develop its gills fully, and which soon became abortive. Branching then took place. In some cases the branches arose from the edges of the pileus (Figs. 28 and 29). After a time the branches developed more abortive pilei (Fig. 29). One monstrosity even produced primary, secondary, and tertiary stipes and primary, secondary, and tertiary abortive pilei (Fig. 28).

Branching may also take place from the stipe. A fruit-body produced a small pileus at the end of a stipe three inches long. After the withering of the pileus about twenty branches grew out from the stipe simultaneously. Some of the gills also produced tiny outgrowths on their edges, which had the appearance of abortive pilei, although no gills were developed on them. The young pileus of another fruit-body (Fig. 27) became injured by being pressed against the glass wall of the damp-chamber. The pileus ceased to develop and several branches arose on the stipe.

I pinched off the ends of a number of rods upon which pilei had not been formed. A new growing point was developed at the end of each rod, but the injury did not induce any branching.

Two out of a considerable number of rods, grown entirely in the dark, each developed a very short lateral branch. In these two instances branching appeared to be due to internal causes, for the rods had grown apparently without injury under very constant conditions. A branched monstrosity looking very much like an elk's horn I found growing out of a block, which with others had been removed from a street and piled up at a wharf (Fig. 18).

From the observations recorded in this paper it is evident that the

fruit-bodies of *Lentinus lepideus* are extraordinarily variable. We have seen that under some conditions they are practically without a stipe; that under others they possess stipes three inches long; that sometimes the pileus is excentrically developed; that in the dark the fruit-bodies assume the form of tapering rods, attaining a length of some six inches but with no trace of a pileus, and that finally in weak light they often develop into curious branched monstrosities (cf. Figs. 15, 11, 16, 26, 25 and 28).

Agaricus campestris in comparison with *Lentinus lepideus* is a very stable form. Its fruit-bodies grow quite normally both in sunlit fields and in completely darkened cellars. They always develop a stipe and a symmetrical pileus bearing gills, which produce spores. The stipe and pileus are never branched. One may inquire why the one Agaric should be so variable, and the other comparatively stable. I will venture the suggestion that the difference is connected with the habitat of the two Fungi. *Agaricus campestris* is a ground Fungus. The orientation of its substratum is in so far definite that the surface is on the average horizontal. This being so, when a fruit-body has once begun to form on the surface of the ground, suitable reactions to the stimulus of gravity alone are necessary to bring about the development of the stipe and pileus in vertical sequence, and the subsequent uplifting of the latter into the air. Hence we find that the development of the Mushroom is controlled by the stimulus of gravity and is practically unaffected by light. On the other hand, *Lentinus lepideus* is a tree Fungus. The orientation of the surface of its substratum is indefinite. It may be in any position whatsoever with regard to the fruit-bodies. In order that the latter may be brought into the open air, they are provided with the power of reacting to the stimulus of light, the advantage of which has already been explained (p. 433), as well as to the stimulus of gravity. The adaptation of the two Fungi to different conditions of life has thus led to the acquirement of different physiological properties, with the result that *Lentinus lepideus* has become physiologically much more complex than *Agaricus campestris*, and, therefore, much more liable to variation. If the foregoing remarks upon the physiological differences between the two Fungi hold good, further investigation should show that in general tree-Agarics (and possibly also dung-Agarics) behave to light and gravity like *Lentinus lepideus*, whilst ground-Agarics behave like *Agaricus campestris*.

Summary.

The fruit-bodies of *Lentinus lepideus* were grown upon rotten paving blocks, taken from the streets of Birmingham. The Fungus belongs to the Agaricini.

The papillae, from which the fruit-bodies arise, are not somatotropic, so far as the wooden substratum is concerned, but grow out perpendicularly

to the surface of the mycelial layer on which they develop. Their formation takes place equally well in light and darkness.

Before the development of the pileus, the stipe is perfectly indifferent to geotropic stimuli. In the absence of light it is rectipetal, and in its presence positively heliotropic.

In the absence of light the stipe may continue to grow for weeks or months, and may attain a length of six or more inches, but no signs of a pileus make their appearance. The development of the pileus depends on the presence of sufficient illumination. Grown in the dark, therefore, the fruit-bodies are all monstrous and abortive.

Whilst the pileus is developing, the stipe alters its reactions to external stimuli. It becomes negatively geotropic and ceases to be heliotropic.

The pileus is sometimes developed unequally in fruit-bodies with oblique stipes. The longest gills are always found on the side continuous with the lower side of the stipe. The inequality of development is induced by the stimulus of gravity.

The gills begin their development in such manner as to become perpendicular to the surface of the pileus from which they are formed. They are never heliotropic, but become positively geotropic.

Fruit-bodies grown in weak light are prone to branching and often become grotesque. Branching is often induced by the formation of abortive pilei.

It may be shown that the reactions of the fruit-bodies to external stimuli are admirably adapted for the economical distribution of the spores.

DESCRIPTION OF THE FIGURES IN PLATES XXIII, XXIV AND XXV.

Illustrating Professor Buller's paper on *Lentinus lepideus*.

PHOTOGRAPHS.

Figs. 1–5. Nat. size. All the same fruit-body and illustrating reaction to geotropic stimuli.

Fig. 1. The stipe grew towards the light at an angle of about 45° with the vertical. When the pileus began to develop the end of the stipe became negatively geotropic, and curved upwards through an angle of 45° so as to become vertical as in figure.

Fig. 2. The fruit-body grown in the position shown in Fig. 1 was turned through a right angle so as to assume the position shown in this figure.

Fig. 3. The same fruit-body after three days. The end of the stipe had curved through a right angle so as to bring the pileus into the erect position once more.

Fig. 4. The fruit-body was then turned through another right angle so as to take up the position shown in this figure.

Fig. 5. The same fruit-body after four days. The end of the stipe has turned through an angle of 45° and growth has ceased. The pileus is, therefore, only partially brought into the erect position. The gills have all curved towards the earth.

Fig. 6. Nat. size. A number of papillae and conical protuberances arising on a mycelial layer covering the end of a piece of a rotten paving block. The development took place in moderate light.

Figs. 7–9. Nat. size. All the same fruit-body and illustrating the positive geotropic reaction of the gills.

Fig. 7. The fruit-body developed in the normal vertical position (as in Fig. 1). It was then turned through a right angle and placed on its side as in this figure. All light was then carefully excluded from it.

Fig. 8. The same fruit-body after two days. The gills have grown towards the earth. They have become much longer, and their edges have developed the torn appearance characteristic for the genus *Lentinus*. The whole pileus has turned upwards slightly.

Fig. 9. The fruit-body of Fig. 8 photographed from below. The gills were pointing vertically downwards.

Fig. 10. Nat. size. Three young fruit-bodies grown in moderate light. The uppermost is aborted and has ceased its development. The lowest one, although still growing, shows no signs of a pileus. The middle one is producing a pileus symmetrically. Drops of water have been excreted upon it. The rudimentary gills are scarcely visible. The whole group is photographed from below.

Fig. 11. Nat. size. The middle fruit-body of Fig. 10 at a later stage and photographed from the side. The pileus has become erect owing to the curvature of the end of the stipe.

Fig. 12. Mag. $\frac{1}{4}$. The top of a paving block kept in complete darkness in a damp-chamber. From the mycelial layer two rods have grown out. That on the right has grown upwards at an angle of about 45° with the vertical. The other one has grown more or less parallel to the upper surface of the block. There are no signs of pilei.

Fig. 13. Mag. $\frac{2}{3}$. Paving block kept in complete darkness. A white layer of mycelium formed upon the surface of the wood and gave rise to numerous papillae, a number of which have developed in the course of about five weeks into monstrous fruit-bodies showing no traces of pilei.

Fig. 14. Nat. size. The stipe grew from the wood towards the light. A pileus is in a very early stage of formation. As yet there are no signs of gills.

Fig. 15. Nat. size. A fruit-body with short stipe and broad decurrent gills. Drops have been excreted upon the gills, which have torn edges.

Fig. 16. Nat. size. Another fruit-body with a long stipe. The pileus is chiefly developed on the side towards the observer. The gills are not yet fully grown, and have intact edges.

Fig. 17. Mag. $\frac{1}{3}$. Monstrous fruit-bodies photographed from above after growing from the mycelial layer on the wood for six weeks. There are no signs of pilei.

Fig. 18. Mag. $\frac{5}{12}$. Paving block, found among a pile of blocks which had been removed from a street in Birmingham. A monstrous fruit-body, branched like an elk's horn and devoid of pileus, had grown upon it.

Fig. 19. Mag. $\frac{1}{3}\frac{9}{7}$. Another photograph of the block shown in Fig. 17, again taken from above. The longest rod had attained a length of nearly 7 inches during nine weeks' growth. It had turned a rich rusty brown colour. Three young white protuberances are to be seen towards the centre of the block. They are growing out almost parallel to the surface of the wood.

Fig. 20. Mag. $\frac{1}{8}$. The same block and monstrosities as in Fig. 13. The rods had been growing in darkness for about nine weeks. There are no signs of pilei. The lower part of the rods had turned brown. The rods had become depressed owing to their own weight. The damp-chamber, with glass walls, was situated in a dark room used for photography. The occasional light was sufficient to give a heliotropic stimulus to the rods. They all grew towards the source of light.

DRAWINGS.

Fig. 21. Nat. size. A diagram to illustrate direction of outgrowth of fruit-bodies. *w*, block of wood; *m*, mycelial layer; *p*, papillae growing out perpendicularly to the mycelial layer and incidentally perpendicularly to the surface of the wood; *o*, o^1, papillae growing outwards perpendicularly to the edge of the mycelial layer and incidentally obliquely to the surface of the wood.

Fig. 22. Nat. size. To illustrate heliotropic curvature. The light was at first direct on to the block in the direction indicated by the arrow *a*. The rods *o*, *p*, *r*, and *s* grew towards the light. The block was then moved round in a horizontal plane through two right angles, so that the light was directed as indicated by the arrows *bb*. The rods *o* and *p* gradually grew in a curve through

a right angle so as to place their axes parallel to the incident rays. The rods *r* and *s* had withered up and are directed as were the rods *o* and *p* before the change in the direction of the light. The rod *p* has developed an abortive pileus. The rods *t*, *v*, *w*, &c., grew out from the block after the light had been changed in direction.

Fig. 23. Nat. size. Another heliotropic experiment. The rod first grew from the block *Bl*, so as to place itself parallel to the incidental rays of light which had the direction indicated by the arrow *a*. When the rod had attained the length *ll*, the block was turned in a horizontal plane through two right angles, so that the light came to have the direction indicated by the arrow *b*. The rod gradually grew in a curve so as once more to place its axis in the direction of the incident rays.

Fig. 24. Nat. size. A rod which grew for three months towards weak light without forming any pileus.

Fig. 25. Nat. size. Drawing of monstrosity shown in Fig. 19. For convenience the centre part of the rod has been represented straighter than it actually was. For the exact shape see Fig. 19. The monstrosity had grown for nine weeks in the dark. There is no trace of a pileus. *w*, part of wooden block; *m*, mycelial layer; *c*, another monstrosity cut off.

Fig. 26. Nat. size. Section through a fruit-body, the pileus of which is very unequally developed. The gills are longest on what was originally the under side of the stipe. On the opposite side the gills have not grown beyond the most rudimentary stage.

Figs. 27–30. All nat. size. Branched monstrosities, produced in weak light.

Fig. 27. The pileus touched the glass of the damp-chamber at *i*, and ceased its development. Some days afterwards branches began to grow out of the stipe at *s*.

Fig. 28. The fruit-body produced a rudimentary pileus with abortive gills at p^1 and a stipe s^1. From the edges of the pileus new branches (s^2) were developed. Some of them produced rudimentary secondary pilei (p^2) upon secondary stipes (s^2). One stipe (s^2) gave rise to a tertiary stipe (s^3) upon which a rudimentary tertiary pileus (p^3) was formed. The fruit-body was growing for about four months.

Fig. 29. The primary stipe (s^1) produced the primary pileus (p^1). The latter became abortive. Secondary stipes (s) arose from its edges and produced secondary pilei (p^2).

Fig. 30. Another branched monstrosity.

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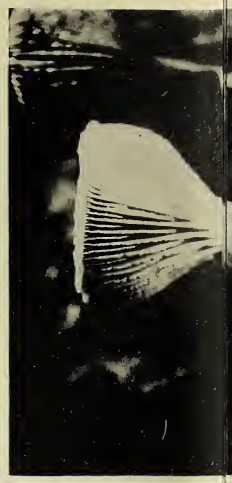

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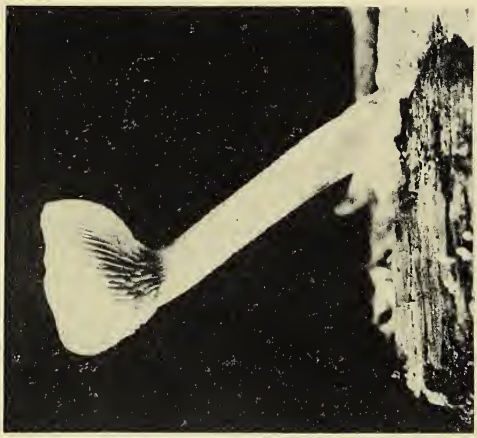

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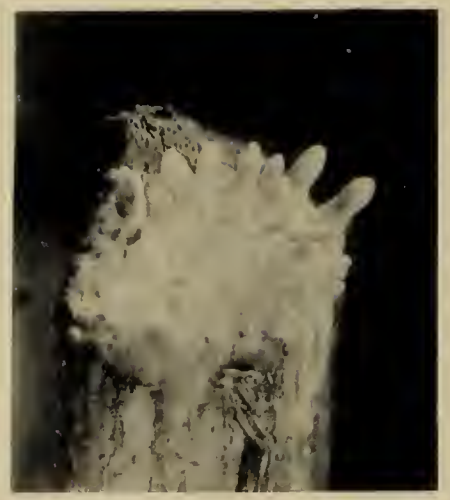

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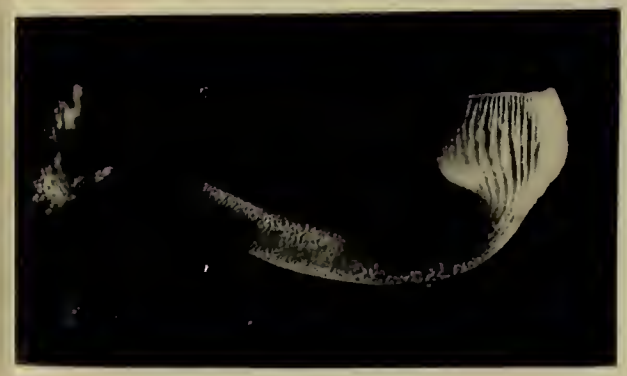

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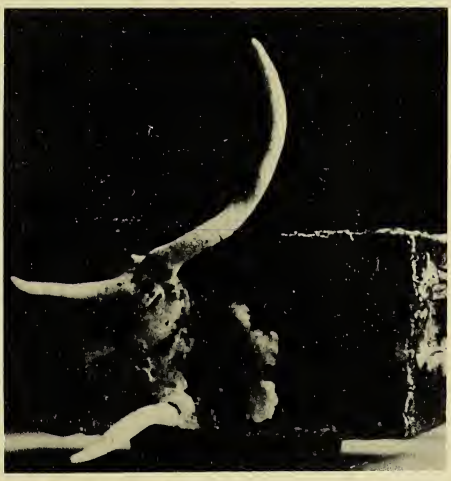

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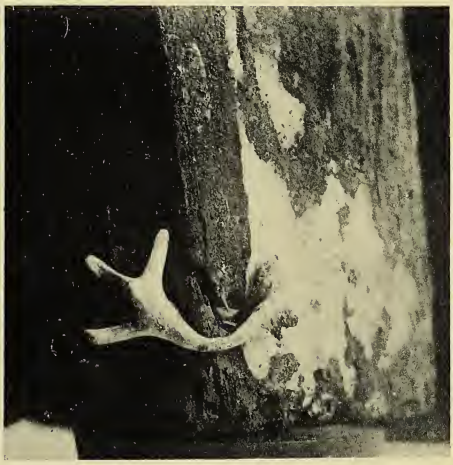

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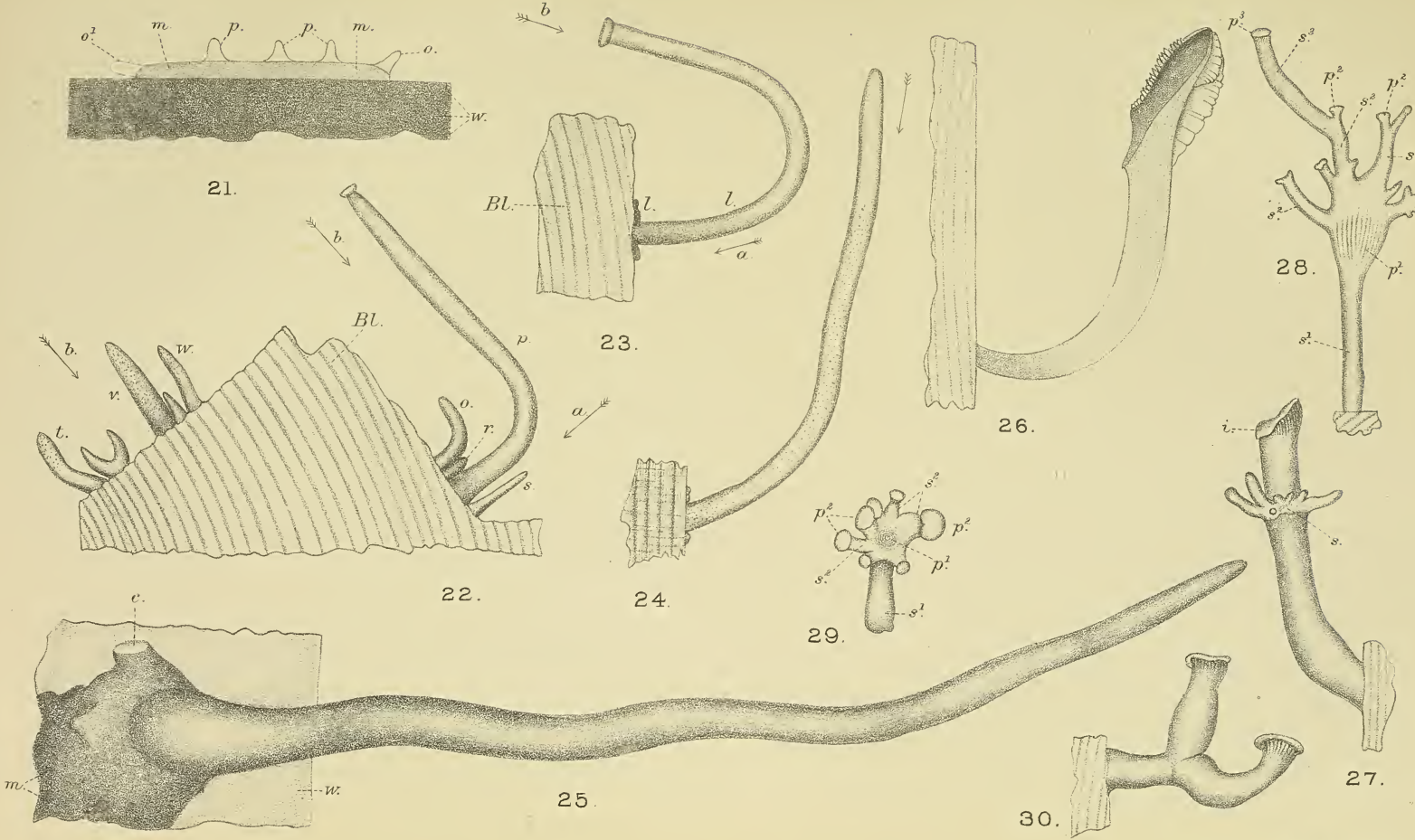
BULLER.—ON LENTINUS LEPIDEUS.



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BULLER.—ON LENTINUS LEPIDEUS.

NOTES.

ON PHLOMIS LUNARIFOLIA, SIBTH. ET SMITH, AND SOME SPECIES CONFUSED WITH IT.—At least two species have been confused under the name *Phlomis lunarifolia*. A comparison with Sibthorp's type, kindly lent by the authorities at the Oxford University Herbarium, shows that the plant figured and described in the *Botanical Magazine* (1900), t. 7699, is a distinct species (to which I give the name *Phlomis grandiflora*), differing in the following points:—

P. lunarifolia.—Bracts lanceolate or ovate-lanceolate; calyx glabrous between the ribs, with five long ascending mucros, and nearly truncate; hood of corolla semicircular in outline; lower lip scarcely longer than the upper; middle lobe of lower lip entire; nutlets slightly glandular above; leaves crenate, and veins of leaf conspicuously impressed above.

P. grandiflora.—Bracts ovate; calyx stellately pubescent all over, with five membranous, broad and emarginate lobes produced into mucros which are shorter and more spreading than in *P. lunarifolia*; hood of corolla more gradually curved and larger than in *P. lunarifolia*; lower lips conspicuously longer than the upper; middle lobe of lower lip obcordate; nutlets glabrous; leaves entire (or very minutely and obscurely crenulate) and veins *not* conspicuously impressed above.

Phlomis lunarifolia was first described in Sibthorp and Smith's *Prodromus Florae Graecae* (1806), vol. i, p. 414, as follows:—'foliis cordatis crenatis, subtus tomentosis; bracteis ovato-lanceolatis fasciculato-ciliatis mucronatis. P. Samia herbacea, lunariae folio, Tourn. Cor. 10. . . . In variis Peloponnesi locis; etiam in monte Atho.'

Sibthorp and Smith give it the habit of *P. Samia* but with broader bracts; and as Boissier points out in *Flora Orientalis*, vol. iv, p. 785, their statement that it was found in the Peloponnesus and on Mt. Athos probably arose from a confusion of the species with *P. Samia*, Linn., which actually occurs in the Peloponnesus and on Mt. Athos. At any rate *P. lunarifolia* has not since been recorded from Greece, and as Sibthorp visited Chrysoku in May, 1787 (see Unger and Kotschy, *Die Insel Cypern*, p. 148), where Kotschy found the plant, we may assume that Sibthorp's specimen came from Cyprus and that the label was lost.

Bentham, who says (*DC. Prod.* 1847, vol. xii, p. 541) 'exemplar in herb. Sibthorp. pessimum,' places it next to *P. Russeliana*, Lag., and he distinguishes it from *P. Russeliana* by the bracts being three times as broad.

In the *Botanical Magazine* (1825) t. 2542 is figured and described *Phlomis lunarifolia β Russeliana*, which was thought by the sender, Mr. Lambert, to be the *P. lunarifolia* of the *Prod. Flor. Graecae*. Dr. Sims, the Editor, had no opportunity of comparing the plant with Sibthorp's specimen, but he considered it a variety of it,

and suggested that should it afterwards be determined a distinct species, Lagasca's name *Russeliana* would be appropriate, as he had 'no doubt but that it is the same species of which Dr. Russell has given a figure in his history of Aleppo, and which he thought might perhaps be a yellow-flowered variety of *Phlomis Herba-venti*.' *P. Russeliana*, Lag., has, however, since been reduced by Boissier to *P. viscosa*, Poir., and it is totally different from both *P. lunarifolia* and *P. grandiflora*.

Boissier (*Flora Orientalis*, 1879, vol. iv), who did not see the type specimen of *P. lunarifolia*, confused two species under that name; but his description is better adapted to *P. grandiflora*, e.g. 'bracteis ovatis, . . . calyx stellatim puberulo.' He quotes Kotschy, no. 678[1], and Péronin, no. 71, from Cilicia, which are the true *P. lunarifolia* of Sibthorp, and Bourgeau, no. 296, from near Elmaly in Lycia, which is *P. grandiflora*. Sir J. D. Hooker, in the *Botanical Magazine* (1900), t. 7699, alludes to the confusion of islands [see footnote], and states that Boissier overlooked the record in Unger and Kotschy's *Die Insel Cypern*, published in 1865, where on p. 275 we read—'In Cypern bei Chrysoku im Thale gegen Chrysoroodissa nicht selten bis 6' hoch, n. 678, Peloponnesus.'

Boissier quotes as a synonym '*P. imbricata*, Boiss., in Bourg. Pl. Lyc. 1860.' This refers to the specimen mentioned above (no. 296) which clearly belongs to *P. grandiflora*. He gives *P. fruticosa*, Linn., as the nearest ally, and distinguishes it thus—'ab ea [*P. fruticosa*] indumento tenui, bracteis membranaceis ciliatis glabris calyceque distincta.' *P. fruticosa* may be separated from both *P. lunarifolia* and *P. grandiflora* by its tomentose bracts and calyx, and by the short and recurved calyx-teeth; and from *P. lunarifolia* in having entire leaves.

Halácsy, the most recent writer on the Greek flora, in his *Conspectus Florae Graecae* (1902), vol. ii, p. 509, simply quotes the description of Sibthorp and Smith, and remarks that the species is unknown to present-day authors.

Phlomis Cypria, Post (in *Mém. de l'Herbier Boissier*, 1900, p. 99), which has somewhat broad bracts, is distinguished by its smaller heads of flowers, which are solitary at the top of the branches, by its woolly bracts and calyx and very small lanceolate leaves. In the Kew Herbarium there are two fruiting pieces of what may be *P. Cypria*, which were distributed with flowering specimens of *P. viscosa* var. *cretica* [wrongly labelled *P. fruticosa*, Linn.] from waste places at Canea in Crete, by E. Reverchon (no. 143). The former match Post's type specimens of *P. Cypria* in Herb. Kew. very well, and as Reverchon states the specimens were collected in May and July (1883), it is probable that the two pieces in question were gathered in July, the collector supposing them to be fruiting specimens of *P. viscosa* var. *cretica*.

I now give descriptions and synonymy of both *P. lunarifolia* and *P. grandiflora*.

Phlomis lunarifolia, Sibth. et Smith, *Prod. Florae Graecae*, vol. i, p. 414.

Suffrutex 2–6 pedalis, ramis junioribus stellatim tomentosis. *Folia* 2–5 poll. longa, $\frac{3}{4}$–3 poll. lata, apice rotundata, crenata, supra sparse stellatim pubescentia, subtus stellatim tomentosa, venis et venulis supra conspicue impressis, inferiora anguste ovata, basi cordata, petiolis usque ad $8\frac{1}{2}$ poll. longis, superiora ovato-oblonga, basi rotundata vel obtusa, subsessilia vel breviter petiolata. *Bracteae* lanceolatae vel

[1] Erroneously from Rhodes instead of from Cyprus (Chrysochu).

ovato-lanceolatae, acuminatae, extra stellatim puberulae, marginibus tuberculato-setosis. *Calyx* 5–7 lin. longus, inter costas glaber, margine ciliato, mucronibus et costis primariis setosis, costis secundariis stellatim pilosis, lobis plane obscuris, unde calycis ore truncato, mucronibus inaequalibus circa 2–4 lin. longis, ascendentibus. *Corolla* flava, extra stellatim tomentosa; galea sub apice retuso obtuse bicarinata, semicircularis, 8 lin. longa, $3\frac{1}{2}$–4 lin. lata; labium inferum quam galea vix longius, lobo medio integro; tubus 7 lin. longus, annulo pilorum uniseriatim dispositorum infra insertionem filamentorum instructus. *Filamenta* ad et infra medium pilosa, postica appendiculata, appendice $\frac{3}{4}$ lin. longa, $\frac{1}{4}$ lin. lata. *Nuculae* apice sparse glandulosae, glandulis minutissimis subsessilibus.

P. lunarifolia, Bentham in *DC. Prod.* (1847) xii, 541.

P. lunariaefolia, Boissier, *Fl. Orient.* (1879) iv, 785, *partim.*

P. Samia, herbacea, Lunariae folio, Tourn. *Cor.* 10.

Distribution :—

Cyprus, near Chrysoku (see above); *Sibthorp* (Oxford University Herb.); *Kotschy*, no. 678, ann. 1862 (Herb. Kew. and Brit. Mus.).

Cyprus, without precise locality, *Miss Samson*, ann. 1904 (Herb. Kew. Leaves rather broader and more lyre-shaped than in the other specimens).

Cilicia, mountain near Anamour, *Péronin*, no. 71, ann. 1872 (Herb. Kew. and Brit. Mus.; sub *P. fruticosa*, Linn.).

Phlomis grandiflora, H. S. Thompson, sp. nov.

Suffrutex 3–4 pedalis, cano-tomentosus, ramis junioribus stellatim tomentosis. *Folia* $2\frac{1}{4}$–$2\frac{3}{4}$ poll. longa, 1–$1\frac{1}{2}$ poll. lata, apice obtusa, basi rotundata vel obtuse cuneata, integra vel minutissime et obscure crenata, supra tenuiter stellatim pubescentia, subter stellatim tomentosa; venis et venulis supra haud vel vix impressis; inferiora petiolis usque ad 2 poll. longis, superiora subsessilia. *Bracteae* late ovatae, breviter acuminatae, extra stellatim puberulae, breviter ciliatae. *Calyx* $6\frac{1}{2}$ lin. longus, ubique dense stellatim pilosus, lobis valde depressis, latissimis, emarginatis, mucronibus e loborum emarginaturis ortis inaequalibus, circa $\frac{1}{2}$–$1\frac{1}{2}$ lin. longis, subpatentibus. *Corolla* aurea, extra (imprimis galea) stellatim tomentosa, galea sub apice retuso obtuse bicarinata, cymbiformis $10\frac{1}{2}$ lin. longa, $3\frac{1}{2}$–4 lin. lata; labium inferum quam galea multo longius, lobo medio obcordato; tubus $8\frac{1}{2}$ lin. longus, annulo pilorum pluriseriatim dispositorum infra insertionem filamentorum instructus. Filamenta ad et infra medium pilosa, postica appendiculata, appendice $1\frac{1}{2}$ lin. longa, $\frac{1}{2}$ lin. lata. *Nuculae* glabrae.

P. lunariaefolia, Boiss., *Fl. Orient.* (1879) iv, 785, *partim*; Hook. f., Bot. Mag. (1900) t. 7699, non Sibth. et Smith.

P. imbricata, Boiss., *Fl. Orient.* (1879) iv, 785.

Distribution :—

Asia Minor, Lycia, Andipholi; Prof. Forbes, no. 449, ann. 1841 (Herb. Kew.).

Lycia, near Elmaly; E. Bourgeau, ann. 1860 (Herb. Kew.).

Pisidia, on Dauros Dagh, SW. of Egerdir; Whitall, ann. 1893 (Herb. Kew.). Also specimens raised in Hort. Kew. from seed sent by Mr. Whitall (Herb. Kew.).

H. STUART THOMPSON.

Kew.

THE RESISTANCE TO FLOW IN WOOD VESSELS.—In vessels filled with water, the resistance to flow depends upon the rate of flow, the square of the radius of the tube, the length of the vessel and the viscosity of water. Although the vessels are relatively narrow tubes, the rate of flow is not very great, so that the total viscosity resistance to flow in an ordinary tree during active transpiration is considerably less than the height of the tree[1]. As air appears in the vessels, however, two new factors are introduced. In intact vessels these bubbles when large and pressed against the sides of the vessels remain approximately stationary, the water flowing past them. Under these circumstances the surface tension film around each bubble forms an additional limiting surface to the ascending stream. If the latter exercises any friction upon the sides of the bubble, and it is difficult to see how friction against a stationary surface tension film around the air-bubble can be avoided, then the bubble must be subjected to some downwardly directed force which counterbalances the frictional and gravitational upthrust upon it.

The explanation of this peculiarity seems to lie in the fact that a true Jamin's chain action is exercised in wood vessels containing air-bubbles and water-columns, in spite of the fact that the walls of the wood vessels are lined internally by a more or less continuous adherent film of water. No Jamin's chain action can be exercised when the liquid wets the smooth walls of a containing vessel equally at all points, since in this case the boundaries of the surface tension films move as readily as the central regions instead of being dragged back, so that the front boundary of each drop becomes convex, and the hinder one concave. As a matter of fact, however, a true Jamin's chain resistance does appear to be shown in vessels lined by a film of water and whose walls are wetted by water. Possibly this may be due to the internal localized thickenings of the walls, the surface tension films coming within the limit of adhesion opposite to these but moving freely between them. In this way a valvular mechanism could be maintained in the vessels, by the aid of which water could be passed from point to point in a vessel by slight local forces, without the total head of water in the tree requiring to be maintained by any pronounced pressure or tension applied at base or apex. Thus in Fig. 8 a slight pressure generated between *b b* would be prevented from acting downwards by the bubble beneath adhering at *a a*, so that the surface tension film became more convex, and would act upwards only. A wave of actions of this kind running up along the vessels would raise the water upwards.

Evidence as to the existence of a true Jamin's chain resistance in the conducting wood of actively transpiring trees is afforded by the fact that the resistance to flow is much greater than would be expected from a viscosity calculation. In addition direct evidence was obtained in the following way. Living branches of the plants previously examined (l. c.) were shaved down at one end until thin enough to examine the uncut vessels under the microscope. The other end was fixed to a pressure apparatus containing a coloured liquid which could be driven slowly

[1] See Ewart on the Ascent of Water in Trees. Phil. Trans. 1905, p. 44 seq.

through the wood. To retard the flow the distal end was imbedded in a mass of vaseline, and in all cases it was seen that where the vessels contained large air-bubbles the front end of each film became more convex when pressure was applied, while the hinder end became flattened or concave. These effects become extremely pronounced when the bubble is passing the constrictions where segment cells join

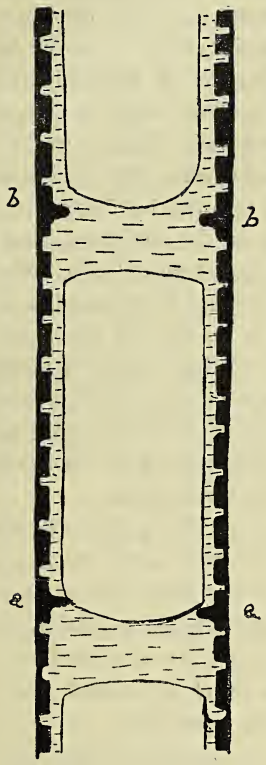

Fig 8

Fig 9

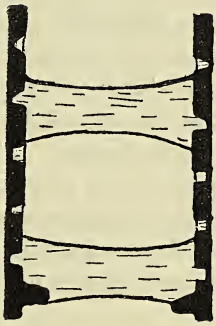

Fig. 10.

FIG. 8. Diagrammatic longitudinal section of a segment of a vessel. The ends of the segments at *a a*, *b b*, form the adhesion points of the surface tension films. FIG. 9. View from beneath of a surface tension film on the under side of an air-bubble at the middle of a segment, showing that water may still be able to pass the air-bubble in spite of the adhesion of the surface tension film to the inner wall of the vessel. FIG. 10. As in Fig. 8, but with less water, so that the films adhere to the inner wall, and as in the true Jamin's chain there is no internal lining of water between the water-columns.

(Fig. 8, *a a*, *b b*). The same differences were maintained as the bubble was driven slowly along the tube, so that it can be safely assumed that the differences of curvature were even more pronounced with velocities too high to permit of direct observation. At the same time the coloured solution appeared in front of the last few air-bubbles in the series [1], so that here we have what appears to be definite proof

[1] These observations can be made on the stem of the *Wistaria* with the aid of a hand lens without injuring the wood.

that the water is able to pass the air-bubbles and surface tension films in the vessels, and yet the films are able to exert a back pressure increasing the resistance to flow. Further research must determine whether the explanation given is a true one, or whether unknown factors enter into play in the vessels.

When the walls of the vessels dry, or become covered internally over the whole or parts of their length by films of oily or similar substances, a true Jamin's chain may be set up in the vessel (Fig. 10). This, when once formed, is difficult to remove and interposes a relatively enormous resistance to flow when the vessel is narrow and the air-bubbles numerous. Even then, however, the presence of longitudinal pores containing water and longer than the air-bubbles may allow water to pass slowly even when the films appear to be adherent to the internal wall. The air in dry wood vessels always tends to break into shorter or longer bubbles when the wood is soaked in water, so that the normal conductivity can only be restored by forcing in water under such pressure as either to break the adhesion of the surface tension films to the walls of the wood vessels, or to render their attachment more labile. Naturally the conductivity restored in this way will be rapidly lost again if the wood is allowed to become depleted of water, and even if the supply is maintained by suction, the presence of any films of oily or impermeable material on the interior of the vessels will tend along with the increasing air-bubbles to produce an ordinary high resistance Jamin's chain. The latter involves an interrupted column of water, rapid upward flow requiring a movement of the entire series of air-bubbles and water-columns, which is impossible in vessels with imperforate partitions across them.

On the other hand, the valvular type of Jamin's chain with a peripheral adhesion film of water, allows local feeble pumping actions to be maintained from point to point in a staircase fashion. The least resistance to flow is shown when the vessels are filled with water, and continuous water-columns may support a tension of over 200 atmospheres in the absence of air[1]. The transpiring leaves cannot exert the tensions required even momentarily in the wood vessels of the tallest trees (150 metres), since these are very much greater than is usually supposed[2].

ALFRED J. EWART.

BIRMINGHAM UNIVERSITY.

ON ENDOPHYTIC ADAPTATION SHOWN BY ERYSIPHE GRAMINIS, DC., UNDER CULTURAL CONDITIONS[3].—In recent papers by the author the fact has been pointed out that certain species of the *Erysiphaceae* are able, under cultural conditions, to infect their host-plants vigorously when their conidia or ascospores are sown on the cells of the internal tissues exposed by means of a wound, although the fungi in question are confined normally to the external surface of the epidermal cells.

The present paper gives the results of investigations carried out in the laboratory of Professor Marshall Ward at Cambridge, with the object of ascertaining the details

[1] Berthelot, Ann. de Chimie, xxx, 1852. [2] Ewart, loc. cit., p. 55.

[3] Abstract of a paper read before the Royal Society on April 6, 1905.

of growth of the fungus under these abnormal conditions, and of discovering to what extent the hyphae penetrated into the intercellular spaces of the internal tissues, and whether haustoria (normal or otherwise) were produced by these hyphae.

A rapid survey is first made of our present knowledge of the mycelial characteristics of the *Erysiphaceae* in relation to their parasitic habit. The species of the *Erysiphaceae* were regarded since De Bary's time as strict ectoparasites, until in 1899 Palla discovered the semi-endophytic habit of the genus *Phyllactinia*. With this exception the species of the *Erysiphaceae*, so far as they have been investigated, have been found to be strictly ectoparasitic in habit, the hyphae of the mycelium being confined to the external surface of the epidermal cells (never gaining access to the intercellular spaces of the internal tissues), and merely sending haustoria either into the epidermal cells alone, or, in the case of one species, into the sub-epidermal cells as well.

The experiments carried out and the methods employed in the present investigations are then described. The fungus used was the conidial stage of *Erysiphe graminis*, DC., a strict ectoparasite under normal circumstances. Young leaves of oats and barley were cut off from seedling plants, and a minute piece of tissue was cut out with a sharp razor from the upper surface of the leaf. In this operation the upper epidermis was removed, and often a considerable amount of the mesophyll also, so that in inoculation the conidia were sown on the sub-epidermal or deeper layers of the exposed mesophyll, or even on the internal surface of the lower epidermis. After inoculation, the leaves were placed on damp blotting-paper in a Petri dish. By the sixth to eighth day vigorous infection had nearly always resulted, the surface of the wound bearing patches of clustered conidiophores. The leaves were then fixed in Flemming's fluid or in chromacetic, and subsequently embedded in paraffin, microtomed, and stained with Diamant fuchsin and Lichtgrün.

It was found on examining such wounded leaves that the fungus had invaded the internal tissues to a remarkable extent. Where the mesophyll-cells remaining uninjured were several layers deep, the hyphae had penetrated inwards, winding through the intercellular spaces as far as the internal surface of the lower epidermis. Haustoria were sent into the cells of the superficial layer of the mesophyll by the hyphae creeping on the surface of the wound, and into all the deeper layers of the mesophyll by the hyphae running in the intercellular spaces. The cells of the lower epidermis were also attacked, the internal wall having been penetrated. The sheath-cells of the vascular bundles were much invaded by very vigorous haustoria. The haustoria formed in the cells of the internal tissues resemble in every way those which occur normally in the epidermal cells.

The hyphae enclosed in intercellular spaces, either just below the surface of the wound or deep down in the internal tissues, struggle to produce conidiophores. The respiratory cavities over the stomata of the lower epidermis were in a great number of cases full of vigorous hyphae producing young conidiophores. When the intercellular space, where the young conidiophore was produced, was shut off from the open air by only a thin membrane consisting of the walls of collapsed mesophyll-cells, the young conidiophore growing upwards, sometimes proved able to break through it and continue its growth. The direction of growth of the young

conidiophores produced in the respiratory cavities and other intercellular spaces was usually vertical, and towards the surface of the wound. Examples were observed, however, of young conidiophores growing horizontally in intercellular spaces between the mesophyll-cells, or, in a few cases, vertically, with the apex of the conidiophore directed away from the surface of the wound.

In several cases hyphae had penetrated laterally, in a direction parallel to the surface of the leaf, from the edge of the wound, and occurred in the intercellular spaces in the middle of the mesophyll, at places where all the tissues, including the epidermis above and below, were uninjured. In such places both haustoria and young conidiophores were produced.

Figures are given illustrating the details of the growth of the hyphae in the interior of the leaf, and the production of haustoria and intercellular conidiophores.

The author, reviewing the results of the investigations, points out that they afford proof that *E. graminis* is not, as perhaps might have been expected, so highly specialized as an ectoparasite as to be necessarily restricted for its food-supply to cells of the epidermis; but shows itself capable of immediate adaptation to conditions closely resembling those obtaining in endophytism.

This fact suggests the possibility that under some circumstances the mycelial hyphae of species of the *Erysiphaceae* which are normally ectoparasites may penetrate into the internal tissues of their host-plants exposed through wounds caused in nature by the attacks of animals or by physical agency. It is pointed out, however, that the successful entry of the hyphae might be prevented, either by the drying up of the superficial layers of cells, or by the healing processes shown by many actively growing leaves.

ERNEST S. SALMON.

KEW.

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ANNALS OF BOTANY

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ASSISTED BY OTHER BOTANISTS

London

HENRY FROWDE, AMEN CORNER, E.C.

Oxford

CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1905

Printed by HORACE HART, *at the* CLARENDON PRESS, *Oxford.*

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The Embryology of some Anomalous Dicotyledons.

BY

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With Plates XXVI and XXVII.

Historical.

WITHIN the past few years, the discovery of an anomalous character in the embryo of some species of certain dicotyledonous families has stimulated interest in the question of the phylogeny of the Angiosperms, especially as these anomalies have been regarded as throwing some light upon the origin of the two divisions of the higher seed plants. The several observers have approached the subject from two standpoints, some dealing with the development of the embryo, while others have devoted themselves to the study of the comparative anatomy of seedlings. Of the publications that have recently appeared, dealing with the embryological evidence, that of Lyon ('01) was among the first. This observer has shown that *Nelumbo lutea*, one of the Nymphaeaceae, is fairly typical of the known anomalous dicotyledons. The development of the embryo is described under four stages, namely, the 'spherical stage,' the 'monocotyledonous stage,' the 'dicotyledonous stage,' and the 'mature embryo.'

The early development in *Nelumbo* results in a spherical embryo, consisting of several hundred cells. Growth now takes place such that a flattened or button-shaped mass of tissue results. From the distal side of this mass the plumule, or rather the apex of the stem, appears as a small protuberance, as does also the single primordium of the cotyledons, which arises as a crescent-shaped mound of tissue around the rear of the embryo, its wings (points of the crescent) extending forward even with the plumule. Judging from Lyon's Fig. 8 (l. c.), it seems that, at this stage, the plumule lies between the points of the crescentic cotyledonary primordium, but later it becomes situated within the space enclosed by the crescent. This closes the monocotyledonous stage. The subsequent development, immediately following, results in the cotyledonary primordium becoming two-lobed through the localization of growth at two nearly opposite points. With the final development of the embryo, these two lobes grow into the two large cotyledons. The sinuses between the cotyledons are not of equal

[Annals of Botany, Vol. XIX. No. LXXVI. October, 1905.]

depth, that representing the original cleft of the primordium being deeper. The two cotyledons have, therefore, a common base, having arisen from a common primordium. From these facts Lyon concludes that the embryo of *Nelumbo lutea* is monocotyledonous, placing the Nymphaeaceae among the Monocotyledons, in the series Helobieae.

In a preliminary note on the embryo of three species of *Nymphaea*, *N. odorata*, Ait., *N. coerulea*, Sav., and *N. Lotus*, L., Conrad ('02) asserts that the development up to the stage of the spherical embryo coincides with that of *Nelumbo*, as described by Lyon, but 'the spherical embryo, however, unlike that of *Nelumbo*, gives rise to two opposite and symmetrical outgrowths near its lower end. These become the two equal cotyledons. The intervening apical portion of the sphere becomes the plumule, with the rudiments of two unequally developed leaves. The basal portion of the sphere becomes the radical.'

In the same year Schmid ('02) gave an account, though somewhat incomplete as to certain details, of the embryology of two very remarkably anomalous dicotyledonous species, namely, *Ranunculus Ficaria*, L., and *Corydalis cava*, Schw. and Kte., one of the Fumariaceae. In *Ranunculus Ficaria*, after the embryo has become somewhat pear-shaped, growth takes place in such a manner that the central part of the apex, i. e. the truncated distal end of the embryo, seems depressed, while the edge grows up in the form of a thick ridge, or rampart, concave within and kidney-shaped in cross-section. This ridge Schmid calls the *Anlage* of one cotyledon. During the germination of the seed, this cotyledon unfolds as a single broad lamina with a rather deep notch at its apex. There is no trace of a second cotyledon. Schmid's Fig. 38, *a-e*, shows that this cotyledon arises as a kidney-shaped primordium, which seems to be exactly like that giving rise to the two cotyledons in *Actea alba* and in other species to be mentioned later on. The same origin is figured also for the single cotyledon of *Bunium Bulbocastanum*, one of the Umbelliferae. In *Corydalis cava* the cotyledon arises as in *Ranunculus Ficaria*. Here the stem-apex is pushed to one side by the terminal position taken by the cotyledon. As in *Ranunculus Ficaria*, the base of the cotyledon is crescentic in cross-section, thus appearing with a narrow channel or groove down one side (Schmid, l. c., Fig. 16). This condition resembles a cotyledonary tube open on one side. While Schmid regards this structure as one cotyledon, he states on p. 214 (l. c.) that the end of this cotyledon is slit near the apex, giving reference to his Fig. 49. Judging from this figure and from the fact that, in a number of other plants, the two unquestionable cotyledons have a single primordium, and inasmuch as Schmid has not given a more complete history of the embryo, it does not seem improbable that we have here two cotyledons with a common base, or in which one sinus is very much deeper than the other; but as the writer has not examined this species, he prefers

to withhold judgement in the matter. It may be remembered that in two other species of *Corydalis* studied by Schmid, *C. nobilis* and *C. lutea*, the embryo is dicotyledonous, but these species do not form tuberous stems, a phenomenon that will receive further attention in a later paragraph. Schmid speaks of these plants as pseudo-monocotyledons, but he does not discuss the question of phylogeny. In *Ranunculus Ficaria*, *Eranthis hiemalis* and *Corydalis cava*, the embryo, which is incompletely developed when the seed is ripe, continues its development after the latter falls from the plant, and this author carried out experiments which showed that the phenomenon in question is due rather to favourable physical conditions than to any specific property of the soil. Schmid's study tended to show that the anomalous character of the embryo is closely associated with a geophilous habit and a short and tuberous stem.

Cook ('02) states that only one cotyledon is present in *Castalia odorata* and *Nymphaea advena*, members of the Nymphaeaceae, as he did not find a bifurcation of the cotyledonary primordium, as described by Lyon ('01) for *Nelumbo*. Cook's investigation does not seem to have been very exhaustive. Series of transverse sections through the cotyledonary origin at several stages in its early development, which are so necessary for a definite conclusion, are not given. He concludes that the Nymphaeaceae should be classed among the Naiadales.

The same observer has shown that the embryo of *Claytonia virginica* ('03) is remarkably anomalous in character. The embryo of the mature seed possesses one large cotyledon, with only the mere rudiment of the second. The nature of the cotyledonary primordium is, however, not figured.

Contrary to Cook, Schaffner ('04) finds that, in *Nymphaea advena*, the cotyledons arise from a crescentic primordium, as described by Lyon for *Nelumbo*.

Working under the direction of the writer, Lewis ('04) made a careful study of the development of the embryo of three species of anomalous Dicotyledons, namely, *Podophyllum peltatum*, *Caulophyllum thalictroides*, and *Jeffersonia diphylla*, of the Berberidaceae. In each case the cotyledonary primordium is a broad, crescentic ridge open at one side, which develops from the truncated distal end of the usually pear-shaped embryo. From the centre of the truncated end enclosed by the cotyledonary primordium, develops the plumule, or apex of the stem. This ridge soon bifurcates at a point opposite the opening or primary sinus to form the two lobes, the cotyledons. With further growth, the two sinuses become of almost equal depth, so that the older stages do not reveal the true nature of the origin of the cotyledons. In *Podophyllum* there is a rather long cotyledonary tube formed by the intercalary growth of the common base of the cotyledons.

Approaching the subject of the phylogeny of the Angiosperms, with the anatomy of seedlings as data, Miss Sargant has brought together in a

very skilful manner an immense mass of facts, derived from a thorough study of the anatomy of a large number of seedlings, chiefly of the Liliaceae. As will be more explicitly indicated in a later paragraph, this observer regards the Angiosperms as monophyletic in origin, the Dicotyledons being the more primitive, from which the Monocotyledons have been derived.

Observations.

The writer's observations were made upon *Actea alba* (L.), Mill., *Delphinium tricorne*, Michx., *Aquilegia canadensis*, L., *Syndesmon* (*Anemonella*) *thalictroides* (L.), Hoffmg., of the Ranunculaceae, and *Sanguinaria canadensis*, L., and *Stylophorum diphyllum*, Michx., of the Papaveraceae. In all of these species the development of the embryo was carefully traced from a very young stage to that found in the ripe seed. Although all show certain well-marked anomalies in the origin of the cotyledons, yet it will be seen from what follows that a gradual transition from a clearly anomalous type to that which is typically dicotyledonous is to be found in the same family, and even in the same species of a genus. Owing to the fact that the anomalous character is more pronounced in certain individuals of *Actea alba*, a detailed account of the embryo in this species will be given first.

Actea alba.

No effort was made to find the earlier cell-divisions in the young embryo, as that part of the process was not considered important in this study. The youngest embryos observed in *Actea* are represented in Plate XXVI, Figs. 1 and 2. At this stage it is clear that the embryo consists of a short suspensor about two cells broad and a somewhat club-shaped or cylindrical body, as indicated in both longitudinal and transverse sections (Figs. 1, 2, and 3). The cells of the suspensor are characterized by the presence of starch in greater or less abundance, while those of the rest of the embryo contain little or no starch. Whether Fig. 2 is an older stage than Fig. 1, or merely a section at right angles to the plane of Fig. 1, was not determined. Fig. 3 is a cross-section through the middle of an embryo of the stage shown in Fig. 1. The superficial cells now undergo periclinal and anticlinal divisions, whereby the epidermis is differentiated, while the cells within divide in three planes (Fig. 4). With further growth the embryo becomes symmetrically pear-shaped (Fig. 5). The distal end is either nicely rounded as in the figure just mentioned, or it may be somewhat flattened. The suspensor has grown only a little. Its cells contain starch, although this substance is seen in a few neighbouring cells of the remaining embryo.

At this stage begins the development of the cotyledonary primordium. As the true and exact nature of this can be better ascertained in series of

cross-sections taken at right angles to the longitudinal axis of the embryo, a series of outlines of similar sections of embryos in different stages of development will be given. These sections not only show the shape of the embryo, but also that part involved in the formation of the cotyledons, and the changes taking place in their early formation. Fig. 17, *a* to *p*, includes the whole embryo, with the exception of the first two cells of the suspensor and fragments of some cells at the distal end. The sections of this embryo were cut 7·5 microns in thickness. *a* to *k* represent alternate sections; *l* to *p* successive sections. Sections *m* to *p* of this figure show unmistakably that the formation of the cotyledonary primordium involves almost the entire end of the pear-shaped embryo, and that this end grows out into a thick crescentic mound or ridge. The entire embryo at this stage (Fig. 6) is a pear-shaped object with a curved indentation on one side at the broad end, which extends quite, or nearly, to the centre. Fig. 6 was carefully constructed from the series of sections shown in Fig. 17. The anatomical details of the embryo at a slightly later stage, as seen in a transverse section passing through the base of the cotyledons and including the tip of the stem, are shown in Fig. 14. At this stage, and even earlier, the formation of the plerome-strands (*pl*, Fig. 14) in each cotyledon is very evident, and the central position of the apex of the stem (*st*, Fig. 14) is clearly indicated. With further development of the embryo, growth becomes localized in the cotyledonary primordium in such a way that a cleft is formed exactly opposite the original sinus, as indicated in Fig. 19, *a–k*. In this manner the two opposite and symmetrical halves of the cotyledonary primordium develop into the two cotyledons. At the stage of development represented in Fig. 19, we have an embryo with two cotyledons possessing a common base, and in which one of the sinuses between the cotyledons is twice as deep as the other. A median longitudinal section of an embryo similar to that of Fig. 19, taken at right angles to the flat surface of the cotyledons, is shown in Fig. 7. In this and similar sections there is nothing to indicate definitely that the two cotyledons have arisen from a common primordium, for the figure seems to coincide in every respect with that from a typical dicotyledonous embryo. At this stage (Fig. 7), it will be seen that the plerome-strands in the cotyledons and hypocotyl are becoming well defined. The suspensor is short, being one or two cells wide at the free end, but more massive at its juncture with the body of the embryo. As the embryo continues its development in the seed, the inequality in the depth of the sinuses, as shown in Fig. 19, is maintained in some individuals until a later stage, but growth is such that this difference in depth is somewhat equalized when the seed is mature. However, in the ripe seed, embryos are frequently found in which the sinuses are of unequal depth, and we have then cases of anomaly of a pronounced character (Fig. 8). This figure was constructed in the same

manner as Fig. 6, from an embryo of a mature seed. The sinuses are of unequal depth, the primary cleft being deeper. There is no protruding stem-apex or plumule. The cotyledons are crescentic in cross-section, being concave on the inner surfaces. There is no cotyledonary tube formed in *Actea alba*. Although the cotyledons have a common base, yet they cannot, as will be shown in the following paragraph, be regarded as one cotyledon which has bifurcated. Such an interpretation might be defensible if all embryos of *Actea alba* followed closely the line of development as detailed in the foregoing.

It will now be shown that there is in this species what may be reasonably regarded as typical dicotyledonous development. Instances were found in which the cotyledonary sinuses appear at about the same time. Fig. 18, *a–h*, represents in outline a series of transverse sections, beginning just below the base of the cotyledons. Of course it cannot be stated with absolute certainty that the cotyledonary primordium was not slightly crescentic in its earliest stage, but from younger stages of a similar condition, and from the evidence obtained in *Syndesmon*, to be mentioned below, it is highly probable that the primordia of the cotyledons were separate or nearly so. As in the former case, it will be seen from *b*, Fig. 18, that the origin of the cotyledons involves almost the whole truncated end of the embryo. Fig. 16, which is *b*, Fig. 18, drawn in detail, shows the same condition of things as indicated in Fig. 14. Fig. 16, *b*, is taken at a level corresponding with that of Fig. 14. In Fig. 18 is shown what is of frequent occurrence, namely, the unequal length of the two cotyledons. In several cases examined, both in *Actea* and in the other species to be mentioned later, the two cotyledons are not only of unequal length, but one may be more massive as well. Cases are also met with in which the embryo was obliquely symmetrical (Fig. 15). It may be mentioned further that, in ripe seeds of the same ovary, the embryos are frequently of different sizes, and may have attained appreciably different stages in development.

Sanguinaria canadensis.

The earliest stage observed in the development of the embryo of *Sanguinaria canadensis* is shown in Fig. 9, which is a median longitudinal section. At this stage the embryo is cylindrical, as shown in cross-section (Fig. 10), with a very short suspensor of about two or three cells. The cytoplasmic contents of the cells are regular and uniform, little or no starch being found in the embryo at this or later stages. The resemblance of this embryo to that of Monocotyledons in similar stages of development is noticeable, but no special significance can be attached to this fact. As has been pointed out in *Actea alba*, the immediate subsequent development results in a pear-shaped structure (Fig. 20, an outline of a median longitudinal section). As this figure indicates, the suspensor is short, merging

insensibly into the remainder of the embryo, unless the three cells mentioned above are to be regarded only as the suspensor. In Fig. 20, the embryo shows a slight depression in the centre of the broad, flattened end. The part surrounding the depressed centre now grows faster to form the primordium of the cotyledons. Serial cross-sections of a slightly older stage (Fig. 21) show beyond a doubt that the thick, crescentic ridge-like primordium of the cotyledons obtains here as in *Actea*. Fig. 21, *a*, which is the first successive section (7·5 microns in thickness) below this primordium, shows that the embryo is somewhat flattened at right angles to the plane of the cotyledons, a phenomenon of usual occurrence in the embryos of the several species with which we are dealing. Later the embryo becomes more cylindrical. In *c*, Fig. 21, it will be seen that the apex of the stem is recognized as a very small rounded protuberance exactly at the centre of the space enclosed by the cotyledonary primordium. Fig. 22, *a–d*, is a series similar to Fig. 21, taken from an embryo only a little further developed. Section *d* of this figure includes the distal end of the embryo, and shows what would be seen by looking directly upon this end. The bifurcation of the primordium opposite the primary sinus is just perceptible in the section, being indicated in the figure by dotted lines. The apex of the stem can also be recognized as a small rounded elevation. A median longitudinal section, at right angles to the plane of the cotyledons, is shown in Fig. 23. It is apparent now that the growth of the embryo of *Sanguinaria canadensis*, immediately following the pear-shaped stage, results in a very marked increase in the width of the distal end with scarcely any increase in length. The suspensor is comparatively very short. With the final development of the embryo, culminating in the ripe seed, the same proportion of increase in size is maintained (Fig. 24, *a–h*). *a* to *c* of this figure represent consecutive sections, beginning at the base of the cotyledonary primordium, while *d* to *h* are alternate sections. It is clear from this series, which was made from an embryo in the ripe seed, that the common base of the two cotyledons has undergone intercalary growth, the result of which is that the primary sinus is deeper (22 microns) than the other. The apex of the stem, although central, extends slightly upward, as if adnate to that part of the cotyledonary primordium directly opposite the primary sinus. The cotyledons are crescentic in cross-section at the base, but become gradually more flattened near their tips. Fig. 25 has been constructed from all the sections of the embryo, represented only in part in Fig. 24, and it shows the condition of the embryo in the ripe seed. We have therefore in the mature seed of *Sanguinaria* an embryo broader than long, in which the difference in depth of the two sinuses of the short and widely divergent cotyledons is only slight. The suspensor is short, consisting of only a few cells. The apex of the stem is recognizable as a small, rounded protuberance at the centre of the area surrounded by the

base of the cotyledons. In conclusion it may be said that the embryo of *Sanguinaria canadensis* shows a well-marked, though not very pronounced, degree of anomaly in the development of the cotyledons, and that this anomalous character may persist in the embryo found in the ripe seed. Variations in the degree of anomaly are also to be observed.

Stylophorum diphyllum.

The development of the embryo of *Stylophorum* is similar to that of *Sanguinaria*; consequently attention will be directed to certain features only. The earlier stages, in so far as they were observed, were similar to those of *Sanguinaria*. Beginning with the development of the cotyledons, we find that they originate in the familiar crescent-shaped ridge (Fig. 27, *a–f*). In this figure I have indicated the plerome of the cotyledons and hypocotyl by broken lines. After what has been said concerning *Sanguinaria*, this series of outline drawings is self-explanatory. It is worthy of note, moreover, that the anomaly, in so far as it is present, is even less pronounced in *Stylophorum*, the bifurcation of the crescentic primordium occurring earlier, and in the mature embryo the depth of the sinuses is almost, if not quite, equal. Not only this, but cases were observed (Fig. 26, *a–c*) in which no anomaly was present. In these we have before us apparently the development of a typical dicotyledonous form. In such a typical dicotyledonous form, however, it must be remembered that, as stated in a foregoing paragraph, the primordia of the cotyledons involve nearly the whole end of the embryo as in the most pronounced anomalous types (Fig. 26, *a–b*). The difference between Figs. 26 and 27 lies mainly in the fact that, in the former, the primordium bifurcates to form the two sinuses at exactly the same time, and it is doubtless proper to speak of primordia rather than of a single primordium; but these primordia are not so distinctly isolated as one might suppose from the descriptions usually given of the origin of the cotyledons. In the nearly mature seed, although the cotyledons are relatively short, the embryo is much less in the form of a low broad goblet than that of *Sanguinaria*, i. e., the longitudinal axis is greater than the transverse (Fig. 28). The suspensor is also short. *Stylophorum diphyllum* may be regarded, therefore, as typically dicotyledonous, showing a tendency only to a slightly anomalous character in the formation of the cotyledons.

Delphinium tricorne.

We shall now follow the development of the embryo in two or three more species of the Ranunculaceae for comparison with *Actea alba*. Agreeing with the other species already described, the pear-shaped embryo of *Delphinium tricorne* is somewhat flattened at right angles to the plane of the cotyledons (Fig. 29). The first indication of the cotyle-

donary primordium appears in the form of a narrow, elongated depression, which is just perceptible at the truncated end, extending from one side to the centre. The shaded part of *b*, Fig. 29, represents this depression. The primordium here is more nearly kidney-shaped than crescentic. Fig. 11 represents a more detailed drawing of *a*, Fig. 29. The larger cells in the centre indicate the apex of the stem. There is at this stage no indication of the plerome strands; these appear later, as shown for the species already described.

As growth continues, the primordium becomes typically crescentic, and the embryo is more cylindrical (Fig. 30, *a–e*, representing alternate sections of a part of the embryo). Whether the embryo represented in this figure was not cylindrical from the beginning cannot be stated. However, this matter is unimportant. During succeeding development, the crescentic primordium soon bifurcates, but the difference in depth of the two sinuses is increased rather than diminished, so that in the mature seed the anomaly is more pronounced (Fig. 31, *a–g*). This figure does not include all of the embryo beyond the base of the cotyledons, but only alternate sections from that point to the bottom of the second sinus. This series of sections, taken from an embryo in the mature seed, shows that a cotyledonary tube is present, a phenomenon that has been reported for two other species of *Delphinium*, *D. nudicaule*, and *D. hybridum* (Sargant, '03, p. 73). Fig. 32 is intended to show the form of the embryo as it is in the mature seed. As it was found impracticable to dissect out the embryo on account of its small size and the firmness of the endosperm, this figure was constructed, as in the other species, from a complete series of cross-sections, and the proportions are reasonably accurate. It may be noted that the embryo has a relatively longer hypocotyl than in *Actea*, and the cotyledons diverge towards the apex. These are crescentic in cross-section. The two sinuses are of unequal depth, and the cotyledonary tube is formed by the intercalary growth of the common base of the cotyledons. In Fig. 32, the relative depth of this tube is indicated by the dotted line, which shows also the outline of the space enclosed by the cotyledons. In *Delphinium*, as well as in the other species under consideration, there is no plumule in the ordinary conception of that term. Here the apex of the stem does not even project as a recognizable protuberance, at least, so far as my observations have extended.

Aquilegia canadensis.

The youngest stage of the embryo of *Aquilegia* observed was an elongated cylindrical structure, somewhat bent near the middle, and possessing a suspensor of three or four cells (Fig. 12). At this stage, it was not possible to determine the exact limits of the suspensor. With

subsequent growth the pear-shaped form is assumed, and the beginning of the cotyledons is soon manifested (Fig. 33, *a–d*, representing four consecutive sections only of the embryo). A comparison of this figure with figures 29 and 30 will show the similarity in the origin of the cotyledons with that in *Delphinium*. It is evident that, in this case, the primordium was kidney or bean-shaped, but as it bifurcates soon after being laid down, the anomalous character can be said to exist only as a mere trace. This is made clear in Fig. 34, *a–f*. In this embryo the cotyledonary primordia seem to have arisen separately, and not as one piece, the two involving, however, nearly the whole end of the embryo. In *d*, Fig. 34, the base of one of the young cotyledons is more massive than the other. In fact, in all species of the two families examined, it not infrequently happens that one cotyledon is larger than the other, being sometimes longer or thicker (see also Fig. 18). Fig. 35, *a–g*, refers to an embryo of a mature seed. This series is not consecutive throughout, one or more sections being omitted between *b* and *c*, *d* and *e*, and *f* and *g*. The base of each cotyledon is almost cylindrical, a feature in which *Aquilegia* differs from the other genera mentioned. The nature of the embryo in the mature seed is shown in Fig. 13. This figure was constructed from a series of cross-sections as mentioned for the preceding species, with the exception that the middle portion of each cotyledon has been omitted in order to show that, while the cotyledons are somewhat cylindric at the base (*e*, Fig. 35), they soon become concave within, and consequently crescentic in transverse section. No indication of a plumule was observed in the embryo of the ripe seed.

Syndesmon thalictroides.

Measured by the standard of endosperm-formation, the development of the embryo in *Syndesmon* is relatively slow; for, in a large percentage of the preparations made from the ripe seed, the cotyledons had just put in an appearance. In some cases no indication of these were to be seen, while in others the cotyledons had attained a length equal to about one-third of the length of the entire embryo. The origin of the cotyledons seems to be typically dicotyledonous, even more so than in *Aquilegia*, so that it was not considered necessary to give illustrations. In only one case was the slightest indication of an anomaly observed. The first appearance of the cotyledonary primordia, it may be added, is almost identical with that in *Aquilegia* in which no anomaly was observed, and this fact is probably due more to a similarity in habit than to genetic relationship. The suspensor of the embryo seems to be a little more massive than in the species hitherto mentioned, the whole embryo, however, resembling that of *Aquilegia*.

Theoretical.

It now becomes necessary to examine the evidence revealed by a study of the embryology of the several anomalous dicotyledons, in order to show what light knowledge thus obtained throws upon the question of phylogeny of the Angiosperms, and what the relative value of such knowledge is as compared with that derived from other sources.

Any consideration of this subject brings the investigator face to face with the ever recurring questions, which have received various answers at the hands of the most competent observers: Have the Angiosperms been derived from the Gymnosperms or directly from a pteridophytic ancestor? Have the two classes of Angiosperms had a common or an independent origin? If monophyletic, which are the more primitive, Monocotyledons or Dicotyledons?

It must be admitted that many, perhaps the majority of the known facts, may be reasonably interpreted in the light of opposing doctrines. In fact, this has been done in the several theories that have been advanced in the past. No matter what answer may be given to the first of these questions, the status of the other two will remain about the same. Some observers may prefer to adhere to the older view, that the Angiosperms have been derived from the Gymnosperms, with *Gnetum* as the nearest living representative of a transitional condition, yet others will undoubtedly be inclined to seek the ancestry of the Angiosperms among the Pteridophytes. The dicotyledonous character of certain Gymnosperms has lost much of the importance formerly attached to it in the consideration of the origin of the Angiosperms, since it has become known that very important similarities in structure have appeared independently in different organisms widely separated in phylogeny, and because of the incomplete geological evidence in regard to *Gnetum*. The writer prefers to regard the gymnospermous origin of the higher seed plants as less probable, and believes that the most important evidence points to a pteridophytic ancestry. Now, if we assume that the Angiosperms have sprung from the Pteridophytes, we have in *Isoetes* what may be regarded as a transitional condition leading to the Monocotyledons, and in *Selaginella* certain embryological characteristics suggestive of a dicotyledonous nature. Professor Campbell ('91) has shown in his thorough and exhaustive study of *Isoetes* the striking embryological resemblance between this genus and certain Monocotyledons, and the facts there set forth point strongly to the great antiquity of the monocotyledonous ancestors. Whether the evidence will justify the assumption of a separate pteridophytic ancestry of the two classes of Angiosperms may be seriously questioned, especially if we do not regard the character of the female gametophyte and the seed-habit of equal phylogenetic value. The writer

is quite willing to admit the probability of the seed-habit as having arisen independently in Gymnosperms and in the Angiosperms, but it is certainly difficult to understand how a structure like the embryo-sac, which is exactly alike in the vast majority of both groups of Angiosperms, could have arisen otherwise than from a common ancestor. The embryo-sac is certainly as deeply seated as any known structure, and if morphological characters have any great value in determining phylogeny, the embryo-sac tells no uncertain story. This may not be said with equal emphasis for the seed-habit or for the organs of an embryo. The embryo in the mature seed of Zamia, for example, bears a marked resemblance to that of a Dicotyledon, yet the difference in the early development of each is very great. Furthermore, it may be said that the first step toward the seed-habit was taken when the female prothallium of a Pteridophyte was retained within the macrospore, and the greatest progress toward seed formation found in any living Pteridophyte is the female prothallium with its enclosed embryo in the macrospore of *Selaginella* and *Isoetes*. Let it be understood, however, that the writer does not imply that the change from the habit now existing in *Selaginella* or *Isoetes* to an angiospermous seed has been a direct one, but conditions like these seem to indicate the probable line of development leading towards seed-formation.

The embryo-sac is still our greatest stumbling-block, and, as stated above, if deep-seated morphological characters count for much, Monocotyledons and Dicotyledons have had a common ancestor, or their parent stocks were very closely related. Probably the majority of recent writers agree in the monophyletic theory of the origin of the Angiosperms, but they differ as to which of the two classes is the more primitive, and as to the relative value of the evidence brought forth in support of their respective views.

Lyon ('01), in a well-written essay, argues in favour of the monophyletic theory, regarding the Monocotyledons as the more primitive stock from which the Dicotyledons have been derived. The monocotyledonous stock, according to his view, is derived from pteridophytic ancestors, the foot of the embryo of a Fern being homologous to the cotyledon. 'It (i. e. the foot) is to the pteridophyte embryo in a simple way what the cotyledon is to the embryo of a Monocotyledon, and is in fact to be considered as a more primitive type of cotyledon' (l. c., p. 66). The period of life of the sporophyte of Bryophytes and Pteridophytes within the calyptra is regarded as comparable to the intra-seminal life of the angiospermous embryo, and the single cotyledon of the monocotyledonous embryo is considered as having bifurcated to give rise to the two cotyledons in a manner similar to the bifurcation of the cotyledonary primordium of *Nelumbo*. Lyon's point of view is well stated, and his theory is suggestive, but it seems to the writer that the facts do not indicate that the foot has undergone the morphological

changes demanded by the theory. The writer feels that a detailed statement explanatory of his view would far exceed the purpose of this paper, and he prefers to let the matter stand at present with the above expression of his opinion.

An argument in favour of the monophyletic origin of the Angiosperms has also been advanced by Miss Sargant ('03), but this observer considers the Dicotyledons as the more primitive stock from which the Monocotyledons have been derived, suggesting that the anomalous character of certain dicotyledonous embryos indicates the probable line of transition. Miss Sargant approaches the subject from the standpoint of the anatomy of seedlings, examining into the anatomical details of a large number of species, chiefly of the Liliaceae, and bringing the great mass of details together in a very careful and praiseworthy manner.

Using *Anemarrhena* as a type, she finds two opposed vascular bundles in the terminal cotyledon. These extend down into the hypocotyl, where each divides, and the four plerome-strands thus formed are continuous with those of the tetrarch primary root. This behaviour of the bundles suggests, it is pointed out, that the single cotyledon of *Anemarrhena* is the homologue of two, which were separate in some dicotyledonous ancestor. It is further suggested that *Eranthis hiemalis*, one of the Ranunculaceae, may be illustrative of such an ancestor.

A summary of the views of Coulter and Chamberlain in regard to the phylogeny of the Angiosperms is expressed by these authors (Morphology of Angiosperms, pp. 287, 288) as follows:—'The Monocotyledons and Dicotyledons represent two independent lines derived directly from Pteridophyte stock, probably from the Filicales. At the same time, the arguments in favour of the monophyletic origin of Angiosperms are strong; and if this view be accepted, the derivation of Monocotyledons from primitive Dicotyledons seems to rest on stronger evidence than the reverse relationship. It must also be said that the Gymnosperm origin of Angiosperms is not to be discredited so much now as formerly.'

With the brief statement of the two views concerning the relative antiquity of Monocotyledons and Dicotyledons, under the assumption of a monophyletic origin, we are now prepared to examine more directly the evidence furnished by the embryology of anomalous Dicotyledons in the light of recent investigations. In basing conclusions upon morphological data, indeed the most reliable of all, the first requisite is to determine whether the structures dealt with represent primitive or derived characteristics, as it is upon this point, of course, that observers will be found to differ most, especially in the presence of a few isolated facts. With a detailed knowledge of only a few anomalous Dicotyledons, observers were quick to regard this character as primitive, and consequently conclusions were easily reached. With the crescent-shaped primordium of the cotyledons as

found in certain Nymphaeaceae and Ranunculaceae as a basis, it is easy to show how, during phylogeny, this primordium, by a bifurcation opposite the primary sinus, might have given rise to two cotyledons. Or if the primordium should develop as one piece without undergoing a bifurcation, the resulting cotyledon might then be looked upon as a union of two cotyledons. The former is the view held by Lyon, the latter, the interpretation given by Miss Sargant.

As a matter of fact, the writer believes that the investigations of Schmid ('02) on species of *Ranunculus* and *Corydalis*, and the results of similar studies upon species of the Ranunculaceae and Papaveraceae as detailed in the foregoing paragraphs, together with the observations of Lewis ('04) on *Podophyllum* and *Jeffersonia*, show conclusively that the anomalous character of the embryo is not a primitive, but purely a derived condition. In the genera forming the basis of this paper, it is seen that there is a transition from the anomalous embryo to that which is typically dicotyledonous, and that this transition is seen in different species of the same genus. Schmid points out that, in *Ranunculus Ficaria* and *Corydalis cava*, only one cotyledon develops, leaving us to infer that the second is not formed, but in both *Corydalis nobilis* and *C. lutea* two cotyledons are typically formed. Are we to infer, therefore, that *Corydalis cava* represents a primitive condition in the development of its embryo, while *C. nobilis* and *C. lutea* do not? Schmid states further that the seedling of *C. cava* forms a tuberous stem, while *C. nobilis* and *C. lutea* do not. Cook ('03) has shown also that in *Claytonia virginica*, a geophilous plant, only one cotyledon develops, the other remaining abortive. It is probably true without exception that dicotyledonous plants possessing anomalous embryos are either partly or wholly geophilous in habit, having stems either in the form of a rhizome, tuber, or a short, squat axis. In other words, the anomalous character is in all probability correlated with an hypogean habit. There can be no doubt, I think, that the cotyledonary tube is merely an adaptation to a geophilous habit, as seems to be shown in all plants possessing the same. If, on the contrary, the cotyledonary tube and the anomalous embryos represent primitive characters, then it must be shown that the geophilous habit is a primitive condition, but the writer does not believe that that has been done up to the present, at least. If the conclusion arrived at here is correct, anomalous Dicotyledons throw little or no light upon the relative antiquity of the two classes of Angiosperms; for as embryological evidence is of greater value than anatomical, it seems as reasonable to conclude, using the anomalous character as a basis, that Dicotyledons have been derived from Monocotyledons as the reverse.

Summary.

Of the species investigated, all except *Stylophorum diphyllum* and *Syndesmon thalictroides* show a certain well-marked anomalous character in the development of the embryo of numerous individuals, while in others the anomaly may be only slightly manifested. In *Stylophorum* and *Syndesmon* the embryo is typically dicotyledonous, except in certain individual cases observed in which slight anomalies were present.

In the anomalous forms in question, the primordium of the cotyledons arises as a thick crescent-shaped ridge of tissue, open at one side, growing out of the truncated end of the pear-shaped embryo. The opening of the crescent becomes the primary sinus of the cotyledons. With further growth, the primordium bifurcates at a point opposite the primary sinus and thus forms the two cotyledons. With subsequent growth of the embryo, the depth of the two sinuses may or may not become equalized.

The anomalous character represents a derived and not a primitive condition. Consequently the anomalous Dicotyledons do not show that one class of Angiosperms was derived from the other.

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EXPLANATION OF FIGURES IN PLATES XXVI AND XXVII.

Illustrating Professor Mottier's paper on the Embryology of Anomalous Dicotyledons.

Unless otherwise stated, all figures were drawn from carefully prepared microtome sections with the aid of the camera lucida.

Figs. 1–8. *Actea alba.*

Figs. 1 and 2. Longitudinal sections of young embryos, the cells of the suspensor contain starch. × 425.

Fig. 3. A transverse section through the middle of an embryo in the stage of development of Fig. 1. × 425.

Fig. 4. Median longitudinal section. The embryo is becoming pear-shaped. The suspensor is short, its cells contain starch. × 425.

Fig. 5. A typical pear-shaped embryo just prior to the appearance of the cotyledonary primordium. × 425.

Fig. 6. View of an embryo after the appearance of the cotyledonary primordium, which is a thick crescentic ridge formed at the margin of the truncated distal end. The opening of the crescent faces the observer. This opening, or primary sinus, is an indentation at one side of the embryo extending towards the centre, but becoming shallower at that point. This figure was constructed from the series of cross-sections shown in Fig. 17.

Fig. 7. Longitudinal section of an embryo from a nearly mature seed, at right angles to the plane of the cotyledons. Only one cell of the suspensor is omitted from the drawing. The plerome strands in hypocotyl and cotyledons are being differentiated. × 250.

Fig. 8. View of an embryo from a mature seed. The cotyledonary sinuses are of unequal depth, the primary sinus which is nearest the observer being deeper. Constructed in the same manner as Fig. 6.

Figs. 9, 10. *Sanguinaria canadensis.*

Fig. 9. Median longitudinal section through young cylindrical embryo with very short suspensor. × 425.

Fig. 10. Transverse section through a similar embryo. × 425.

Fig. 11. *Delphinium tricorne.* Transverse section through broad end of pear-shaped embryo before the appearance of the cotyledonary primordium. Tissues undifferentiated. × 300.

Figs. 12, 13. *Aquilegia canadensis.*

Fig. 12. Longitudinal median section through young embryo. × 425.

Fig. 13. View of embryo from mature seed. The middle part of each cotyledon is omitted. Constructed in the same manner as Figs. 6 and 8.

Figs. 14–19. *Actea alba.*

Fig. 14. Transverse section through the base of the cotyledonary primordium, showing primary sinus; *st.* apex of stem; *pl.* plerome strands. × 300.

Fig. 15. Section similar to Fig. 14, but from an embryo showing no anomaly. The two sinuses of the cotyledons were formed simultaneously. This embryo was obliquely symmetrical. × 300.

Fig. 16. Similar to Fig. 15, but from a symmetrical embryo.

Fig. 17. A series of transverse sections of an embryo shown in outline, at the time of the appearance of the cotyledonary primordium. Sections were cut 7·5 microns in thickness. *a–k*, alternate sections; *l–p*, successive sections. × 300.

Fig. 18, *a–h*. A series of transverse sections beginning just below the cotyledons. This embryo showed no perceptible anomaly; one cotyledon, however, was longer than the other. × 125.

Fig. 19, *a–k*. Similar series, but of an older embryo. The anomalous character is typical and the most pronounced at this stage for this species. × 180.

Figs. 20–25. *Sanguinaria canadensis.*

Fig. 20. Outline of a median longitudinal section of a pear-shaped embryo. The slightly depressed centre is an indication of the beginning of the cotyledonary primordium. × 180.

Fig. 21, *a–c*. Three cross-sections, beginning below the primary sinus of the cotyledonary primordium. The stem apex is only a perceptible protuberance at the centre of the space enclosed by the cotyledonary primordium. × 125.

Fig. 22, *a–d*. Successive sections of an embryo, beginning below cotyledonary primordium. *d* includes the end of the embryo, giving the view obtained by looking directly at the end. The second sinus and the tip of the stem indicated by the broken lines are just perceptible. It is seen that the primary sinus is deeper, and a well marked anomaly exists. × 125.

Fig. 23. Outline of a longitudinal section of an embryo similar to the above. × 125.

Fig. 24, *a–h*. Series of alternate sections of an embryo from a mature seed, beginning below the primary sinus. The common base of the two cotyledons is quite marked. The base of each cotyledon is crescentic in cross-section, but their diverging ends become flattened as shown in *g* and *h*. × 125.

Fig. 25. View of an embryo constructed from the same series of sections shown in part in Fig. 24. The tip of the stem is a very small protuberance. This is the only indication of a plumule.

Figs. 26–28. *Stylophorum diphyllum*.

Fig. 26, *a–c*. Three transverse sections through base of cotyledons of an embryo from a nearly mature seed. The two cotyledonary sinuses are of equal depth, and the two primordia seem to have been separate from the beginning, if there is such a thing as quite separate primordia. In *a*, the plerome of the stem and cotyls are indicated by broken lines. The stem apex is quite central. × 125.

Fig. 27, *a–f*. Series of an embryo similar to the foregoing. A slight anomaly is manifested here. The stem apex is central, although appearing (*d*) slightly adnate to the base of the cotyledons below the secondary sinus. × 125.

Fig. 28. Outline of a longitudinal section of an embryo. × 125.

Figs. 29–32. *Delphinium tricorne*.

Fig. 29, *a–b*. Two consecutive transverse sections from the broad end of a pear-shaped embryo in which the kidney-shaped cotyledonary primordium has just appeared. The primordium comprises almost the whole end of the embryo. × 125.

Fig. 30, *a–e*. Series from an older embryo. The anomaly is quite well marked. × 125.

Fig. 31, *a–g*. Series (alternate sections) from an embryo of a ripe seed, beginning at the base of the cotyledons, *a*, and extending to secondary sinus, *g*. The anomaly is marked; the common base of the cotyledons by intercalary growth has formed a cotyledonary tube. × 125.

Fig. 32. View of embryo constructed from same embryo as Fig. 31. The broken line indicates depth of cotyledonary tube.

Figs. 33–35. *Aquilegia canadensis*.

Fig. 33, *a–d*. Four consecutive sections including base of cotyledons. The cotyledonary primordium seems to have been kidney-shaped. × 125.

Fig. 34. From an embryo similar to the preceding; anomaly less marked. × 125.

Fig. 35, *a–g*. Sections of an embryo from mature seed. *c*, *d* and *e*, *f* are consecutive. The base of each cotyledon is somewhat cylindrical. × 125.

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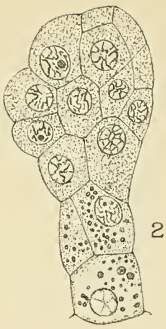

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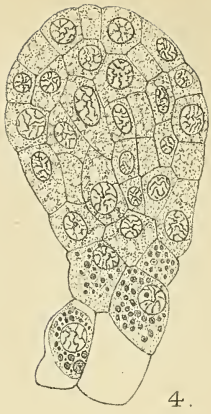

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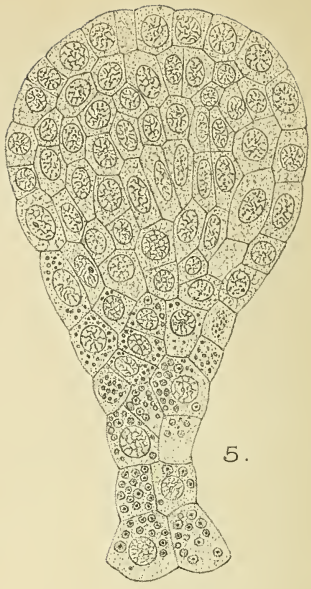

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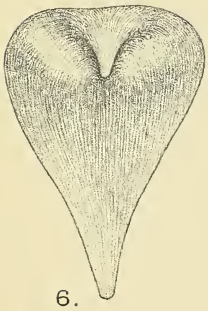

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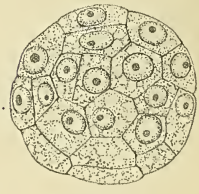

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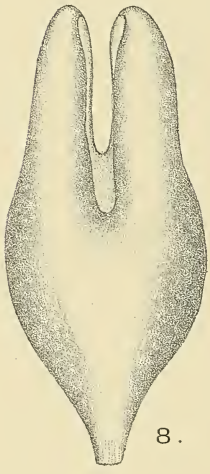

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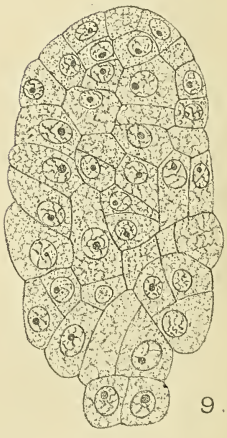

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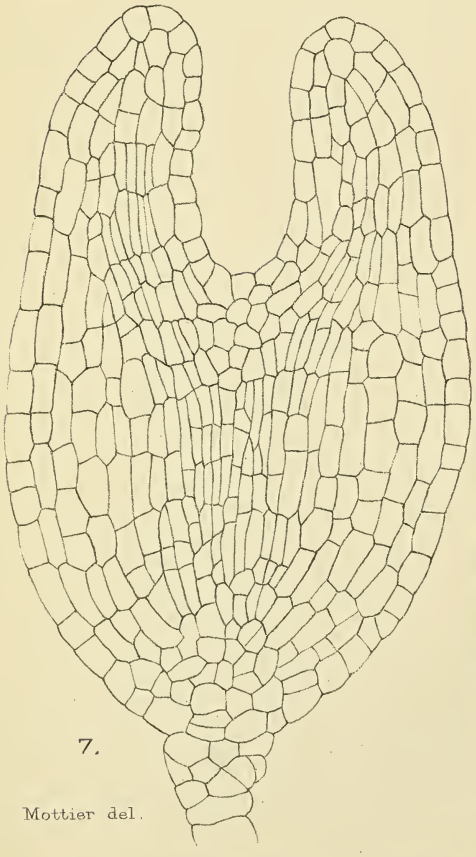

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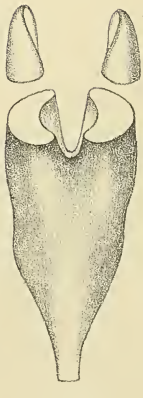

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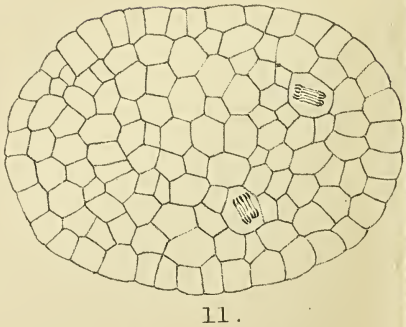

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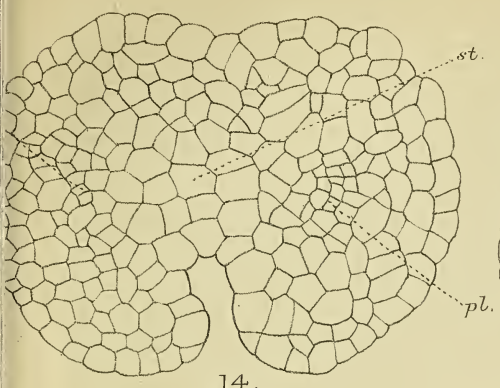
MOTTIER, —— EMBRYOLOGY OF DICOTYLEDONS



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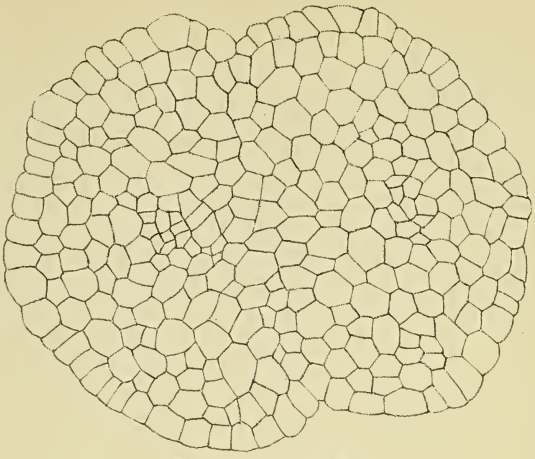

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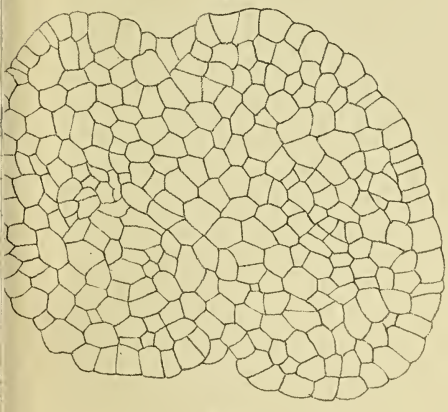

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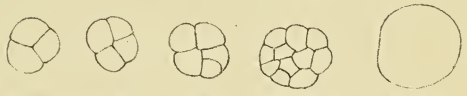

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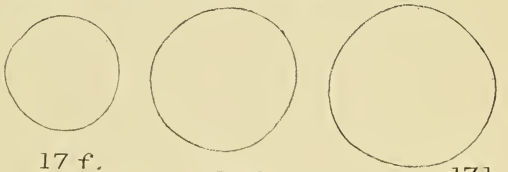

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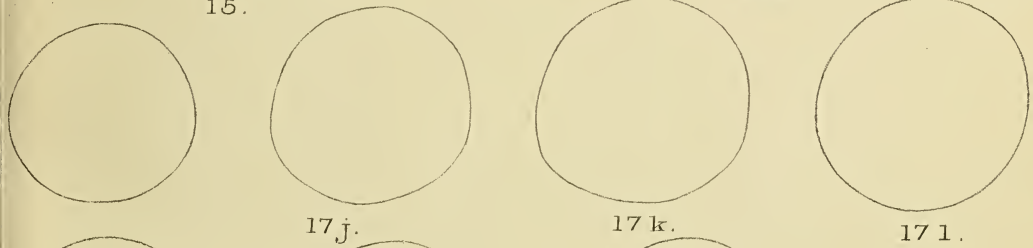

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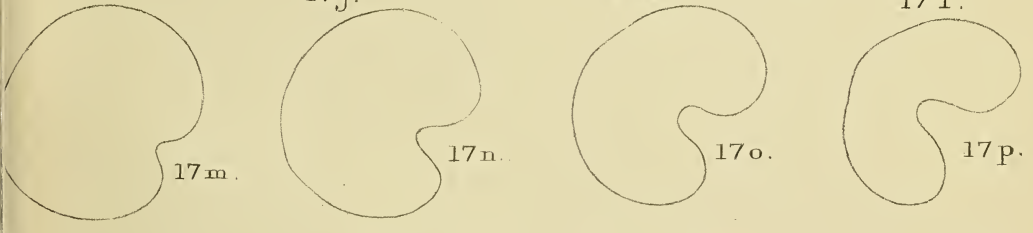

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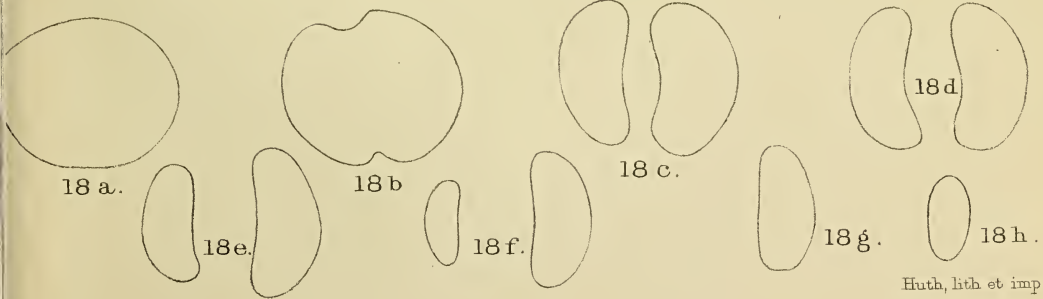

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18e. 18f. 18g. 18h.

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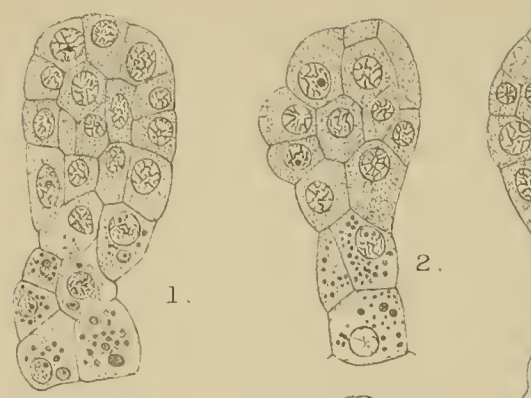
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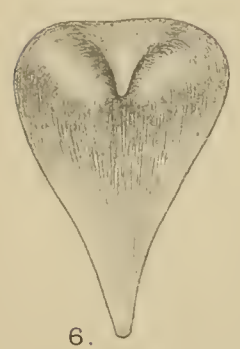
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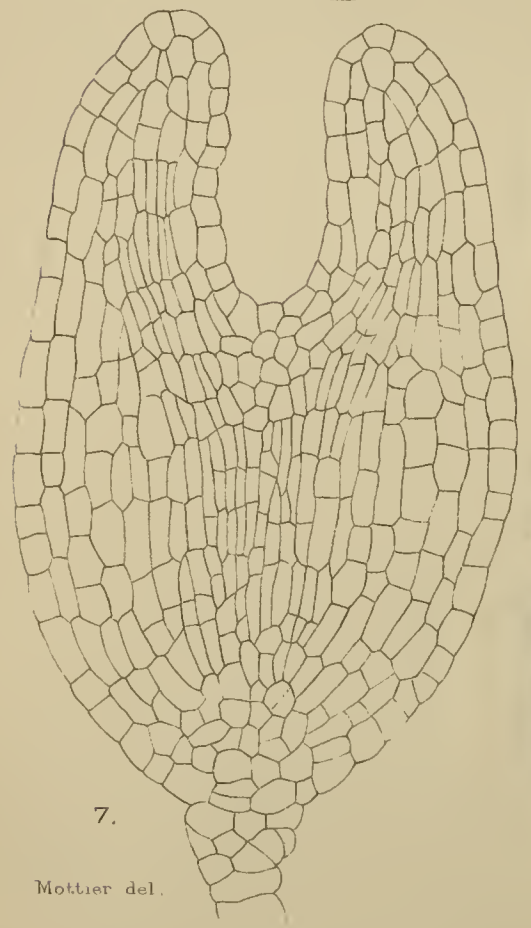
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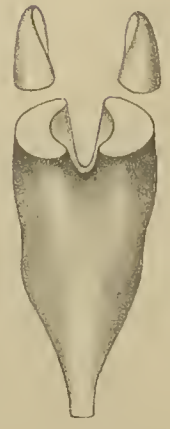
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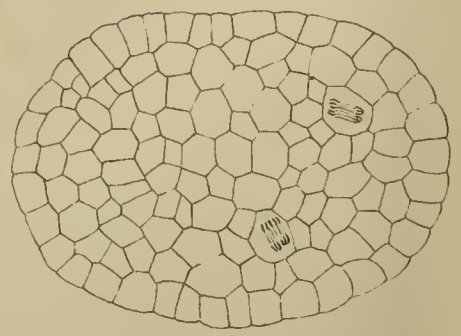
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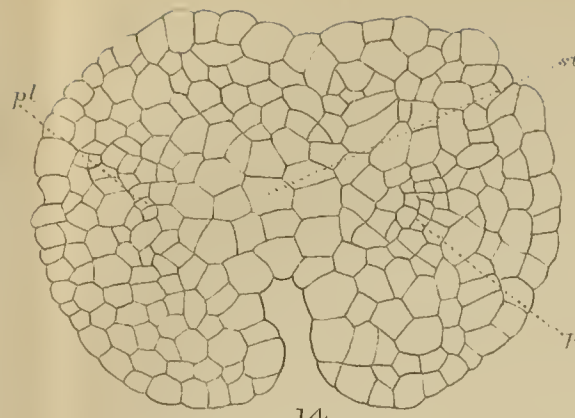

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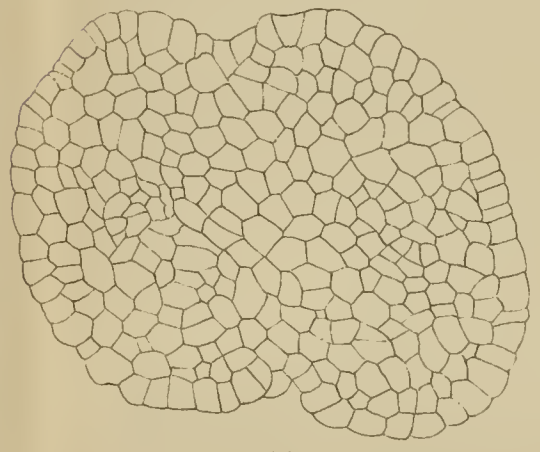
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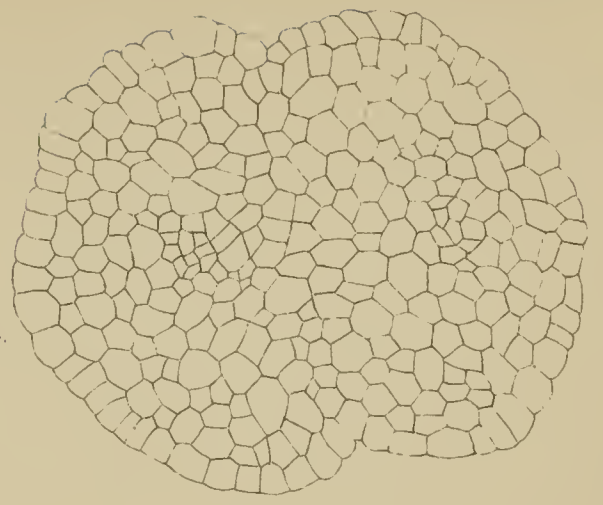
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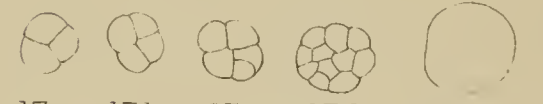
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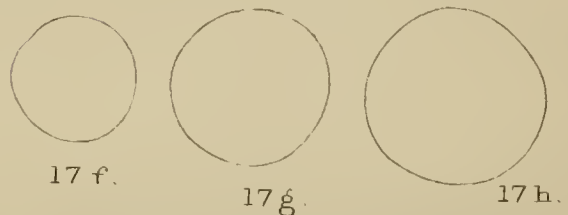
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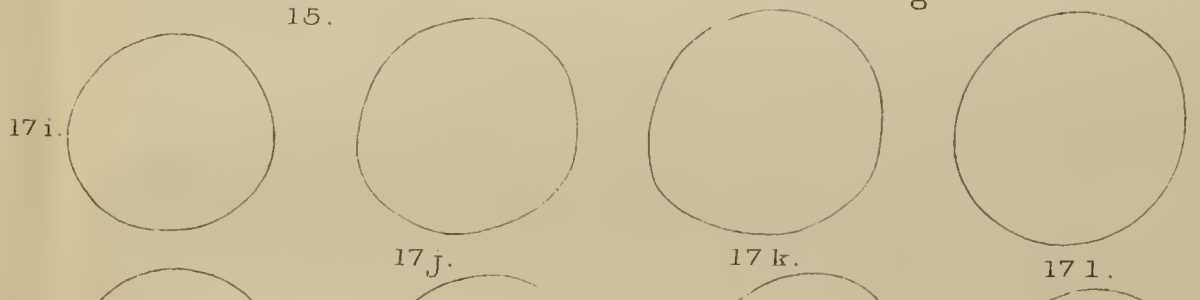
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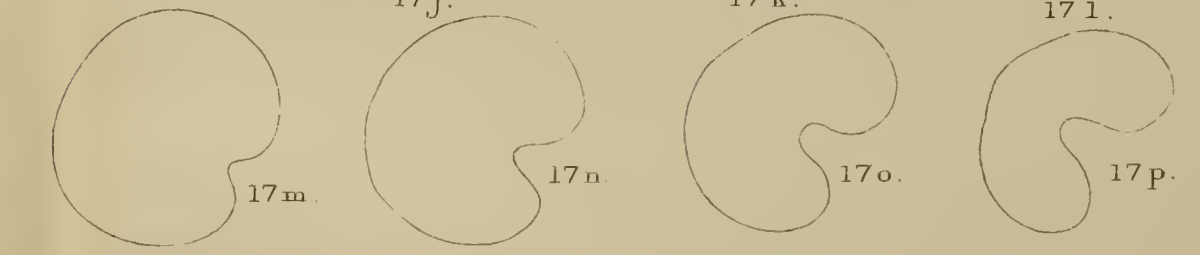
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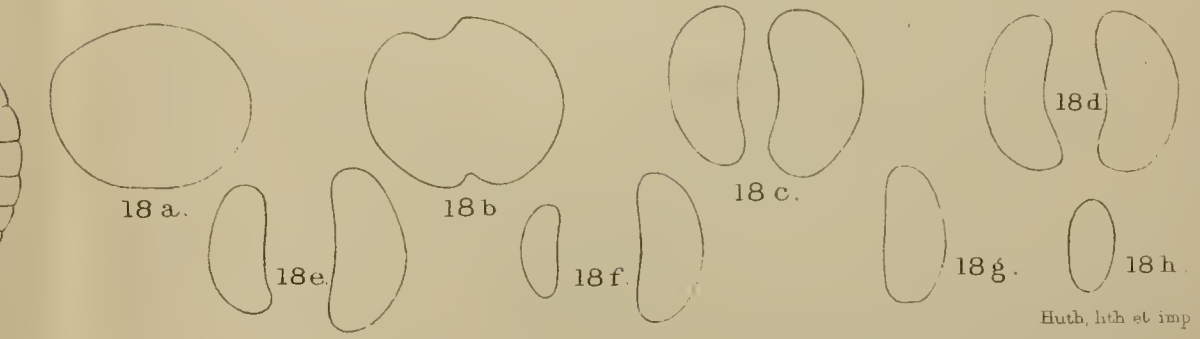
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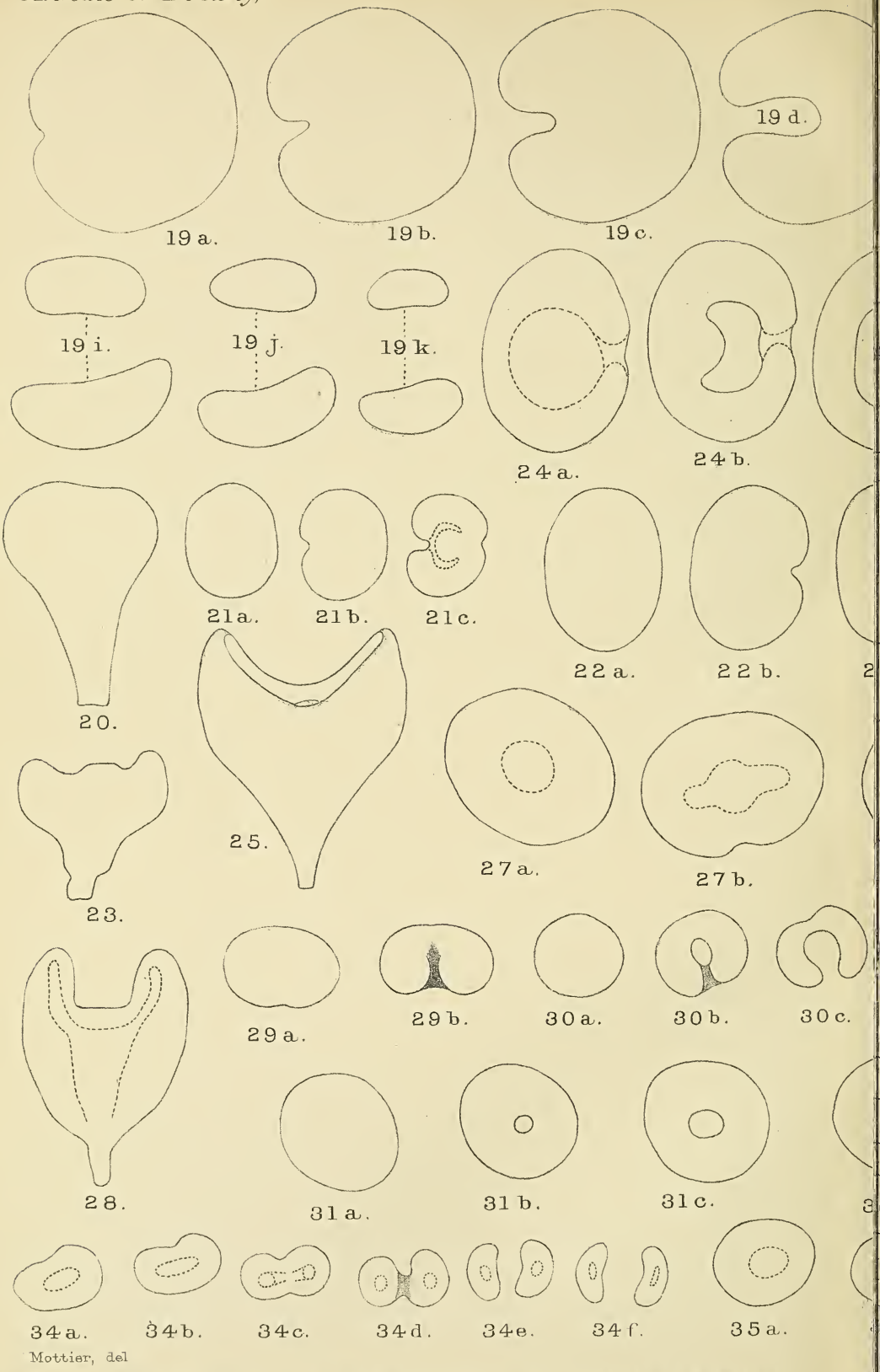
Mottier del.

Huth, lith et imp

MOTTIER, — EMBRYOLOGY OF DICOTYLEDONS



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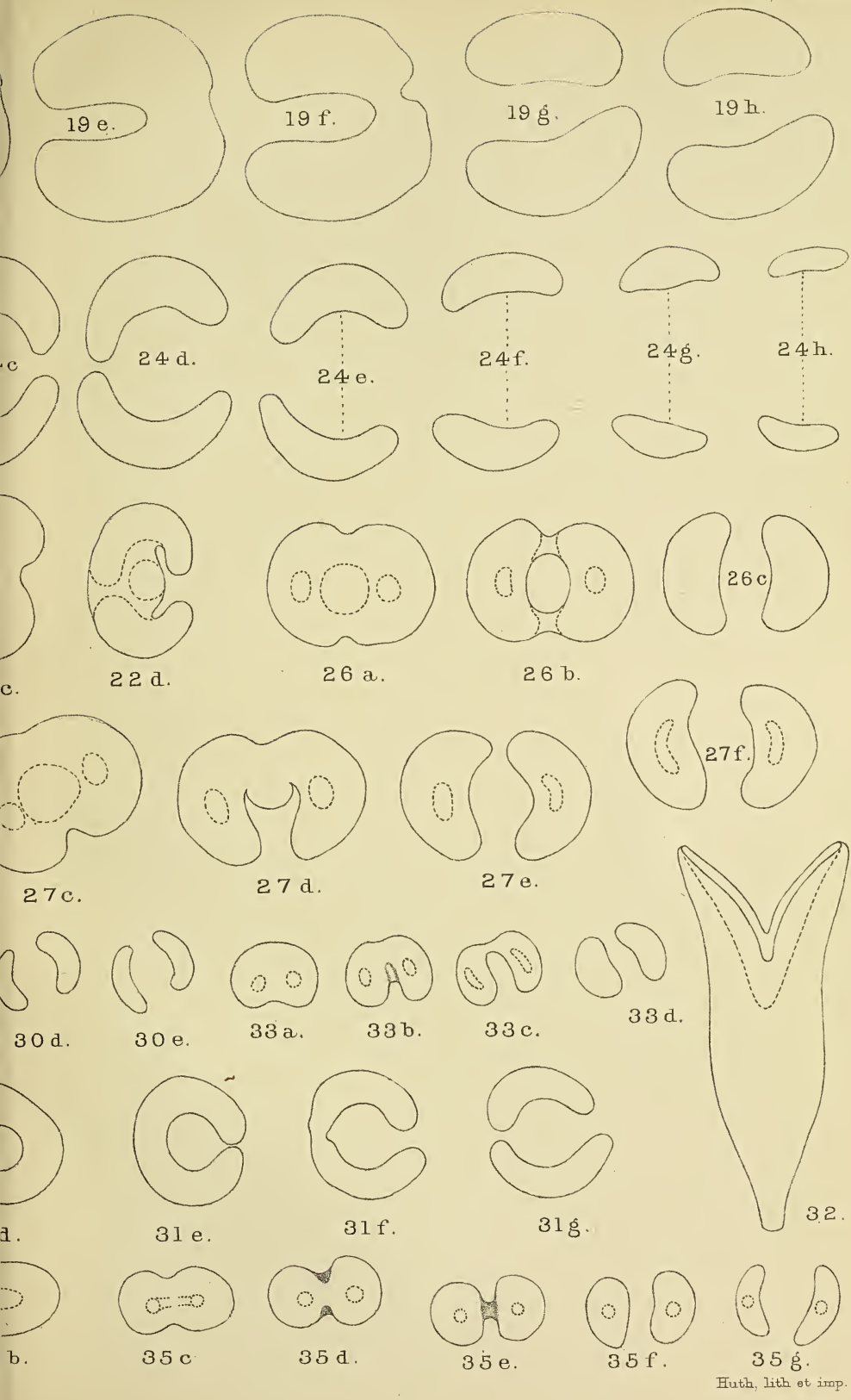
MOTTIER, — EMBRYOLOGY OF DICOTYLEDONS.



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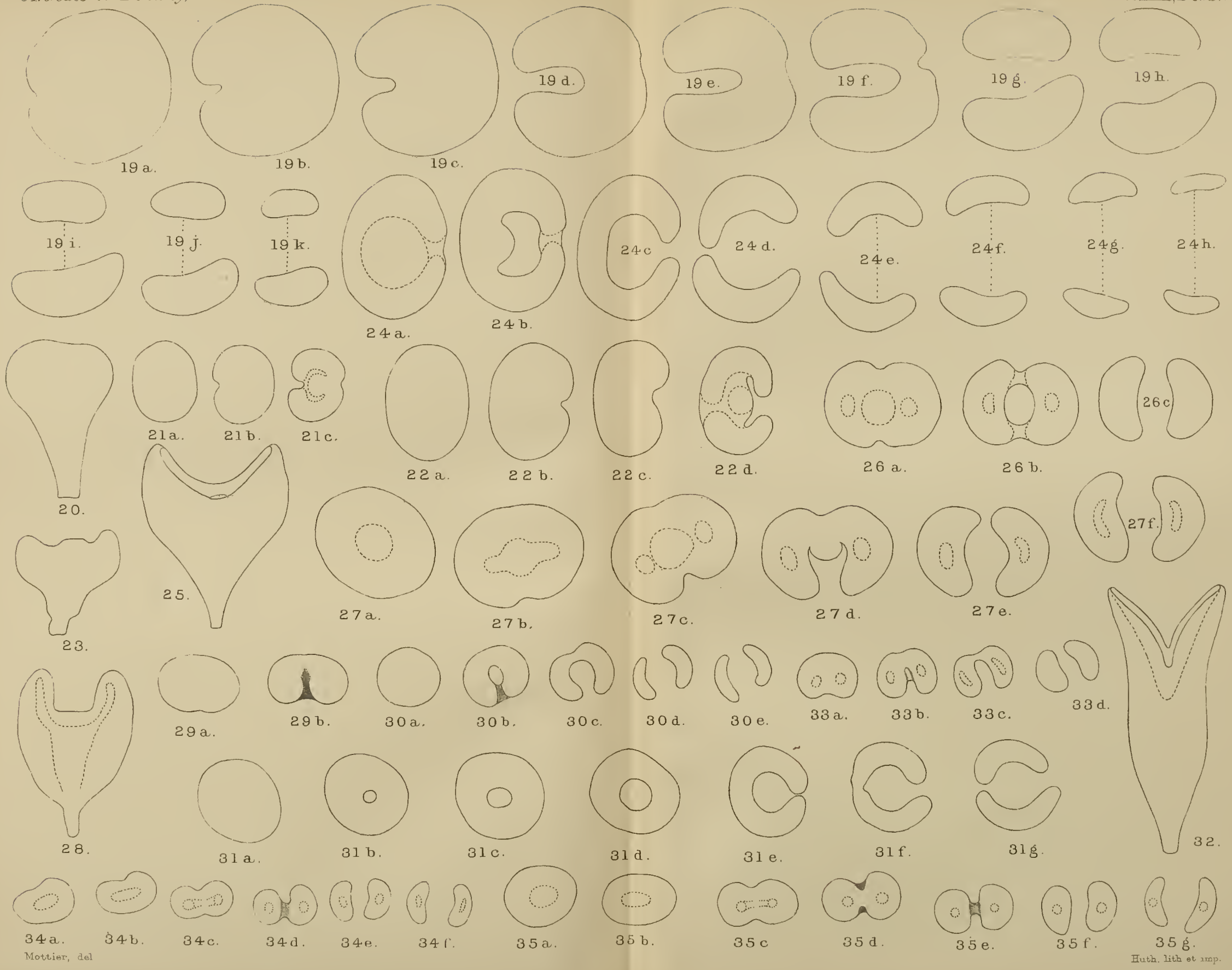

Mottier, del

Huth. lith et imp.

MOTTIER, — EMBRYOLOGY OF DICOTYLEDONS.

Spore Formation in Botrychium virginianum.

BY

WILLIAM C. STEVENS, M.S.,

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With Plates XXVIII, XXIX, and XXX.

IN *Botrychium virginianum* the development of the sporangia begins a year previous to their ripening. Plants which are dug up about the time of the shedding of the spores show next year's leaf with sporangia just forming, enclosed by the stipule-like sheath at the base of the leaf of the present year. The youngest sporangia are then in the form of slight elevations. At the apex of each of these is a pyramidal cell which divides by a periclinal wall, and the inner of the two cells thus formed is the archesporium which, by repeated divisions, gives rise to the spore-mother-cells (Campbell, '95).

As soon as the leaves come above the ground in the spring the spore-mother-cells are found in the condition represented in Fig. 1, evidently in the early prophase of their first division. The spore-mother-cells are then seen to be demarked from each other and from the tapetum by a delicate plasmatic membrane. They are not, as a rule, united into a single mass, but are separated into groups of four to thirty-five. It is possible, however, that the separation into groups in this early stage is done in the preparation of the sections, but, at any rate, we may be sure that the tendency to separate exists, since in later stages we find like groups immersed in the plasmodium of the tapetal cells that has flowed in between them. At this stage the nuclear thread is rather loosely-coiled (Pl. XXVIII, Fig. 1), its windings, where applied to the nuclear wall, showing some degree of symmetry (Fig. 2). With the safranin-gentian violet-orange method the nuclear thread is at this stage stained a uniform purple or violet, showing no differentiation into chromatin and linin disks. Usually but one nucleolus is present, but as many as two or three are sometimes found.

The tapetum in this early prophase consists of two layers of cells of varying form and size, each cell being deliminated by a plasmatic membrane merely, which, in some instances, is incomplete, so that there is a fusion of the cytoplasm of adjacent cells. This fusion is progressive

[Annals of Botany, Vol. XIX. No. LXXVI. October, 1905.]

and results in a common tapetal plasmodium by the close of the first prophase. The cells of the tapetum are easily distinguished from the spore-mother-cells by their coarser-grained cytoplasm, their irregular size and form, and their smaller nuclei with close-meshed reticulum and numerous nucleoli.

Following now the behaviour of the spore-mother-cells: as the prophase advances the nuclear thread becomes thicker, evidently by shortening through longitudinal contraction, and clearly not by the approximation and apparent fusion of parallel parts of the thread as described by Berghs ('04) for the microspore-formation of *Allium fistulosum*. I have examined my preparations carefully to see if this fusion occurs, and while I can find numerous instances of the close approximation of the parts of the nuclear thread, I do not find these parts fusing, but do find them thickening without fusion until the stage represented in Fig. 3 appears. While the nuclear thread is thickening it is becoming more and more erythrophil, and retains the safranin in preference to the gentian-violet (when the triple stain safranin-gentian violet-orange is used) some time before its separation into chromosomes. Although my preparations show a close succession of stages in the prophase I have not been able to find the gamosomes recently described by Strasburger ('04) for *Thalictrum purpurascens*. It may be that in *Botrychium* their isolation from the linin framework takes place earlier during the long winter rest of the spore-mother-cells.

When the nuclear thread first segments into chromosomes the latter appear from various points of view, as seen in Fig. 4, where *a*, *b*, *c*, and *d* show clefts that have evidently arisen from longitudinal fission; *e*, *f*, *g*, *h*, and *i* show chromosomes bent or doubled in various degrees; and *j* shows an end view of such a figure as *d* or *h*, one arm being longitudinally split and the other not. On comparison with the work of Farmer and Moore ('03), Strasburger ('04), Gregory ('04), and preparations illustrating spermatogenesis in the Acrididae which Dr. McClung has kindly shown me, where the different prophase stages can be seen with remarkable clearness, I am led to the conclusion that these bent chromosomes are or become divided by transverse fission at the apex of the bend. The chromosomes of Fig. 21, culled from stages represented by Figs. 16 and 17, indicate that this fission has taken place, and they are very similar to Strasburger's Fig. 5, where with a better subject he is confident of a transverse fission. My Fig. 18 indicates that in the anaphase the separation of the chromosomes is to take place in the plane of transverse fission. The longitudinal division begun in the early prophase, and clearly seen in Fig. 4, is lost sight of and only slightly indicated in the cross-shaped chromosomes of Fig. 18. In the anaphase (Figs. 22 and 24) all trace of the longitudinal division is lost. It will be seen that *Botrychium* is not a good subject to bring in evidence regarding the vexed question of the manner of chromosome-division. So far

as its evidence is clear, however, it agrees with the recent conclusions of Farmer and Moore, Strasburger, and Gregory.

Soon after the nuclear thread segments into chromosomes the nuclear cavity is seen to be traversed by numerous fine threads which at first take on a grey and later a violet colour by the three-colour-method ; the nuclear membrane also becomes fibrillar and is stained violet. These characteristics become progressively more pronounced and the fibrils begin to press out at various points into the cytoplasm in the form of a multipolar spindle (Figs. 7, 8, and 9). In this way the nuclear membrane loses its identity in the spindle (Figs. 10 to 14 inclusive). In the meantime the cytoplasm has become progressively less dense and has evidently contributed of its substance to the growth of the spindle. Later the several cones of the spindle collect at two opposite poles and fuse, forming the usual bipolar spindle (Figs. 15 to 17 inclusive), a polar view of which near the equator shows that the fibrils are about equally distributed throughout the space occupied by the spindle (Fig. 20). No indications of centrosomes can be seen.

Investigations into the formation of the kinoplasmic spindle have revealed two prevailing types. In one type the beginnings of the spindle appear first in the cytoplasm, and in the other first in the nucleus before the breaking down of the nuclear membrane, or in the nuclear region simultaneously with or just after the disappearance of the membrane. To the first type belong *Equisetum* (Osterhout, '97), *Lilium* (Mottier, '97), *Gladiolus* (Lawson, '00), *Osmunda* (Smith, '00) *Agave* (Osterhout, '02), *Iris*, *Disporum*, *Aloe*, *Hesperaloe*, *Hedera* (Lawson, '03). To the second type belong *Cobaea* and *Passiflora* (Williams, '99), *Lavatera* (Byxbee, '00), and *Nymphaea alba* (Strasburger, '00). With this second type *Botrychium virginianum* must now be classed.

There does not, however, appear to be any important significance in the existence of these two types. Strasburger ('00, p. 121) suggests that they may be due to differences in the amount of extranuclear kinoplasm available, and he calls attention to the fact that, as a rule, extranuclear kinoplasm takes an important part in the formation of the first spindle of pollen-mother-cells, and a subordinate part in the formation of the second spindle. It may be also true, it seems to me, that the force which causes the differentiation of the spindle emanates from the nucleus, and that in some cases this force penetrates the cytoplasm with sufficient energy to make itself manifest, and in others it does not. It might well be that the second division, following as a rule close upon the heels of the first, and with the reduced amount of chromosome material, does not have at its disposal sufficient nuclear energy to dominate the cytoplasm, with the result that the second spindle is chiefly intranuclear in its origin.

Evidently cytopolarity (meaning by this term the polarity of the cell

mapped out by the kinoplasmic spindle) is not a constant state, but is engendered at the time of nuclear division. The gradual evolution of the spindle-fibres, in many cases without definite orientation at first, may be interpreted in this way. The force in play during cytopolarity seems to emanate from the nucleus, and particularly from the chromosomes, whether united into the nuclear thread or distinct during the later prophases and thereafter. The evidence for this is found in the facts that the axis of the poles always passes through the nucleus, and the spindle-fibres arise first in the nucleus or in the cytoplasm in definite relation to the nucleus; and in pollen- and spore-mother-cells after the second maturation-division kinoplasmic connecting fibres spring up between and connecting the pairs of granddaughter nuclei preparatory to the formation of the cell-plates (see my Figs. 37 to 40 inclusive, and, without seeking further, Mottier's Fig. 50 in Cytologische Studien). That the chromosomes have the dominant part in this appears from the fact that the nucleus as an entirety has in so many instances disappeared, leaving only the chromosomes to represent it, before polarity has become completely established (see my Figs. 9 to 15 inclusive, and Osterhout's and Mottier's Figures in Cytologische Studien). This conjecture receives further support from those cases where the second division takes place without the formation of a resting daughter-nucleus.

In *Botrychium*, as the chromosomes of the first division approach the poles the spindle-fibres form a dense cone, as shown in Fig. 24. Apparently the chromosomes are being drawn toward the pole by a contraction of the fibres. I see nothing in my preparations to indicate that the anaphase translocation of the chromosomes is brought about in any other way. (For a summation of the evidence on which he grounds his opinion of chromosome translocation by a contraction of the spindle-fibres see Strasburger, '00, pp. 140–2; and for a *résumé* of the different theories regarding this subject see Häcker's Praxis und Theorie der Zellen- und Befruchtungslehre, pp. 73–8).

Arrived at the poles the chromosomes fuse together, and the daughter-nuclei enter upon a resting-stage in which the chromatin becomes again cyanophil, forming a fine-meshed reticulum, and one or more nucleoli appear (Figs. 25–8).

While the daughter-nuclei are entering into the resting-stage the connecting fibres are spreading out along the equatorial plane (Fig. 26), and in the equatorial zone, deposits of granules are accumulating which are stained from grey to brownish by the three-colour method (Figs. 26–8). The connecting fibres disappear soon after they have traversed the equatorial plane, and the resting-stage then appears as in Fig. 28. It is seen that no cell-plate of the usual kind is formed, but in its place is a broad and dense zone of cytoplasm.

In the fact that the daughter-nuclei resulting from the first division pass through a resting-stage, *Botrychium* differs from *Pteris* as reported by Calkins ('97), and *Scolopendrium* as described by myself ('98). According to Calkins, in *Pteris*, when the chromosomes of the first division have arrived at the poles they again divide and pass through the stages of the second division without having fused with each other. In *Scolopendrium* I found that the chromosomes of the first division fuse together after they have arrived at the poles, and form a thick thread, but that no other steps towards the formation of a resting-nucleus are taken.

The processes of the second division do not seem to differ essentially from those of the first so far as concerns the formation of the spindle. The thread of the reticulum thickens and the reticulum loses its netted character (Fig. 29); the chromatin gathers together in clumps (Fig. 30); the nucleoli disappear, and the nascent spindle becomes visible within the nuclear cavity, and finally presses outwards in the form of a multipolar spindle (Figs. 31–2). These prophases evidently come rapidly to completion, for they are not frequently found, even in preparations in which all other phases abound. The manner of the separation of the chromosomes in the second division I have found it impossible to determine (see Figs. 30 to 36 inclusive).

In the second division the daughter-nuclei may divide in the same plane or in planes at right angles to each other (Figs. 33 to 36 inclusive). In either case, soon after the chromosomes have arrived at the poles, and before the resting granddaughter-nuclei have been formed, the thick plate of cytoplasm which was laid down in the equator of the first division becomes gradually transformed into kinoplasmic connecting fibres (Figs. 37 to 41 inclusive). If the planes of division are at right angles to each other the fibres connect the granddaughter-nuclei as shown in Figs. 37 to 40; but if both nuclei divide in the same plane the granddaughter-nuclei become joined by the connecting fibres as shown in Fig. 41. Fig. 37 represents a slightly later stage than Fig. 36. At *a* in Fig. 37 is the bundle of connecting fibres remaining at the close of the anaphase of the second division, while at *b* and *b* are nascent connecting fibres arising out of the dense cytoplasm laid down in the equatorial region at the close of the first division, as in Fig. 28. These may be called secondary connecting fibres to distinguish them from the primary connecting fibres at *a*. Both primary and secondary connecting fibres take part in the formation of the cell-plates that are to demark the spores, as is clearly shown in Fig. 41, where both of the last nuclear divisions took place in the same plane. Figs. 40 to 44 inclusive show a progression of events in the formation of the cell-plates, with attendant changes in the condition of the cytoplasm. Reviewing the stages thus far described we find a striking confirmation of the opinion of Strasburger ('97) that the trophoplasm and kinoplasm are mutually interdependent, one increasing at the expense of the other as the require-

ments of the cell demand. The evidence which *Botrychium* affords for this may be summarized as follows:—In the prophases of the first division the density of the trophoplasm diminishes as the kinoplasmic spindle increases in size (Figs. 9 to 18). There is a gradual transition, both in structure and reaction to stains, from the kinoplasmic connecting fibres to the dense granular trophoplasm occupying the equatorial zone in the first telophase (Figs. 25 to 28). The trophoplasm as a whole becomes regenerated after the daughter-nuclei have entered into the resting-stage, and all kinoplasmic differentiations then disappear (Fig. 28). Later, the dense equatorial zone of trophoplasm gradually disappears as the kinoplasmic secondary connecting fibres are generated between the pairs of granddaughter-nuclei (Figs. 37 to 41). And, finally, after the cell-plates demarking the granddaughter-cells or spores have been laid down, we find a gradual transition from the filar kinoplasm to the alveolar trophoplasm (compare Figs. 41 to 44 inclusive).

The plasmodium formed by the fusion of the tapetal cells continues to flow in between the groups of mother-cells while the division phases are progressing, until only a very thin layer of the plasmodium remains at the exterior. The plasmodium appears to be quite fluid, for very minute rifts between the mother-cells, sometimes not more than one micron in diameter, become filled by it. The nuclei of the plasmodium are unable to pass into the smaller crevices and so accumulate in large numbers where the spaces are larger, as shown in Fig. 47.

After the disappearance of the connecting fibres of the second division the cell-plates thicken, nucleoli appear in the nuclei and the mother-cells become more or less dissociated from their previous grouping (Fig. 44). The middle lamella of the cell-plates separating the granddaughter-cells now becomes soluble and the granddaughter-cells or young spores become detached from each other while still enclosed within the membrane of the mother-cell (Fig. 45). Later, the granddaughter-cells become separated from the membrane of the mother-cell also and lie free within it (Fig. 46). The membrane of the mother-cells soon disappears and the granddaughter-cells then lie embedded in the tapetal plasmodium. The young spores now enlarge and build a nodulated wall about themselves which is differentiated into a relatively thick exosporium stained red by the safranin, and a thin endosporium stained violet by the gentian-violet of the three-colour-method (Fig. 48). As the spores advance in their development the tapetal plasmodium becomes strikingly depleted. Instead of the dense cytoplasm of the earlier plasmodium shown in Fig. 47 we find the vacuolate and attenuate structure of Fig. 48. The nuclei of the plasmodium possess at the same time a reticulum which also is depleted, the threads being thinner and taking the stain with less intensity. Their nucleoli appear, however, as in the earlier stages.

The massing of the plasmodial nuclei in the lakes of cytoplasm which occupy the larger clefts between the groups of mother-cells may have an important significance in the nutrition of the developing spores. Loeb ('99) came to the conclusion that enucleated cells lose their capacity for regeneration because the power of oxidation departs with the nucleus, and the synthesis which must depend upon the preliminary exercise of this power can no longer take place. From Lillie's observations ('02) it seems clear that 'the oxidative activities of the organs must be largely a function of their extent of nuclear surface,' and 'the same conclusion applies also to synthetic processes in so far as they depend on oxidations.' In the light of these conclusions the massing of the nuclei of the plasmodium may not be merely an incident following the inflow of the cytoplasm. It may well be that there, where they occur associated in large numbers (see Fig. 47), they exert a powerful influence in the nutrition of the developing spores at the cost of the substance of the plasmodium itself.

Methods.

Small pieces of the sporophyll bearing only a few sporangia were fixed in the field as soon as they were cut off in the following manner:—The pieces of sporophyll were placed in a phial containing a 0·5 % solution of chromic acid, from which the air was then removed by a portable air pump. When the atmospheric pressure was again turned on the infiltration of the pieces by the fixative was shown by their immediately sinking in it. The material was then transferred at once to Flemming's fixative. Both the stronger and weaker solutions were employed, and both seemed to give equally good results. The material was embedded in paraffin, and the sections, which were cut 5 microns thick, were stained by Flemming's three-colour method.

NOTE.

After this paper was sent to the publishers a contribution by Ira D. Cardiff on 'The Development of the Sporangium in *Botrychium*' appeared in the Botanical Gazette, xxxix, 5, p. 340. As the title indicates, Cardiff has made a study of the development of the sporangium rather than of the spores, and our papers cross the same ground chiefly as regards the tapetum. Cardiff worked with two species of *Botrychium* (*B. virginianum* and *B. ternatum*), and he states that the two are found to be essentially the same in their sporangial development. The details of the paper and drawings do not refer to either species in particular, and I assume that they are intended to apply equally well to either. On this assumption it is interesting to note the differences that occur in members of the same species growing in different localities (Cardiff's *B. virginianum* was taken in the vicinity of Woodville, Ind., and mine near Lawrence, Kans.). He finds that the tapetal

layer becomes as much as four or five cells in thickness, while I find two cells in thickness the rule. His material shows an excessive growth of the tapetum about the time of the first prophase and after, while mine does not. I have measured the volume of the tapetum before it begins to flow in between the groups of spore-mother-cells, and find it more than two-thirds the volume of the sporogenous tissue; and since the sporangia do not essentially increase in size while the inflow is taking place, this amount is evidently sufficient to fill the gaps between the groups and still leave the thin layer that remains at the exterior. He finds frequent amitotic nuclear division in the tapetum, while my material does not show this. Our subjects agree in showing that none of the spore-mother-cells is restrained from forming spores.

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EXPLANATION OF PLATES XXVIII–XXX.

Illustrating Professor W. C. Stevens's paper on *Botrychium*.

PLATE XXVIII.

Fig. 1. Spore-mother-cell in early prophase. × 1800.

Fig. 2. The same, showing spireme applied to nuclear wall. × 1800.

Fig. 3. Later prophase-stage showing greatly thickened nuclear thread. × 1800.

Fig. 4. Chromosomes from various points of view immediately after the segmentation of the nuclear thread. × 1800.

Figs. 5, 6. Later prophases than Fig. 4, showing chromosomes contracted and thickened and lines of cleavage no longer recognizable. × 1800.

Figs. 7, 8. Prophases showing the beginning of the formation of the spindle within the nuclear cavity. × 1800.

Figs. 9–12. Various stages in the development of the multipolar spindle. × 1800.

Figs. 13–15. Stages in the fusion of the multipolar spindles to form bipolar spindles. × 1800.

Figs. 16, 17. Late prophases with bipolar spindles. × 1800.

Fig. 18. First metaphase. × 1800.

Fig. 19. Polar view of nuclear plate. × 1800.

Fig. 20. Polar view above nuclear plate showing trans-section of the spindle-fibres. × 1800.

Fig. 21. Chromosomes culled from stages represented in Figs. 16 and 17. These indicate that transverse fission has taken place following the stage shown in Fig. 4. × 1800.

Fig. 22. Anaphase of the first division. × 1800.

Fig. 23. Late anaphase of the first division. × 1800.

Fig. 24. Polar view of anaphase of the first division. × 1800.

PLATE XXIX.

Fig. 25. Two spore-mother-cells in telophase of the first division. × 1800.

Fig. 26. Later stage showing daughter-nuclei demarked by nuclear membrane, and beginning of dense equatorial zone of cytoplasm. × 1800.

Fig. 27. A stage still later than Fig. 26. The nucleoli have appeared in the daughter-nuclei, and the connecting fibres have nearly disappeared. × 1800.

Fig. 28. A group of spore-mother-cells after the close of the first division. The daughter-nuclei are in the resting-stage. The cytoplasm has become entirely regenerated and the dense equatorial region is very apparent. × 1800.

Fig. 29. Early prophase of the second division. × 1800.

Figs. 30, 31. Later prophase stages of the second division. In Fig. 31 the spindle-fibres are becoming evolved within the nuclear cavity. × 1800.

Fig. 32. A succeeding stage showing a multipolar spindle. × 1800.

Figs. 33, 34. Metaphases of the second division. In Fig. 33 the nuclei are dividing in the same plane, and in Fig. 34 in planes at right angles to each other. × 1800.

Figs. 35, 36. Anaphases of the second division.

Figs. 37, 38. Telophases of the second division, showing the origin of the secondary connecting fibres from the dense equatorial cytoplasm of Fig. 28. × 1800.

Fig. 39. Completion of formation of granddaughter-nuclei. × 1800.

Fig. 40. Beginning of formation of cell-plate. × 1800.

Figs. 41–43. Completion of cell-plates of second division, and gradual transition from the fibrillar condition of the connecting fibres to the alveolar condition of the general cytoplasm. × 1800.

PLATE XXX.

Fig. 44. Group of mother-cells after the completion of the second division, showing the granddaughter-cells or young spores still connected with each other and closely invested by the membrane of the mother-cell. × 1800.

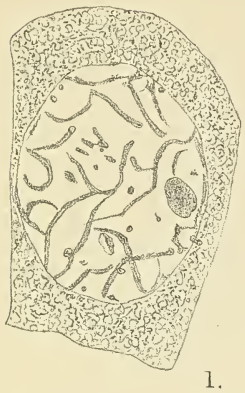
Fig. 45. Granddaughter-cells separating from each other, but still adhering to the membrane of the mother-cell. × 1800.

Fig. 46. Granddaughter-cells lying free within the membrane of the mother-cell. × 1800.

Fig. 47. Portion of tapetal plasmodium that has flowed in between the groups of spore-mother-cells, as it appears at the close of the last division. × 900.

Fig. 48. Mature spore embedded in the plasmodium which is now much depleted. One nucleus of the plasmodium also shown. × 900.

Fig. 49. Nucleus of the plasmodium on a larger scale embedded in the depleted cytoplasm of the plasmodium. × 1800.



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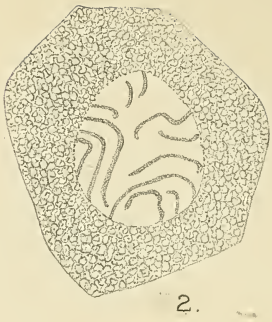

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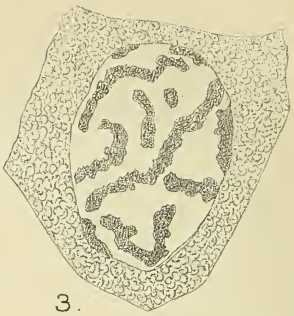

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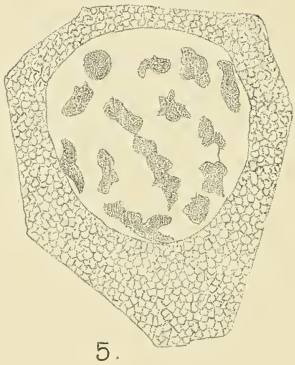

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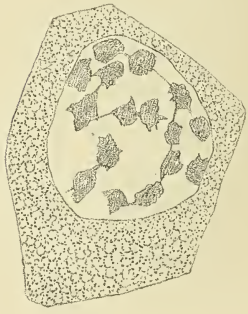

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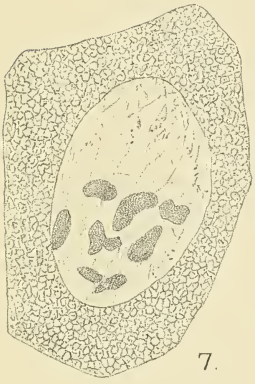

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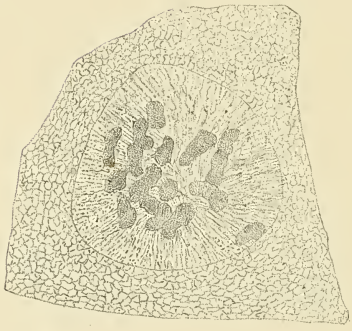

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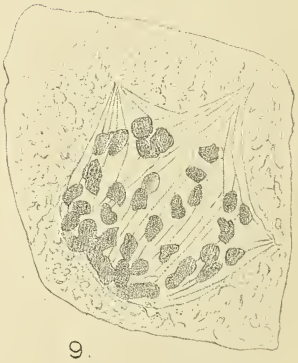

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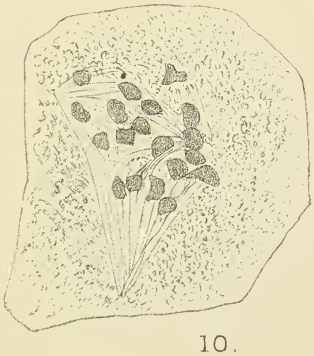

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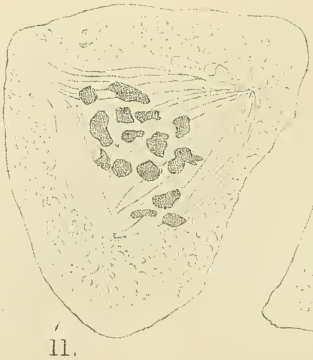

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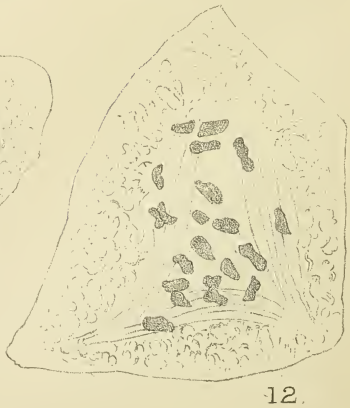

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Stevens del.

STEVENS.—SPORE FORMATION IN BOTRYCHIUM VIRGINIANUM.

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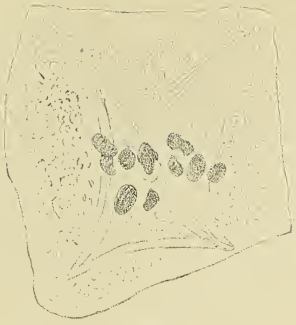

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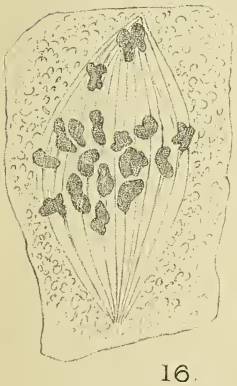

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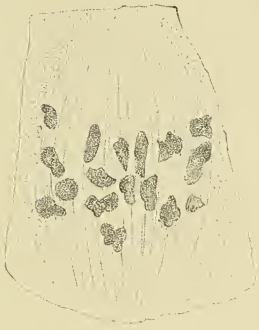

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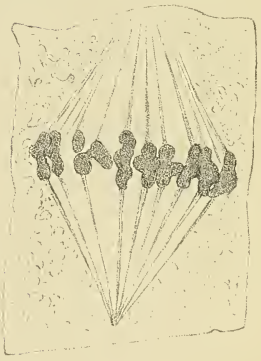

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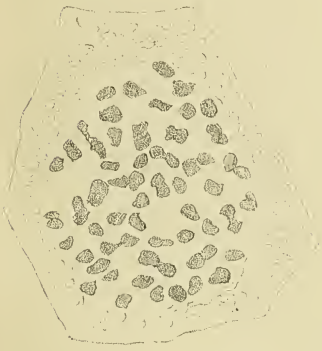

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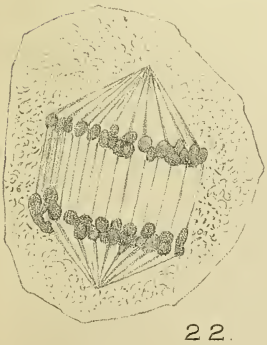

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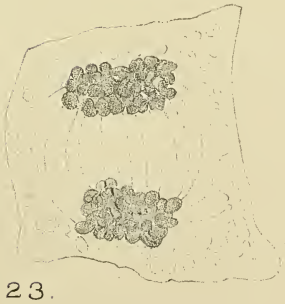

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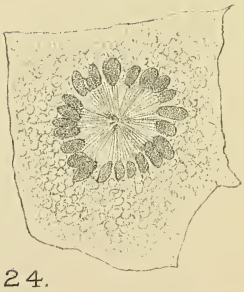

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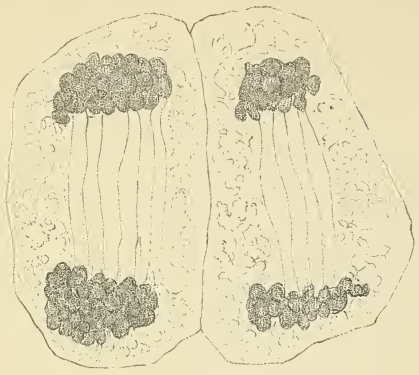

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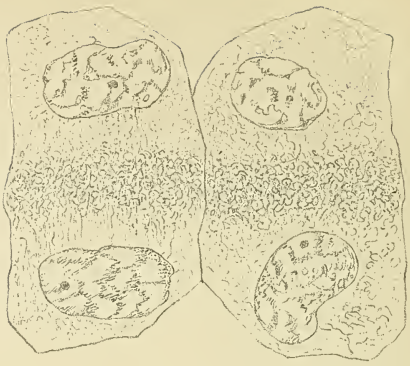

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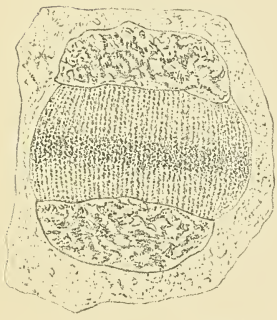

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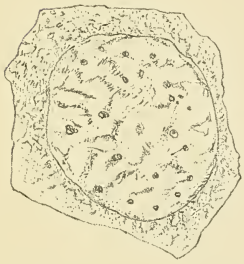

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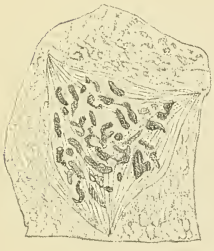

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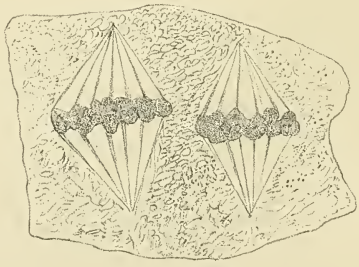

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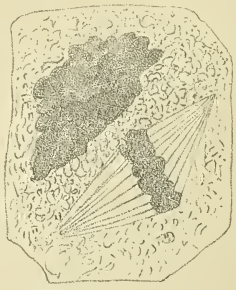

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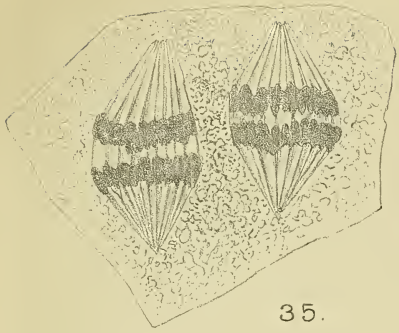
Stevens del.

STEVENS—SPORE FORMATION IN BOTRYCHIUM VIRGINIANUM.



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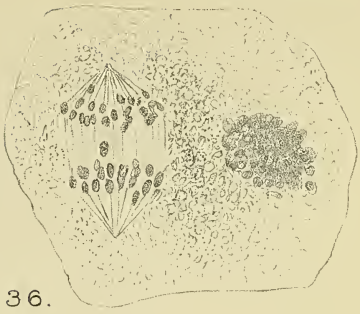

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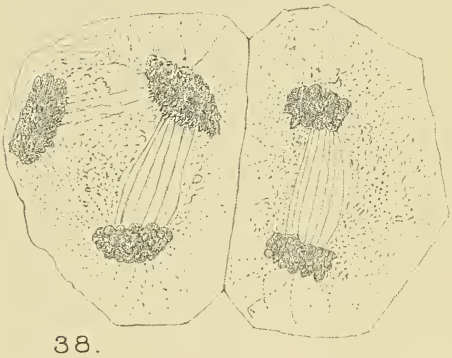

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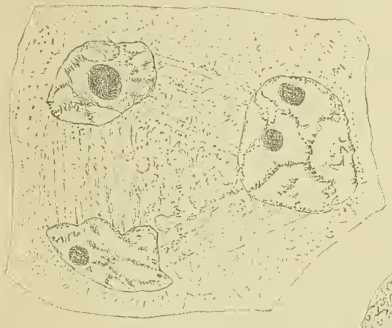

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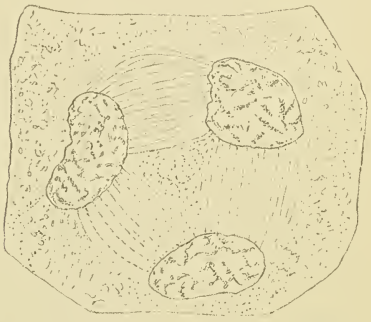

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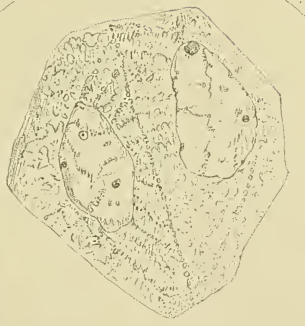

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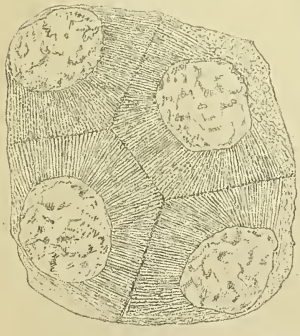

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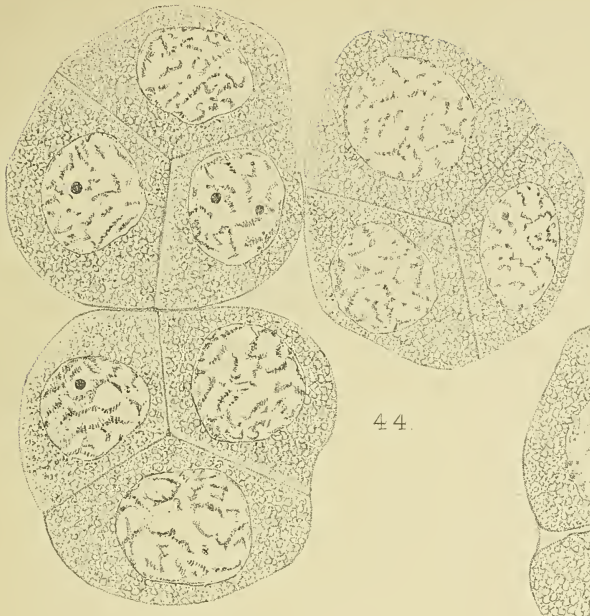
Huth lith. et imp.



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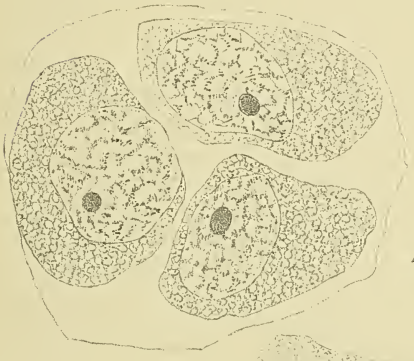

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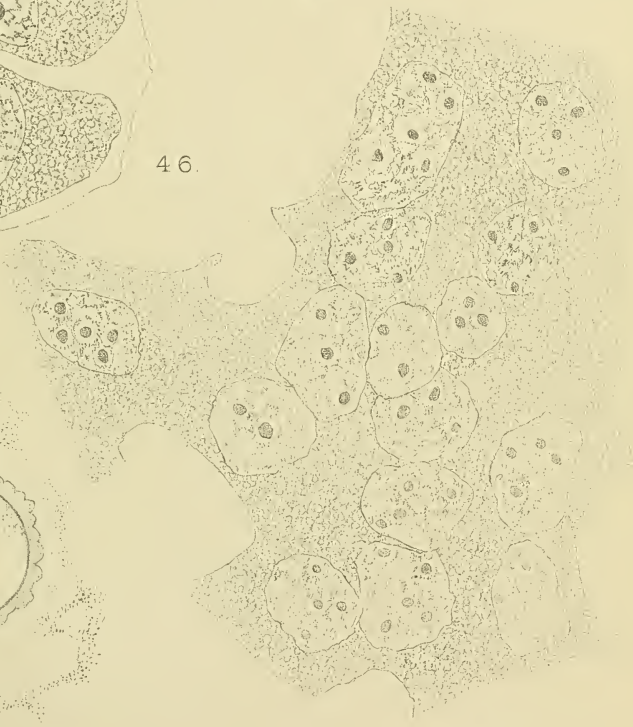

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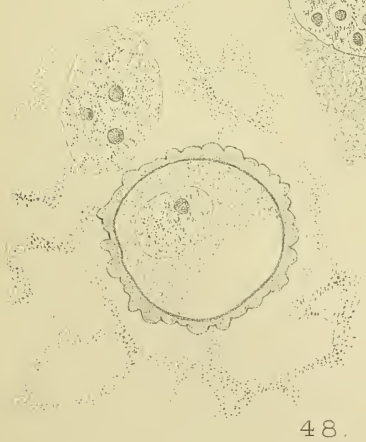


48.

Stevens del. Huth lith. et imp.

STEVENS.—SPORE FORMATION IN BOTRYCHIUM VIRGINIANUM.

A Study of the Vascular System of Matonia pectinata.

BY

A. G. TANSLEY, M.A.,

Assistant-Professor of Botany, University College, London,

AND

MISS R. B. J. LULHAM, B.Sc.

With Plates XXXI, XXXII, and XXXIII, and five Figures in the Text.

CONTENTS.

INTRODUCTORY.

EVER since the meeting of the British Association at Bristol in 1898, at which Mr. Seward described[1] the complicated and striking vascular anatomy of the comparatively rare Malayan fern *Matonia pectinata*, the problem of relating this apparently unique vascular skeleton to those of other Ferns has been a matter of considerable interest to students of Filicinean vascular morphology.

[1] Subsequently published in full, Phil. Trans. B., vol. cxci, p. 171, 1899.

[Annals of Botany, Vol. XIX. No. LXXVI. October, 1905.]

It was already evident in 1898 that a study of the young plants would throw light on this question[1], though the importance of the vascular structure of the first-formed stem, especially in Ferns, as furnishing an ontogenetic parallel to the process of evolution of the adult structure, was not at that time so clearly recognized as it is to-day.

It was largely for this reason that I welcomed the opportunity, in January, 1901, of joining Dr. W. H. Lang in his visit to Mount Ophir in the Malay Peninsula, the original locality[2] for *Matonia pectinata*, which is particularly abundant on its upper slopes. I can confirm Wallace's description of the habitat of this magnificent Fern, as quoted by Seward[3]. The *Matonia* is almost confined to open situations on Ophir, covering the comparatively shallow soil overlying the flat or gently sloping rock faces in dense thickets. Its most abundant congener in these thickets is *Gleichenia linearis* (*dichotoma*), while *Gleichenia flagellaris, Dipteris conjugata*, and a little *Pteris aquilina* are also constantly associated. All these Ferns are characteristic of the open, or of slightly shaded situations. *Matonia* scarcely penetrates the thick dwarf woods of the upper part of Ophir, though an occasional clump occurs where an opening in the trees allows more light than usual to reach the ground. In Borneo *M. pectinata* occurs on Santuborg and Matang, 3,500 to 3,800 feet above sea-level, situations which seem quite comparable to the Ophir one, though curiously enough it is said to be there confined to old jungle[4], quite contrary to its occurrence on Ophir. It is also known from various Malay islands at sea-level, and thus seems to belong to the coast-mountain flora to which Ridley has called attention[5].

Young Plants of Matonia pectinata.

After considerable search I discovered three young plants embedded in moss and humus on a shaded ledge of rock, a few feet below the rocky top of Gunong Ledang, which is the principal summit of Mount Ophir. These are represented on Plate XXXI, Figs. 4, 5, 6, and 7. The strong resemblance of their fronds to those of older but starved specimens of the plant growing in a deeply shaded crevice below an overhanging rock on the other side of the summit, left no doubt of the identity of these plants, while their small size and the shortness of their rhizomes indicated their comparative youth.

Dr. Lang afterwards found three even younger specimens, which he very kindly handed over to me, in the damp soil by the side of a small stream on Padang Batu. These are represented on Plate XXXI, Figs. 1, 2, and 3, and here again the form and venation of the fronds, when compared with the

[1] Seward ('99), pp. 180, 181.
[2] Wallace, The Malay Archipelago, 1886, p. 30.
[3] Seward ('99), p. 171.
[4] Seward ('99), p. 174.
[5] Ridley ('01).

older specimens, left no doubt of their identity. The rarity of the young plants of this species compared with the comparative abundance of those of *Gleichenia linearis* and *Dipteris conjugata* in similar situations was rather striking. Neither Dr. Lang nor I succeeded in finding any specimens with the prothallus attached, in spite of continued hunting. In addition to the six young plants, I subsequently found some plants with thin rhizomes and small comparatively simple fronds growing in the deep shade of a rock-cleft. These were evidently much older than the oldest of the six, but had been starved owing to want of light and perhaps also to lack of soil.

The specimens thus obtained form a fairly good series, both as regards the gradually increasing complication in the form of the frond, and also, as we shall see presently, in vascular structure.

During my stay on Ophir I naturally took the opportunity of laying in a good stock of material of the adult plant, and after my return to England I sent, at Prof. Weiss's request, a few nodes preserved in spirit to the Owens College, Manchester. In this material Miss Wigglesworth, a pupil of Prof. Weiss, discovered a distinctly more complicated type of structure than that described by Mr. Seward, a type with *three* concentric amphiphloic siphonostelic cylinders in the rhizome[1]. It thereupon seemed desirable to work through the whole of my stock of material, and Miss Lulham undertook this laborious task.

The results of the investigation, together with those obtained from the young plants[2], are now presented. The considerable delay in publication has been due to a variety of causes, not the least being the lengthy character of the work. No less than fifty nodes have been examined from the adult plants alone, several thousand sections have been cut, and numerous wax models made. It is hoped that the fairly complete account, which we are now able to give, of the progressive complication in the vascular structure of this unique type, may be a sufficient justification.

A. G. TANSLEY.

The Morphology of the Leaf.

A comparison of the fronds borne by the different young plants (Plate XXXI, Figs. 1–8) shows considerable variety of form, and, in the larger fronds at least, a mixture of the dichotomous and monopodial types of branching. There is, however, as we shall see, good reason to believe that dichotomy is the primary method.

Plant A (Fig. 1) has one tiny frond which is simply bifid. The larger frond is trifid, and might be considered to consist of a central

[1] Grace Wigglesworth, New Phytologist, vol. i, 1902, p. 157.

[2] I am indebted to my wife and to Miss E. N. Thomas for preparing sections of the six young plants.

phyllopodium with a right and a left lateral. A microtome series of the top of the petiole and base of the frond, however, reveals a clear dichotomy of the single petiolar strand into two equal trunks, one of which (that belonging to the central lobe) increases in size somewhat, and sends off a branch nearly at right angles, which becomes the midrib of the left-hand lobe. This behaviour of the vascular strands certainly suggests a primary dichotomy, one of the members of which sends off a single branch from its base.

Plant B (Fig. 2) has a small frond with apparently a primary dichotomy, one of whose members remains small, while the other undergoes a secondary dichotomy. The larger frond, on the other hand, if we may judge from the course of the vascular strands, has a middle lobe, from the base of which arise two laterals, each of which branches, whether dichotomously or monopodially it is hard to decide. This frond, assuming the laterals to dichotomize, may be regarded as a sort of prototype of the adult frond.

Plant C (Fig. 3) has both its fronds, and Plant D (Fig. 4) two of its three fronds, clearly showing a primary dichotomy, while the subsequent branching, especially in C, appears to be very largely dichotomous.

Fig. 5 (a detached leaf of Plant E) shows an unusual type of leaf. Fig. 6 (another leaf from E) returns to the type of the larger frond of B (Fig. 2), i.e. a trifid leaf. Here, however, all three lobes are branched, partly at least dichotomously, the middle one most extensively.

Plant F (Fig. 7) has all its leaves of this trifid type, and the branching of each lobe is for the most part monopodial, though a tendency to dichotomy is still observable in some cases. In the weaker fronds of the starved plants mentioned above, this trifid character is the rule; but in one case at least (Fig. 8) there is a clear primary dichotomy with no middle member, and each branch apparently forks again, three of the four resulting members branching monopodially. The stronger fronds of the starved plants are simply feeble expressions of the adult type. (Cf. Fig. 9.)

The adult type of frond in this species is quite unique among the Ferns. 'The long petiole branches to the right and left, giving off lateral members from the upper faces of its two recurved arms in a scorpioid manner' (Seward, '99, p. 175). It is well illustrated by Seward ('99, p. 176, Fig. 1). In all the fronds we have ourselves seen there is a middle 'pinna' arising from the angle of the dichotomy. This is commonly the longest of all the pinnae.

The following solution of the problem as to the real nature of this curiously branched frond is suggested. The primary branching of the frond is assumed to be dichotomous. Many of the leaves of the young plants are difficult or impossible to interpret as monopodially branched, and a primary dichotomy appears to be the simplest explanation of the

main fork in the adult. In the angle of the dichotomy, however, a 'middle lobe' is developed in all but some of the simplest fronds, just as is often the case, for instance, in the branching of the thallus of the Liverworts. It is this middle lobe which gives to so many of the fronds of the young plants their trifoliate character (Figs. 2, 6, and 7). In the adult the middle lobe is represented by the median pinna[1]. The subsequent branching of the primary forks and of the middle lobe at first (i.e. in the young plants) shows a tendency to be dichotomous, but the dichotomous systems easily pass over into the monopodial type, and the three lobes of the trifoliate leaf are in most cases pinnately branched and correspond with the 'pinnae' of the adult. Some of the starved plants have trifoliate fronds whose three lobes resemble in all respects the 'pinnae' of the adult frond.

The origin of the adult frond from this type of structure is not at first sight quite obvious. We suggest that it arises by the dichotomy of the primary forks and the repeated dichotomy of the *lower member only* of each successive fork, the upper member in each case, and the lower member also of the last fork, becoming a pinna. If this be so, the 'recurved arm' is a sympodial axis composed of the bases of the lower members of successive dichotomies. To suppose that it is a monopodial axis giving off lateral members from the upper face only would be to assume a type of branching unparalleled, so far as we know, in the fronds of Ferns, while the 'scorpioid' character of each half-frond certainly suggests a sympodium. Furthermore, the hypothesis of dichotomy as the fundamental type of branching in the frond as a whole, enables us to bring it into relation with the fronds of *Gleichenia*. Many of the species of this genus, as is well known, have long straggling fronds with repeated dichotomous branching. 'Pinnae' may be borne on the primary forks, or may be confined to those of a higher order. A bud normally arises from the angle of the primary dichotomy. This bud is sometimes developed, forming a main rachis of the whole frond, and itself dichotomizing and bearing pinnae to a greater or less extent. Boodle ('01 B, p. 705) has already suggested that it is possible to derive the pinnate type of frond, so common among the Ferns, from such a dichotomous frond as is found in these straggling *Gleichenias*, by imagining the successive forks of this main rachis to be limited in growth so that they become pinnae. Such a mode of origin is all the more likely since some species

[1] It is possible to regard the 'middle lobe' not as a structure *sui generis*, so to speak, as it apparently is in the thalloid Liverworts, but rather as *in origin* the inner branch of the second dichotomy, becoming in the adult a pinna which, like the other inner branches, has lost the power of further dichotomy. Such an interpretation is indeed suggested by Figs. 1, 2, and 5, and is perfectly compatible with the structure of the adult frond. No light is thrown upon this question by any of the adult fronds of *Gleichenia* we have examined (*vide infra*), though an extended comparative examination of the fronds of *young* Gleicheniaceous plants might be expected to illuminate the whole subject.

of *Gleichenia*, e. g. *G. circinata, speluncae*, and *elongata*, exhibit intermediate forms in which the bud of the main rachis grows more strongly than the primary forks, and the successive forkings of the rachis appear as pinnae, while *G. Boryi* actually shows a typical pinnate structure of the whole frond, the main rachis itself ending in a 'pinna.'

We are inclined to regard the frond of *Matonia pectinata* as having also taken origin from a primitive dichotomous type, but in another direction. The middle lobe or bud in the angle of the primary fork has here been at once limited in growth so that it forms a pinna, while the primary forks dichotomized repeatedly, the upper member of each successive fork forming a pinna, while the lower branched again. Thus the *Matonia*-frond became moulded into a fan-shaped structure with a power of *peripheral* growth, while the ordinary Fern-frond acquired a pinnate structure retaining a power of *terminal* growth. Here again certain species of *Gleichenia* furnish evidence of intermediate stages in such a process of evolution. *G. flabellata*, Br., *G. Cunninghami*, Hew, and *G. quadripartita*, Hk., show the relevant phenomena. Seward ('99, p. 192, Fig. 7) has already noticed the 'superficial resemblance' of the frond of *G. Cunninghami* to that of *Matonia pectinata*. In the frond of the former species which he figures there is no middle bud evident, but the primary forks of the frond (which bear pinnules from their base) fork again close to their base, and each of the four secondary members at once forks again. The three upper members on each side so produced become simple pinnae, undergoing no further branching, but the lowest (peripheral) member on each side dichotomizes once more. This already gives us an indication of what has taken place in *Matonia pectinata*. *G. flabellata* sometimes produces fronds (Plate XXXI, Fig. 10) which are even closer to the *Matonia*-type, for here *only the lower members of the secondary forks* dichotomize, and the bases of the primary forks are bare of pinnules, while a median bud is present, though it has become adherent to one of the primary forks[1]. If this bud were developed into a pinna (a condition which is sometimes realized in *G. cryptocarpa*, Hk., though in that species the frond is more complicated and less compact), and the peripheral dichotomizing were carried further, we should have the type of the adult *Matonia pectinata*. In *G. quadripartita*, Hk., the arrest of the branching of the frond after the second dichotomy is characteristic, as the specific name indicates, but the lower branch on each side here also sometimes branches again, as in the frond of *G. flabellata* figured.

Fig. 9 shows a frond of *Matonia* from one of the starved plants alluded to above. The secondary dichotomy has occurred on the left,

[1] On the hypothesis suggested in the footnote on p. 479 this middle bud would represent the arrested inner branch of the first dichotomy of the left-hand member of the primary fork. Its position here would then be more primitive than when it occupies the actual angle of the fork, as in most *Gleichenias*.

while on the right there is a suggestion of it, the lowest pinnule on the peripheral side being isolated and much longer than the others.

It seems difficult to resist the conclusion that the adult frond of *Matonia pectinata* represents a further development of the tendency seen in these *Gleichenias* with compact flabellate fronds. Though growth in length of the axes of the frond has been arrested, the extent of lamina borne by each frond has been kept, along with the compact habit, by the peripheral growth brought about through the retention of the power of branching of the outer (lower) member of each successive dichotomy.

It is interesting to note that the rachis of each 'pinna' retains at its base the characteristic form of the leaf-trace as it leaves the stele of the rhizome, though without the final curl, while the sympodium formed, on the view just expressed, by the crowded bases of successive members of the dichotomizing system, shows a vascular system consisting of a closed cylinder with an internal band-shaped vascular strand. This partial simulation of the rhizome-type of vascular structure by the sympodium is probably due to the exigencies of the crowded insertion of successive pinna-traces.

The form of the frond in the other species of the genus, *M. sarmentosa*, should be mentioned in this connexion. Here the frond possesses a long unbranched rachis, continuous with the petiole, and bearing distant groups of two, or sometimes three, narrow strap-shaped laminae which reach a length of several inches. The members of each group of laminae are inserted on a slightly swollen common base, from which often arises a structure like an arrested bud. The laminae themselves are often dichotomously branched, and then resemble very closely the dichotomizing laminae of the young *M. pectinata* (e. g. as seen in Plate XXXI, Fig. 6). In the only intact frond we have seen, the rachis also ends in a similar lamina dichotomizing in the same manner.

The type of branching of the frond of *M. sarmentosa* is thus clearly different from, and appears to be as exceptional as, that of *M. pectinata*. Its morphology is puzzling, though it might possibly be elucidated with the help of young plants, which have not yet been seen. The adult frond scarcely gives any help with that of *M. pectinata*, but there is certainly no clear indication of derivation from a typical pinnate form. The resemblance of the dichotomously branched strap-shaped laminae to those of the young *M. pectinata* is very striking [1].

If the above considerations are sound, it follows that the morphology of the leaf in *M. pectinata* furnishes strong confirmation of the suggested

[1] It is of interest to note that the vascular system of the rhizome of *M. sarmentosa* is organized on the same lines as that of a young *M. pectinata*. Of two fragments which we were able to examine both showed the dicyclic arrangement. In one case the second cylinder was protostelic, in the other solenostelic. In the second case a dorsal gap in the second cylinder was still open. The base of the petiole, however, could not be examined.

Gleicheniaceous affinities of *Matonia*, recently expressed by Christ, who includes both in his class Oligangia, as well as by Bower, who places both families in his group of 'Simplices,' and concludes that the affinities of *Matonia* are with Gleicheniaceae rather than with Cyatheaceae ('00, p. 45). The evidence for this view, which must be based primarily on the sporangial and soral characters, is difficult to impugn, and it seems to us to be strengthened by the morphology of the leaf, which appears to have much in common with the Gleicheniaceous type and nothing with the Cyatheaceous.

Detailed Account of the Progressive Complication in the Vascular System of the Rhizome and Leaf-Trace[1].

(1) *The six young Plants.*

A transverse section of the proximal end of Plant A (Pl. XXXI, Fig. 11) shows a very simple protostele, consisting of about twenty-four elements, surrounded by a pericycle and endodermis which are obviously sister-layers. The whole of the tissue of the stele apparently consists of xylem, which is made up of about ten scalariform tracheids and fourteen parenchyma-cells, exclusive of the pericycle. No phloem can be distinguished. The cortex is only three or four cells thick.

This structure is maintained for a distance of about ·5 mm., both cortex and stele increasing in diameter, but undergoing no alteration of structure. The cortex then extends itself on the dorsal side of the rhizome preparatory to the origin of the first leaf[2], while on each side, in connexion with the leaf base, a root is given off. The insertion of the steles of these roots on the stele of the stem takes place in front of (about ·1 mm. nearer the apex of the stem than) the insertion of the leaf-trace.

The leaf-trace itself is of the simplest, consisting merely of a strand of tracheids, which are inserted almost perpendicularly on those of the stem-stele, surrounded by pericycle and endodermis, but, like the stele, apparently without phloem. The structure of such an early leaf-trace is in fact indistinguishable, except for its smaller size, from that of the stele of the rhizome. In neither is any trace of spiral elements to be seen.

Immediately in front of the origin of the first leaf-trace and before the insertion of the two root-steles is reached, the stele of the rhizome is found

[1] It has been thought well to publish full details of the phenomena met with during the progressive complication of the vascular system. A shorter and more general account, accompanied by explanatory tables and diagrams, will be found on pp. 496–502.

[2] It is of course possible that earlier leaves originally existed in this, the youngest specimen obtained. No definite indication of approach to the structure of the primary root was observed in the transverse section of the proximal extremity, .5 mm. below the first leaf; but, on the other hand, the extreme simplicity of the structure of this part of the rhizome and of the leaf-trace indicates that the latter belongs at least to a very early leaf.

to have increased considerably in diameter, and now possesses some fifty or sixty elements (Fig. 12). In its centre is a group of about half a dozen cells of rather smaller diameter than the tracheids and parenchyma surrounding them, possessing relatively thin walls which stain dark blue with haematoxylin, and no nuclei. This is the first appearance of unmistakable phloem. The cells in question are no doubt sieve-tubes. Associated with the sieve-tubes are an approximately equal number of parenchyma-cells, with nuclei which fill the greater part of the lumina, and dense granular protoplasm (*prot. c*?). These are probably the 'Eiweisszellen' of Strasburger, so widely distributed among Pteridophytes in association with sieve-tubes. The first two root-steles have no connexion with the strand of internal phloem. In the neighbourhood of the points of insertion of these steles, however, there are indications of the appearance of external phloem elements at various points on the periphery of the rhizome-stele, and these are connected with the phloem of the root-steles. Just in front of the second of these two roots the rhizome-stele stretches itself at right angles to its axis preparatory to giving off the second leaf-trace, and the internal strand of phloem follows the outline of the stele, and itself sends a contribution to the trace (Fig. 13, *int. ph. tr.*). The external phloem of the stele, which by this time has become a fairly well-defined layer, also contributes to the trace. The phloem of the leaf-bundle during its passage through the cortex appears to surround the xylem completely, though it is mainly massed on the adaxial side, and consists largely of parenchyma. A third root is inserted just behind and a fourth just in front of the origin of the second leaf-trace. Beyond this point the stele increases greatly in diameter, the central phloem-strand becoming particularly bulky. In the middle of the latter appear certain large, more or less isolated cells, which resemble the cells of the endodermis both in size and contents, and represent the first appearance of an internal endodermis. The stele becomes greatly elongated dorso-ventrally and three roots, which have not yet penetrated the cortex, arise from its ventral side.

The tissue of the dorsal side of the stele now draws together to form the third leaf-trace (Fig. 14), which consists of an arc of xylem incompletely surrounded by phloem, that in the adaxial concavity of the arc derived from the internal phloem of the stele, while the sieve-tubes occurring on the lateral and abaxial faces of the trace are continuous with the external layer of the stelar phloem. The endodermis of the concavity of the trace is also continuous with the internal endodermal cells of the stem-stele. The external phloem of the stele beyond the point of departure of the trace is, of course, continuous with the adaxial phloem of the trace. We have here, in essentials, the relations of external and internal phloem of the stem-stele with leaf-bundle-phloem characteristic of the *Lindsaya*-type[1].

[1] Tansley and Lulham ('02).

Another point is to be noted in connexion with the origin of this third leaf-trace. For a distance of ·1 mm., during the early stages of the separation of the trace, the internal phloem of the stem-stele has a strongly curved form, into the concavity of which the xylem projects on one side of the base of the trace. This is the very simplest form of that 'local dilatation of the edge of the leaf-gap' which, as Gwynne-Vaughan has shown, forms the starting-point of the formation of internal accessory vascular strands.

After the departure of the third leaf we have reached a point in this specimen so close to the apex of the stem that the whole of the tissue on the ventral side of the stele is in a meristematic condition, the xylem on the dorsal side of the central phloem being alone lignified. In the course of the next ·1 mm. the whole of this dorsal tissue of the stele, including the central phloem, is seen to be continuous with the fourth leaf-trace, while the ventral meristematic tissue ends in the stem-growing point. The actual growing apex is forced downwards by the early differentiation of the fourth leaf, so that it appears as a curved knob of meristematic tissue adhering to the ventral surface of the petiole.

Plant B (Fig. 2) is very slightly more advanced than A. Its tissues were not very well preserved and present no new anatomical feature. The stele of the rhizome shows the same features as in the more advanced part of A, and the leaf-bundles are of corresponding type.

Plant C, as may be seen from Fig. 3, possessed two leaves of considerably greater size and elaboration than those of A and B. A length of rhizome of a little over 1 mm. is preserved. The structure of the proximal end is essentially the same as the structure of B and that of the distal end of A, i. e. it consists of a hollow cylinder of xylem, with external phloem, pericycle, and endodermis, and enclosing a central strand of phloem which itself contains more or less connected endodermal cells. Passing forward towards the apex, the internal endodermal cells are continuous with a constant though irregular strand, consisting, on the transverse section, of several cells. The origin of the leaf-traces is, in this specimen, decidedly inconstant and irregular. Sometimes they are attached at right angles to the course of the stem-stele, while in other cases they pass off slowly, so that successive sections of the rhizome cut both stele and trace transversely. This form of variability is common in all the young plants. The cross sections of the different traces also vary considerably in form. The local dilatation of the xylem of the stele on one side of the attachment of the leaf-trace, already described in the case of the third trace of Plant A, occurs again in several of the traces of the present specimen. In one case the dilatation is continued forward as a ridge in front of the separation of the trace for a distance of ·09 mm.; it then becomes separated from the rest of the xylem of the stele and continues for ·2 mm. as a free strand surrounded by the internal phloem. It becomes gradually thinner, but does

not die out, and eventually enters into connexion with the dilatation at the base of the next trace. The apex of the rhizome in this specimen had died and its tissues partly perished. A young leaf with undifferentiated tissue and showing its circinate vernation is attached to this portion of the stem.

Plant D (Fig. 4), as will be seen, bears leaves of a type no more complex than those of C, nor does its rather bulkier stele show any definite advance in structure. The transverse section of the proximal end shows essentially the same type that we saw in the distal end of C, i.e. the central phloem of the stele encloses both internal endodermal cells and also a free strand of xylem. But here the xylem-strand is comparatively bulky and centrally situated, while the endodermal cells are few and more or less scattered (Plate XXXII, Fig. 15). Presently the endodermal cells unite to form a strand one or two cells thick and crescent-shaped in cross-section on one side of the central xylem, while the latter comes into connexion with the external xylem, probably in the mid-dorsal line. A leaf-trace is now gradually given off on one side of this connexion. The strand of endodermal cells in the concavity of the leaf-trace is perhaps[1] unconnected with the internal stelar endodermis, but the corresponding phloem is certainly connected both with internal and external stelar phloem. The leaf-trace, after it becomes free from the stele, is concentric in structure. It is kidney-shaped in section and its wings contain large scalariform elements, while its centre is composed of a strand of small tracheids. After the departure of the leaf-trace, there is a gap left in the xylem-ring[2], and this is closed by the external xylem of the stele, the inner xylem-strand becoming immediately again detached from the outer ring. Later it again enters into connexion with the outer ring, and the next trace arises from the other side of the point of connexion. Though rather bulkier, it arises in identically the same way and has evidently the same structure as the last. The endodermis of its concavity is certainly connected both with the internal and with the external stelar endodermis. Before the trace is fully detached, the tissues of the stele cease to be fully differentiated, and the stele shortly becomes promiscuously invaded by cells like those of the inner cortex, the rest of the stelar cells having meristematic characters. The stele is then lost in a uniform mass of brown cells, while a curved knob of meristematic cells, representing the actual growing point, is found attached to the ventral surface. The explanation of this appearance is probably due to a checking in this particular plant of apical growth beyond the point of insertion of the last leaf, and the consequent passing over of the majority of the undifferentiated cells into a passive condition without differentiation.

[1] Owing to defects in the series this point could not be absolutely determined.

[2] Where such a gap occurs it may be called a 'xylem leaf-gap,' or a 'xylem-gap,' as distinguished from a true leaf-gap in which the tissue of the pith is put in connexion with that of the cortex owing to an interruption in all the stelar tissues.

Plant E had most of its leaves broken off. The one nearest the apex (Fig. 5) was considerably larger and more complex than any we have yet met.

A transverse section of the proximal end of the rhizome (Fig. 16) shows a state of things rather different from any we have hitherto seen. While in D there is present an internal endodermis (either in the form of scattered endodermal cells or of a strand having a crescentic section) not associated with ground-tissue-pith ('internal cortex' of Van Tieghem and Jeffrey), together with an internal ridge or strand of xylem, in the present case there is an internal endodermis enclosing a ground-tissue parenchymatous pith of about twenty cells; we have, in fact, a simple solenostele with a comparatively slight local internal dilatation of the xylem-ring. The first leaf-trace goes off gradually, apparently in the form of a closed cylinder. The pith included within this ring almost certainly communicates with that of the stele, though the connexion was not actually seen. There is no leaf-gap. A group of small tracheids (not spiral) occurs in the middle of the abaxial side of the trace. After the departure of the first trace the internal xylem-dilatation becomes more marked. The second leaf-trace goes off like the first. The connexion of its pith with that of the stele is here indubitable. There is no leaf-gap, but the xylem of the trace is interrupted by the junction of the external with the internal endodermis of the trace, at the point corresponding with the opening in the horseshoe type of trace.

The internal xylem ridge now becomes cut off from the ring by an extension of the internal phloem. It runs past the third node without joining the external xylem. This is the first instance of such a behaviour in this species, a behaviour which is quite exceptional, but which we have found in one case in the relation of the third cylinder to the second in the adult plant [1]. The internal endodermis also extends at the node itself round the internal strand, but immediately afterwards the latter rejoins the external xylem, and at the point of junction tracheids are seen running from the central strand to the point from which the trace has just departed. In the course of the next internode the xylem of the central strand again becomes separated from the external xylem by an extension of the phloem. Soon, however, the junction of the xylem is re-effected, and this is maintained through the fourth node. The fourth leaf-trace goes off similarly to the third. The fifth trace now follows on the other side. It goes off almost perpendicularly to the axis of the stele, is considerably bulkier than the preceding traces, and is the first which makes a definite gap in the stele, so that the ground-tissue of the cortex comes into connexion with that of the pith (Fig. 17). This gap, however, is very short, i. e. it does not extend in front of the base of the leaf-trace (Fig. 18). Immediately after the closure

[1] Seward ('99), p. 186, also records such a case.

of the gap, tracheids can be seen running upwards and forwards from the internal strand to the point in the external xylem-ring from which that wing of the leaf-trace which is turned towards the mid-dorsal line of the stele has just departed, showing that the internal xylem-strand is acting as a 'faisceau réparateur' (Fig. 19).

In the course of the next internode, which is considerably longer than the last, the internal xylem-strand becomes completely separated from the external xylem by phloem, and eventually by endodermis also (Fig. 20). The sixth trace shows a reversion to the earlier type. It departs very slightly to one side of the dorsal line of the stele and makes no leaf-gap. At its base it has an internal endodermis only, which is in connexion with the dorsal part of the endodermis surrounding the internal strand of the stele, the pith of the stele being still confined to the ventral side of the internal strand. The inner wing of the trace (i. e. next the mid-dorsal line) departs as usual slightly earlier than the outer. Immediately after the separation of the trace, the usual connexion is made between the xylem of the internal strand and the outer xylem.

In the course of the next internode the xylem of the internal strand again becomes separated from the outer xylem, in this case by the phloem alone. The seventh trace also departs without making a gap; it has no pith, but an internal endodermal strand only, connected with that of the stele. The seventh internode is like the sixth and the eighth trace like the seventh. Towards the end of the eighth internode the internal strand becomes again completely separated from the stele by endodermis as well as phloem. At the same time a dilatation of the pith extends dorsally and connects with the pith of the ninth trace, which is however insignificant, and confined to its base. The usual connexion of the xylem of the internal strand with the outer xylem occurs just after the departure of the xylem of the trace.

In the course of the ninth internode the xylem of the internal strand again becomes separated from the external xylem, at first by phloem alone. The endodermal separation is barely made before the internal strand again connects with the external xylem, this time *before* the departure of the (tenth) trace. This is the first occasion on which the xylem of the internal strand is directly connected by obliquely running tracheids with the xylem of the leaf-trace (Fig. 21). Just as this xylem-connexion is made two or three phloem-elements appear in the centre of the internal xylem-strand (Fig. 21), and almost immediately become connected laterally with the phloem surrounding the internal strand. This phloem-connexion is widened as the trace departs, so that the internal phloem becomes a branch of the external, *and the whole of the dorsal half of the internal xylem-strand helps to fill the gap in the external xylem left by the departure of the trace*, while the ventral half continues through the tenth internode separated from the

outer xylem. Just before the eleventh trace departs the internal strand of xylem again enters into connexion with the outer, just at the point where the dorsally turned wing of the trace is about to separate and appears to close the xylem-gap, as in the case of the preceding trace. A transverse section of the trace close to its point of origin is represented in Fig. 22.

The twelfth trace is considerably bulkier, and a considerable gap occurs in the side of the stele, placing the pith of the stele in connexion with the cortex. This gap, however, opens before the departure of the trace and is not actually made by the latter, the xylem immediately dorsal to the gap not departing with the trace, but remaining behind in the stele. The xylem of the internal strand also enters into connexion with the external xylem on the opposite side to the gap, but does not directly contribute to the trace. As the second (ventrally turned) wing of the trace is departing, the internal xylem-strand loses its first connexion and makes a new one with the external at the point from which the first (dorsally turned) wing has gone off, the tracheids again running up into the external xylem and taking part in the closure of the xylem-gap (to be distinguished from the *lateral* gap in the stele as a whole, which is quite an exceptional phenomenon).

In the course of the twelfth internode the xylem of the internal strand becomes, as usual, separated from the external by phloem, but for a very short space. It becomes reconnected again considerably before the departure of the thirteenth trace. The stele has not only increased considerably in diameter, but also in xylem-elements in proportion to its bulk, so that the pith is reduced in one place to three or four cells. The latter rapidly increases again, however, at the expense of the internal xylem-strand, so that from a crescentic cross-section it acquires a triangular and then a circular one, the internal xylem-strand completely disappearing. The thirteenth trace now arises from a diametrically opposite position on the stele to that at which the internal strand has been absorbed. The trace goes off as usual with one wing slightly in advance of the other. It has a small pith and is distinctly less bulky than the immediately preceding traces. Its departure makes no gap in the xylem of the stele. This trace then shows a striking reversion to the state of things existing at the proximal end of the plant under description.

As a preliminary to the origin of the fourteenth trace, a new local dilatation arises on the inside of the xylem. The trace departs much like the last and the tracheids run from the new internal strand to the point of departure of its second wing. The tissues now begin to show the same appearance that was noted at the apex of D and the growing point is reached.

The most advanced (F) of the six young plants available (Plate XXXI,

Fig. 7) has at its proximal end a structure practically identical with that seen in the neighbourhood of the tenth trace of E, i. e. an internal xylem-strand enclosing a few phloem-cells in its midst, and separated from the xylem of the stele by phloem, endodermis, and pericycle. The first trace is bulky and its base occupies nearly the whole of the dorsal surface of the stele, but it goes off in the usual way with one wing distinctly in advance of the other. Before either wing has become separated from the stele, however, the xylem of the internal strand comes into connexion with external xylem, and tracheids are seen in abundance running up towards the bases of the xylem-wings of the trace. The trace is distinctly horseshoe-shaped; its pith, directly it becomes free from the stele, coming into connexion with the cortex of the stem, so that the latter is in connexion with the pith of the stele through the pith of the trace. In the course of the next internode the internal strand of phloem comes into connexion with and is absorbed in the external, and at the same time the internal strand becomes shut off from the stele by endodermis. This separation persists considerably longer than in Plant E.

The second trace arises like the first, the xylem of the inner strand again coming into connexion with the outer xylem while the trace is departing. It is again distinctly horseshoe-shaped in section, and maintains this shape in the petiole. During the origin of the trace a new strand of phloem arises in the centre of the xylem of the internal strand, but dies out again very shortly.

There is no need to describe in detail the origin of the whole of the twelve traces of this plant, since they all arise in fundamentally the same way. It is to be noted, however, that as in the advanced traces 10, 11, and 12 of Plant E, the bulkier leaf-traces here take off so much of the dorsal side of the stelar xylem that the internal strand actually fills, or helps to fill, the gap thus made in the external xylem, whereas in the earlier type of origin the gap is closed by the external xylem itself, the internal xylem merely coming into connexion with the external laterally to the point of closure [1].

The complete separation of the internal strand, which we may now call the *second cylinder*, takes place regularly after the departure of the leaf-trace, and is maintained throughout the internode. Pith as well as endodermis often extends right round this second cylinder. The first indication of the origin of a leaf-trace is the thickening of the pith dorsal to the second cylinder, and the correlated pushing out of the xylem-ring on that side; the thickened region of pith now becomes disconnected from the rest on the side towards the mid-dorsal line, causing the second (inner) cylinder to become confluent with the outer one, first by its phloem and then by its

[1] It is to be understood that usually no *actual* xylem-gap is formed in either case, the tracheids of the internal strand moving up *pari passu* with the moving out of the leaf-trace tracheids.

xylem. Soon after this the first arm of the trace (turned towards the mid-dorsal line of the stele) departs, and meanwhile the thickened portion of the pith disconnects with the rest on the outer side and becomes the pith of the leaf-trace, the xylem of the inner cylinder becoming confluent with that of the outer immediately below the base of the outer arm of the trace. This outer arm of the trace now departs and the trace-pith is opened to the cortex of the stem. Shortly afterwards the inner cylinder again separates from the outer.

After the departure of the fourth trace, the strand of phloem in the centre of the second (inner) cylinder becomes constant, and increases in size, being sometimes isolated and sometimes in connexion with the external phloem of the second cylinder. In the course of the seventh internode a strand of endodermal cells appears in the midst of this internal phloem, but disappears again before the departure of the next leaf-trace. The eighth trace is the first in this plant showing a true ' leaf-gap ' in a single transverse section, i. e. the pith of the base of the trace opening to the cortex before it is disconnected from the pith of the stele (Pl. XXXIII, Figs. 27, 28 right). Before the eighth node is passed another endodermal rod appears in the internal phloem, consisting this time of but a single cell in cross-section. It dies out again almost at once. The ninth trace shows a reversion to an earlier type, passing off much more suddenly and showing no gap, while the xylem of the inner cylinder joins that of the outer between the bases of the arms of the trace (Pl. XXXIII, Fig. 28 left). The tenth internode shows a third endodermal rod, which rapidly increases in size, joining the outer endodermis of the inner cylinder, and then disappears again. The eleventh internode shows yet another, which persists through the rest of the stem.

(2) *Starved Intermediate Plants.*

The next advance in complexity we found in certain plants, already alluded to, which were growing in a deeply shaded crevice below an overhanging rock close to the top of Gunong Ledang. The smallest of these scarcely exceeded F in stoutness of rhizome or size and complexity of leaf, though they have longer petioles; while others are considerably larger, though in all respects much smaller than typical vigorous plants growing in the open. They were, no doubt, plants of some age which had been unable to attain anything like their full growth, owing to want of soil and light. Their vascular structure is of interest, since it leads up from that of the six young plants to that of the typical adults.

In the simplest one, which we will call for convenience G, the stelar system of the rhizome is identical with that of the distal part of F, i. e. it consists of an outer solenostele and an inner cylinder, the xylem

of which is sometimes solid and sometimes in the *Lindsaya*-phase, with occasionally an endodermal rod enclosed in the central phloem. The leaf-trace at a short distance from its origin shows in cross-section the form of a flat-topped arch (Fig. 23) with the free edges of the xylem sometimes merely thickened and sometimes with an incurved hook structure, which has been recently shown to be very common in the petioles of Ferns with a comparatively simple vascular structure[1]. The free edges of the arch are approximated, and the cavity of the arch is occupied by ground-tissue in continuity with that of the petiolar cortex by an isthmus passing between the free edges. The whole structure is much like that of *Gleichenia*, § *Mertensia*, and even more closely resembles that of *Loxsoma*[2].

The behaviour of the inner cylinder at the nodes is the same, in essentials, as in the plant F, i. e. it becomes attached to the outer cylinder dorsally towards the end of the node, just before the end of the departure of the wings of the leaf-trace. When the leaf-trace departs symmetrically the inner cylinder sends up a broad column between the bases of the arms, closing the gap in the outer cylinder and at the same time contributing a strand of tracheids to the thickened end of the leaf-trace arm on each side. Immediately after the closure of the gap, the inner cylinder again becomes free, its internal phloem and endodermis (where present) remaining unaffected throughout the node. When the leaf-trace departs to one side of the dorsal line and asymmetrically, i. e. with the dorsal arm leaving before the lateral one, the connexion of the inner cylinder with the outer takes place below the dorsal arm, before the closure of the gap. In some cases the junction only takes place (just as is normally the case in many of the nodes already described in the young plants) after the departure of the leaf-trace is completed. It might be thought that in such a case the function of the inner cylinder was confined to closing the leaf-gap, as it undoubtedly is in many of the nodes of the younger plants, but in this case an actual continuity can be traced between the tracheids coming up from the inner cylinder, backwards through the thickened edges of the gap, and up the edges of the leaf-trace arms. This backwardly running supply of the incurved arms of the trace is, as we shall presently see, much more extensively developed in the more complex types.

The next stage of complexity is to be found in one of the 'starved' plants (H), with larger, stouter leaves, a rhizome of 2 mm. and an outer stele of 1 mm. diameter—a considerably larger plant than G.

The inner cylinder is here solenostelic, or sometimes, after the departure

[1] Cf. Boodle ('01 B), Pl. XXXVIII, Fig. 7; ('01 A), Pl. XX, Fig. 17, Pl. XXI, Fig. 44. Gwynne-Vaughan ('03), Pl. XXXIV, Fig. 25, Pl. XXXV, Fig. 26.

[2] Gwynne-Vaughan ('01).

of the compensation-strand, it forms a gutter, open on the dorsal side, which does not close for some time. The leaf-trace close to its origin is an arch whose ends are curved inwards and then backwards towards the general curve of the arch. The whole thickness of the arch is involved in this curvature, so that the filling of ground tissue extends into the lateral concavities. The cross-section of the trace is very much like that of the solenostelic species of *Gleichenia* (*G. pectinata*) as figured by Boodle [1].

The connexion of the inner cylinder with the outer at the node takes place as before towards the end of the node, just as the extremities of the wings are departing. Tracheids from the inner cylinder supply the whole of the backwardly directed limbs of the leaf-trace, and also of course fill up the dorsal gap in the outer cylinder formed by the departure of the trace. As in the simpler case just described, when the trace is asymmetrical, i. e. turned slightly towards the median dorsal line of the stele, the connexion is first made below that wing (the dorsal one) which goes off first.

(3) *The adult plants.*

We now come to the 'adult' plants, with steles varying from 5·2 to 1·6 mm. in diameter. The term 'adult' may be considered ambiguous in a case like this, where the degree of complication attained varies within such wide limits, and is no doubt wholly determined by external conditions. The term may, however, be fairly applied to plants with leaves and leaf-bundles showing their full complication, i. e. in which the forwardly directed limbs of the lateral loops are present (Seward, '99, Plate XIX, Fig. 31, &c.).

The complication of the vascular system of the rhizome varies, however, from the case in which there are two siphonostelic cylinders only (Seward, '99), through various cases marked by increasing complexity of the third cylinder, to the case in which there are three complete siphonostelic cylinders (Wigglesworth, '02). This increasing complication is pretty strictly correlated with the diameter of the rhizome, which also determines the diameter of the outer cylinder and that of the leaf-trace. In the large leaf-traces the lateral loops are much more extensively developed than in the smaller ones, and it is this last feature no doubt which is connected with the increased complexity of the vascular system of the rhizome.

The 'adult' rhizomes may be divided into two groups. First, those in which the connexion of the second cylinder with the first occurs relatively far back in the node, just below the point at which the backward curl

[1] Boodle ('01 B), Figs. 26, 27.

passes into the forward. In this group the connexion takes place by the more or less sudden passing up of a column of tissue from the second cylinder to this point. Secondly, those in which the connexion takes place further forward, sometimes after the complete departure of the trace. In this group the connexion occurs by the gradual raising of the roof of the second cylinder till its sides come into contact with the bases of the backward curls of the leaf-trace.

The first type of connexion between the rhizome cylinders and the petiolar bundle in 'adult' plants is found, as might be expected, in the smaller dicyclic forms. It is essentially similar to cases G and H. In the simplest case of this group (Stage X) the final 'forward curl' of the leaf-bundle is not continued down to its actual insertion on the rhizome stele, and, as in the last-described case, the second cylinder merely sends up a column of tissue (median, or first on one side, according as the trace is attached symmetrically or asymmetrically) to supply the backwardly directed limbs of the trace and to fill the gap [1]. The tracheids at the outer limits of this column, on each side, sometimes end in a kind of notch (equivalent to the 'gutter' in more advanced forms), which is continuous above with the forward curl of the trace.

In the next case (Stage XI) the forward curl of the leaf-bundle is continued down to the point of attachment, and then the ascending column of tissue from the second cylinder branches on each side into an inner and an outer limb, supplying respectively the backward and the forward curl of the trace (Plate XXXIII, Fig. 29).

The three cases of this group are found in quite small rhizomes, with an outer cylinder of 2·1 mm. average diameter and an inner of ·8 mm.

The second group of cases (Stage XII) represents the great majority of the adult rhizomes. It numbers forty-four nodes, and the outer vascular cylinder has an average diameter of 3·7 mm. A third cylinder occurs in forty-two out of the forty-four cases. The cylinders are much further apart in this group than in the preceding one. Consequently the connexion of the second cylinder, owing to the gradual raising of its roof, with the first, takes place much further forward, often in front of the anterior roots of the leaf-trace; and the tracheal supply from the second cylinder to the leaf-trace is consequently directed sideways (or even backwards) and upwards instead of forwards and upwards. A final elaboration of the leaf-trace connexions must now be mentioned. The bases of the free forward curls of the trace are continued forwards as outwardly turned

[1] The mode of connexion between the second cylinder and the lateral loops of the leaf-trace is decidedly variable in this type. In one case, for instance, the second cylinder sends up a mass of tissue in the form of two arms which pass *back* and join together to form a median bulge in connexion with the lateral loops of the trace; this then passes forward again, still in connexion with the loops, but above the original arms, and finally becomes the compensation-tongue which closes the gap in the outer cylinder.

gutters (hooks in vertical section of the node) attached to the bases of the backward curls. After the backward curls have become continuous with the compensation-tongue (roof of the second cylinder) and the gap is formed in the second cylinder, the outer limbs of these gutters are left attached to the free edges of this gap, and are continued forward for some distance as flanges, ultimately dying away before the gap in the second

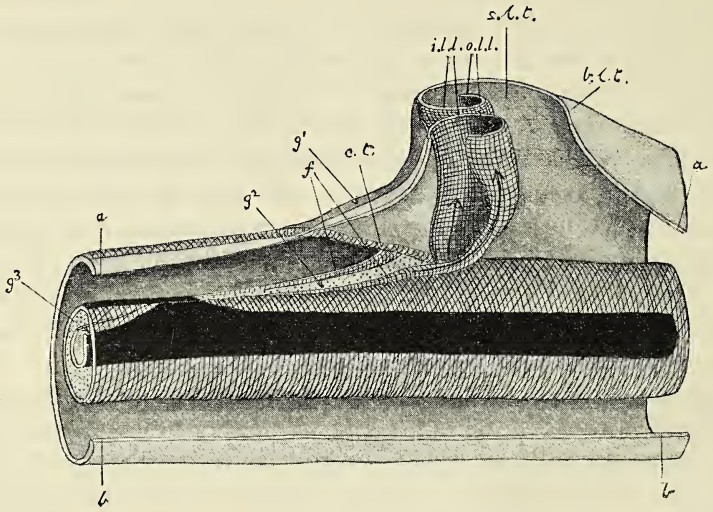


FIG. 11. Side view of vascular system of node of large tricyclic form. The side of the outer (first) cylinder and base of the leaf-trace towards the observer is cut away, exposing the left lateral loop of the leaf-trace. *Outer cylinder* with *uniform shading*. *Middle cylinder* together with lateral loops of leaf-trace, compensation-tongue, and mid-dorsal strip of outer cylinder in front of node, i. e. parts supplied from middle cylinder, *cross-hatched* outside, *dotted* inside. *Inner cylinder* with mid-dorsal strip of middle cylinder in front of node, *black* outside, *white* inside. The middle cylinder is represented as if transparent, so that inner cylinder is seen through it. *a–a*. upper cut edge, *b–b*. lower cut edge of outer cylinder. *b. l. t.* back, *s. l. t.* side of main arch of leaf-trace; *i. l. l.* inner limbs, *o. l. l.* outer limbs of lateral loops of leaf-trace; *f.* flanges on edges of gap in middle cylinder, forming the forward continuations of *o. l. l.*; *c. t.* compensation-tongue passing upwards and forwards from middle cylinder attached to bases of lateral loops, and behind cut edge of outer cylinder, to form mid-dorsal strip of outer cylinder; g^1, g^2, g^3, gaps in outer, middle, and inner cylinders respectively. The arrows indicate the direction of the protoxylem-channels of the left lateral loop.

cylinder is closed (Plate XXXIII, Figs. 30, 31, and Text-Fig. 11). A study of the course of the tracheids shows that they run up from the side of the second stele and *back* along these flanges into the forward curls of the trace, while the existence of continuous protoxylems in these flanges and gutters (p. 508) shows that this water-channel is laid down early, and is presumably well established and important in the economy of this most complicated type of vascular system.

Again, the leaf-gap (i. e. the space between the backward curls of the trace) is considerably wider, and hence the gap left in the second

cylinder is much wider also. The third cylinder is developed in connexion with the closure of this second gap, which takes place well in front of the node[1]. Of forty-two cases in which a third cylinder occurred, only six cases of discontinuity were found. In the first of these cases the third cylinder arises as an internal thickening of the mid-dorsal region of the second cylinder, immediately after the closure of the gap. Traced forwards it soon becomes detached as a protostelic strand which quickly dwindles and dies out in the ground-tissue. A little further forward in the same internode a third cylinder again arises as an internal thickening of the same region, but a little to one side of the mid-dorsal line. It becomes free and remains so to the end of the series. The third case in another rhizome was exactly like the first. In the fourth case, after passing through a node, and coming into connexion with the second cylinder at the closure of its gap, the third strand separated again, dwindled and died out, appearing again after the next node by a dorsal thickening of the second. The fifth and sixth cases were connected with the branching of the rhizome; at the origin of the weaker branch the third cylinder, formed from the branching of that of the parent axis, died out, but shortly afterwards a fresh one arose freely in the pith. In all these cases of discontinuity the third cylinder is protostelic in structure. In nineteen cases the third cylinder remains attached to the second, as an internal ridge, for some distance in front of the connexion. In all of these also the third cylinder is protostelic. So far as they go, the cases just cited tend to support the view that the third cylinder of *Matonia* is in its origin an internal elaboration of the gap formed in the second, that it bears in fact the same relation to the second as the second does to the original stele. The blind ending of the third cylinder is not without parallel in other Ferns possessing internal accessory vascular strands. It occurs, for instance, in the young plants of *Alsophila excelsa* and in *Cyathea*[2].

We found twenty-two cases altogether in which the third cylinder is protostelic in structure, four in which it exhibits, for part of its course at least, the *Lindsaya*-type, and fourteen in which it is solenostelic. Its structure frequently varies in a single rhizome from one of these types to another, the complexity being greatest in the region of the node. Thus in one case the third cylinder is protostelic as far as the node, in the course of which phloem appears in the middle of the xylem. In front of the node its structure becomes solenostelic, and finally, before its junction with the edge of the gap of the second cylinder, it opens and becomes protostelic again, though it has enlarged considerably in the course of these changes. The following table will show the close relation

[1] Seward ('99), p. 187.

[2] Gwynne-Vaughan ('03).

of the complexity of structure of the third cylinder with the diameter of the whole vascular system :—

Structure of Third Cylinder.	*Cases.*	*Horizontal diameter of First Cylinder in mm.*
Third cylinder absent (adult type)	5	1·6 — 2·2 Average 1·9
Third cylinder protostelic alternating with its absence	2	3·0 ,, 3·0
Third cylinder protostelic throughout	19	1·9 — 4·0 ,, 3·1
Third cylinder solenostelic at nodes only (simpler in internodes)	10	3·5 — 5·1 ,, 4·1
Third cylinder solenostelic throughout	11	3·8 — 5·2 ,, 4·6

Summary of the Progressive Complication in the Vascular System of Rhizome and Leaf-Trace.

The following paragraphs summarize the preceding section. The successive complications in the structure of the vascular system are treated as forming a single series with the exceptions noted in the course of the summary; they are illustrated by the accompanying tables (pp. 498–501).

The simplest structure available consists of a slender cylinder of xylem surrounded by pericycle and endodermis, with no characteristic phloem present. The first leaf-trace is a strand of similar structure. The stele of the rhizome now increases considerably in diameter and in number of elements, and a well-marked strand of phloem containing sieve-tubes and parenchyma appears in its midst. Immediately afterwards external phloem appears at several points on the periphery of the xylem, and is connected with the phloem of the first roots, which arise in this region. The second leaf-trace consists of a slender strand of xylem surrounded by a thin layer of phloem, which is contributed to by both the internal and the external phloem of the stele. The internal phloem now increases considerably in bulk, and there arise in it a few large isolated endodermal cells. The third leaf-trace is an arc of xylem covered with a layer of phloem, of which that on the abaxial and lateral faces is continuous with the external, and that on the adaxial concavity with the internal phloem of the stele, while the adaxial endodermis of the trace is continuous with the internal endodermal cells of the stele. An internal ridge of the xylem projects into the internal phloem of the stele on one side of the base of the third trace. The internal endodermal cells eventually join to form a continuous endodermal strand. At subsequent nodes the internal ridge of xylem again appears, and at length a node is reached in which the ridge is continued forward in front of the departure of the trace for a short distance, and then becomes free in the internal

phloem. Traced forward towards the next node it becomes gradually thinner, but eventually connects with the xylem-dilatation of the next node.

In subsequent internodes there may be a free internal strand of xylem, which is relatively bulky and centrally situated, while the internal endodermis, at first still occasionally represented merely by isolated cells, comes to have the form of a strand, crescentic in cross-section and one or two cells thick, on one side of the internal xylem-strand. The leaf-traces are now concentric in structure and kidney-shaped in cross-section. The xylem consists of a central strand of narrow tracheids (probably representing the protoxylem) and two wings of wider scalariform tracheids. The free internal xylem-strand enters into connexion with the external xylem-ring in the mid-dorsal line, in the neighbourhood of the node, sometimes before, sometimes during, and sometimes just after the departure of the leaf-trace, and may remain in connexion with it for the greater part (rarely the whole) of the internode. Where a 'xylem-gap' is formed by the departure of the trace, this is not closed by the internal strand, but by the approximation of the free edges of the external xylem. Tracheids from the internal strand never, at this stage, contribute to the leaf-trace.

The stele increases in diameter and a pith appears, enclosed within the internal endodermis, and consisting of cells like those of the inner cortex. The leaf-traces now often have the form of a closed ring, enclosing a pith which is sometimes, but not always, connected with the pith of the stele at the node. On the adaxial side of the trace the xylem of the ring is not continuous, the space between the free xylem-edges being occupied by phloem or pericycle, or by infolded endodermis.

The internal xylem-strand still varies in its behaviour (in one case running freely past the node without making any connexion with the external xylem), but it is now normally, though by no means invariably, separated from the external xylem during the greater part of the internode, either by phloem only or also by endodermis, and sometimes makes its connexion only at the very end of the node. Tracheids often run from the internal strand to the point in the external xylem from which the last tracheids of the trace depart, but do not contribute to the trace itself.

True leaf-gaps now appear for the first time in connexion with traces, having a horseshoe-shaped cross-section, but they are very short, not extending in front of the actual base of the trace [1].

Phloem extends from one side into the middle of the internal xylem-strand, and the dorsal half of the strand, above this phloem, frequently moves up and actually helps to close the xylem leaf-gap. Further on another internal phloem-strand appears, and this internal phloem shortly becomes

[1] Reversions to earlier types of trace without leaf-gaps are common at this stage.

TABLE SHOWING THE DIFFERENT STAGES OF PROGRESSIVE COMPLICATION IN THE RHIZOME AND LEAF-TRACE.

(1) *The young plants.*

Stage.	*Plants in which it occurs.*	*Rhizome.*		*Leaf-trace.*
		Internode.	*Node.*	
I.	A, node 1.	Protostelic structure; no distinguishable phloem.	As in internode.	Simple, like stele of stem.
II.	A, node 2.	*Lindsaya*-type.	Internal and external phloem in continuity.	'Concentric'; phloem mainly adaxial.
III.	A, node 3. B.	*Lindsaya*-type, with scattered endodermal cells in internal phloem.	As in II, with local thickening of edge of 'xylem-gap.'	'Concentric'; gutter-shaped strand.
IV.	D.	*Lindsaya*-type, with internal (often scattered) endodermis, and free internal xylem-strand.	Internal xylem-strand joining external xylem.	'Concentric'; kidney-shaped strand.
V.	E, nodes 1, 2.	Solenostele with local internal dilatation.	As in internode.	Closed ring in cross-section, with sclerized pith.
VI.	E, nodes 3–9, and 13–14.	Solenostele with internal xylem-strand often free (separated from external xylem by phloem and also often by endodermis) = *second cylinder*.	Internal strand in connexion with external xylem at node. True leaf-gap sometimes present (diagram).	Variable in structure. Sometimes a closed ring with sclerized pith, with or without internal endodermis, and xylem interrupted on adaxial side; sometimes crescentic in section.
VII.	E, nodes 10–12. F (10 nodes).	As in VI, but with local internal phloem and sometimes endodermis in second cylinder.	True leaf-gap constant; dorsal half of second cylinder often fills gap caused by departure of trace (diagram).	Horseshoe-shaped in cross-section.

DIAGRAMS SHOWING THE DIFFERENT STAGES OF PROGRESSIVE COMPLICATION BY TRANSVERSE SECTIONS OF CORRESPONDING NODES, INTERNODES, AND LEAF-TRACES. [The diagrams represent actual sections × 45.]

(1) *The young plants.*

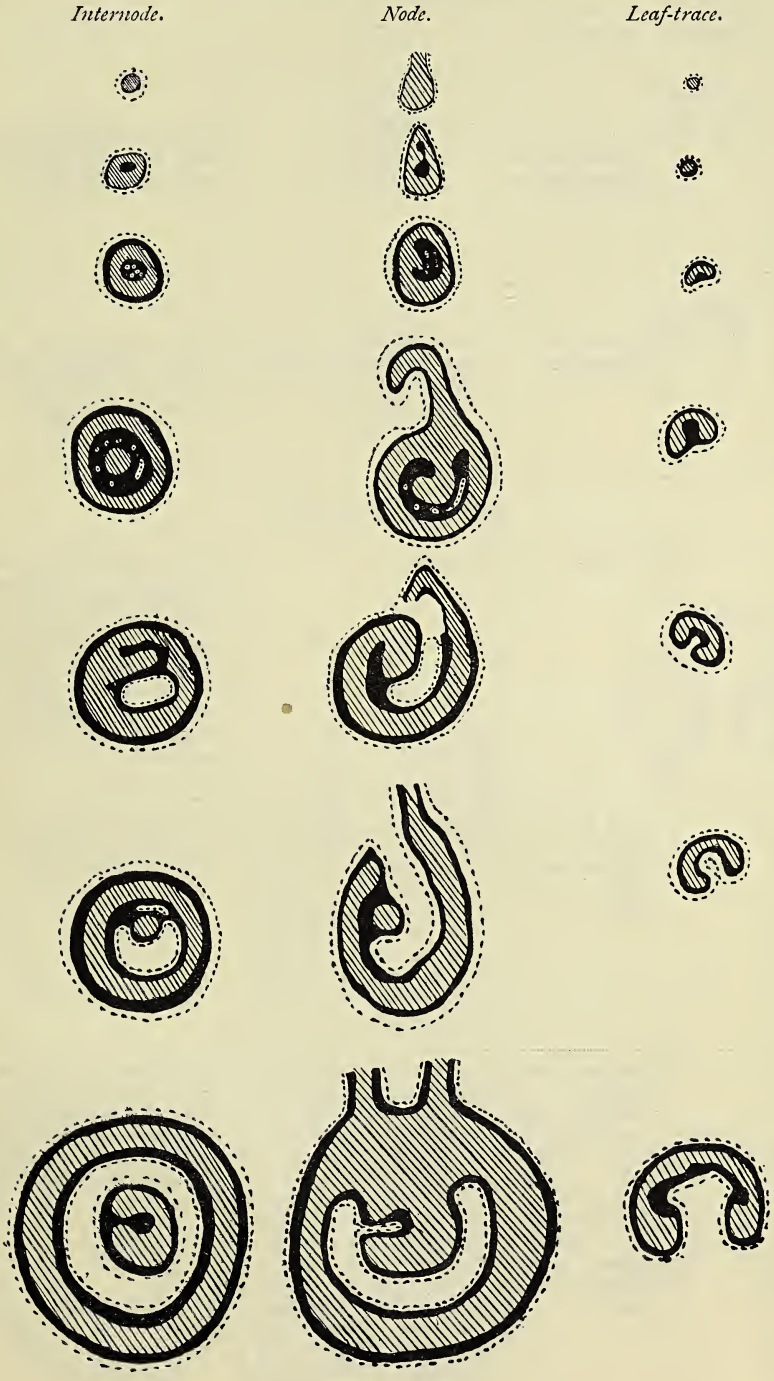


FIG. 12. *Xylem* diagonally lined, *Phloem* black, *Endodermis* dotted.

TABLE SHOWING THE DIFFERENT STAGES OF PROGRESSIVE COMPLICATION IN THE RHIZOME AND LEAF-TRACE (*continued*).

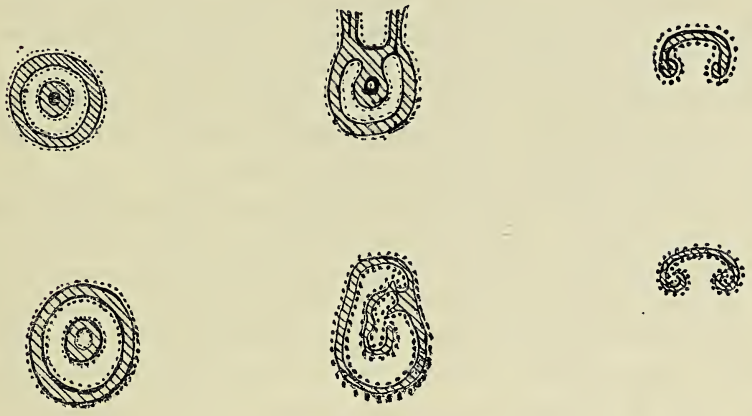
(2) *Starved intermediate plants.*

Stage.	*Plants in which it occurs.*	*Rhizome.*		*Leaf-trace.*
		Internode.	*Node.*	
VIII.	G.	As in VII: but internal phloem of second cylinder constant.	As in VII.	Horseshoe-shaped in section, with incurved abaxially directed xylem-hooks.
IX.	H.	Second cylinder solenostelic.	Second cylinder filling gap made in first and entirely supplying the lateral loops of the trace.	Horseshoe-shaped in section, with incurved abaxially (backwardly) directed limbs forming the *lateral loops*.
(3) '*Adult*' *plants.*				
X.		As in IX.	As in IX.	Adult type; i. e. horseshoe-shaped section with incurved abaxially (backwardly) directed limbs continued by again incurved adaxially (forwardly) directed ones, so that the lateral loops are spirally twisted on the sides of the primitive arch. Adaxially directed limbs not continued down to actual insertion of trace.
XI.		As in X, with sometimes a third protostelic cylinder (diagram).	Supply of lateral loops of trace (arising from second cylinder) branches on each side into two; an inner division which supplies the abaxially directed limb, and an outer division which supplies the adaxially directed limb of the lateral loop.	Adult type; adaxially directed limbs continued down to the insertion of the trace.
XII.		Three concentric cylinders of which the third (innermost) is either protostelic (with solid xylem), of *Lindsaya*-type (with internal phloem), or solenostelic (diagram).	The nodal connexion of the second cylinder with the first takes place far forward in the node (often in front of the area of insertion of the trace), by the gradual raising of the roof of the second cylinder till it comes in contact with lateral loops of the trace or their continuations forward (diagram). The third cylinder joins the second in front of the node and closes the gap formed in the roof of the second.	Adult type; the ultimate adaxially directed limbs of the lateral loops continued downwards and forwards as flanges attached to the edges of the gap in the second cylinder.

DIAGRAMS SHOWING THE DIFFERENT STAGES OF PROGRESSIVE COMPLICATION BY TRANSVERSE SECTIONS OF CORRESPONDING NODES, INTERNODES, AND LEAF-TRACES (*continued*). [The diagrams represent actual sections x 15.]

(2) *Starved intermediate plants.*

Internode. *Node.* *Leaf-trace.*



(3) '*Adult*' *plants.*

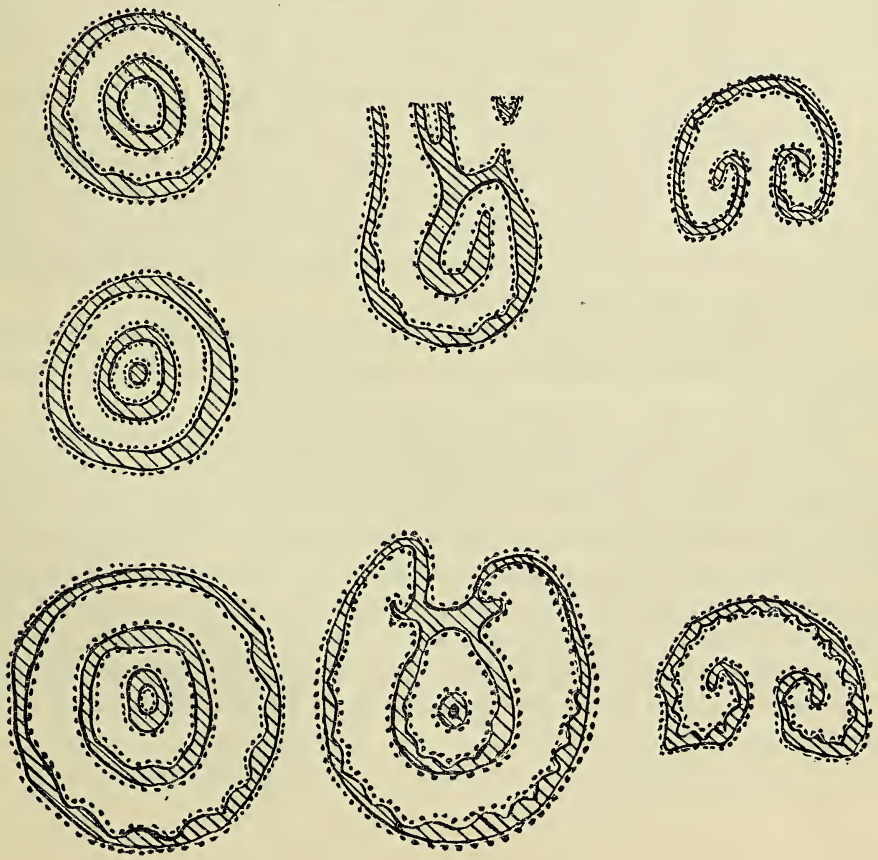


FIG. 13. *Xylem* diagonally lined, *Phloem* black, *Endodermis* dotted.

a constant strand in the centre of the second cylinder. After a time an endodermal rod appears in the central phloem and dies out again ; then a second, which likewise disappears, and so on. The stele is now much greater in diameter, and the space between its dorsal side and the internal cylinder considerably more. At the node the latter therefore sends up a distinct obliquely or vertically running column of tissue, which now invariably closes the gap and contributes a strand of tracheids to each of the free edges of the leaf-trace, which is horseshoe-shaped in section with an incurved hook of xylem on each side, or with actual incurved backwardly directed limbs. In the last case the tracheids running up from the inner cylinder supply the whole of these limbs.

The adult type of trace, with its free edges again incurved and running forward, is now reached. At first this structure is only found at a short distance above the actual point of insertion of the trace and does not reach down to its base. In more advanced nodes, however, the forward curls do reach to the base and the ascending column of tissue from the second cylinder branches on each side into an inner and an outer limb, which supply the backward and the forward curl respectively. In the largest and most advanced nodes the diameter of the second cylinder has increased very considerably and the connexion with the outer cylinder and the base of the leaf-trace is made, not by the sending up of a column of tissue, but by the gradual raising and flattening of the roof of the second cylinder, till, as a broad almost horizontally running plate, its sides join the bases of the backwardly directed limbs of the trace. These last are often extended forward, so that the junction is effected at a point considerably in front of the last section in the transverse series showing the departure of the front of the trace itself, a state of things which carries us back to the condition in which the simple internal xylem-strand only joined the external xylem after the departure of the trace.

The forward curls of the trace are, in the largest forms, continued forward as flanges attached to the edges of the gap in the second cylinder.

The third cylinder, which may vary, according to the diameter of the whole vascular system, from a solid protostelic condition through the *Lindsaya*-type to a solenostelic condition, probably first appeared as a local internal thickening of the gap in the second cylinder. With the exception of one case, we have always found it attached to the second cylinder at or near the point of closure of this gap, which in the vascular systems of the largest rhizomes is, on an average, about 8 mm. in front of the anterior roots of the leaf-trace, while in the absence of the third cylinder the gap in the second closes at an average distance of 1·6 mm. in front of these anterior roots.

Protoxylems.

The question of the morphological value of protoxylems is one of considerable interest. There is no doubt that in many cases at least the centres of differentiation of the xylem (Bertrand's *pôles trachéens*) are of morphological importance, affording a kind of basis, or skeleton, on which the whole xylem-system is built up. Not only is this clearly the case in the roots of most vascular plants, where the position and often the number of protoxylems within considerable circles of affinity is very constant, but it is certainly also the case in the vascular strands of the shoots of many groups. We need only refer to the universal exarchy of the steles of Sphenophyllales and Lycopodiales, and to the endarchy of the Angiosperms, or to Scott's important demonstration of the gradual transition from exarchy to mesarchy in the leaf-trace, and to endarchy in the stele, of the great Cycadofilicinean series, in order to emphasize the point.

In the Filicales proper the case is rather different. The exact position of the spiral protoxylems in relation to the metaxylem of the vascular strands of the stem is decidedly variable. Exarchy, endarchy, and mesarchy are all found within comparatively small groups, and the actual course of evolution seems to affect the position of the protoxylems much more freely and rapidly than in the other great groups of vascular plants. Furthermore, as the researches of Boodle showed in Hymenophyllaceae [1] and Schizaeaceae [2] and those of Gwynne-Vaughan in *Loxsoma* [3] and various Davalliaceae [4], the spiral protoxylems of many Ferns are confined to the leaf and are absent from the stem altogether. This phenomenon, as Boodle showed, is connected with the slow growth in length of the stems of the Ferns in question, and in *Trichomanes* and *Dicksonia* at least we get the presence and absence of spiral protoxylem in the stem in different species of the same genus. It is in fact quite likely, though this has never been definitely established, that spiral protoxylem is not formed except where definite growth in length occurs after the differentiation of the first formed tracheal strands. Moreover, spiral protoxylems in the stems of Ferns are always, according to Gwynne-Vaughan [5], and so far as his observations extend, continuous with those of the petiole. In those cases in which there is no spiral protoxylem in the stem there may be a localized non-spiral protoxylem-band round the stele, which is exarch (*Loxsoma*, various Davallias, &c.) or endarch (*Schizaea malaccana* [6]), but which has no connexion with the leaf protoxylem; or the differentiation of tracheids may be more or less irregular (*Gymnogramme*, *Lindsaya*, &c.). In *Dicksonia* we have in the stems of different species the three cases—endarch or

[1] Boodle ('00). [2] Boodle ('01 A). [3] Gwynne-Vaughan ('01), p. 79, and ('03), p. 727.
[4] Gwynne-Vaughan ('03), p. 728. [5] l. c. ('03). [6] Tansley and Chick ('03), p. 508.

mesarch spiral protoxylem continuous with that of the leaf (*D. rubiginosa*, *davallioides*, *adiantoides*), exarch non-spiral protoxylem (*D. apiifolia*), and irregularity of xylem differentiation (*D. Barometz*) [1]. These facts, taken in conjunction with those obtaining in *Trichomanes* [2], are sufficient to show that the existence of spiral protoxylem and even of localized protoxylem in any form is a highly variable phenomenon as between species and species in certain genera of Ferns, in striking contrast to its constancy in many other vascular plants. It is highly probable that a careful developmental study of the relations would generally reveal specific adaptation.

In *Matonia pectinata*, the distribution of the spiral protoxylems is of some interest, and so far as it goes certainly tends to show that the development of these structures is strictly dependent on the elongation of the stem after the beginning of tracheal differentiation.

In none of the six young plants A to F, nor in the weaker starved plant (type G), can spiral protoxylem be detected in rhizome or petiole. We cannot state that no localized protoxylem of any kind occurs, because we have not succeeded in finding a good case of partly differentiated xylem in stele or leaf-trace. Apical growth appears to be very slow, and the complete differentiation of the vascular tissues extends to within a very short distance of the growing point. In the type of leaf-trace characteristic of stages IV to VI (see table, p. 498) the xylem usually consists of a central (abaxial) strand of narrow tracheids and two lateral wings of wider tracheids (Pl. XXXII, Fig. 22). The central strand is in some cases very clearly distinct from the wings. It seems probable that this central strand may be formed before the wings, and thus represent protoxylem, but we have not succeeded in establishing spiral elements; in most cases the narrow tracheids are certainly scalariform. As the arch of the trace becomes broader (VII and VIII) the tracheids tend to become grouped in a series of curved bands as in the adult type, and small groups of narrow tracheids are distinguishable between the bands (Fig. 23), i.e. in the position of protoxylems in the adult; but these are not constant.

In stage IX (H), however, a plant which, it will be remembered, had a much stouter rhizome and much longer petioles than any of the simpler ones, we meet with undoubted spiral protoxylems, both in the rhizome and petiole. The stelar system here consists of a solenostele, with an internal accessory solenostelic cylinder (Text-Fig. 14, A) supplying the lateral loops of the leaf-trace. The external cylinder has its xylem arranged in not very well defined arcs, like those of the adult (Seward ('99), Plate XVII, Figs. 8 and 9), with unmistakable spiral protoxylems (eight or nine in all) at their points of junction. On the ventral side these arcs are longer and broader than on the dorsal, so that the cylinder of xylem is thinner and has more protoxylems on the dorsal side, which

[1] Gwynne-Vaughan ('03), p. 727.

[2] Boodle ('00).

is destined to form the arch of the leaf-trace. The internal cylinder has one distinct spiral protoxylem a little to one side of the mid-dorsal line. As the leaf-trace goes off five of the dorsal protoxylems enter the arch of the outer cylinder (by the time the trace is separated this number has been increased to seven by branching), while the single protoxylem of the inner cylinder takes up a median dorsal position, and runs up embedded in the tongue of xylem, which becomes separated

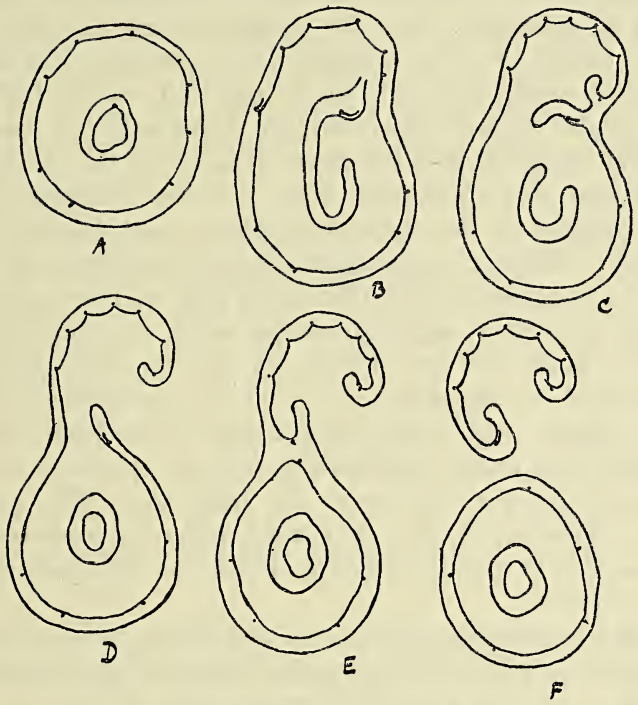


FIG. 14. Diagrams of a series of transverse sections through the node of a dicyclic rhizome (Plant H), showing the course of the protoxylems. *A*. Internode; nine protoxylems in outer cylinder (five dorsal and four ventral) and one dorsal protoxylem in inner. *B*. Beginning of node; dorsal protoxylem of inner cylinder now in compensation-tongue sending off a branch to the right to run into right lateral loop of leaf-trace. *C*. Protoxylem of right lateral loop of leaf-trace separate. *D*. Dorsal protoxylem derived from second cylinder (now running in outer cylinder) branching to the left to run into left lateral loop. *E*. Protoxylem of left lateral loop separate. *F*. Leaf-trace detached from stele. Protoxylem of left lateral loop branching. Five protoxylems in outer cylinder, of which the dorsal one is derived, with the compensation-tongue, from the inner cylinder; the four ventral are as they were before the node was reached.

from the inner cylinder to form the lateral loops of the trace and close the gap in the outer cylinder. On the formation of the first lateral loop (the trace departs asymmetrically) the protoxylem branches, sending off a strand of spiral elements which enters the loop at its lowest point (Text-Fig. 14, B and C). After the detachment of the first loop the protoxylem sends a branch into the loop on the other side, itself continuing forward in the remainder of the tongue, which has now become

part of the outer stele (Text-Fig. 14, D–F). The internal cylinder, which has thus lost its dorsal part and become gutter-shaped, is now without a protoxylem, but it soon closes up and another protoxylem appears in its dorsal region. The behaviour at the next two nodes is apparently similar.

In the smaller rhizomes showing the 'adult' type of leaf-trace (stages X and XI) the protoxylems are often less developed than in stage IX. In some cases, indeed, we have found it impossible to detect spiral protoxylems in connexion with the second (inner) cylinder at all. In other cases strands of spiral elements are sometimes confined to the leaf-trace itself, where they are to be detected on the insides of the two limbs of the lateral loops, but are apparently not continued, at least as spiral elements, into the inner cylinder of the rhizome. Here we may suppose that elongation, after the first formation of xylem-elements, is confined to the base of the petiole, and does not affect the rhizome itself. In other cases, again, these protoxylems are continued downwards and backwards into the dorsal part of the second cylinder, either joining to form a single strand, as in H, or remaining apart as two distinct strands, one on either side of the mid-dorsal line. A protoxylem-strand is sometimes found in the compensation-tongue of the second cylinder, which fills up the gap in the outer cylinder, but this, in the cases now under consideration, does not appear to be connected with the protoxylems which enter the leaf-trace. The protoxylems of the outer cylinder, on the other hand, are more numerous than in H, and a number of those occupying the dorsal side pass up into the arch of the leaf-trace.

Stage XII represents the largest and most complicated type of vascular system, in which the connexion between the second cylinder and the outer one takes place far forward, often in front of the actual departure of the trace. Here the protoxylem-system of the dorsal region of the second cylinder and of the gutters which continue forward the lateral loops of the trace is much more developed, in correlation with the much more considerable development and longitudinal extension of these structures, which form the apparatus of connexion between the trace and the second cylinder.

In the outer cylinder of this stage as many as eighteen protoxylems occur in a large rhizome. These are arranged closer together in the dorsal than in the ventral portion of the stele (Text-Fig. 15, A). A number of the dorsal protoxylems pass off with the main arch of the leaf-trace. The second cylinder may contain as many as nine protoxylems, though it often has only one or two, even in quite large forms. Of these there is always either one occupying the mid-dorsal line or a little to one side of it, or there are two, one on each side of the dorsal line.

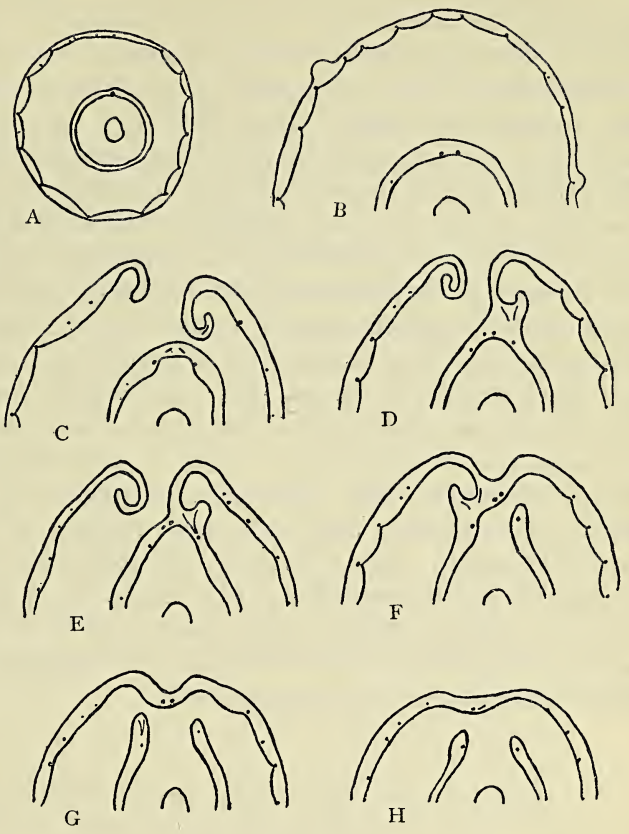
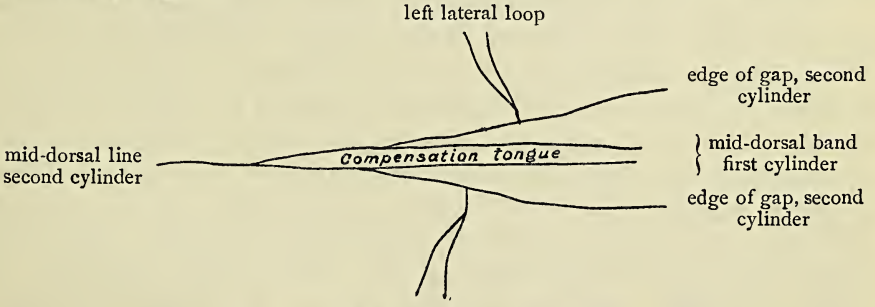


FIG. 15. Diagrams of a series of transverse sections through the node of a tricyclic rhizome. *A*. Internode shortly before node; dorsal protoxylems dividing up preparatory to formation of leaf-trace. Second cylinder with one dorsal protoxylem. Third with none. *B*. Second cylinder with three protoxylems. *C*. Two dorsal protoxylems of second cylinder have divided to form four. Protoxylem of right lateral loop branching. *D*. Second cylinder in connexion with right lateral loop. Two protoxylems in lateral loop. *E*. The two protoxylems of right lateral loop in connexion with a branch from right outer protoxylem of dorsal group of second cylinder. *F*. Two central protoxylems of dorsal group of second cylinder now in compensation-tongue, which has filled gap in outer cylinder. Right outer protoxylem remains in edge of gap of second cylinder. Two protoxylems in left lateral loop of leaf-trace. *G*. The two protoxylems of left lateral loop in connexion with a branch from left outer protoxylem originally derived from dorsal group of second cylinder (now in edge of gap of second cylinder). Two median dorsal protoxylems of this group seen in median dorsal position in outer cylinder. *H*. Protoxylems of left lateral loop have joined protoxylem in edge of gap.



J. Plan of protoxylems seen from dorsal side.

N.B. These figures are taken from a series through a very young node in which the protoxylems are the only parts of the vascular tissue differentiated. The main arch of the leaf-trace, which would be attached between *B* and *C*, is not yet laid down, the forward extensions of the lateral loops (seen in *C* to *F*) being the only part of the attachment of the trace to the stele as yet formed. The protoxylems of the left lateral loop (that nearest the apex of the stem and therefore the youngest) are not yet formed in *C* to *E*, but only in *F*, where the loop is in immediate connexion with the upstanding flange on the edge of the gap of the second cylinder.

In the case which we have been able to trace out most fully (Text-Fig. 15), the behaviour of the dorsal protoxylem of the second cylinder is as follows. The single dorsal protoxylem-strand of the second cylinder branches into two, right and left (Text-Fig. 15, B). Each of these then again divides into a dorsal and ventral branch (Text-Fig. 15, C). The dorsal branches continue straight forward in the compensation-tongue, which eventually fills up the gap in the outer stele (D–G), while the ventral branches each send off a strand which runs straight up and divides into two, an outer and an inner division, of which the former enters the 'gutter,' i. e. the continuation forwards of the final curl (outer limb) of the lateral loop of the leaf-trace, while the latter enters the inner limb (D and E, F and G). From this point these two last-mentioned strands on each side run backwards and upwards into the trace, while the original ventral strands run on forwards in the free edges of the gap in the second cylinder (F, G, H). A comparison of other cases shows an essentially similar condition of things. Sometimes the protoxylems supplying the lateral loops are in direct connexion, not only with those running in the free edges of the gap of the second cylinder, but also with those belonging to the compensation-strand which closes the gap in the outer cylinder. In any case the important point is that the protoxylem as well as the metaxylem supply of the lateral loops of the leaf-trace runs *backwards* and upwards from xylem belonging to the second cylinder.

The Nature and Phylogenetic Relations of the Vascular System in Matonia.

Morphological Position and Origin of the Matonia-type.

The first general result obtainable from the foregoing observations is the apparently definite solution of the problem of the nature and origin of the internal vascular cylinders in the rhizome of the adult *Matonia pectinata*, and that in the sense already indicated by Gwynne-Vaughan [1]. On the basis of a series of cases represented by *Dicksonia apiifolia*, *D. adiantoides*, *D. rubiginosa*, and *Pteris elata*, var. *Karsteniana*, Gwynne-Vaughan concludes that the internal vascular cylinder in the last-named plant has arisen as the result of progressive elaboration from a local thickening of the margin of the leaf-gap of the original solenostele. He further concludes that the vascular system of *Matonia pectinata* is 'essentially similar.' The detailed account we have given of the origin of the internal cylinders in the young plants of *Matonia* and in the weak

[1] Gwynne-Vaughan ('03), pp. 703–5. The suggestions of Seward ('99, p. 180) and Boodle ('01 B, p. 739) as to the splitting off of an internal ring from the original solenostele must be dismissed as untenable.

rhizomes of relatively starved plants can leave no doubt whatever of the correctness of this view. Indeed, no more complete illustration of Gwynne-Vaughan's hypothesis could be given than that afforded by the origin of the first internal accessory cylinder from the original stele. Of the two alternative suggested methods, however, by which a solid internal strand might be converted into a closed hollow cylinder (the enlarging, curving round and meeting of the edges, or the branching and subsequent fusing of the branches into a cylinder [1]), neither will hold for *Matonia*.

In the case of all three cylinders, i. e. the original stele, and the two internal accessory cylinders, the solenostelic structure is arrived at by means of the development first of internal phloem within the xylem, then of internal endodermis within this phloem, and finally of internal 'extra-stelar' parenchyma or pith within this endodermis. In other words, the cylinders pass through the *Lindsaya*-stage on their way from protostelic to solenostelic structure.

The appearance of the *Lindsaya*-phase in the 'ontogeny' of all three cylinders of *Matonia* adds another to the growing series of cases in which this type precedes the solenostele in the development of the individual Fern-stem [2], and serves to strengthen the hypothesis we put forward in a previous note [3], that the *Lindsaya*-type is also the phylogenetic precursor of the solenostele. This hypothesis has been strongly supported by Gwynne-Vaughan, who has brought forward much new evidence in its favour [4], and we have now little doubt that it may be taken as of general application.

There is such a strong *prima facie* case for holding that the stages of vascular structure met with in passing up the stem of a Fern which has attained a certain degree of complication represent, in a general way, the phylogenetic stages through which the adult stem has passed in arriving at its present structure, that we need have no hesitation in interpreting the one in the light of the other, but we must remember that it does not follow that this parallelism will be complete in all its details. In the present case, for instance, the local internal thickening of the xylem in the region of the leaf-gap, which is the precursor of the formation of internal cylinders, appears at an extremely early stage in the third node of the youngest plant we found, when the leaf-trace consists of a very slightly curved arc of tissue. At a very slightly more advanced stage the internal ridge of xylem actually becomes free from the external, and runs for some distance isolated in the internal phloem. The beginning of the formation of an internal endodermis is seen at an even earlier stage. Whatever may be

[1] Gwynne-Vaughan ('03), p. 704.

[2] According to Chandler (New Phytologist, vol. iii, p. 123), the *Lindsaya*-stage is general, preceding the solenostelic stage, in the bases of the young stems of many species of Ferns he has investigated, mostly dictyostelic forms. This is quite borne out by Chandler's full paper in the last issue of this journal (Ann. of Bot., xix, p. 365).

[3] Tansley and Lulham ('02).

[4] Gwynne-Vaughan ('03).

the causes which led to the formation of an internal endodermis and internal accessory vascular cylinders, there can be no doubt that they did not arise at a correspondingly early stage in phylogeny to that in which they are seen in the young *Matonia*. We must suppose that in accordance with Darwin's well-known principle, this character has been inherited at an earlier and earlier stage of development, till it has appeared in the young plant at a very early period indeed.

Judged by the comparative method there is a distinct correlation between the type of leaf-trace and the type of stele with which it is associated. In general terms we may say that a compact circular, oval, or kidney-shaped trace is associated with the protostelic type (*Gleichenia*, *Lygodium*, Hymenophyllaceae), while the passage from the protostelic to the solenostelic type is marked by a broadening and opening out of the trace into the arch-shaped form (*Lindsaya*, *Davallia repens*, *D. pinnata*, *D. aculeata*). It is only after the definite establishment of this last type that we begin in some cases to get the development of accessory strands within the solenostele, and the further evolution of these is associated with a further elaboration of the leaf-trace (*Dicksonia rubiginosa*, *Pteris elata*, var. *Karsteniana*, *Pteris aquilina*, &c.). It is obvious that in the ontogeny of *Matonia pectinata* the appearance of internal accessory strands is much in advance of corresponding elaboration in the leaf-trace.

Functional Relations of the Vascular System of Matonia in connexion with its Evolution.

In order to attempt to understand the factors which have led to the evolution of the *Matonia*-type of vascular system, we must endeavour to realize the conditions existing at each stage of the progressive complication described, which, we may now assume, took place along the lines indicated by Gwynne-Vaughan's *Dicksonia*-series, and confirmed by the history of development in *Matonia* itself.

Starting with a hypothetical solenostelic ancestor and the arch-shaped type of leaf-trace, we have to consider first of all the cause of the origin of the local thickening of the xylem of the leaf-gap, such for instance as Gwynne-Vaughan has described in *Dicksonia apiifolia*, &c. This is clearly of the nature of a reinforcement of the water-conducting tissue at a point in the xylem-cylinder immediately beyond that at which the drain of water consequent on the diversion of the transpiration-current up the leaf-trace is first felt. It may be supposed that the additional tracheids provided serve as an addition to the water-channel supplying the tracheids of the stele beyond the node, which have their supply diminished by the relatively bulky leaf-trace. This development of an additional xylem in connexion with the leaf-gap assumes greater proportions in *Dicksonia adiantoides*,

where the ridge is continued throughout the internodes, and the xylem is separated from the xylem of the stele except at the nodes, and greater still in *D. rubiginosa*, where, according to Gwynne-Vaughan, it takes the form of from one to three separate strands, which only come into connexion with the stele at the leaf-gap. Here we may suppose that three separate internal xylem-strands serve as a reservoir of water to supply the tracheids of the stele, when they are depleted, during active transpiration, by the diversion of the current passing along the stele. This view is supported by the fact that the xylem of the accessory internal strands is in connexion with the xylem of the stele only at the point where the supply of water, to compensate for that diverted to the leaf-trace, is required. This is simply the physiological expression of the view that these internal accessory strands correspond to some extent with compensation-strands (Ersatzstränge, faisceaux réparateurs) serving to 'compensate' the vascular system of the stem for the loss caused by the departure of the leaf-trace. This idea also finds support in the phenomena seen in the young plants of *Matonia*, as detailed on pp. 486–8, and summarized on p. 497, in which the internal accessory strand either supplies tracheids to the xylem of the stele at the point from which the forward wing of the leaf-trace has departed, or sends up its whole dorsal half to fill the gap in the xylem made by the departure of the trace. It may be pointed out that the internal tracheal reservoirs have here no independent connexion with the roots, and therefore presumably no independent water-supply. We must suppose that they are filled from the xylem of the stele, when the water-supply is in excess of the needs of transpiration, and the pressure in the tracheal system consequently increased; depleted again when transpiration is active, and the pressure in the tracheids in the neighbourhood of the base of the leaf-trace consequently lessened. There seems no objection on physical grounds to such an assumption of reversible action, and no other way of giving a functional meaning to the internal accessory strands.

The tracheal system thus originating cannot, however, to speak metaphorically, long resist being drawn into supplying the leaf-trace itself. At least such a diversion obtains in the young *Matonia*, in *Dipteris conjugata*, where a small part of the conspicuous thickening of the leaf-gap edge forms the incurved hook of the leaf-trace [1], and it has probably occurred extensively in many of the advanced polycyclic Ferns [2], though it is not mentioned by Gwynne-Vaughan as existing in his *Dicksonia*-series. In *Gleichenia pectinata*, on the other hand, the thickening of the leaf-gap edge appears from Boodle's figures and descriptions [3] to be *mainly* a decurrent extension

[1] Seward and Dale ('01), Pl. XLVII, Fig. 4.

[2] e.g. *Pteris aquilina*; see Tansley and Lulham ('04). It is possible that in some cases the development of the internal accessory system may be exclusively related to elaboration of the leaf-trace.

[3] Boodle ('01), Pl. XXXIX, Figs. 26, 27.

of the incurved edges of the trace[1]. In *Matonia* the case seems to be that as the leaf-trace increases in size, a number of tracheids from the internal strand run up into the wings of the leaf-trace, as these are departing from the stele. This diversion does not, however, interfere with what appears to be the original function of the internal strand, namely the compensation of the external xylem-cylinder, a compensation which from this time forward takes the form of an actual filling of the leaf-gap by the dorsal xylem of the internal strand (pp. 488–9). The free edges of the leaf-trace at this stage are distinctly thickened, and their further progressive elaboration into the complicated lateral loops characteristic of the adult type goes hand in hand with the progressive elaboration of the internal cylinder, the whole of both limbs of the lateral loop on each side being supplied from this cylinder, which itself becomes solenostelic and increases greatly in size, while its roof regularly closes the leaf-gap of the outer cylinder.

The second cylinder now finds itself in the position of the original stele at an earlier stage of development. Not only has the whole of its roof gone to supply the lateral loops of the trace, and to fill the gap in the outer cylinder, but the tracheids occupying the edges of the gap made by this departure are in actual continuity with those of the free edges of the trace, the water-supply here running *backwards* from the second cylinder to the trace. This channel is established at an early period of development, as is shown by the continuity of protoxylems between the flanges of the cylinder and the gutters of the trace (p. 508), and must aid considerably in the depletion of the second cylinder just in front of the node. It is this state of things, no doubt, which gives the condition for the development of the *third* cylinder as an appendage of the edge of the gap of the second, bearing exactly the same relation to it as the second originally bore to the first, and serving in the same way as a supplementary reservoir of water, drawn upon when transpiration is active. If this view be accepted, we need not suppose that those cases in which the third cylinder ends blindly in the pith represent cases of reduction and loss of function; they can be interpreted as earlier stages in the evolution of the third cylinder, which starts as an elaboration of the edge of the gap in the second and extends in both directions through the internodes till it becomes continuous throughout the rhizome. It has been shown (p. 495) that the complication of the third cylinder is nearly always greatest in the region of its junction with the second, where its evolution may be supposed to be furthest advanced, and is correlated with the diameter of the outer cylinder and consequently with that of the leaf-trace. The increase in the diameter of the leaf-trace gives space for the increase in size and importance of the lateral loops, and these in their turn increase the

[1] In specimens of this plant obtained by Mr. Boodle since the publication of his paper, the thickening of the edges of the leaf-gap is *entirely* a downward extension of the leaf-trace edges.

demand on the second cylinder, thus leading to the appearance and increase in complexity of the third.

It is legitimate to inquire why precisely should the complicated vascular system whose development we have described be developed in this particular species. Such a question is never easy to answer satisfactorily, but the following considerations may be put forward. It is necessary, in the first place, to assume a progressively increasing demand by the leaf on the conducting and storage capacity of the rhizome. If the frond of *Matonia pectinata* has originated from the compact *Gleichenia*-type existing in *G. flabellata*, *G. quadripartita*, &c., as we have suggested (pp. 479–82), the greatly increased extent of lamina produced by the repeated addition of peripheral pinnae would certainly involve such an increased demand on the conducting-system, as compared with that of the hypothetical protostelic ancestor. Such an increased demand might be met by a corresponding increase in the breadth of the arch of the leaf-bundle and a correlated increase in the diameter of the stem-cylinder after it had become solenostelic. Up to a certain point no doubt it has been so met; but it is easy to see that an indefinite expansion of the leaf-bundle and rhizome-stele would involve a correspondingly indefinite increase in thickness of petiole and rhizome, and thus a constantly increasing thickness of relatively useless pith and a wasteful accumulation of material. An increase of the leaf-bundle by the development of its incurved free edges into the lateral loops, and a correlated development of accessory internal cylinders (by the elaboration of an original 'compensation thickening' on the edge of the leaf-gap) connected with the lateral loops would certainly economize space and material. The initiation of such a line of evolution may be attributed to the ease with which the original compensation-strand (second cylinder) can be drawn into supplying the free edges of the trace.

Roots. Nothing has hitherto been said about the connexions of the root-steles, which are presumably the sole means of the original supply of water, with the vascular system of the rhizome. The root-steles are attached to the outer cylinder exclusively, opposite the protoxylems. The roots are scattered along the whole length of the rhizome, without any particular relation to the nodes, except in the young plants, where there are nearly always two or three roots at the node (two often dorso-lateral with their steles attached close to the actual base of the leaf-trace), and often not more than two along the considerably greater length of internode. In the adult rhizomes the roots are in some cases, though by no means always, mainly attached to the ventral and ventro-lateral portions of the surface of the rhizome. The water-supply thus apparently enters the outer cylinder all along its course, though sometimes mainly from the ventral side. As we have pointed out above, it is clear that water can only enter the second cylinder through its nodal connexion with the outer one, and the third

through its connexion with the second in front of the node. It seems impossible to avoid the belief that the accessory cylinders are filled through these connexions when absorption exceeds transpiration, and drawn upon again when transpiration exceeds absorption.

The Morphological Status of 'Pith'; its Relation to Leaf-Gaps and Cortex.

The questions relating to the morphological status of pith, the conception of 'ground-tissue' in morphology, and the like, which, in recent years, have been so much discussed by various anatomists, particularly by Jeffrey, Farmer, and Boodle, are brought prominently before the mind in considering the ontogeny and phylogeny of the vascular system of a form like *Matonia*, where the axis of the rhizome is occupied successively by tracheids, sieve-tubes, endodermis, and 'ground-tissue pith,' in no less than three successive and complete cycles. Without attempting to discuss the whole of the questions that have been brought into the arena of this discussion, we cannot help expressing our belief that Jeffrey's theory of the intrusion of cortex into the stele is without foundation on developmental and comparative grounds, and has at best a merely metaphorical value; while Farmer's view[1], that the only valid distinction is that between vascular (in the wide sense) and non-vascular tissues, while perfectly good, and, so far as we are aware, never disputed since the time of Sachs and De Bary, as a fundamental physiological classification of the internal tissues of a vascular plant, yet radically ignores the morphological problems with which the anatomist is confronted when he is endeavouring to trace out the evolution of the tissues of vascular plants[2].

In our view, the pith is morphologically an entirely new tissue, formed in the centre of the stele, in place of vascular tissue, which preceded it in ontogeny and phylogeny, but is always, in the Ferns, separated from the latter by endodermis. The fact that its histological characters are very similar to, or even identical with those of the cortex, gives us no information as to how the pith arose in the course of descent, which is the essence of the morphological question; while the general continuity of pith with cortex at the leaf-gaps, and the phenomena of 'endodermal' or 'ground-tissue pockets' considered as probable forerunners of a continuous ground-tissue pith, although they do furnish us with good evidence of stages in the phylogenetic origin of pith, at least in certain cases, do not take us one step towards a demonstration of the *cortical origin* of pith, but merely establish

[1] Farmer and Hill ('02), p. 392.

[2] Farmer's view that tissues cannot be treated as morphological entities in the same way as members of the plant body (*op. cit.*) is interesting, but involves questions far too wide to be discussed here. It appears to strike at the root of the investigation of tissue morphology on evolutionary lines, a subject which has been pursued with so much success in this country during the past decade.

a presumption that pith first arose *in connexion with the cortex* at the leaf-gaps. The only evidence which would establish the origin of pith by *intrusion of the cortex* would be proof that the cells of the cortex actually pushed into the stele during development from the growing point, or had done so at some time during the course of descent. The possibility of this has been admitted by Gwynne-Vaughan [1], but all available evidence points to a formation of pith by meristematic cells which would otherwise have given rise to stelar elements. In default of evidence of actual intrusion, the fact that pith occupies a region of the axis previously occupied by a totally distinct kind of tissue leads, in our view, necessarily to the conclusion that it is a new tissue. The statement about the intrusion of cortex becomes not only metaphorical, but misleading; a true and useful statement, on the other hand, would be that the ground-tissue of the plant has now extended so as to occupy the axis of the stem as well as the periphery, by the development of a new tissue, the pith, whose histological characters and opposition to vascular tissue bring it within the wider concept of ground-tissue.

This view necessarily carries with it the consequence that pith is morphologically part of the stele, since it is the phylogenetic successor of vascular tissue. We find it impossible to understand how this conclusion can be escaped if morphology is to have an evolutionary meaning. The case of the young *Matonia* appears to show, further, that pith does not in all cases arise in connexion with cortex. In nodes 2, 3, 4, 6, 7, 8, 9 of plant E there is *no* connexion between the pith of the stele and that of the cortex, though there is a connexion between the pith of the stele and that of the leaf-trace (when the latter is present), the trace being a closed cylinder with its pith shut off from the cortex, and sometimes consisting of a few cells only confined to the base of the trace. In nodes 6, 7, and 8 the leaf-trace possesses at its base an internal endodermis only which is connected with the internal endodermis of the stem cylinder. In the open type of trace, the pith of the stele is at first in connexion with the cortex only through that of the trace (e. g. E, nodes 10, 11, 13, 14; F, nodes 1 to 7); while later on (F, node 8, &c.) the pith of the stele opens directly to the cortex of the stem at the base of the trace, as is the case in typical solenostelic ferns.

In *Schizaea malaccana* [2] and in *S. dichotoma* [3], where the leaf-trace has no pith, the non-continuous ground-tissue pith of the stem is normally in continuity with the cortex in the form of the endodermal or ground-tissue 'pockets.' Isolated strands of internal endodermis enclosing pith are sometimes met with, and these may, with Boodle [4], be interpreted as the result of reduction from a type with continuous pith; though, for reasons given [5], such an interpretation does not appear to be absolutely necessary.

[1] Gwynne-Vaughan ('03), p. 737, &c. [2] Tansley and Chick ('03). [3] Boodle ('03).
[4] Boodle ('03), pp. 519, 521. [5] Tansley and Chick ('03), pp. 500–2.

In *Anemia coriacea*[1] there is a continuous sclerenchymatous pith not in connexion with the endodermal pockets. This Boodle also interprets as a reduction-phenomenon, and the same applies to *Platyzoma*, where the pith has no connexion either with cortex or leaf-trace. But in the young *Matonia* there can be no reason to postulate reduction, and the evidence cited is, we think, sufficient to show that the pith of the stem does not necessarily arise in connexion with cortex[2].

Boodle has made a suggestion with regard to the teleological cause of the origin of pith in Ferns. 'To admit of the insertion of a number of large arched bundles, the stele increased its diameter beyond the size required by the exigencies of water-conduction, and the central part of the xylem of the stele was transformed into parenchyma or other tissues[3].' This seems to us an extremely probable view, though we should be inclined to regard the *Lindsaya*-type as normally antecedent to the production of a parenchymatous pith[4]. But in certain cases at least the pith may well have had a specific function of its own from the outset. In species with superficial creeping rhizomes and large leaves on long erect petioles, the tissues of the rhizome on which the base of the petiole is inserted must be subject to a considerable strain, which might lead to rupture of the vascular system at the junction of leaf-trace and stele. This danger would certainly be lessened by the insertion of a T-piece of hard material, the stalk of which occupies the centre of the leaf-trace and the cross-piece that of the stele. This T-piece is represented by the strand of sclerenchyma in the concavity of the trace and the sclerenchymatous pith of the stele to which it is attached, as actually found in many Ferns. Additional solidity would be acquired by the connexion of the T-piece with the thick-walled cortex of the stem and base of the petiole, and this condition is actually realized in most cases, where the leaf-trace is open at the base, but it is not essential to the mechanical efficiency of the arrangement which appears to obtain in the young *Matonia*. In some cases the strand of sclerenchyma in the concavity of the trace is continuous with a sclerized pith in one (the basipetal) direction only (*Davallia pinnata*). A study of various living Ferns in their natural habitat is required to confirm or disprove this idea.

If the view taken above, that the pith is to be regarded as a new tissue of the stem, is just, it follows that the same considerations must hold of the internal accessory vascular cylinders of *Matonia* and equally of other poly-

[1] Boodle ('01), p. 387.

[2] Cf. also *Gleichenia dichotoma*. Gwynne-Vaughan ('03), p. 739, has set out the alternative methods of evolution of a pith in Ferns.

[3] Boodle ('03), p. 530.

[4] Partly because of the large number of cases now known (see p. 509, *supra*) in which the *Lindsaya*-type precedes solenostely in ontogeny, and partly because, as Gwynne-Vaughan ('03) has shown, the type of leaf-trace associated with the *Lindsaya*-stele is still compact and would not require a wide medullated type of stele for its attachment.

cyclic Ferns in which internal strands arise in the same manner. These accessory vascular strands are to be regarded as essentially new developments, which partially replace the pith, just as the pith at an earlier stage replaced the internal vascular tissues. They are parts of the stele, just as the pith is part of the stele, which has now become a complicated structure without losing its individuality [1].

[1] It is abundantly clear that the term stele must be restricted to the central cylinder of the axis, if it is to retain a morphological meaning, and that the concept of polystely, at least in the Ferns proper, must be definitely regarded as obsolete. We have expressly excluded the other cases of so-called polystely, *Selaginella*, *Primula*, *Gunnera*, Nymphaeaceae, &c., a discussion of which would be out of place here. The idea put forward by Van Tieghem and Douliot, that several equivalent cylinders in a stem could arise by the successive forking of the original one found at its base, was upset by the work of Leclerc du Sablon and Jeffrey, who showed that the real origin of the so-called 'polystelic' condition was quite different. It is doubtful, indeed, if the concept of homology could in any case be applied to the different vascular strands called steles by Van Tieghem and Douliot, whatever their origin. From the nature of the case they certainly could not be morphologically equivalent in the evolutionary sense, which is the only accurately definable and consistent meaning we can attach to the word homology. But since the state of things on which the theory of polystely was based is, so far as we know, non-existent, there is no need to discuss it further. For the reasons given we have avoided applying the term stele to the internal cylinders of *Matonia*, considering them as accessory developments of the original ancestral stele, and as parts of the present complicated stele.

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DESCRIPTION OF FIGURES ON PLATES XXXI–XXXIII.

Illustrating the paper by Mr. Tansley and Miss Lulham on *Matonia pectinata*.

PLATE XXXI.

Figs. 1–7. Young plants of *Matonia pectinata* and detached leaves from the same. Natural size. (For details see text, pp. 477–9.)

Fig. 8. Very small dichotomously branched leaf from a starved plant. Nat. size.

Fig. 9. Simple leaf of adult type from a starved plant, showing 'middle pinna,' a single dichotomy on the left, and a tendency to dichotomy in the single pinna on the right. Nat. size.

Fig. 10. Frond of *Gleichenia flabellata* from the Kew Herbarium, showing the *Matonia*-type of frond-branching, with bud (adherent to one of the forks of the primary dichotomy) representing the 'middle lobe.' Reduced.

Fig. 11. Transverse section of stele of proximal end of rhizome of youngest plant, A (Fig. 1). *end.* endodermis; *per.* pericycle; *tr.* tracheids. Protostelic structure without phloem. × 400 (p. 482).

Fig. 12. Transverse section of stele of rhizome of A immediately in front of first node. *s. t.* sieve-tubes; *prot. c.* proteid cells; *ext. phl.* external phloem. *Lindsaya*-phase. × 500 (p. 483).

Fig. 13. Transverse section of stele of rhizome of A at origin of second leaf-trace. *x. tr.* xylem of trace; *int. ph. tr.* internal (adaxial) phloem of trace; *int. ph. st.* internal phloem of stele; *ext. ph. st.* external phloem of stele; *per. rt.* pericycle of root (cut tangentially). × 400 (p. 483).

Fig. 14. Transverse section of third leaf-trace of A, while it is passing through the cortex, showing phloem massed mainly on adaxial side. Letters as before. × 400 (p. 483).

PLATE XXXII.

Fig. 15. Transverse section of proximal end of rhizome of plant D, showing central phloem enclosing a detached xylem strand and scattered endodermal cells uniting to form a crescentic strand. Sieve-tubes showing typical granules. × 160 (p. 485).

Fig. 16. Transverse section of stele of proximal end of rhizome of plant E, showing a solenostele with local internal dilatation of xylem. × 130 (p. 486).

Fig. 17. Transverse section through leaf-gap of fifth node of E. Internal xylem-strand free. × 100 (p. 486).

Fig. 18. Next section but one; closure of gap; internal xylem-strand connected with outer xylem. × 100.

Fig. 19. Five sections further on; tracheids running obliquely from central strand to close xylem-gap. × 150 (p. 487).

Fig. 20. Transverse section through fifth internode of E, showing internal strand separated from outer cylinder dorsally by endodermis, ventrally also by pith; *px?* probably protoxylem of leaf-trace continued down into stele. × 120 (p. 487).

Fig. 21. Transverse section of the beginning of the tenth node of E, showing tracheids from the internal strand running up into the leaf-trace, and phloem-elements in the interior of the internal strand. × 130 (p. 487).

Fig. 22. Transverse section of the eleventh leaf-trace of E, near its base; sieve-tubes mainly round the convexity of the trace; *ph.f.?* probably fibres developed in phloem of concavity of leaf-trace; *px?* probably protoxylem; *int. end.* internal endodermis. × 500 (pp. 488, 504).

Fig. 23. Transverse section of a leaf-trace of G, near its base, showing the initiation of lateral loops by thickening of the free edge of the trace (left) and xylem-hook formation (right). *px?* probably protoxylems. In the phloem covering the sides of the trace the sieve-tubes are normal; round the middle of the convexity, thick-walled; in the concavity of the trace and in the loop of the xylem-hook on the right, fibres are developed instead of sieve-tubes. × 350 (pp. 491, 504).

Fig. 24. Diagram of transverse section of tricyclic stem in front of node, just before closure of gap in second cylinder. × 8.

Fig. 25. Connexion of third cylinder with edge of gap in second, effecting closure. × 8.

Fig. 26. Gap closed; detachment of third cylinder from second. × 8.

PLATE XXXIII.

Drawings of wax models of the stelar system.

Fig. 27. Side view of eighth node of F. × 25.

Fig. 28. Front view of eighth and ninth nodes of F, showing connexion of second cylinder with outer one in front of insertion of ninth trace. × 40.

Fig. 29. Segment of vascular system of rhizome and base of leaf-trace including front part of node, in simple dicyclic type, showing branching column of tissue rising from second cylinder to supply the lateral loops of the trace and close the gap in the first cylinder. Seen from behind. × 12.

Fig. 30. Segment of vascular system of rhizome and base of leaf-trace including front part of node in most advanced tricyclic adult type, from behind. *a.* Roof of second cylinder rising to fill gap in first and coming into connexion with lateral loops of leaf-trace which bulge back freely above connexion; *b.* these backward bulges; *c.* free edge of forwardly directed limb of lateral loop; *d.* downward and forward continuation of *c*, forming flange on edge of gap in second cylinder. × 10.

Fig. 31. The same from in front; same lettering. × 10.

TANSLEY & LULHAM.—MATONIA PECTINATA.

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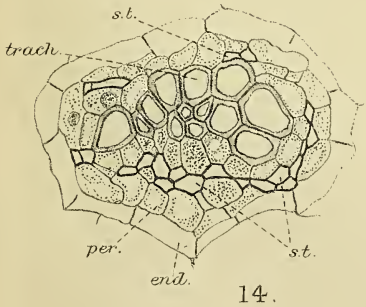

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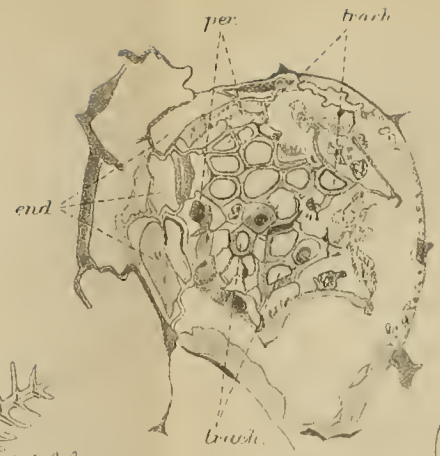

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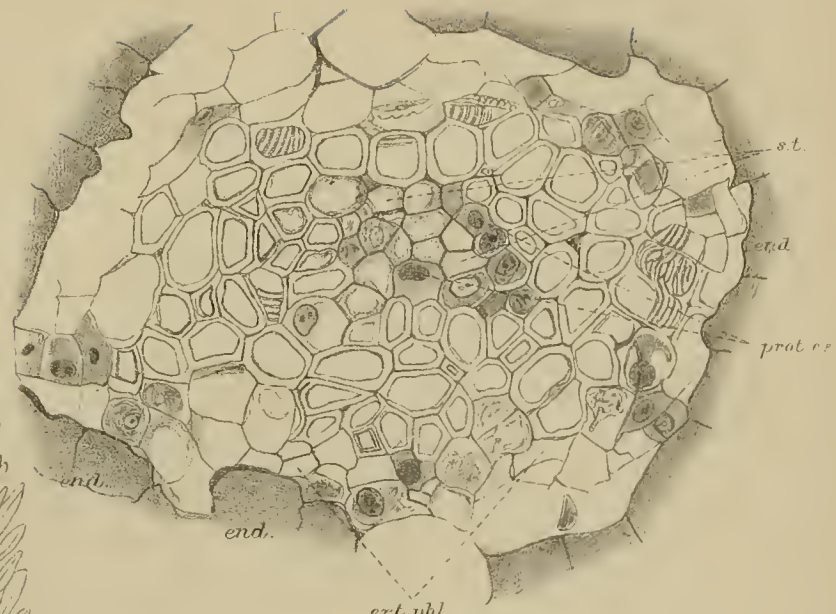

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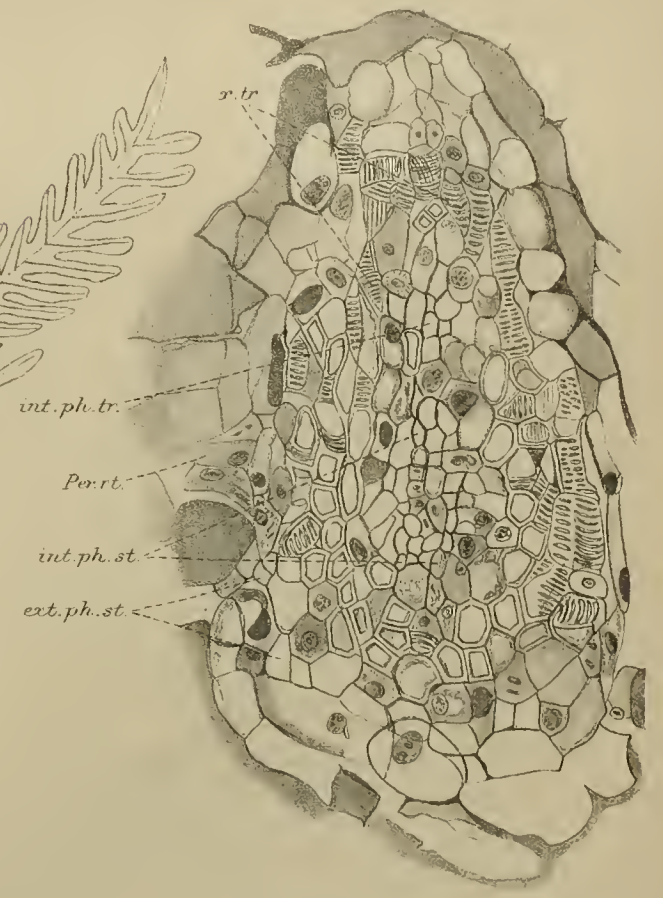

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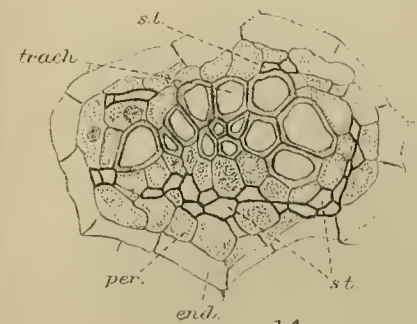

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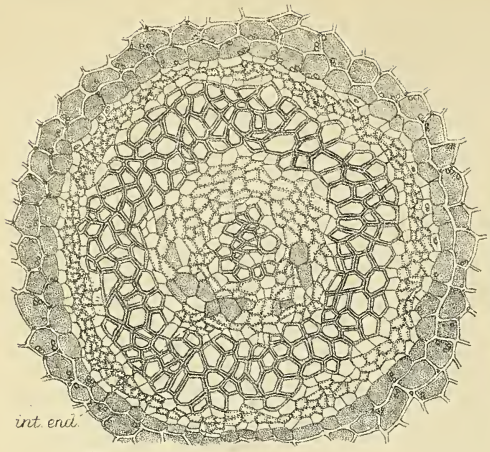

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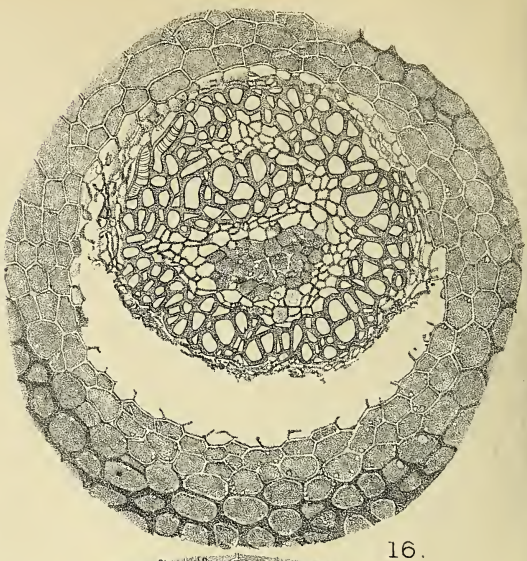

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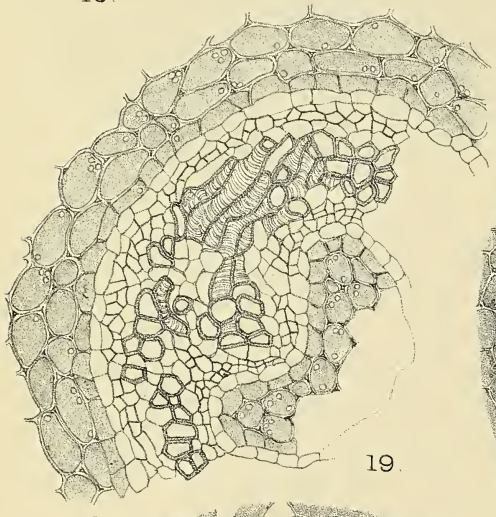

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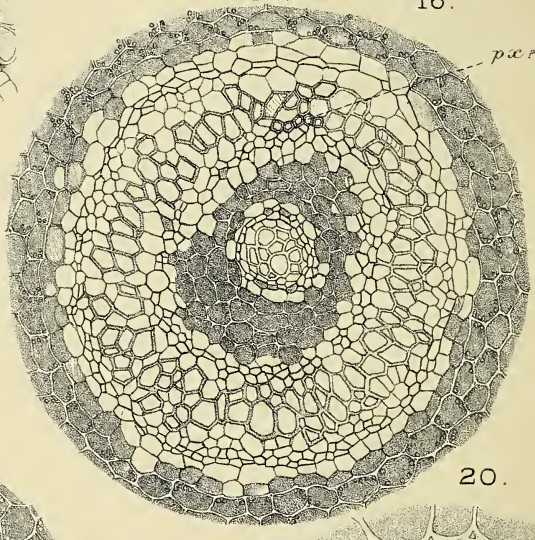

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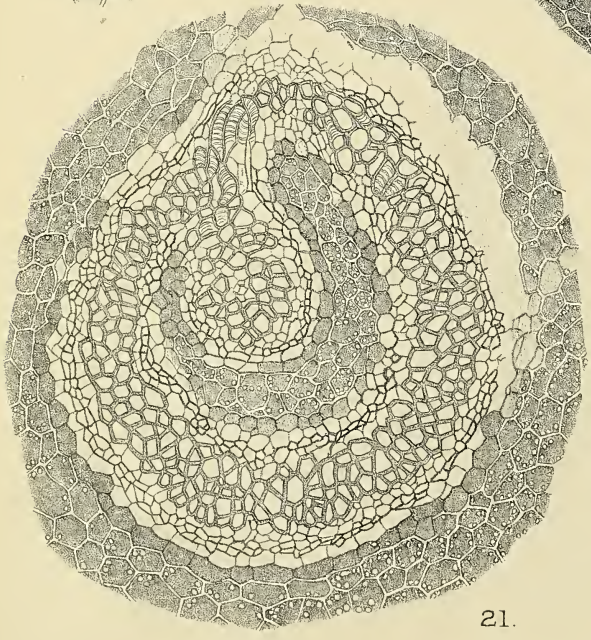

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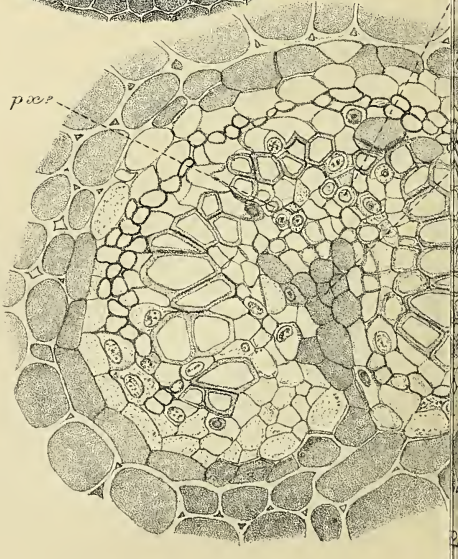

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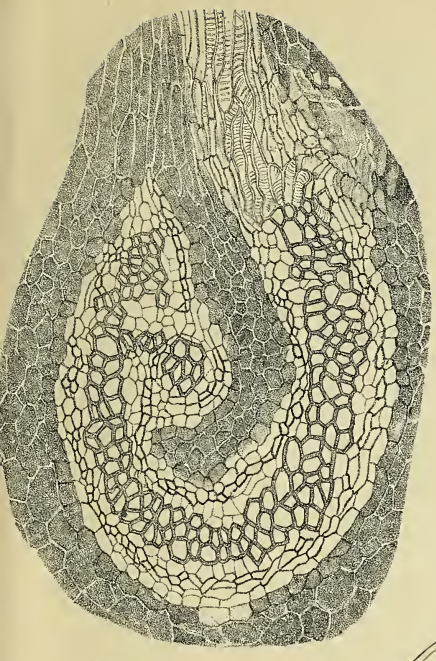

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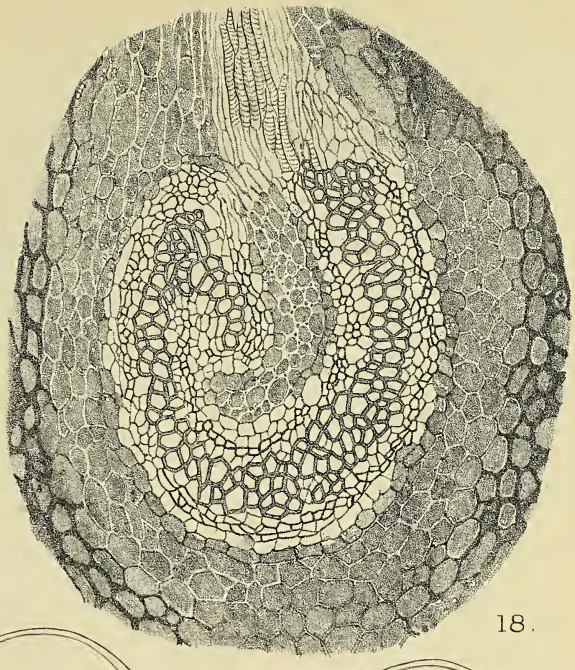

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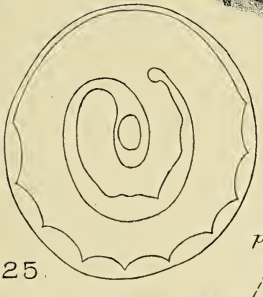

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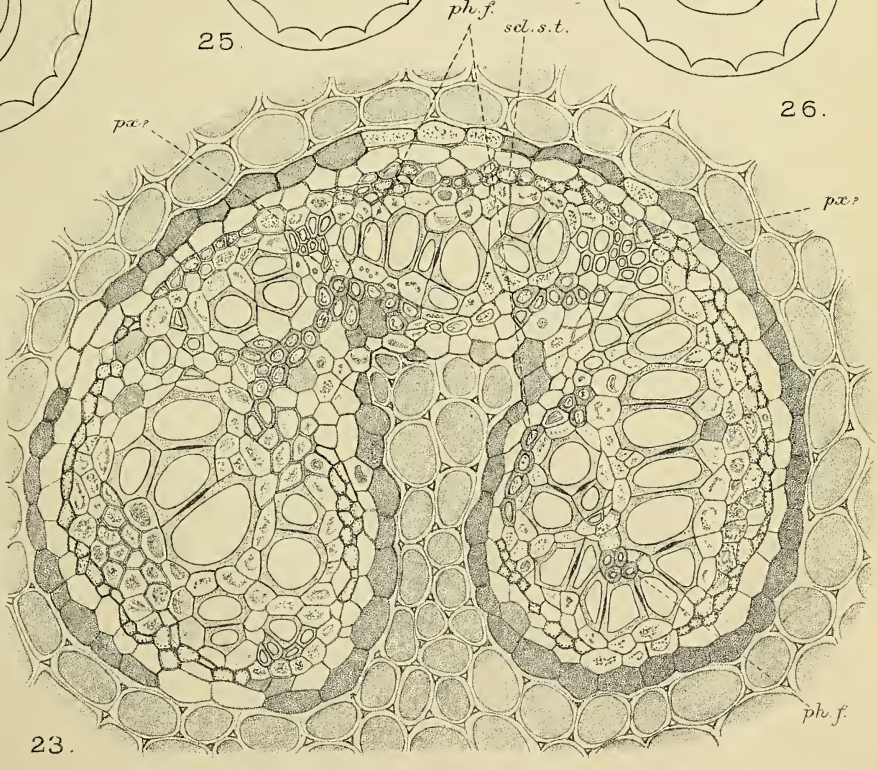

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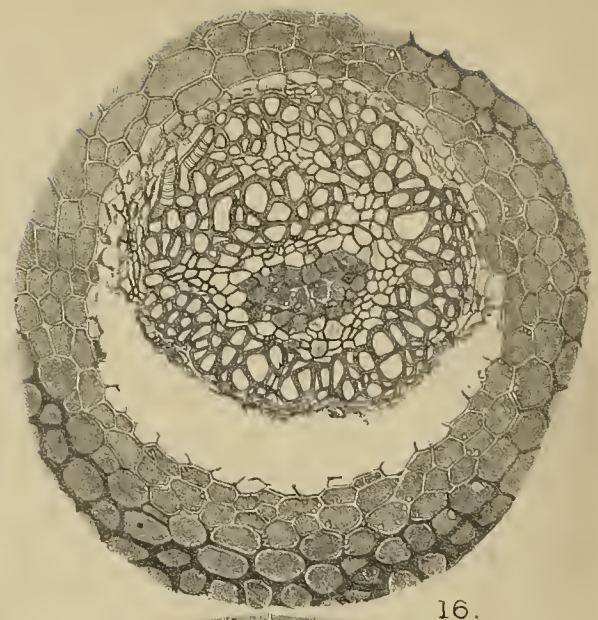

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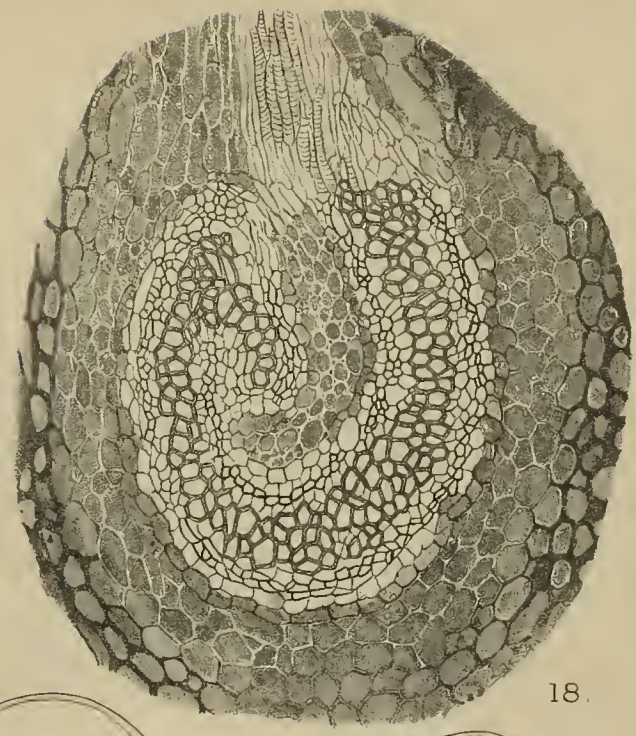

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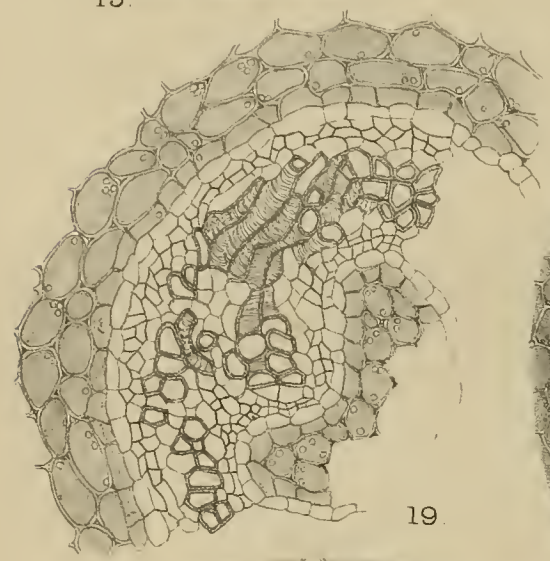

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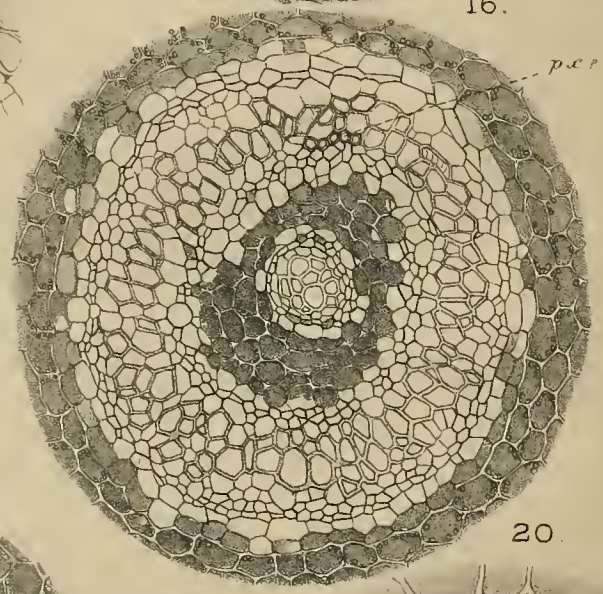

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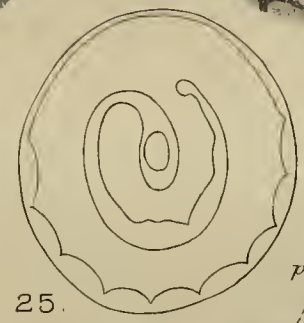

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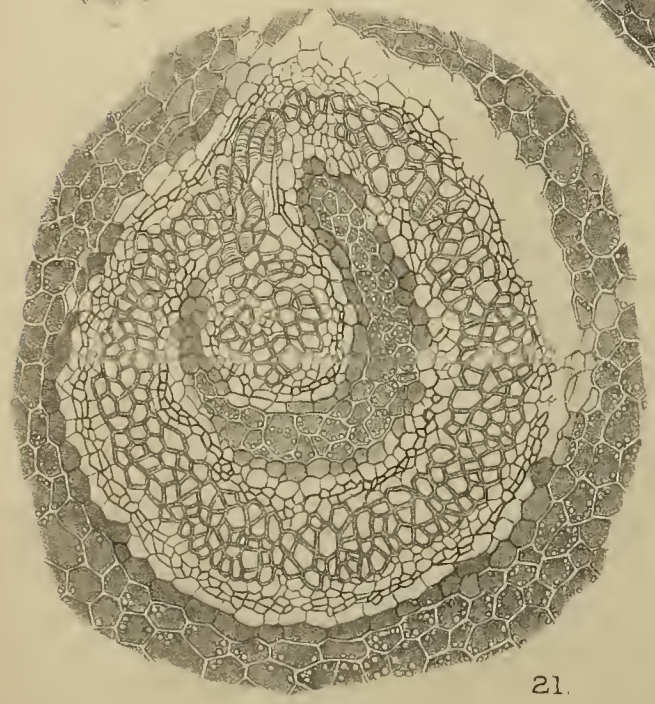

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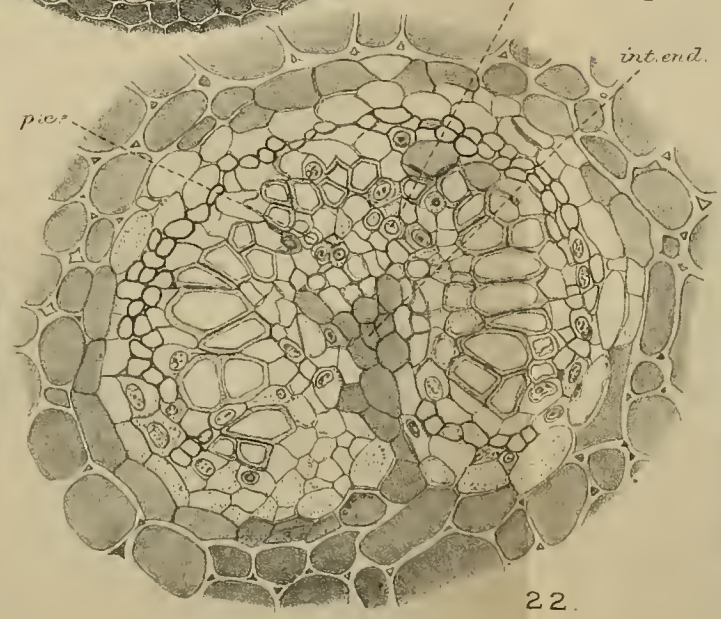

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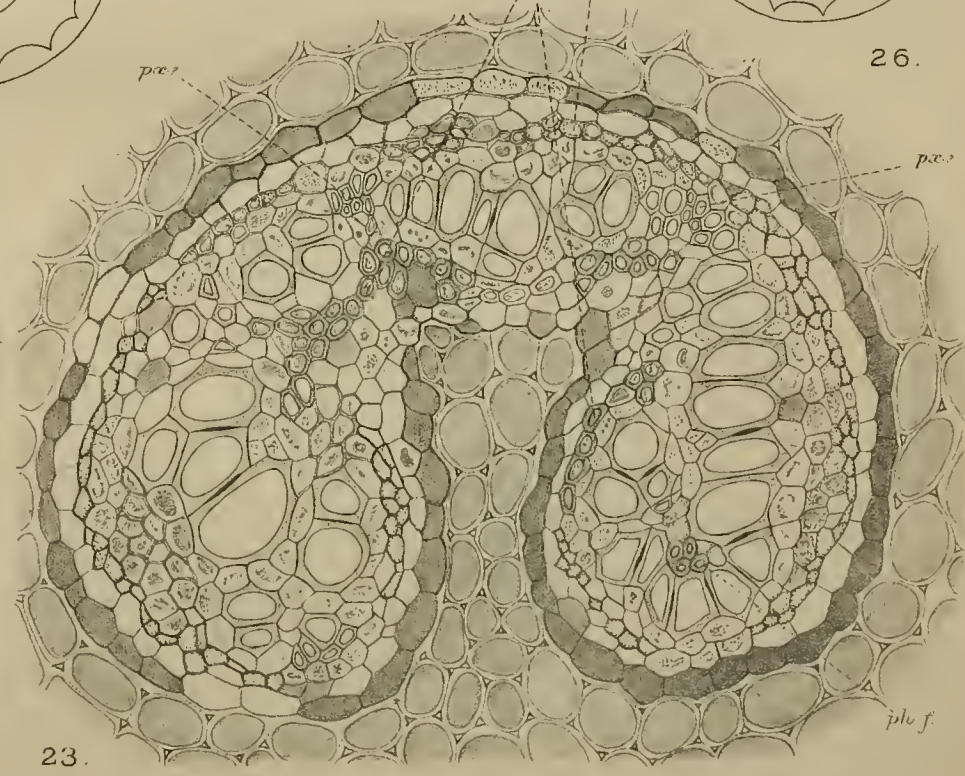

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TANSLEY & LULHAM.—MATONIA PECTINATA.

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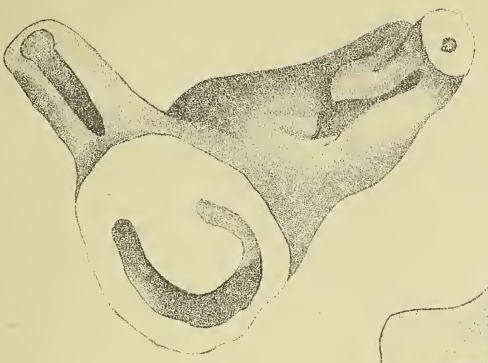

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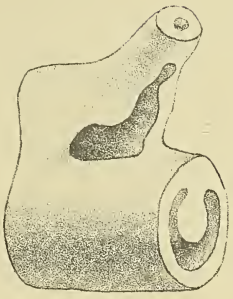

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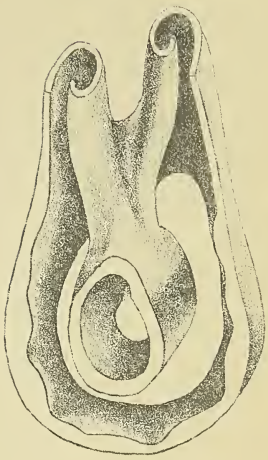

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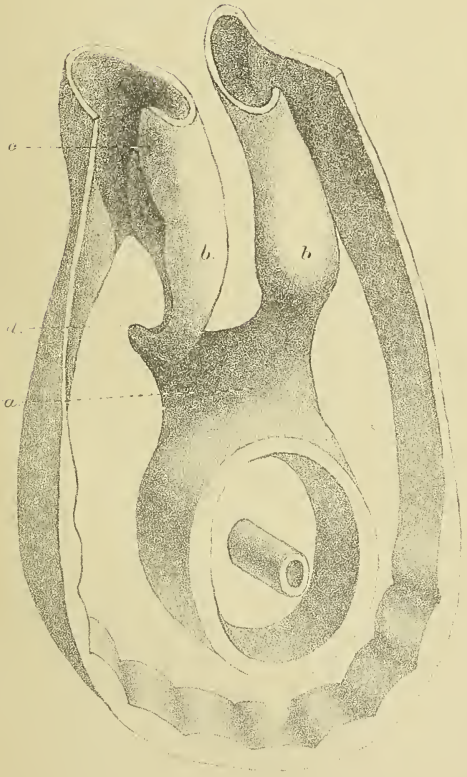

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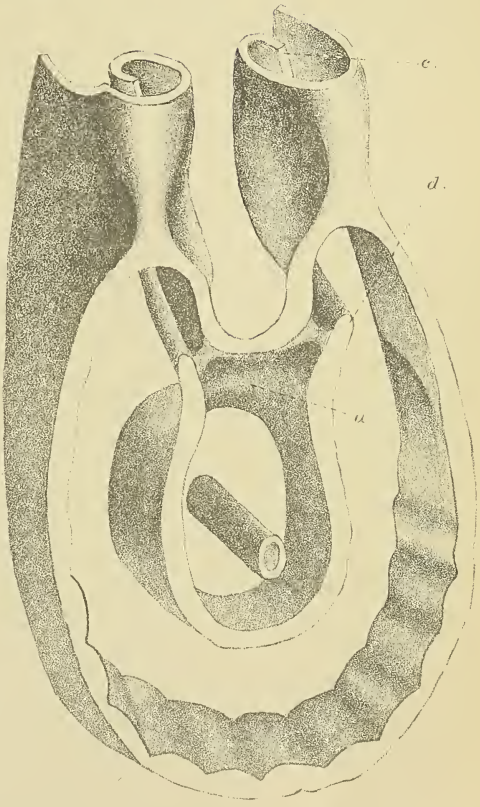

31.

TANSLEY & LULHAM.—MATONIA PECTINATA.

The Effect of Gases on Nuclear Division.

BY

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Assistant Professor of Botany in Indiana University, Bloomington, Ind., U.S.A.

With a Figure in the Text.

EVER since the appearance of Demoor's paper [1] on the physiology of the cell, considerable doubt has been expressed as to the exactness of many of his experiments and conclusions [2]. It was in the hope of removing this doubt that I began an investigation at the suggestion of Prof. Pfeffer, during my study in Leipzig in 1902, to ascertain the correctness of Demoor's work. My experiments, to be described presently, do not include the whole of the work mentioned in the paper of Demoor cited below, but deal only with Chapter IV (pp. 30–54). This chapter contains the results of his experiments on the staminal hairs of *Tradescantia virginica*, especially under the influence of different gases, with reference to protoplasmic and nuclear activity. It is, however, his statement that the nucleus can be divided independently of the protoplasm that is most important and has caused most controversy, and it is this question that I have tried especially to decide by my experiments.

Methods.

Momordica Elaterium was used only for a few experiments on the movements of protoplasm. The best as well as the most certain living object, however, in which nuclear and cell-division can be directly followed is *Tradescantia virginica* [3], and accordingly this plant was chosen for my investigations. The younger staminal hairs, and those containing cells whose nuclei had not yet divided, were selected and put in a 3 per cent. solution of cane sugar to see the division take place. This solution is not concentrated enough to produce plasmolyzation, nor does it apparently interfere in any way with the vital activities of the cell—at

[1] Contribution à l'étude de la physiologie de la cellule. Archives de Biologie, tome xiii, 1894.

[2] Pfeffer, Pflanzenphysiologie, 2. Aufl., Bd. ii, p. 46. Samassa, Ueber d. Einwirkung von Gasen auf d. Plasmaströmung, Bot. Zeitung, 1898.

[3] Strasburger, Botanisches Practicum, 4. Aufl., 1902, p. 589.

[Annals of Botany, Vol. XIX. No. LXXVI. October, 1905.]

least within the limits which concern us here[1]. A stronger solution of cane sugar (5 per cent.) may, as Hörmann[2] has shown, cause a retardation of protoplasmic movement in a cell of *Nitella*. It was also very noticeable that the movement of the protoplasm in the younger cells of the staminal hairs was more readily and quickly affected by the influence of various gases than in the older cells whose cell-walls were, as one would naturally suppose, less permeable. This disregard of the age and condition under which the plant-cells live, and the attempt to apply the same tests to different plants and obtain similar results, has led to numerous errors on the part of many investigators.

Great care has been necessary in these experiments. The perfection of the apparatus, and the nature of the plant-material it was necessary to use, would make it at once evident to any one at all conversant with such a subject as this how very difficult it often is to arrive at a definite and certain result. In order to be sure that my results were correct, I have repeated each experiment several times.

It is not surprising then that Demoor made mistakes in some of his experiments and conclusions. While his general methods were good, he did not take sufficient precaution to obtain gases that were absolutely pure. The same lack of care in obtaining absolutely pure gases caused Lopriore[3] to make some mistakes in his experiments and to arrive at entirely incorrect conclusions.

The only way to ascertain that no oxygen remains about the specimen under investigation is by means of the Bacterium-method of Engelmann. Pure cultures were obtained according to the method given by Detmer[4], and these were used in all my experiments with hydrogen. It is certain, as I shall show later on in this paper, that even a very small quantity of oxygen is sufficient to allow the nucleus to commence and continue its division normally. Since, however, by the excessively delicate reaction shown by the Bacterium-method the billionth part of a milligram of oxygen can be detected[5], I was thus able to ascertain the slightest trace left in the apparatus or if any should be evolved by the plant-cells under observation.

The natural evolution of oxygen by chlorophyll-bearing cells introduced another difficulty, but nevertheless made it absolutely necessary to avoid using any cells containing even a trace of chlorophyll.

Since absolute purity of the gases employed is essential for obtaining correct results, the utmost precaution was taken to construct apparatus and to use only those chemicals that would attain that end.

[1] Ewart, on the Physics and Physiology of Protoplasmic Streaming in Plants, 1902, p. 59.

[2] Studien über die Protoplasmaströmung bei den Characeen. Jena, 1898, pp. 51–5.

[3] Jahrb. f. wiss. Bot., 1895, Bd. 38, p. 531.

[4] Pflanzenphysiologie, 2. Aufl., 1895, p. 32.

[5] Pfeffer, Pflanzenphysiologie, 1897, 2. Aufl., Bd. 1, p. 292 and literature cited there.

Figure 16 will give an idea of the apparatus used to obtain pure hydrogen. It consists of a gas generator *A*, containing zinc and sulphuric acid covered with paraffin[1], which connects with a U-tube *B*, filled with pumice stone and saturated with caustic potash for removing the hydrochloric acid, sulphuric dioxide or hydrogen sulphide, some one or more of which may be present in hydrogen prepared by the action of sulphuric acid on zinc. The U-tube *B* is connected with *C* containing silver nitrate for absorbing the arsenic according to the formula:—

$$6AgNO_3 + AsH_3 + 3H_2O = 6Ag + H_3AsO_3 + 6HNO_3.$$

The U-tube *C* is joined to *D* containing pyrogallol for removing the oxygen. *D* communicates with *E* containing potassium permanganate, and *E* with the bottle *F* containing water for washing the hydrogen and saturating it with moisture. This latter precaution is absolutely essential, for if dry gas were allowed to enter the gas chamber *G* on the microscope *P*, the shallow drop of water containing the specimen for observation would be evaporated before the termination of the experiment. In order to ascertain the rate of flow of the gas, as well as to prevent any back flow of air into *G*, a bottle *K* is attached containing water. The glass tubes connecting the U-tubes and bottles are made secure at their points of union by rubber stoppers covered with a thick layer of sealing wax as at *L*. The glass tubes *M*, *M'* are long enough to allow sufficient movement of *G*. They are fastened to the metal tubes of the gas chamber *G* by rubber tubing covered with sealing wax. Such connexions as are here mentioned will not allow any gas to diffuse, as I have found by long-continued experiments. No leakage whatever could be detected, even when the gas in the apparatus was subjected to a pressure of half an atmosphere under water.

Another excellent method is to generate hydrogen by electrolysis and collect this in a vessel for use.

I. The Effect of Hydrogen.

1. The action of Hydrogen on the streaming of Protoplasm.

In each case after the staminal hair was placed in the 3 per cent. solution of cane sugar a few of the aerobic Bacteria, above referred to, were transferred to the drop by means of a sterilized platinum needle. These Bacteria move when oxygen is present. They also instantly cease moving when oxygen entirely disappears, and are therefore a very delicate test to show that no oxygen was around the cells with which I was experimenting. The use of the various chemicals mentioned has, by the

[1] If, as sometimes happens, the sulphuric acid does not attack the zinc readily, so as to cause a rapid evolution of hydrogen, this may be brought about by the addition of a small quantity of platinum tetrachloride or copper sulphate to the sulphuric acid.

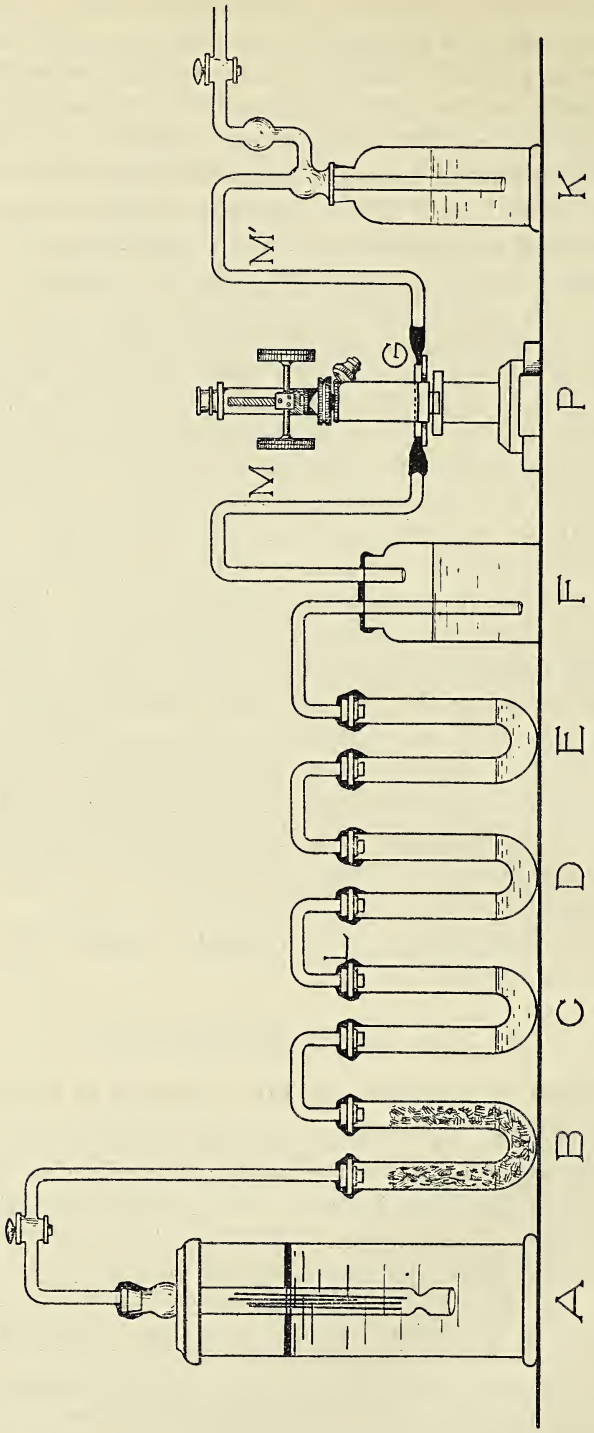

FIG. 16.

time hydrogen reaches the cell enclosing the material for investigation, removed all other gases and impurities, leaving pure hydrogen.

In the first experiment a cell of *Tradescantia virginica* was placed in pure hydrogen and in ten minutes the movement of the protoplasm ceased. To ascertain just when the protoplasm stops moving requires great care and accurate observation, since a very slight movement is easily overlooked. One hour and four minutes afterwards oxygen was introduced and the movement began again in five minutes. This experiment is verified by the action of hydrogen on the cells of *Momordica Elaterium*, for when the cells of this plant were subjected to pure hydrogen the movement of the protoplasm ceased in ten minutes and began again in five minutes as before. When, however, the cells of *Momordica Elaterium* were left in pure hydrogen for six hours they were not killed, but the time of recovery and recommencement of movement was thirty minutes. That a longer time should be required when left in pure hydrogen is not surprising, but shows that if all other conditions were perfect in every way a very long time would be required to kill these plant-cells in pure hydrogen.

The three preceding experiments on non-chlorophyll cells of *Tradescantia virginica* and *Momordica Elaterium* were carried on in light; but others exactly the same, except that they were kept in darkness, were performed with no appreciable difference in the results.

2. Influence of Hydrogen on Nuclear Divisions.

When a nucleus in the resting stage is left in pure hydrogen it cannot begin division. This was verified by bringing resting nuclei of *Tradescantia virginica* into pure hydrogen for four and one-half hours, at the end of which time they still remained in the resting stage and not the slightest advance toward division had taken place. At the end of this time (four and one-half hours), when air was admitted, the nuclear division commenced and was completed.

When a nucleus was near the close of the prophase stage and was brought under the influence of pure hydrogen, indirect division was completed and the formation of the daughter-nuclei occurred, but no cell-wall was ever formed.

Such nuclei finished dividing in two hours. The nuclei which I used as controls showed that this is somewhat longer correspondingly than is required by nuclei of *Tradescantia virginica* under normal conditions to divide. It further shows that the cessation of protoplasmic activity and passage of the hydrogen through the protoplasm to the nucleus is comparatively slow for such a gas, and thus nuclear division is able to advance considerably before being checked by its influence. In all these ex-

periments the motionless aerobic Bacteria showed the total absence of oxygen.

When oxygen was admitted, after each of these experiments, a cell-wall was produced in fifteen minutes. These and many other experiments performed in this way show that it is impossible for the nucleus to divide independently of the protoplasm. That the nucleus could not begin division in pure hydrogen, whereas in the control experiments it could, shows this to be true. That nuclear division advanced in pure hydrogen without the formation of the cell-wall is to be explained simply and only on the ground that the hydrogen could not penetrate quickly enough to prevent a certain advance in nuclear division or interfere with the vital processes of the nucleus, as it had with those of the more exposed cytoplasm.

II. The Effect of Carbon Dioxide.

In the second set of experiments, where carbon dioxide was used, the apparatus shown in Fig. 16 was not employed. This gas was purified and freed from all traces of oxygen and then washed with water and subsequently tested to see that no oxygen remained. Its interference both with protoplasmic streaming and nuclear division was more marked than that of hydrogen.

1. Effects of Carbon Dioxide on the Movements of Protoplasm.

In the cells of the hairs of *Momordica Elaterium* the streaming of the protoplasm ceased in six minutes after admitting carbon dioxide. The specimens were left in a constantly flowing stream of carbon dioxide for three hours, during which time no movement of the protoplasm was evident. When oxygen was allowed to enter, the movement began again in ten minutes. In *Tradescantia virginica* the same effect and time-relation was observed.

2. Effects of Carbon Dioxide on Nuclear Division.

Nuclei of *Tradescantia virginica* in the resting stage were left in pure carbon dioxide for two and one-half hours, and did not commence division. They seemed at the expiration of this time to be slightly disorganized. Recovery of the nucleus was possible if the disorganization had not proceeded too far. Whenever nuclear division did occur after this disorganization it was always by karyokinesis.

A cell of *Tradescantia virginica*, whose nucleus was near the close of the prophase stage, was left in carbon dioxide for three hours, but no further division of the nucleus occurred. After thirty minutes, oxygen was again admitted, when the protoplasm first began to move, then the nucleus to divide, completing its division and forming a cell-wall in one hour and forty minutes. This, as in the similar case of hydrogen, was somewhat

longer for the nucleus to finish division than was required by the control specimens.

In a third series of experiments with carbon dioxide, a cell of *Tradescantia* was left in the gas for fourteen hours. At the expiration of this time, as would be expected, the cell was dead. This disproves the statement of Lopriore[1], who states that carbon dioxide may act for days (together) before causing a permanent cessation of protoplasmic movement. His error was evidently due to the use of impure carbon dioxide.

III. Effects of a Vacuum on Nuclear Division.

As stated, a very small quantity of oxygen is sufficient to allow nuclear division. By using a vacuum pump a pressure of 3 mm. of mercury was reached and maintained. Even at this slight pressure, resting nuclei, as well as those farther advanced, completed division and formed a cell-wall. The movement of the protoplasm does not cease even at this low pressure, showing the incorrectness of Demoor's statement, that it stops at a pressure of 6 to 12 cm. of mercury. Whenever, for whatever reason, the activity of the protoplasm is made impossible, that of the nucleus is also suppressed.

IV. Effects of Ethyl Ether.

In the experiments with ethyl ether and chloroform the specimens were not put directly in the solution mentioned, but the vapour from these solutions was drawn over the cells, while in the sugar solution, by means of an aspirator.

In the vapour of a 1 per cent. or stronger solution of ethyl ether resting nuclei of *Tradescantia virginica* cannot begin division. Those nuclei, however, which had begun division, completed it or advanced towards completion according to the concentration of the solution, as the following experiments will show.

In the vapour of a 1 per cent. solution of ethyl ether the nucleus, when in the prophase stage, finished division about ten minutes sooner than the control specimen. A series of experiments were sufficient to establish the fact that, as in the case of the movement of the protoplasm, a slight acceleration in rapidity of nuclear division was produced with a 1 per cent. solution of ethyl ether.

The formation of the cell-wall also commenced even before the chromosomes began to separate and to move to their respective poles. A somewhat similar cell-division has been observed by Gerassimoff[2] in *Spirogyra* under certain conditions. The resulting nuclei were normal in appearance.

In a 3, 4, and 5 per cent. solution of ethyl ether the nuclei, when near

[1] Jahrb. f. wiss. Bot., 1895, Bd. 28, p. 571. [2] Pfeffer, Pflanzenphysiologie, 2. Aufl., Bd. II, p. 46.

the end of the prophase stage, finished division and formed a cell-wall as soon as the chromosomes had separated. The movement of the protoplasm was slightly accelerated for a short time, as was also the division of the nucleus, as shown by the control specimen. The daughter-nuclei, which at first were not regular in form, became so after a time.

In a 6 per cent. solution of ethyl ether the nuclei finished cell-division and formed a cell-wall. An hour later the protoplasmic movement was still noticeable, but the daughter-nuclei appeared vacuolated and did not become round as usual. At the expiration of twelve hours the cells which had been left in this solution of ethyl ether were dead.

In a 7 per cent. solution of ethyl ether the nuclei in the prophase stage made no advance towards completing division, and the protoplasm, which showed at first a feeble movement in the older cells, stopped in fifty-five minutes. When not left under the influence of a 7 per cent. solution too long, the nuclei began to divide again, but always by karyokinesis. In all the experiments with ethyl ether, as with the other reagents, it was seen that if the activity of the protoplasm was accelerated or retarded, or if the protoplasm was killed, the nucleus was in a short time likewise affected. My experiments further show that the nuclei of the staminal hairs of *Tradescantia virginica* cannot, as stated by A. Nathansohn[1], be made to divide amitotically through the influence of ethyl ether. It may, as just stated, only hasten slightly or prevent division according to the strength of the solution used. When sprays of *Tradescantia virginica*, with cells of the right age for cell-division, are, as Nathansohn states, placed under a bell-jar in water along with a dish containing a 2–2·5 per cent. solution of ethyl ether, the nuclei do not divide amitotically. Karyokinesis is only arrested, and begins again in a short time after admitting fresh air.

V. Effect of Chloroform.

When the cells of *Tradescantia virginica* were placed in a drop of a 3 per cent. solution of cane sugar and the air from a solution of chloroform-water diluted one-half with water was drawn over the cells, the nuclei in the prophase stage completed division and a cell-wall was formed. The movement of the protoplasm, which finally ceased, recommenced when brought into fresh water after three hours. Resting nuclei in a solution of chloroform-water diluted one-half with water did not divide. The air drawn from pure chloroform, as expected, killed all cells.

VI. Effect of Cold.

It is impossible for a nucleus at -3° or -4° C. to divide under any circumstances, as stated by Demoor. Even at 0° C. a nucleus cannot

[1] Jahrb. f. wiss. Bot., 1900, Bd. 35, p. 70.

divide. When, however, nuclei were subjected to a temperature of 1·5° C., they finished dividing and formed a cell-wall when in the prophase stage. Resting nuclei do not divide at less than 7° C., and then very slowly. When the nucleus is kept at 0° C. for a time and then gradually brought back to warmer air, as for example 20° C., the division will be completed and a cell-wall formed after three hours. The movement of the protoplasm was not visible at a temperature at which the nucleus could not divide.

VII. Effect of Heat.

When nuclei in the prophase stage were subjected to a temperature of 34° C. they finished dividing, but no cell-wall was formed. The time for division was increased by one half as shown by the controls. When the temperature was lowered to between 20° C. and 30° C. the cell-wall was formed. The nuclei always appeared normal, and no tendency to direct division, with either heat, cold, or any other agency, was observed.

VIII. Effects of Ammonium Carbonate.

Placed directly in a $\frac{1}{4}$ and $\frac{1}{2}$ per cent. solution of ammonium carbonate, the nucleus of *Tradescantia virginica*, when in the prophase stage, finished dividing and formed a cell-wall in fifty minutes. The daughter-nuclei were irregular in form.

In a 1 per cent. solution of ammonium carbonate the nucleus which had begun to divide stopped almost immediately. When, after one minute, fresh water was again supplied, it began again and completed division. If left longer than one minute in a 1 per cent. solution the protoplasm and nucleus were killed.

Summary.

The foregoing experiments warrant the following conclusions concerning nuclear division in *Tradescantia virginica* and the movements of the protoplasm in this plant and *Momordica Elaterium*:—

1. The protoplasm of these plants cannot move in a pure atmosphere of hydrogen or carbon dioxide.

2. Nuclei in the resting stage cannot divide in pure hydrogen or carbon dioxide. When, however, a nucleus is near the close of the prophase stage, it may complete division but never forms a cell-wall. The further division of the nucleus is only continued till the hydrogen or carbon dioxide kills or disables the protoplasm or penetrates to the nucleus, for when the nucleus is just beginning division it cannot complete division in pure hydrogen or carbon dioxide.

3. A very slight pressure (3 mm.) of oxygen is sufficient for the nucleus to divide.

4. Resting nuclei in a 1 per cent. or stronger solution of ethyl ether cannot begin division. In 1, 2, 3, 4, 5, and 6 per cent. solutions of ethyl ether, nuclei in the prophase stage finished division and formed a cell-wall, both processes taking place in somewhat less time than the controls. In a 7 per cent. solution of ethyl ether no advance towards completing division was made.

5. In a ½ per cent. solution of chloroform water, nuclei in the prophase stage divided and a cell-wall was formed. Resting nuclei could not divide.

6. Nuclei in the prophase stage can divide at 1·5° C. Resting nuclei cannot divide below 7° C.

7. Nuclei in the prophase stage can finish dividing, but form no cell-wall at 34° C.

8. Nuclei in the prophase stage in a ¼ or ½ per cent. solution of ammonium carbonate can divide and form a cell-wall. In a 1 per cent. solution of ammonium carbonate nuclei in any stage cannot divide.

9. Whenever nuclear division occurred in these experiments it was always by karyokinesis.

10. These experiments show that, contrary to the opinion of Demoor, the nucleus cannot divide independently of the protoplasm, and that if the protoplasm is killed or temporarily disabled the nucleus is also soon likewise affected. The only reason that the nucleus continues to divide is that a little time is naturally necessary for the reagent to affect the protoplasm or to reach the nucleus. Even when the activity of the protoplasm just ceases, nuclear activity ceases also. It is just as impossible for the nucleus to divide after the protoplasm has been killed or its activity annulled as it is for either the nucleus or protoplasm when separated to continue to live, no matter how perfect all other conditions may be.

In conclusion it is my pleasant duty to express my thanks to Prof. Pfeffer for his constant kindness and advice in these experiments.

Studies in the Dictyotaceae.

III. The Periodicity of the Sexual Cells in Dictyota dichotoma.

BY

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With six Diagrams in the Text.

WHILE studying the development of the sexual cells in *Dictyota* the interesting discovery was made that the process was practically simultaneous, not only for a given plant, but for all the plants of the locality; that the crop so produced took but little time to mature and become liberated, and that a succession of many of these crops were produced during the course of the fruiting season. A closer study enabled one to see that each crop was initiated, matured, and discharged all within a fortnight, and that a general liberation of the oospheres and antherozoids of the locality took place on a certain day, or sometimes two or three days, immediately after the highest spring tide.

Although the main facts were put beyond any doubt at an early period of the inquiry, there were slight variations in the times of maturation of some of the crops, and of particular sori, as well as of occasional plants. These deviations from the general rule took a longer time and more careful study to account for. Records have been systematically kept of the details relating to these crops from 1897 to the present year, and these enable us to come to a definite conclusion with regard to the factors concerned in bringing about the periodicity, as well as in producing local departures from the normal course of events.

In order to understand the facts, a general description of the sexual plant will be useful. *Dictyota* is an annual. Germlings, and, in all probability, small vegetative shoots from the preceding autumn which have remained dormant during the winter and spring, commence to elongate during May, and here and there fruiting plants may be met with about the end of June, but reproduction is not by any means common until the end of July. During August and September the process is general and perfectly

[Annals of Botany, Vol. XIX. No. LXXVI. October, 1905.]

regular in its periodicity, but towards the end of October it becomes slower, finally ceasing about the middle or end of November. In very mild and bright winters new sori may be produced even at Christmas ; this, however, is exceptional, and the sori sometimes seen on the plants in mid-winter are in most cases arrested ones from October.

The proximal portion of the thallus as well as the edges are always free of sori ; and when a plant is in its earliest crops and still actively elongating, the distal parts are also without reproductive cells. The remaining portions have on both surfaces numerous sori, which are all of the same age, except a few at the distal end of the reproductive area, which are always younger. The reason for the latter fact is evident : this portion of the thallus being younger and only just removed from the meristematic condition, arrives at the stage capable of bearing sori a few days later than the general initiation of the crop.

The gametangia arrive at maturity about the highest spring tide, and, as will be explained in detail in another section, they are liberated during a very short period of time soon afterwards.

At the period of liberation, or a few tides after, the faint rudiments of the sori of the new crop begin to make their appearance. They are not localized on any definite region as are the tetrasporangia of *Padina,* but are scattered over the parts occupied by the preceding crop together with a short distal extension of it. With each successive crop the clear distal area becomes smaller and smaller, apical elongation becoming gradually slower, until at last the reproductive area reaches close to the apical cell itself.

In a very short time after the escape of the gametes the walls of the gametangia degenerate and disappear, but, as already described [1], the position of the liberated sorus is always indicated by the old basal cells, which are continuous with the cortex or limiting layer of the thallus, but rendered conspicuous by their increased size and different colour. At first they are much paler, owing to the small number and size of the chloroplasts and the paucity of colouring matter in them. They gradually acquire a deeper colour, still the scars of the two last crops can always be distinguished from each other. In the case of the male plants the raised borders of sterile cells is a further indication of the position of the old sori. These basal cells are incapable of giving rise to new gametangia, and when the whole or nearly the whole surface has been used up in this manner the plant dies, the process being hastened in some localities by the attacks of endo- and epi-phytes as well as of various animals.

The following examples illustrate some of the above points a little more clearly :—

1. A male plant 12 in. long and $\frac{1}{4}$ in. wide below the first dichotomy. The distal $\frac{1}{2}$ in. was quite clear of sori, and the current crop reached $1\frac{1}{2}$ in.

[1] Ann. of Bot., xviii, 1904, p. 184.

beyond the scars of the last crop. Thus the elongation during the last fortnight was only one-third of that for the preceding fortnight.

2. Female plant in its third crop. The proximal clear area was 1½ cm. long, that occupied by the scars of the first crop 3 cm., the second crop 7 cm., and the current crop 13·5 cm., while the remaining 3 cm. at the distal end had not started reproduction. There is in most cases a progressive increase in the extension of the reproductive area in the case of the first three or four periods.

Vigorous plants produce such a number of sori that they soon exhaust themselves and decay. Many plants that start fruiting early are found in this condition by the end of September, and the first violent gale sweeps many of them away. The fact that we find many young plants in their first crop in early October proves that they must be the offspring of tetrasporic plants of the current season. There is thus a continuous succession of young plants commencing the process of reproduction from the end of June to mid-October.

The History of a single Crop of Sexual Cells.

In order to understand the course of events, it will be convenient to follow in detail the development of a single crop from the first appearance of the rudiments to the discharge of the mature gametes. Diagram I shows the results of observations at the Swillies in the Menai Straits during the early part of August, 1901, plotted down in such a manner as to show the relation borne by the various stages in the development of the crop to the heights of the tides. The vertical columns represent the days of the month, while the curve in the upper part shows the hours of high water and the heights of the tides in feet. The lower part of the diagram is utilized to denote the stage of division of the antheridium as seen in surface view. Thus, a small cross placed on the lowermost line indicates an undivided rudiment, and a similar one on the sixth line shows that there are sixty-four cells in surface view. After the final division of the antheridium a short time elapses before maturation is completed and liberation sets in, and during this interval the only division that occurs in the oogonium rudiment—that which separates the stalk-cell—is accomplished. In the diagram this is indicated by means of the dotted line. Finally, the stage at which liberation of gametes takes place is denoted by inserting the marks above the tenth line.

The first observations were made on the sixth of the month, when most of the antheridial rudiments were found to be undivided; a few were two-celled and a very small number four-celled. On the eighth and tenth the plants gathered showed hardly any advance, but by the twelfth the great majority of the antheridia were in the two- or four-celled stage. By the fifteenth many were apparently mature, and

some which were taken into the laboratory and kept in a damp chamber overnight and immersed in sea-water the following morning yielded motile antherozoids. On the seventeenth there was a general liberation of gametes in the Straits. In order to obtain abundance of swarming antherozoids it was not necessary on this occasion to place the male plants in a damp chamber overnight. By the evening of this day most of the female plants could be seen even with the naked eye to be clear of oogonia excepting for a few at the distal ends. By the eighteenth at least three-fourths of the crop had been cleared off: on the nineteenth only a few sori were left—most of these were the distal belated ones, others had been arrested just before maturity. On this day the first faint and undivided rudiments of the succeeding crop made their appearance. By the twentieth the whole plant was clear of the old crop, and the new antheridia, though still undivided, were more prominent in size and colour than before.

In this particular case, general liberation took place about three tides after the highest spring. If in the diagram we connect this and the middle of the line of average development for each day, we get a curve which we may call the *optimum of development.* This we may safely regard as expressing the time of development of a single sorus, and the great majority of the sori in the locality coincide with it in their times of initiation and rate of development.

In the case of this crop the diagram further shows that although some of the rudiments appear eight or nine tides before the lowest neap, and others as late as one tide after it, the majority are initiated about four tides before the lowest neap. The life of an average sorus from its first appearance to its discharge would be about nineteen tides (ten days); the width of the 'crop-band' is from eight to ten tides, and the whole time taken by the crop from the appearance of the earliest of the rudiments to the liberation of the latest is about twenty-eight tides. The presence of younger sori on the distal part of the reproductive area of certain plants has already been explained, and it is clear that such cases should be excluded from the diagrams, for in the case of plants which have ceased to elongate, and where the reproductive area reaches to the very apex, all the sori are practically in the same stage. It should be observed, however, that most of the distal sori thus belated in their initiation accelerate their development to such an extent that their liberation is not much behind the others, so that even including them the periodicity is still well marked. There are other circumstances which sometimes interfere with the periodicity—these will be dealt with further on—but they do no more than slightly modify the normal process.

Comparison of Different Crops.

If now we study the crop for late August and early September, 1902, and compare the results, as represented in Diagram II, with those just described, we find that although there is general agreement between the two, there are several differences in matters of detail. We first observe that general liberation is delayed until about the fifth tide after the highest spring. Now as maturation and liberation are so evidently dependent on the spring tides, one would naturally expect the process to be accelerated directly as the height of the spring tides. Here, however, although all the spring tides in the second series are higher than those of the first, liberation, instead of being earlier, is actually later. This state of things occurred so frequently that it was at first conjectured that the increase in the amount of exposure during low water of the higher spring tides subjected the plants to more than their optimum of light or of heat, and in that way brought about their retardation. Though it is quite true that the plants are very sensitive to light, and possibly to variations of temperature, we now know that the above suggestion is not the correct one.

There are other variations also which appear in the tabulated results of the observations. The length of the optimum line may be nineteen tides, as in the diagram quoted, or any number up to twenty-five tides. The width of the crop-band may vary from five tides to about ten, while the whole time occupied by a crop may range from twenty-four to thirty-four tides.

In order to explain these variations it was natural to look for their causes in the fluctuations of the amounts of light and heat. Consequently careful records were kept of the state of the weather, especially as to amount of sunlight and the temperature of the sea. It is now found that the influence of these factors is quite secondary. Thus the liberation of the crop of September 23, 1899, took place five tides after the highest spring tide, the weather being very dull, cold, and stormy during the whole period. The crop of August 25 in the same year was liberated exactly at the same interval after the highest spring, though the weather during this period had been continuously bright and warm. Particulars relating to the weather are consequently omitted from the tables, and only such data are included as have been found to have an influence in deciding these variations. At the same time it must be remembered that extremes of illumination or of temperature may assist other causes in producing acceleration or retardation.

When all attempts to explain these variations on meteorological grounds had thus proved abortive, the solution was sought for in the fluctuations of the tides themselves. Now, although the main fact of

periodicity is clearly dependent upon the difference between spring and neap tides, it was not anticipated that the slight differences between one spring tide and another would be reflected in variations in the time of appearance of the different stages of the crops. But, unlikely as it may seem, it is here that we must look for the primary causes of the differences, while meteorological phenomena of various kinds may occasionally either restrain or intensify them. When looked at from this standpoint the results are so simple and consistent that it may be of some interest to state that all the data for the North Wales coasts had been collected and tabulated in chronological order before the right clue to their meaning was discovered. This makes the record more valuable than if the explanation had been in one's mind while studying the facts.

The tidal peculiarities here referred to are either differences of rise between successive spring tides, or differences in the intervals of time separating them from each other.

1. There is generally a fairly regular alternation of higher and lower spring tides. Sometimes the higher ones occur at the New Moon, and the lower at the Full: at other times the reverse is the case. It is but rarely that the two spring tides occurring in a month are the same height. During the fruiting period of *Dictyota*, this inequality always obtains, and there is the further consideration that the equinoctial tides, which occur about this time, are higher than ordinary spring tides. Thus, to take two examples from the year 1899:—

	The spring tide on	August	9	was	18 ft. 4 in.
	„	„	„	25	„	21 ft. 8 in.
Again	„	„	October	12	„	18 ft. 11 in.
While	„	„	„	27	„	21 ft. 7 in.

In the following discussion the spring tides will be designated simply 'lower' or 'higher' springs, as the case may be, whether they are ordinary or equinoctial.

2. The interval between two highest spring tides, corresponding to and very nearly coinciding with the interval between New and Full Moon (or between Full and New Moon), and technically called a 'semilunation,' varies in length. In the tables it is sometimes as low as twenty-five tides, while at other times it is as high as thirty-one tides.

Tables I and II give the most important of the data relating to these two groups of tides. The records of the other tides are fragmentary, but as far as they go they are in complete harmony with the results here quoted.

The following are some of the most obvious deductions from the tabulated results.

1. In the case of the higher spring tides of either August or September

TABLE I. CROPS LIBERATED DURING THE HIGHER SPRING TIDES IN AUGUST AND SEPTEMBER. (MENAI STRAITS.)

	Date of liberation of crop.	1 *Height of highest spring tide.*		2 *Height of lowest preceding neap tide.*		3 *Number of tides from the highest spring tide of the last series to the highest of this.*	4 *Length of crop optimum, i.e. the number of tides taken to develop a single sorus.*	5 *Ratio of crop optimum to tidal period (col. 3), the latter taken as 100.*	6 *Time of maximum liberation, in tides after the highest spring tide.*	7 *Breadth of 'crop-band' in tides.*	8 *Number of tides from the earliest liberation to final clearance of crop.*
		ft.	in.	ft.	in.						
1	August 25, 1899	21	8	11	8	27			5		
2	September 23, 1899	21	9	11	4	28	21	75	5	6–8	24
3	August 15, 1900	21	4	11	3	29	22	76	5	10	
4	September 3, 1901	21	1	10	10	31	24	77	5	6–7	32–34
5	August 8, 1902	20	4	13	0	27	20	74	4–5	10	
6	September 6, 1902	20	5	12	11	25	19	76	5	5–6	
7	August 27, 1903	20	10	12	4	27	20	74	5	10	27

TABLE II. CROPS LIBERATED DURING THE LOWER SPRING TIDES IN AUGUST AND SEPTEMBER.

8	August 20, 1898	18	11	10	6	30	22	73	3	8–10	30
9	August 9, 1899	18	4	11	11	30			2		
10	August 28, 1900	18	8	12	1	27	21	78	3	10–12	28–30
11	August 17, 1901	19	3	12	11	25	19	76	3–4	8–10	29
12	August 23, 1902	19	3	11	0	31	25	81	3	10–12	32–34
13	August 3, 1903	18	1	12	2	29	22	76	3	10	

TABLE III. CROPS LIBERATED DURING OCTOBER AND NOVEMBER.

14	October 3, 1901	21	6	10	9	31			7	7–8	
15	October 12, 1899	18	11	10	5	29	22	76	12		
16	October 27, 1899	21	7	12	3	27	20	74	14	6	27
17	November 3, 1901	21	5	11	6	29			12		

liberation of gametes nearly always takes place about five tides after the highest, while in the case of the lower springs there is an apparent acceleration, and it occurs about *three* tides after the highest.

2. The above rule for the time of liberation is quite independent of the length of the interval from one spring tide to another. Thus in the table of higher tides, whether the semilunation includes twenty-five, twenty-seven, twenty-nine, or thirty-one tides, maximum liberation is effected about the *fifth* tide after the highest, and, excepting in the case of No. 12, there is the same uniformity in Table II.

3. Though the length of the interval between two highest spring tides does not determine the exact time of liberation, it has a direct influence upon the length of the period taken by an average sorus to develop; in other words, the length of the optimum line varies directly as the number of tides included in the semilunation. Thus in No. 6 the interval between the spring tides is twenty-five tides, while in No. 4 it is thirty-one. In the former case the optimum line is nineteen, while in the latter it is twenty-four. In Col. 5, where this relation is shown in percentages, this is seen more clearly. Out of thirteen recorded cases, nine are either 74 or 75 %, the average of the whole being 76 or, excluding No. 12, 75·4.

This confirms in a striking way the conclusion arrived at from other facts—that the development of the crops is chiefly dependent on the spring tides, and that the process goes on but slowly during neaps.

4. In the following table the crops are arranged according to the number of tides in the semilunation.

	Date of crop.	*Length of semi-lunation.*	*Height of lowest neap.*	*Length of crop optimum.*
			ft. in.	
1	September 6, 1902	25	12 11	19
2	August 17, 1901	25	12 11	19
3	August 8, 1902	27	13 0	20
4	August 27, 1903	27	12 4	20
5	August 28, 1900	27	12 1	21
6	September 23, 1899	28	11 4	21
7	August 15, 1900	29	11 3	22
8	August 20, 1898	30	10 6	22
9	September 3, 1901	31	10 10	24
10	August 23, 1902	31	11 0	25

While it confirms what has been said before, it also brings out another fact which must have some influence upon the length of the optimum or the rate of development. As a general rule, the longer the interval the poorer will be the neap tides, and in consequence the activity of reproduction will be correspondingly diminished.

5. It was at first thought possible that just as the spring tides were advantageous for maturation, so there might be some special virtue in *neap*

tides for initiation and the earlier stages. The above shows this idea to be erroneous. The earliest rudiments are generally formed while the liberation of the preceding crop is still going on ; hence there is a slight but distinct overlap. The optimum of initiation takes place generally from four to two tides before the lowest neap. It seems then that as soon as the old crop has ceased its demands upon the plant, the 'reproductive energy' becomes directed to the formation of new sori ; these make their appearance immediately. At first their progress is fairly rapid, but with the gradual lowering of the tides at neap, development becomes somewhat slower, to be once more greatly accelerated as the spring tides come on. Hence, if the optimum line were drawn with absolute accuracy, it would show a slight curve towards the vertical at each end, while the middle portion would be very nearly horizontal. This cannot be well shown in the diagrams, as the data are not sufficiently full.

6. We have taken the divisions in the antheridium as indications of the rate of development of the crop. As already explained, there is only one division in the oogonium rudiment—the one which separates the stalk-cell. In the case of the tetrasporangium this division takes place at a very early stage ; here it is delayed until a few tides before the highest.

7. In most of the cases which are fully recorded there is a fairly close and significant correspondence between the semilunation and the number of tides occupied by the whole crop from the very earliest initiation to the final clearance. In Tables I–III this is seen when columns 3 and 8 are compared for the crops numbered 4, 7, 8, 10, 12, and 16. Thus, although any given sorus takes much less than a semilunation for its development, the whole crop occupies practically the whole of the interval from a given tide to the corresponding one in the next series.

8. Initiation and liberation during the earlier crops each occupies about ten to twelve tides. Later, in September and October, there is far greater uniformity as to stages of development, and the width of the crop-band is reduced to from six to eight tides, sometimes even fewer. This can be well seen by comparing Diagrams I and II. The principal reason for this is probably the cessation of growth, for elongation, as has already been shown, exercises a slightly disturbing influence on the regularity of the process. It is possible also that the recurrence of the crop-periods impresses the rhythm more distinctly upon the individual ; this can be conceived of as possible whether we regard the periodicity as inherited or merely induced during ontogeny.

9. Reverting to a point already mentioned, the existence on the distal portion of an elongating plant of sori very much younger than on the remainder of the thallus naturally suggests an inquiry as to their subsequent fate. Do they mature and liberate with the current crop, or remain until the succeeding one? Very careful observation of a large

number of cases makes it certain that the great majority mature very soon after the others. In some cases, particularly when the conditions are unfavourable, they are arrested shortly before maturity, degenerate *in situ*, and very soon fall off. In very exceptional cases, more especially when very late in starting, they may remain on the plant till the succeeding crop. It is evident that in the first case there must be considerable acceleration as compared with the crop-optimum. This is probably due to the greater vigour of the part. Now, if younger sori can be started in the manner just described, at the 'wrong period,' so to speak, why could similar ones not be interpolated among the older sori on the other parts of the thallus? The probable answer is that the formative reproductive material or energy is diverted into the adjacent sori, which being already started form centres of reproductive metabolism for the neighbouring cells. When a plant gets old it frequently happens that the distal sori, instead of being later, actually liberate before the others. The reason for this would probably be found in the senility of the older parts and the greater metabolic activity of the distal portions, which have not as yet produced many crops.

10. In most cases the crop-band, if accurately plotted, would show a greater width at the initiation end than at the other; hence there must be acceleration of the later among the ordinary sori. (Here the exceptional distal sori discussed in paragraph 9 are excluded.) This is clearly shown in Diagram I, where the difference amounts to as many as four tides.

In Diagrams III and IV the optima of the two sets of crops above discussed are compared. The interval between the highest spring tide and the discharge of the gametes in the two cases is well shown, and the agreement between the crops in the respective groups is most striking. The diagrams also show the variation in length of the optima, but it does not indicate the corresponding variation in the length of the tidal periods.

In the above discussion the crops of October and early November are left out of consideration. In view of the regularity of the August and September crops those of the later months disclose a very remarkable retardation, which progressively increases until initiation coincides with the rising spring tides, and liberation is effected at, or just before, the lowest neaps, thus almost reversing their usual tidal relations. It would only be natural to expect a retardation towards the close of the season when so many unfavourable conditions supervene; the remarkable thing, however, is that *the optimum is not lengthened by a single tide.* This is well shown in Table III, where the percentages in column 5 are 76 and 74. Here, then, although there is great retardation, the periodicity is as clearly marked as ever.

In the second paper of this series I suggested that it would be interesting to find out whether the periodicity, which up to that time

had only been verified for the coasts of North Wales, obtained also in other districts, or was merely a phenomenon induced by circumstances peculiar to the locality. A number of short visits had been paid to Weymouth, Torquay, Sidmouth, and other places, and the sexual plants observed on these occasions clearly suggested periodicity. During the summer of last year, as well as of this, I spent five weeks at the Marine Biological Laboratory in Plymouth, and while there I was able to follow out the whole course of development of a number of crops. The periodicity is as well marked here as in the Menai Straits, but the liberation of the crops is delayed several tides beyond the time when it occurs on the North Wales coasts. This is exceedingly significant, and the reason for it will be discussed later on.

Furthermore, a careful examination of the plants in the herbaria of the Natural History Museum and of Kew shows clearly that periodicity occurs on the coasts of other countries, especially on those of France and Spain, and even in various parts of the Indian Ocean and Australasia. Besides, there are unmistakable indications of its obtaining in the case of several foreign species of *Dictyota.* At the same time specimens were seen where sori of different ages were mixed up together. It would not be wise to generalize too much from herbarium specimens, but we may safely conclude that periodicity obtains generally in the case of several species of *Dictyota* in seas where the rise and fall of the tide is fairly well marked.

Probable Cause of Periodicity.

Whatever factor be suggested as the cause of this phenomenon, it must of necessity be one which varies with the tides and which produces its most potent effect during or soon after the spring tides. There are several which seem to satisfy these requirements to some extent, such as variations in the degree of aeration and of pressure, and particularly differences of temperature and of illumination.

The amount of aeration may possibly have some influence on the form and on the size of the plants, but it is most unlikely that it has anything to do with determining the periodicity of reproduction, for the great difference in amount of aeration between deep-growing plants never exposed, and those which are habitually emergent during low water of spring tides, is not reflected in any way in the condition of the sexual crops.

The alternation of pressure may have a much greater influence as a stimulus than we generally give it credit for, but actual observation of the widely different kinds of habitats in which *Dictyota* flourishes—in rock-pools at half-tide level as well as in deep channels, some never exposed and others left bare by the majority of spring tides—persuade us that this factor does not satisfactorily explain the phenomenon.

As for temperature, though it is a fact that exposure to a very high degree of heat brings about irregularities in the development of the gametangia, or otherwise causes injury to the plants, it is extremely improbable that the particular fluctuations of temperature to which the plants are subjected as a direct consequence of the alternation of neap and spring tides, are the effective causes of the regular succession of crops. If we take the Menai Straits, for instance, the difference between the temperature of the water in the channels where the current is nearly always flowing, and that of the shallow bays and eddies, or the pools left by the retreating tide, is enormous. A few inches below the surface the temperature in the current is fairly constant, showing a mean of about 13° C. in June, 14·5° in July, and 15·5° in August. When plants are left in shallow rock-pools they may in a short time experience a temperature of 21° C. or more; and as for the plants that are left quite uncovered, they will be subject to extremes of temperature that are greater still. It is important to remember that the most luxuriant plants and those which display the greatest regularity in the development of their crops are those which grow in the deep channels, where the temperature shows the least amount of fluctuation.

The lowering of temperature during October and November may have something to do with the retardation of the crops, but only as a secondary cause, as will be shown below.

I have been definitely forced to the conclusion that the stimulus which incites the plants to produce their fortnightly crops of gametes is the periodic change in the amount of light received by them, as a direct result of the alternation of neap and spring tides.

The time and mode in which the rhythmic variation of light takes place varies in different localities according to the time of the tides. Thus in the Swillies (Menai Straits) low water of the highest spring tides always takes place about six o'clock, morning and evening, while that of the lowest neap tides takes place about noon. The result is that during low water of neaps a considerable depth of water covers the plants, and this intercepts much of the light (Diagram V). It would be instructive to measure the amount of light obtained by a plant at various times; hitherto there has been no opportunity of doing this. During low water of spring tides many of the plants are actually emergent, and the others are so near the surface that they suffer but little loss of light. This is shown in Diagram VI. But this is not all, for during neap tides only one low water period occurs during daylight, the other taking place about midnight, whereas in the spring tide periods there are two exposures to the maximum of light—one at six in the morning, the other about the same hour in the evening. If, as is here contended, light be the determining factor in bringing about this rhythmic succession of crops, then it is perfectly evident that in the

Menai Straits we have ideal conditions for securing to the plants a high degree of illumination which at no time is in danger of being excessive. Partly as a result of this, *Dictyota* grows far more luxuriantly and fruits more freely and with greater regularity in the Straits than in any other locality where I have studied it.

If the above hypothesis be true, a locality where the times of the tides are the reverse of those obtaining at Bangor ought to yield different results. At Plymouth, for instance, the low water of springs always takes place about midday, thus giving only one period of strong illumination during the day. Of the three crops which I have most closely followed at Plymouth, two were developed during the higher spring tides of early August—one last year and the other at the corresponding period of the present year. In both cases general liberation did not take place until seven tides after the highest. In the third case, which was a low tide one, the crop was still later; initiation did not take place until after the lowest neap, and the crop was not discharged until at least twelve tides after the highest spring. The temperature was lower and the light poorer during this period than it was during the two preceding ones, so that some portion of the retardation may be due to this cause. Even after allowing for this, it is clear that all the crops are later at Plymouth than they are in the Menai Straits, a result which fits in admirably with the 'light' hypothesis. It may be suggested that inasmuch as the times of the tides are the reverse of those in the Menai Straits, giving two daylight periods of low water at neaps and only one during springs, that the times of initiation and liberation should also be exactly reversed. It must be borne in mind, however, that at the spring low water the plants are very much more exposed to the light than during the neap ebbs, and, what is still more important, that the light at noon is far more intense than at six o'clock. When collecting in the vicinity of Plymouth I observed that luxuriant fruiting plants were never found excepting where they were shaded from the direct rays of the noonday sun during the spring ebbs. The various forms of *Dictyota* are very abundant on the south coast, but when they are compared with those growing on the North Wales coasts one is at once struck with the smaller size of the plants and the greater difficulty of finding well-fruited specimens. The luxuriance of the Menai Straits plants may be partly due to the greater range of the tides and to the continuous circulation of clean sea-water; but it is highly probable that much of the success of the plants there, as compared with those of the south coast, depends on the fact that the total illumination obtained by them in the Straits is greater than that experienced by the Plymouth plants, and at the same time that maximum illumination occurs at a time of the day when there is far less danger of excessive insolation.

It has been pointed out in a preceding section that after the higher

springs the crops in the Menai Straits are later in their liberation by at least two tides than after low springs. The idea that this apparent retardation is due to excessive illumination, or to exposure to the air during the extraordinary low water of these tides cannot be correct, for it obtains in deep channels and in well-shaded rock-pools just as in more exposed habitats. The explanation is to be sought for at the other end of the crop period. A low spring crop liberates early simply because it had a good start during the higher springs of the preceding series, and the comparative lateness of the high spring crop is due to the less favourable light conditions prevailing during its early stages.

In the case of Plymouth it is highly probable that the above relations are reversed. The two higher springs recorded agreed in having the liberation seven tides after the highest, while the low spring crop took as many as twelve tides before the plants were cleared. When the records are so few it is unsafe to generalize, but if the above relation be correct it will be seen that this also harmonizes perfectly with the hypothesis. In these cases the crop periods lie wholly within the respective spring tide periods. It is only natural then that a high spring crop should mature early. It is in accordance with this also that we find the optimum length in the latter shorter than in the lower springs.

The remarkable retardation of the crops in the Menai Straits during October can also be easily explained in the same way. That this reversal of the time is not due to very unfavourable circumstances is shown by the fact that the optimum length of the crop is not increased. As the day shortens, the light during both morning and evening of spring tide ebbs becomes gradually poorer, until at last the midday light of neaps is actually better than morning and evening light of springs. The result is that the crop periods very nearly correspond to those obtaining at Plymouth.

A very striking instance of this is also seen in Cardigan Bay, where low water of spring tides takes place about three o'clock. Here, during August and September, sexual plants are far more liable to injury by excessive insolation than are the Straits plants, while later in the season the light during the early morning hours is missing altogether. These two peculiarities combine to bring the fruiting period of the plant to a close much earlier than in the Straits.

In August and September there is an overlap of the crops amounting frequently to two or five tides. During October, however, there is a gradually widening interval between the clearance of one crop and the first appearance of the succeeding one. A very remarkable circumstance, however, is that in spite of the retardation in the time of initiation there is no lengthening of the whole crop period. This seems to show that the rhythmic influence is so fixed in the plant that when conditions of light (and of temperature) change, the new crop is delayed until there is a sufficient

accumulation of reproductive energy and the necessary amount of light stimulus to start the 'wave' of activity.

By mid-November it generally happens that the antheridial sori become very small, and only a few of the cells in the centre of the sorus divide up to form antherozoids. This is chiefly due to senility (aided, of course, by unfavourable conditions), for one finds even at this time vigorous young plants in their first or second crop where this does not occur, and female plants with very few unshed oospheres. This shows the importance when studying the periodicity of these crops to take into account the age and vigour of the individual plant. In the earliest records also there was another slight error arising from the fact that when plants whose crops are nearly mature are brought into the laboratory, the liberation of gametes may be accelerated. In this way one has frequently succeeded in getting antherozoids to swarm in the laboratory five tides before liberation has started in the sea. How this acceleration is brought about—whether by increase of light, a higher temperature, or increased oxygenation—has not yet been made out, though several attempts have been made to solve the question by direct experiment.

It has been shown in the case of several plants, particularly of Algae, that a mere change of environmental conditions suffices to stimulate them to reproductive activity, or to a change in their mode of reproduction. It is a point deserving of consideration, whether the alternation of extreme conditions experienced during the ebb and flow of the spring tides does not in some way have an effect upon sexual reproduction in *Dictyota*. If it has any influence it clearly can be nothing more than a stimulus, and the evidences in favour of light being the effective agent in controlling periodicity appear overwhelming. In the Menai Straits, where there are two periods of full illumination during the day, liberation takes place very soon after the highest spring tide; at Plymouth, where there is only one such period, liberation is retarded. In the Straits, when the periods of maximum light change owing to the shortening of the day, the whole crop period is correspondingly shifted without incurring any alteration in its length or any other respect. On the North Wales coasts the liberations after low spring tides are earlier than those after high springs, whereas at Plymouth the contrary seems to be the rule. In all these cases the peculiarities are easily correlated with the times of springs and neaps and the consequent effect upon the light.

I have spoken above of light as *controlling* the periodicity of gamete production, and not as causing it. This is because it is shown in the next section that the phenomenon may occur in the total absence of tides and of any fluctuation in light besides the usual diurnal one.

Is Periodicity an Hereditary Character?

The question which naturally arises at this point is—to what extent is the phenomenon of periodicity in *Dictyota* due to heredity? There are two possibilities which may be considered. We may conceive in the first place, of sensitiveness to a light stimulus being transmitted to the sexual generation in such a way that in all cases where there is a rhythmic fluctuation of light there will also be a corresponding periodicity in the production of gametes. Thus the *tendency* would always be present, only requiring the appropriate stimulus to manifest itself. In localities where there are no perceptible tides this stimulus would be absent, and consequently there would be no periodicity of crops. In the alternative case we may conceive of the character being so firmly fixed upon the sexual generation, that periodicity would manifest itself under any conditions, whether there would be any corresponding fluctuation of light or not. Should this supposition be the correct one, then the rôle of the light is merely to control the periodicity by fixing the periods of initiation and liberation, but not in any way to originate it.

At first the facts seemed to point to an ideal case of direct induction of a character by environmental conditions during the ontogeny of the plant. Thus the crops of June and early July appeared very vague and indistinct in their periodicity, for on the same plant would be found sori of several different ages. One might naturally suppose this to be due to the plants being as yet too young to have acquired the rhythm. Following this came the more and more distinctly marked periodicity of August and September, succeeded in October and November by retardation and irregularity, as the conditions became altered. The whole hypothesis seemed to fit the facts in a most admirable manner, but it was soon shown to be illusory. If only the portions of the July plants already mature be considered, the crops are found to be perfectly normal in their time of development, and in the case of the October crops the retardation really follows the changed light arrangements, and the finding of old and young stages together late in the season is chiefly due to the failure of many of the sori to become quite mature. Thus the periodicity is perfectly regular throughout, and there are no evidences of its being induced.

It would be very interesting to ascertain what happens in those seas where there are no appreciable tides; still more instructive would it be to cultivate the sexual plants from tetraspores. This is a most difficult undertaking, but it is to be hoped that some means will be found of getting plants under culture to thrive as in the sea. The history of one of the experiments tried by me is very curious and is exceedingly important in this connexion.

It has already been explained that the plant never fruits in the sea before June, and also that a fruiting plant transferred into a culture vessel in the laboratory may complete and liberate the current crop, but it is very unusual indeed for it to mature another. In October of last year two plants of *Dictyota*, one male, the other female, were placed in a glass dish about eight inches in diameter and containing about an inch of water. The dish was then closed with a lid and left absolutely undisturbed ; the lid was not raised until the beginning of April in the following year. When examined the bottom of the dish was covered with what appeared to be brown dirt, but which on closer examination turned out to be a multitude of healthy little germlings, evidently the result of normal sexual fusion and not of parthenogenesis. The parent plants themselves had disappeared, with the exception of a number of little tips of branches, not more than $\frac{1}{4}$ inch long. When these were examined every piece was found to bear young sori, and *these were all in the same stage of development.* Two days afterwards the oogonia were apparently mature, and in two days more they all were liberated, while on the succeeding day the oospores had completed their first segmentation. In the same manner two more crops were developed. The first and second *took about the usual time* to pass through the various stages, but the last of the three was a weak one: many of the gametangia failed to mature, and after that the little branch-tips died, partly from exhaustion, but partly, it is to be feared, as a result of the interference of the experimenter.

Judging from the fate of numerous other cultures, the following was probably the course of events. The two plants when first transferred into the dish suddenly stopped the production of crops for a considerable period. After a time, however, they were able to adapt themselves to their changed environment, and when the necessary metabolic processes had been going on (very slowly, it is true), there came at last a time when the slow accumulation of reproductive energy caused the simultaneous initiation of sexual cells over the whole plants without the intervention of any kind of external stimulus. Gradually the older, basal portions became exhausted, died and decayed, leaving the bigger branches beyond the first dichotomy free. Still later, the smaller branches became separated in the same way, but each separate branch, however small, continued its rhythmic production of gametes, and on all the separate little pieces *the process was carried on simultaneously.*

After labouring to demonstrate the complete dependence of the periodicity upon the tide-table, it is startling to come upon a case like the above, where there cannot possibly be any circumstance external to the plant to suggest any rhythm in reproduction. It may be argued that inasmuch as these individual plants had been subjected to tidal influence when growing in the sea, they may have acquired the periodicity in such

a way as to 'remember' it, so to speak, after a period of quiescence, and under circumstances when the original stimuli were no longer present, where the temperature was fairly uniform, and when there was no fluctuation in the amount of daylight. This may have some amount of truth in it, but it seems to me far easier to believe that the periodicity is hereditary: that it arose in some ancestor of *Dictyota dichotoma*, and that it was afterwards modelled and fixed by the alternation of neap and spring tides until it became what we now see it. Even should this explanation be correct, it is difficult, however, to see how the crops in the dish should maintain the same optimum length as before, and especially how the small fragments should synchronize so remarkably after their separation from each other.

Briefly stated, then, the conclusion we come to is, that the periodicity of the sexual cells is a hereditary character, and consequently may be expected to manifest itself in seas and habitats where there are no tides. At the same time the sexual cells are so responsive to changes in the amount of illumination that the time of their development in seas where there are tides is regulated by the increased illumination obtained during the low water of spring tides.

Local Conditions and their Effect upon the Crops.

The most favourable conditions for the production of regular crops of gametes are the following:—

(1) Ample light for their needs, without exposure to much direct sunlight at low water.

(2) A plentiful circulation of pure, clean water. As the plants are easily torn away or otherwise injured, the currents or waves must not be violent.

(3) Freedom from epiphytes and from dirt.

A few examples will show how the crops are affected when these conditions are not fulfilled.

Near the Menai Bridge a very shallow pool, four feet above low-water level, had well-grown sexual plants of *Dictyota*, whose sori were very unequal in development, and many of which failed to mature. In the channel below, growing on *Halidrys*, were luxuriant clean plants whose sori were all in the same stage, and of which none failed to mature. Immediately below these, but growing on the bottom of the channel, were other plants which were generally white with mud. At spring tide such plants often showed many sori near maturity, but with oogonia near their edges, which had been arrested and were degenerating, numerous whole sori which had ceased developing, sori that were fairly young, together with a large number of germlings growing *in situ*.

In the first example the irregularity and failure were due to excessive insolation and a high temperature, caused by the high level of the pool and consequent long exposure. In the case of the bottom plants, the same result is brought about by the continual settling of dirt on the surface of the thallus. Most of this foreign matter rests upon the hair clusters of the plant, and any sharp movement of the plant suffices to shake it off. The irregularities caused by the dirt are far greater in the lower portion of the thallus, where movement caused by currents is less felt. It must be kept in mind that even in these cases the periodicity is still sharply marked.

At Gallows Point, near Beaumaris, there are a number of muddy pools where *Dictyota* grows well. When these are covered at the flow, there is an eddy which prevents the current being much felt. The result is that the plants are to a great extent deprived of light by the mud suspended in the water, and especially by that which settles on the surface of the plants, making them appear hoary white. As this dirt is mostly kept from actual contact with the surface cells of the thallus by the hairs, the injury from mechanical irritation must be very slight compared with that caused by loss of light. On the same plant might be found, in addition to the normal condition, a large number of arrested gametangia, others that were decayed, oogonia which had reverted to the vegetative condition and segmented in the manner described in my last paper, and still others apparently mature—probably belated ones from the preceding crop. In addition to this, we find that the plants here are later in starting their reproduction by a fortnight or a month, and that they are slower in maturing their crops, than the cleaner plants of the Swillies.

In several places on the coast of Anglesea and Carnarvon, as well as in the English Channel, there are quiet sheltered bays where, during ordinary springs, there is a sufficient depth of water to protect the plants from excessive insolation. During the extraordinarily low water of the equinoctial springs, however, the plants are nearly or quite exposed; and as in many of these localities the exposure to maximum light takes place at midday or early in the afternoon, the result is that the plants suffer injury, the thallus becomes pale, and the gametangia are killed. The antheridia are fully divided and appear quite mature, but the antherozoids passively float out. It is probable that one reason for the failure of algologists to recognize the motility of the male gametes was that the specimens they studied had been injuriously affected in this way.

Another circumstance that affects the crops unfavourably is the presence of other plants within or upon the thallus, and it is in situations like the above that this is most apt to be troublesome. Endophytes generally grow between the cells, and in some cases their presence is

not so prejudicial to the plant as is that of epiphytes. Spores of small Algae are apt to be caught by the projecting edges of the sori, and if their growth is very rapid they deprive the surface cells of much of the light they would otherwise obtain. At Plymouth a small green Alga particularly affected the antheridial sori, the surfaces of which it covered with branching, creeping filaments before they managed to liberate their contents. When the hair clusters are eaten off the thallus by marine animals, as they frequently are, the access of foreign spores is greatly facilitated. When the plant becomes infested in this way with epiphytes the result is immediately observable in a diminished production of gametangia, a greater inequality in the ages of the sori, and some amount of delay in their maturation. In this connexion the general ecology of the habitats becomes important. In some localities most of the Algae reproduce in winter or spring, while in others the majority are summer- or autumn-fruiting plants. It is clear that *Dictyota* in the former habitats will be freer of epiphytes than in the latter, and their own reproduction will be correspondingly more successful.

During two visits paid to Meadfoot (Torquay) this August, most of the sexual sori were found to be several stages later than the Plymouth plants. The plants were apparently free from epiphytic growth, but on examining them microscopically they were found to be completely covered with exceedingly fine, nearly colourless, filaments, seemingly of a cyanophyceous nature, and it was probably this growth that caused the marked retardation of the crop.

It has been proved above that changes in the meteorological conditions do not determine the main facts of periodicity for any locality, but that they may, and frequently do, bring about slight modifications in the time of development by either accelerating or retarding it. One striking result of continuously favourable weather conditions is to make the development of the sori more uniform. In the diagrams this is visible in the narrowness of the crop-bands. When, on the other hand, unfavourable weather prevails, the sori are less uniform and the crop-band becomes wider.

If meteorological observations were kept, in order to test the effect of weather on the crops, mere daily records of the total amount of sunshine, mean temperature, &c., would not be satisfactory, for the state of the weather during low water of spring tides is manifestly of greater importance to the plant than during any other time. This applies to the amount of sunshine, the temperature of the air and of surface water (in the case of exposed or nearly exposed plants), and also the force of the wind. The effect of the latter on plants in the lower zones is much greater at low than at high water. If the wind is moderate the movement of the waves is beneficial to the plants in causing effective

aeration, and in keeping their surfaces clean ; if, on the other hand, the wind is violent, the waves tear the plants away or injure them by abrasion of the surface cells, causing various kinds of adventitious growths. When *Dictyota* grows on surf-beaten rocks it is generally dwarf, and in female plants most of the oogonia fail to mature, but divide vegetatively until the whole surface becomes thickened with parenchymatous growth.

Why oogonia, when arrested, should sometimes degenerate and ultimately fall off, while at other times they revert to the vegetative condition and form multicellular growths which remain permanently on the thallus, we cannot decide, nor do we know exactly under what particular conditions this takes place, except that it is apt to occur late in the season, and also in laboratory cultures. It is highly probable that in the second case the arrest takes place at an earlier stage than in the first.

Having described habitats where unfavourable conditions prevail, it would not be amiss to describe the one where I have found the sexual plants to flourish best. This is in the Swillies, a part of the Menai Straits already alluded to, where there are deep channels with clean sandy bottoms, through which there are continuous steady currents of clear water. Immense tufts of *Halidrys* grow down in the lowest zone, and on these tufts an abundance of luxuriant *Dictyota* plants flourish which, in consequence of the clearness of the water, and the currents, aided by the continual swaying movement of the long *Halidrys* on which they are borne, are kept perfectly free of both dirt and epiphytes. These plants are of a large size, healthy in appearance, and of a rich deep colour, while their crops are most abundant and regular in their development. On the south coast the habitat most nearly resembling this that has come under my observation is in some deep channels to the landward side of the Mewstone (Plymouth), where clean and luxuriant *Dictyotas* grow, not upon *Halidrys* in this case, but upon *Cystoseira*.

It has been pointed out that one of the results of unfavourable conditions is the arrest and sterilization of gametangia. It is interesting to note that these are nearly always located at the outer edges of the sori. In this way the normally borderless sori of oogonia acquire borders of sterile cells, and in extreme cases all the gametangia are arrested, or only a few in the very centre arrive at maturity. Even in the case of plants where there has been no sterilization the same thing is observable, in the fact that liberation generally starts in the middle of the sorus. This localization of the reproductive energy is interesting. First there seems a 'flow,' as it were, into definite areas which change with each crop; then we have greater vigour in the centre of such an area, as shown in the more frequent failure of the peripheral cells.

While studying the periodicity of *Dictyota* this summer at Plymouth,

I gathered between Penlee and Rame Head a unique specimen. Though I have examined many thousands of plants in different parts of the coast, I have never come across one similar to this. It was apparently a male plant with the antheridia mature, but in the lower part the sori had very numerous oogonia as well. All the sori on the distal third of the plant were male. On the remainder of the plant the number of oogonia progressively increased towards the base, so that they were most numerous on the weakest part of the plant. Some of the sori had a few oogonia at one end or close to the periphery: in a few curious cases the borders of the male sori had been converted into oogonia. Some sori were wholly female and devoid of borders, and in cases where there were a few antheridia these were squeezed up in the centre between the swelling oogonia. Besides the general appearance of the plant, another circumstance pointed to its being a male which had developed female cells, and that was that in a great many cases the oogonia were derived from half-antheridia: that is to say, the antheridium rudiment divided once, or even twice, and then the two, three, or four resulting cells became oogonia. This, of course, resulted in very great inequality of size in the oospheres. While examining the specimen many of the eggs could be seen emerging from the oogonia. Three pieces of the plant were placed in as many culture-vessels, to see if the eggs would be fertilized; and as a control experiment, pieces of ordinary male and female plants were placed in a fourth. The antherozoids were not attracted by the oospheres. Sometimes wandering sperms would approach an egg, stop an instant above its surface and then proceed on their way—a fact which is of some significance in view of recent attempts to prove that there is no attraction in external fertilization. When the cultures were examined a few days afterwards the eggs in the control-culture had become normal germlings in an advanced stage of segmentation, but in the three other cultures a few eggs had divided parthenogenetically (a further proof that the bodies were eggs, and not gigantic sperms), while the rest remained undivided. At the time of writing the control-germlings are growing well, the other three sets died. An examination of the older scars shows conclusively that the previous crops were similarly anomalous.

Periodicity in Haliseris.

During the two past summers strenuous attempts have been made to work out the history of sexual reproduction in the remaining British genera of the Dictyotaceae—as yet, however, with but indifferent success. Though abundance of *Taonia* and *Padina* have been obtained, not a single sexual plant of the former, and but very few of the latter, have been collected. *Haliseris* is a more uncommon plant, but there is not the same disproportion between the numbers of the sexual and asexual individuals. At Plymouth

the plant is only obtainable by dredging, and even then it is so scarce that it is very difficult to hit upon. Though excellent tetrasporic specimens had been obtained, it was not till August 22 that sexual plants were brought up. The gametangia in these specimens showed such uniformity that periodicity was irresistibly suggested. All the antheridia were half-developed, showing 16–32 cells in surface view, and there were no rudiments. Scars of one previous crop were disposed in close proximity to the midrib, while the current crop area extended laterally outwards, and in a proximal as well as distal direction.

The next successful dredging was on August 29. All the antheridial sori were mature, and there were no rudiments. The female plants had numerous mature oogonia, and no young stages ; but the numerous empty oogonia with the covers still undissolved indicated very recent liberation.

On September 1 some more plants were dredged. Of these the male plants had all the antheridia completely discharged, and there were no fresh sori, while the female plants had the mature oogonia liberated, but showed numerous very faint rudiments. The evidence in favour of periodicity is very strong, but without additional data it is impossible to decide to what extent it conforms with the periodicity of *Dictyota.*

While studying *Haliseris* two additional facts were ascertained, which, though not bearing directly on the subject of this paper, are of sufficient interest to justify their inclusion :—

(1) The antherozoids, as surmised by Johnson[1], are ciliated, and their activity is quite as great as that of the antherozoids of *Dictyota.*

(2) The liberated oospheres of *Haliseris*, when not fertilized, segmented a few times parthenogenetically and then died, exactly in the same manner as the unfertilized eggs of *Dictyota* do.

Summary.

1. The reproductive period of *Dictyota dichotoma* in this country begins about the end of June or the beginning of July, and continues till the end of October or mid-November, the most active months being August and September. The antheridia and oogonia are produced in fortnightly crops which synchronize with the spring tides. In any given locality the great majority of gametangia on all healthy, mature plants are initiated at the same time ; they pass through the several stages of development simultaneously, and general liberation and fertilization take place on a particular day, at a fixed interval after the highest spring tide.

2. In the Menai Straits the rudiments appear a few tides before neap, and the mature gametes are liberated from three to five tides (i. e. on the second or third day) after the highest succeeding spring. At Plymouth the crops are later, and liberation does not take place till from seven to twelve

[1] Johnson ('91), Lin. Soc. Journ., xxvii.

tides after the highest. The October crops in the Menai Straits are retarded until the original times of initiation and liberation are almost reversed.

3. Whether liberation in the Menai Straits takes place three tides after the highest, or whether it is delayed till the fifth tide, depends primarily not upon meteorological conditions, but upon the heights of the spring tides. Of the two sets of springs which occur during a month, one is generally higher than the other. This alternation of higher and lower springs is fairly regular, and, during the fruiting period of *Dictyota*, is very well marked, owing to the exceptional equinoctial tides, the difference in some cases amounting to three feet. In the Straits the crop matured during the higher springs is liberated about the fifth tide after the highest, whereas in the succeeding lower springs the crop is liberated about the third tide after the highest. At Plymouth the reverse is the case, the shorter interval (seven tides) coinciding with the higher springs, and the longer (ten to twelve tides) with the lower spring tides.

4. The 'optimum of development' (= the time taken by an average sorus to develop) is variable in length. In the Menai Straits it may be from nineteen to twenty-five tides. This inequality is due not to meteorological conditions, but to the variable length of the 'semilunation,' or the interval between new and full moon, and consequently between the springs. Sometimes this is as low as twenty-five tides, while at others it is as many as thirty-one. The length of this interval does not affect the actual period of liberation, but it determines the length of the 'optimum of development,' the average ratio of the latter to the semilunation being 76 : 100.

At Plymouth it is probable that the length of the 'optimum of development' is directly dependent on the height of the spring tides, and not upon the length of the semilunation.

5. In the Menai Straits, where the crop is started during the waning of one set of springs and matured during the succeeding set, development is at first fairly rapid; it then becomes slower during neap tides, finally being accelerated again with the increasing 'rise' of the spring tides. At Plymouth, where the crop starts at or soon after neap, development is at first slow, but becomes progressively more rapid towards the close of the period. Thus all the facts go to show that development is determined by the time and height of the spring tides, and that progress is very slow during neaps.

6. Owing to this acceleration of development at maturation, the late-starting sori at the distal ends of growing plants are not much behind the rest of the crop in their liberation, and the 'crop-band' is consequently several tides narrower at the liberation than at the maturation end.

7. As the season progresses the crops show greater uniformity in the ages of the gametangia, so that the 'crop-bands,' which at first were ten to twelve tides wide, become narrowed to six tides or fewer.

8. Of the various factors (temperature, pressure, aeration, &c.) which fluctuate with the alternation of neap and spring tides, the one which seems to account most satisfactorily for the facts of periodicity is the increased illumination experienced by the plant during low water of spring tides; and the striking difference between the details of periodicity at Plymouth and in the Menai Straits are due to the difference in the time of day at which low water of spring tides occurs in the two places.

In the Menai Straits (east end) this always takes place between five and six, morning and evening, so that there are two periods of maximum illumination every twenty-four hours, whereas low water of neaps occurs about twelve o'clock. Here there is only one ebb during daylight, and the illumination then is much poorer than during low water of springs. At Plymouth the reverse is the case, low water of spring tides occurring at noon and at midnight; thus the total amount of light obtained during springs is less, and the difference between neap- and spring-tide illumination is not so great as in the Straits. The result is that at Plymouth the plants are poorer, and the crops several tides later in their development.

9. Other peculiarities in the times of the crops may be explained in the same way, e. g. the retardation of the October crops in the Menai Straits is due to the loss of morning and evening illumination owing to the shortening of the day. (This retardation is not accompanied by a lengthening of the crop period.) The earlier liberation after low springs in the Straits is due to the better start obtained by the crops during the preceding higher springs. At Plymouth, where each crop, starting about neap, coincides with a single set of spring tides, the time of liberation (and probably also the length of the crop period) is directly dependent on the height of the tides.

10. The times of initiation and liberation thus fixed primarily by the time and height of the spring tides may be slightly accelerated or retarded by exceptional meteorological conditions. Winds may cause a difference of two to three feet in the height of the water, and a rise of one inch in the barometer may result in a depression of six or seven inches in the tide, while extreme fluctuations of temperature or light may directly influence the rate of development of the crop.

11. So intimate is the relation between the tides and the crops of sexual cells, that a study of the tidal almanac for any locality will enable us to predict the actual days during August or September in any given year on which general liberation and fertilization of gametes will occur; and even should exceptional conditions prevail, the resulting error will only be about a day earlier or later than the one predicted. It follows that in all places on our coasts where low water of springs occurs at the same hour (i. e. in places with the same 'establishment') maximum liberation will take place on the same day.

12. A study of herbarium specimens shows that periodicity in the case of *Dictyota dichotoma* obtains in other seas besides our own, in localities widely separated from each other and extending as far as Australasia, and in several other species of the genus. There is as yet no evidence to show that it occurs in seas where there are no appreciable tides.

13. In the case of the tetrasporic plants of all our British genera of Dictyotaceae there is no trace of periodicity. Throughout the season, and at every stage of the tides, sporangia of all ages may be found together on the thallus.

14. In a laboratory experiment fragments of plants collected in October and left undisturbed in a small glass dish produced a succession of crops the following April. All their stages were quite similar, the length of the crop period was exactly the same as if the plants had been in the sea, and liberation and fertilization took place in a perfectly normal manner. The natural inference is that periodicity is a hereditary character. At the same time the preceding observations show with equal clearness that in seas where there are tides the periods of the crops are directly regulated by the alternation of high and low tides, so as to secure for the crops the maximum advantage possible from the increased illumination during low water of spring tides.

15. The above observations apply to 'healthy' plants. Such plants require for their development:—

(1) Ample light, without exposure to much direct sunlight at low water.

(2) A continuous circulation of pure, clean water.

(3) Freedom from endophytes, epiphytes, and dirt.

Where these conditions are not secured the following results may ensue:—

(1) Delay in commencing the reproductive season.

(2) Retardation of the crop.

(3) Inequality in the ages of the sori.

(4) Reversion of half-developed gametangia to the vegetative condition.

(5) Degeneration of more advanced gametangia.

In quiet bays without much shelter the plants are apt to suffer from excessive insolation, especially if the weather happens to be very bright during low water of equinoctial tides: this is generally associated with the attacks of parasites, which are particularly abundant in such waters. In such cases the antherozoids are discharged from the mature sori in a passive condition.

The lower temperature and diminished light towards the close of the season result in smaller sori and a great increase in the number of aborted gametangia.

16. A solitary specimen of *Dictyota* was collected whose sori had both

oogonia and antheridia. Some of the oogonia were small, being equivalent to half- or quarter-antheridia. When liberated none of the oospheres attracted the antherozoids, but some of them divided parthenogenetically and then died.

17. *Haliseris* seems to have a similar periodicity in the development of the sexual cells, but the details of the process have not as yet been fully worked out. As surmised by Johnson, the antherozoids are ciliate. They are also very active, and the unfertilized eggs show the same parthenogenetic phenomena as do the eggs of *Dictyota*.

Having discovered a plant whose sexual reproduction shows such remarkable response to external stimuli, it is evidently desirable that observations such as have been here attempted should be made on other coasts, and at the same time be made much more detailed and accurate. My apology for the incompleteness of my own is that they were really only incidental to my cytological work on the group, and made under great difficulties in the short intervals obtained during professional work. If such work be taken up by other algologists, I hope this paper will be of some assistance in setting about the initial steps. To perform such a task successfully, it would be necessary to find a habitat such as the Swillies, where an abundance of clean and healthy plants could be obtained. Plants should be collected and examined at least every alternate day, records should be made from healthy mature plants only, and full particulars should be kept of the times and heights of the tides, and—a point which I omitted to mention in the body of the paper—the direction and force of the wind and the state of the barometer should be entered, for the two things frequently modify very materially the tides as given in the tidal almanac. In fact, the only method of dealing satisfactorily with the tides would be to have a self-recording tide-gauge: this would give an absolutely faithful record of the height of the water at any given moment. In addition to this it would be necessary to accurately measure the amount of light obtainable at different periods of the tide and the variations of temperature. Were this sort of accurate work done at a number of stations, it would form a valuable contribution to our knowledge of the response given to external stimuli by the reproductive process in this plant.

I gratefully beg to express my obligation to the Council of the Royal Society for giving me a grant of money to enable me to carry on my study of the Dictyotaceae on the South Coast, and also to the Royal Microscopical Society for nominating me to their table in the Marine Biological Laboratory at Plymouth. To Dr. Allen, the Director of the latter institution, and his assistant, Mr. Smith, I also wish to express my deep obligation for their unfailing courtesy and their readiness to place the resources of the establishment at my disposal. It is a matter of surprise that so few algologists take advantage of such a well-equipped and admirably conducted station.

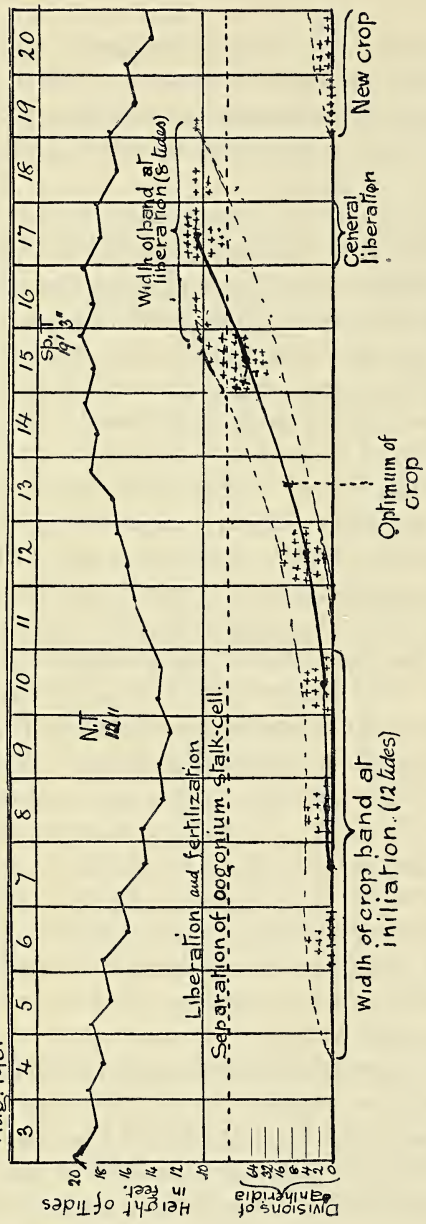


DIAGRAM I. Maturation of crop during lower Spring tide (Menai Straits).

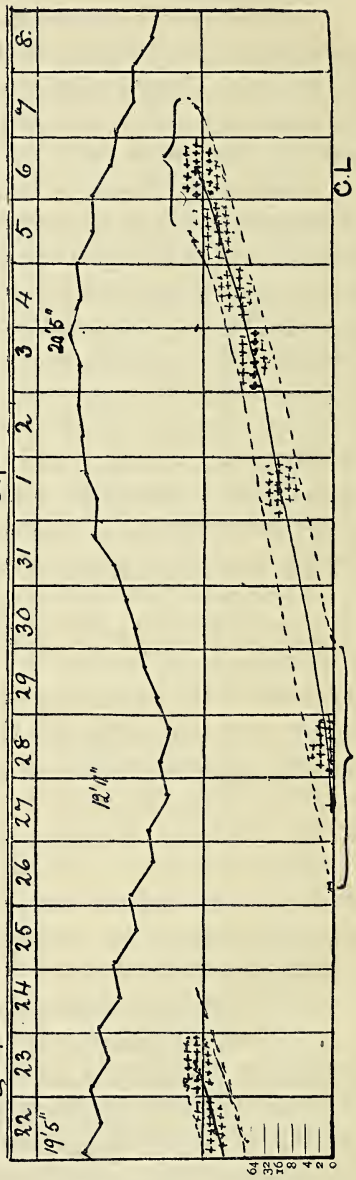


DIAGRAM II. Maturation of crop during higher Spring tide.

DIAGRAM III. Comparison of optima of crops liberated during higher Spring tides in August and September (Menai Straits).

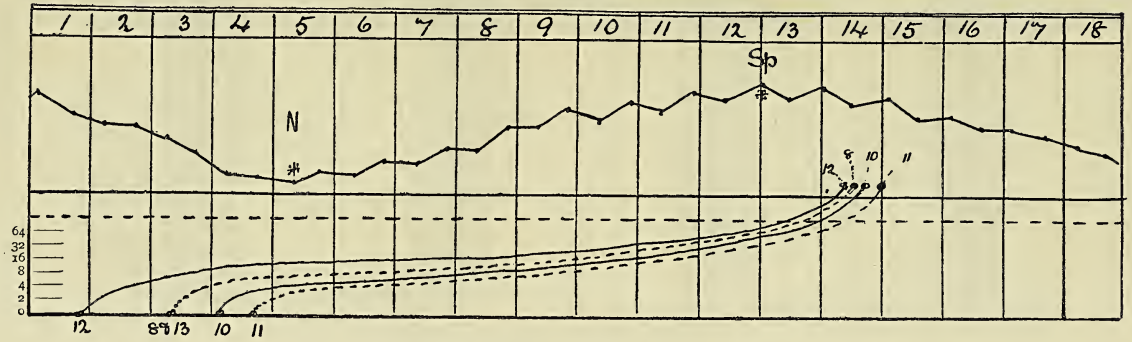
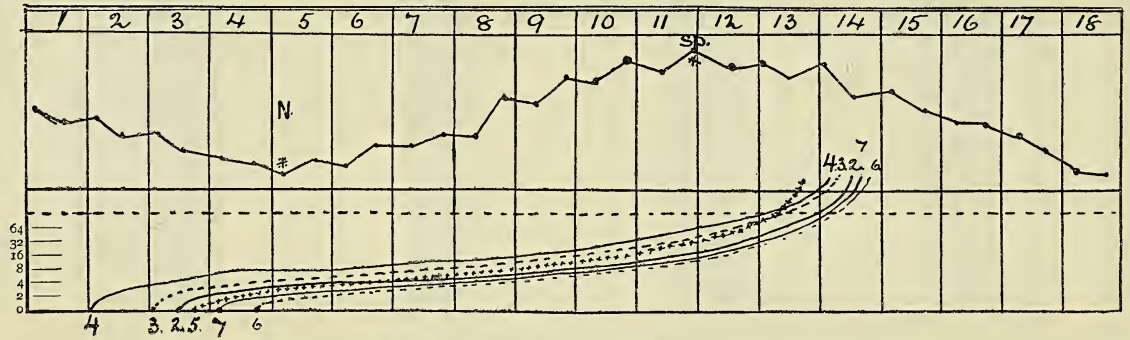


DIAGRAM IV. Crops liberated during lower Spring tides. August and September (Menai Straits).



The numerals under the curves refer to tides in Diagrams I and II.

bb

DIAGRAM V. Heights and hours of low water of neap tides during three days in August in the Swillies (Menai Straits).

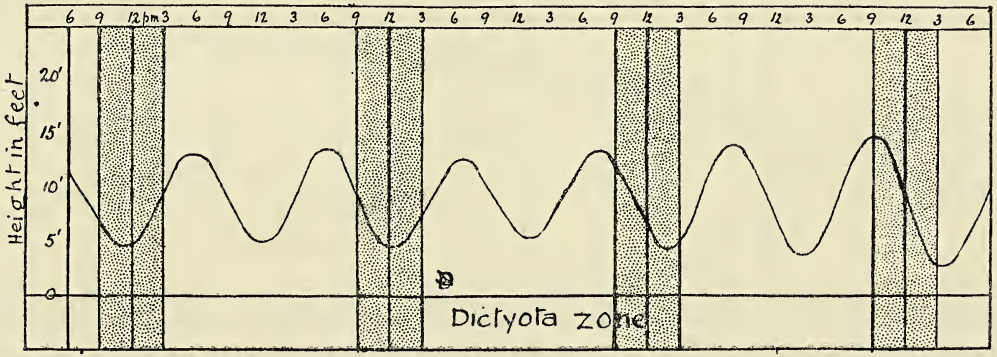
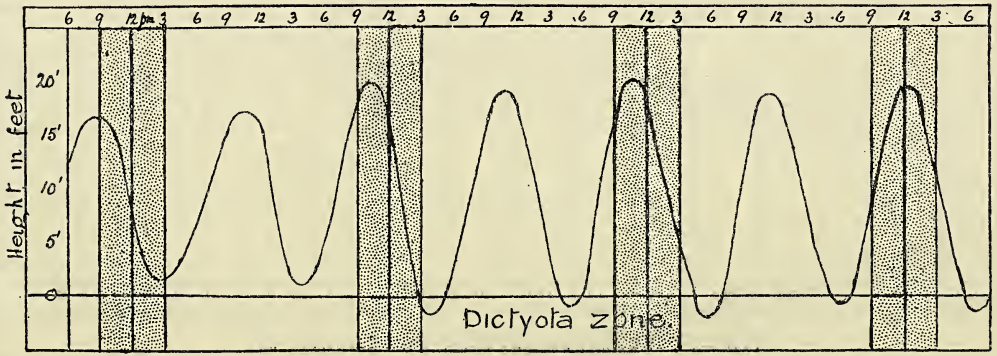


DIAGRAM VI. Heights and hours of low water of Spring tides during three days in August in the same locality.



NOTE.—For Plymouth the times of the tides should be reversed so that in Diagram V low water would occur twice during daylight, and in Diagram VI low water would occur at midnight and midday. For October tides in the Straits if the 'darkness' band is extended, the advantage of the Spring tide low water is obliterated to a great extent.

On the Double Nature of the Cycadean Integument.

BY

MARIE C. STOPES.

INTEREST in the morphology of the ovules of living Cycads has been much stimulated by the recent appearance of several papers dealing with the structure of Cycad-like seeds of Carboniferous age. The structures found in these fossils are not only of great interest in themselves, but also seem to throw light on some of the difficult points in the morphology of recent seeds, and it became clear that a detailed examination of the living forms might be undertaken with advantage. This I attempted to do, publishing the chief details with a short outline of the results in August, 1904[1].

In support of my views on the nature of the Cycad integument, through the kindness of Prof. Oliver, I was enabled to use certain facts discovered by him in the fossil *Lagenostoma*; but my paper was written prior to the publication of the full monograph on *Lagenostoma* which appeared almost simultaneously with it[2]. A large paper by M. Matte[3], in which one of the sections treats of the Cycad ovule, appeared almost at the same time, so that the author had seen neither the full work on *Lagenostoma* nor mine on the Cycads.

In their monograph on *Lagenostoma* the authors state (p. 234): 'A comparison of the seeds of Cycads with *Lagenostoma* is inevitable,' and further: 'In any case, the detailed structure of Cycadean seeds is a subject fully deserving of further attention and elucidation.'

As it happened that the three papers dealing with the Cycads and *Lagenostoma* appeared simultaneously this comparison has not yet been fully made. I will not attempt to do this now in detail, but there are one or two points about which I should like to add something to my published views.

Work on the anatomy and morphology of living Cycads revealed that their integumentary structure is more complex than was generally

[1] Stopes, Beiträge zur Kenntnis d. Fortpflanzungsorgane d. Cycadeen. Flora, Bd. 4, 1904.

[2] Oliver and Scott, 'On the structure of the palaeozoic seed *Lagenostoma Lomaxi*.' Phil. Trans. B., vol. cxcvii, 1904.

[3] 'L'appareil libéro-ligneux d. Cycadacées.' Caen, 1904.

supposed [1], and for the sake of the present argument I will summarize the most important of these points.

The first thing to be established was the fact that the inner of the two series of vascular bundles penetrating the ovule and generally termed the 'nucellar' series was truly integumentary. Facts supporting this were found in a large number of species, the most important case being that of *Cycas circinalis*, where not only are these bundles definitely seen to run in the inner layers of the integument, which is clearly marked off from the nucellus, but they are found to be continued in the integument almost to the micropyle, passing beyond the level at which the nucellus becomes free from the integument. The nucellus itself was found to be entirely devoid of vascular tissue throughout.

In addition, it was found that within the stone are tissues of considerable importance, in which this inner series of bundles runs and which form a very definite inner layer to the integument, although this has been frequently overlooked owing to its liability to get crushed in the ripening seed.

The integumentary nature of the inner vascular strands being established, the course of the bundles of the two systems was examined in detail, the result being the recognition that through all the various specific and generic variations which tend to mask it more or less completely there runs a central plan for their arrangement. This may be shortly described as consisting of a strand which gives off a series of bundles to the outer flesh of the ovule-coat, and continues its way, dividing up to form the inner series which may be further supplemented from the outer series. This is seen best in *Cycas*, *Dioon*, and *Zamia*, though in many cases the bundles appear to come off at the same level. In anatomical structure the main supply bundle is usually either completely concentric or has a strong tendency in that direction; the bundles given off to the outer flesh are collateral and orientated with the phloem outwards and with a considerable development of centripetal xylem; the strand continuing to supply the inner system has a strong concentric tendency, as have also those auxiliary ones derived from the outer series; the actual bundles of the inner series are orientated in the same sense as the outer, but with little, if any, centripetal xylem and seldom with definite spiral protoxylem.

These facts suggested a comparison with *Lagenostoma* and its cupule, in which the details of vascular arrangement and structure are strikingly parallel.

Now that all the facts for *Lagenostoma* are before one, this comparison appears to be justified in the main. One is not 'arguing in a circle,' but rather placing the facts from either side so as to act as props which enable

[1] Stopes, loc. cit.

the theory to stand. To explain *Lagenostoma* without the Cycads or the Cycads without *Lagenostoma* becomes a doubly difficult task.

Matte's work on the recent Cycads appears to have led him to a similar conclusion, though his work is from rather a different point of view. In his description of the vascular system of the ovule he substitutes the adjective 'périnucellaire' for the 'nucellaire' of previous descriptions. He does this, however, not so much as the result of independent observations of the facts of the case, but as he says (p. 168), in the presence of the work of Oliver and Scott and others, 'il est bon d'agir avec la plus grande circonspection en ce qui concerne l'attribution exclusive de cette couronne au nucelle de l'ovule.' He adds, 'En effet, en raison des rapports que les faisceaux de la couronne tégumentaire contractent avec ceux du reste de l'écaille, en raison aussi des rapports que les faisceaux de la couronne périnucellaire contractent avec ceux de la couronne dite tégumentaire, je suis amené à penser que ces derniers correspondent au système nervulaire d'une foliole ou de pinnules d'une penne relevées et concrescentes en cupule et que le système périnucellaire représente une *chose ajoutée* accidentellement insérée sur elles,' suggesting further that the 'système tégumentaire' is the equivalent of the cupular bundles of *Lagenostoma*, and the 'système nucellaire' that of the vessels penetrating the integument. This unites the work from the side of recent Cycads on this point.

The views of Oliver and Scott are expressed in their monograph, where they state (loc. cit., p. 234): 'The canopy of a *Lagenostoma* may well have undergone simplification into the hard integument of a Cycadean seed, and, in that case, the vascular strands, which run in or near the plane of union of nucellus and integument in the latter, should correspond with the integumental bundles of *Lagenostoma*, even though they no longer pass into the free part of the integument.' Now that it is known that in the living Cycads the inner series of bundles runs definitely not 'in or near the plane of union of nucellus and integument' but actually in an inner layer of the integument, and that in addition it is not universally true that they 'no longer pass into the free part of the integument,' but that in certain species they do pass beyond the free part of the nucellus and run in the free integument almost to the micropyle, this rather tentative suggestion may be considered to have accumulated force.

The authors continue: 'Whether the fleshy sarcotesta of *Cycas*, with its vascular strands, corresponds to a completely adnate cupule, may perhaps, be left an open question.' The strengthening of the previous position adds support also to this, and further the marked detailed likeness between the structure and arrangement of the bundles of Cycas and *Lagenostoma* adds weight to the view that they do correspond morphologically if not 'completely.'

One or two further details may be worthy of mention. In the above quotation it is stated that 'The canopy of a *Lagenostoma* may well have undergone simplification into the hard integument of a Cycadean seed.' I had previously looked upon the stone layers as belonging morphologically to the outer flesh, and did so on the ground that it was generally much more difficult to draw a boundary line between these two layers than between the stone and the inner flesh, which are always distinct; that the bundles of the outer flesh are in some cases partly embedded in the stone layers while the bundles of the inner flesh are distinct from it; and also that in the young ovule differentiation and sclerification of the stone cells start in the layers on the inner side and extend in an outward direction. In support of the other view, however, are the facts that the canopy tissue is hard, and that in *Lagenostoma ovoides* there are definite thickened cells in the outer zone of the integument.

It is very clearly marked in many Cycads that the stone has at least two layers, an inner one of mainly vertically running stone cells as in the integument of *L. ovoides*, and an outer one of mainly horizontally running stone cells. It may be that it is in the junction between these two layers of cells that we get the actual plane of fusion between the two integuments; the connexion between the outer stone layers and outer flesh in my eyes is too strong to allow of their morphological separation.

It is true that I have found in *Encephalartos Barteri* and other cases, in the stone freed from the flesh, a strong superficial likeness to the 'canopy' of *Lagenostoma*. This appearance, however, is correlated with the bending away from the stone of the bundles of the outer flesh as they go towards the micropyle, and although superficially it is strongly reminiscent of the canopy, yet as it is connected with the *outer* and not the inner series of bundles it can hardly be homologous with the canopy which belongs to the inner integument. Further, the well-marked ridges in the ripe stone, seen so clearly in *Macrozamia spiralis*, *Encephalartos Altensteinii*, &c., are also the result of the close proximity of the bundles of the outer flesh to the stone, and these ridges always correspond exactly to the bundles, the number thus varying according to the number of the bundles, so that there are but two in *Cycas*, twelve in *Macrozamia* and so on. They do not thus correspond to the joined edges of leaflets as some exponents of the foliar theory of the ovule suggest, but may rather represent the vascular midrib of the lobes of the 'cupule'-like covering. In this way they may perhaps indicate the number of lobes in this covering, except in a case where definite reduction has taken place, as in *Cycas*, where the only remnant of the original radio-symmetry and many-bundled condition of the outer flesh is found just below the base of the seed.

These various considerations, combined with those before stated relative to the development, lead me to consider that at least the outer

stone layers and outer flesh are one morphologically, and hence to look for the plane of fusion of the two integuments either between the inner and outer stone layers, or probably as I had originally suggested between the stone and the inner flesh.

The idea that an outer integument might arise as an independent outgrowth round the ovule in Cycads was suggested by Goebel[1] some time ago, who stated that the 'Wucherungen' of *Ceratozamia* might represent the beginning of a second integument. If the Cycads, however, have already two integuments these 'Wucherungen' may represent a third. I have recently examined fresh material of *Stangeria schizodon*, in which there are upgrowths of the sporophyll which are developed to such an extent that in some cases they unite to completely enclose and cover over the growing ovule, even after it has attained a considerable size. 'Wucherungen' which completely enclose the ovule in this fashion can hardly be termed other than ovular coverings, and it seems to suggest the possibility of a third integument arising on much the same lines as I suppose the second to have done.

Although Oliver and Scott do not definitely state that they consider the cupule of *Lagenostoma* to be the equivalent of the outer flesh of Cycads, yet they suggest (p. 232 loc. cit.) that: 'The outer (i. e. the cupular covering) is probably of later origin, and would appear to have afforded protection to the seed only when the latter was quite young. It is quite possible that the two enclosures have originated very similarly, i. e. as peltate-lobed structures, and that the present integument was once a comparatively unspecialized cupule-like indusium.'

If one can consider the inner integument as having once been a 'comparatively unspecialized cupule-like indusium,' I can see no reason why one cannot suppose the second 'cupule-like' structure to adhere and form the outer integument, nor why the series might not be continued and a third added in the same way. There appears to me to be no fundamental necessity to limit the integuments to two as Celakovsky has done.

The outgrowth I observed in *Stangeria* is entirely free from the outer integument of the ovule; it is undifferentiated in character, and its tissues are those of the sporophyll. It is obviously not split off from the outer integument, but is the free upgrowth round the already doubly integumented ovule, and it seems to me to illustrate markedly the case in point.

I have assumed throughout a sporangial theory of the ovule, an assumption that seems well justified in the light of recent work on fossils and living plants; the question under discussion is therefore only that of the nature of the integuments, and in particular the recognition of the double nature of the normal Cycadean integument.

[1] Organographie der Pflanzen, 1898, p. 786.

On bringing together recent work on this subject as I have attempted to do, it appears to me that it unites in supporting the conclusion that in a *Lagenostoma* with its cupule we see the morphological equivalent of a Cycadean ovule with its complicated integument representing the inner and outer fused. A consideration of the envelopes of the two seeds together thus throws light upon each, and upon the origin of integumentary structures in general.

March 10, 1905.

NOTES.

FERTILIZATION IN SPHAEROTHECA.—In connexion with a course of lectures on the Ascomycetes given by one of us, material of the Hop-Mildew (*Sphaerotheca Humuli*, Burr.) was collected in the summer of 1904 for the purpose of following the development of the perithecium. As the results obtained, incomplete as they are, confirm the work of Harper [1] on the same object, it seemed worth while to put them on record, since the accuracy of his observations has been so directly called in question by Dangeard [2], and also doubted by other workers (Lindau Holtermann, Kuyper).

Fig. 17, 1 shows the oogonium and the antheridial hypha side by side; the actual antheridial cell has not yet been cut off. In Fig. 17, 2 (in which the plane of section is at right angles to that of Fig. 17, 1, and the antheridium is behind) there is actual cytoplasmic continuity between oogonium and antheridial cell, and the male nucleus has obviously just passed in; the contents of both nuclei are somewhat contracted away from the nuclear wall. Four cases were observed in which the oogonium and antheridium were in open communication. In Fig. 17, 3 the two nuclei probably represent the sexual nuclei, as, judged by the branch which has only just begun to grow up from the basal cell, the stage is still quite young; it is, of course, possible that the two nuclei have been produced by division (as in Fig. 17, 6), for the development of the sheath, as some of the figures show, does not always run *pari passu* with the internal development of the oogonium. In Fig. 17, 4 the two nuclei which are in contact must clearly be the sexual nuclei just before fusion; the nuclei here, also, are badly fixed, so that they stain in a homogeneous manner. Neither in this nor in Fig. 17, 3 is the communication between antheridium and oogonium now visible. In Fig. 17, 5 the large single nucleus in the oogonium (oospore) represents, no doubt, the fusion nucleus. The non-nucleate antheridial cell is clearly visible here as well as in Fig. 17, 3, and in both these figures the separate origin of the hyphae which bear the oogonium and antheridium, respectively, is well seen. Fig. 17, 6 shows what are doubtless the first two nuclei formed by division in the fertilized oogonium; the antheridial hypha, with the cell above containing only cytoplasm, is clearly visible on the left, having been pushed aside by the upgrowths from the basal cell which form the sheath. In Fig. 17, 7, a section from an older perithecium, is seen a row of four cells developed from the fertilized oogonium; the penultimate cell is binucleate and is the young ascus.

We observed no cases in which the antheridial cell was without a nucleus while the oogonium was still unfertilized, nor any in which the antheridial cell still contained a nucleus when the oogonium showed two nuclei; neither did

[1] Ber. d. Deutschen Botan. Ges., xiii. 1895, p. [67].

[2] Le Botaniste, 5e série, 1896, p. 245.

we observe any instances of degeneration of the nucleus in the antheridial cell. It is on cases such as these, and on the absence of observed cell-fusion, that Dangeard relies for his refutation of Harper's statements. Furthermore, the row of cells produced from the egg (Fig. 17, 7) was generally found to consist of at least four cells; the three or two cells of Dangeard were very rarely, if at all certainly, observed. Unless Dangeard's material showed a course of development very

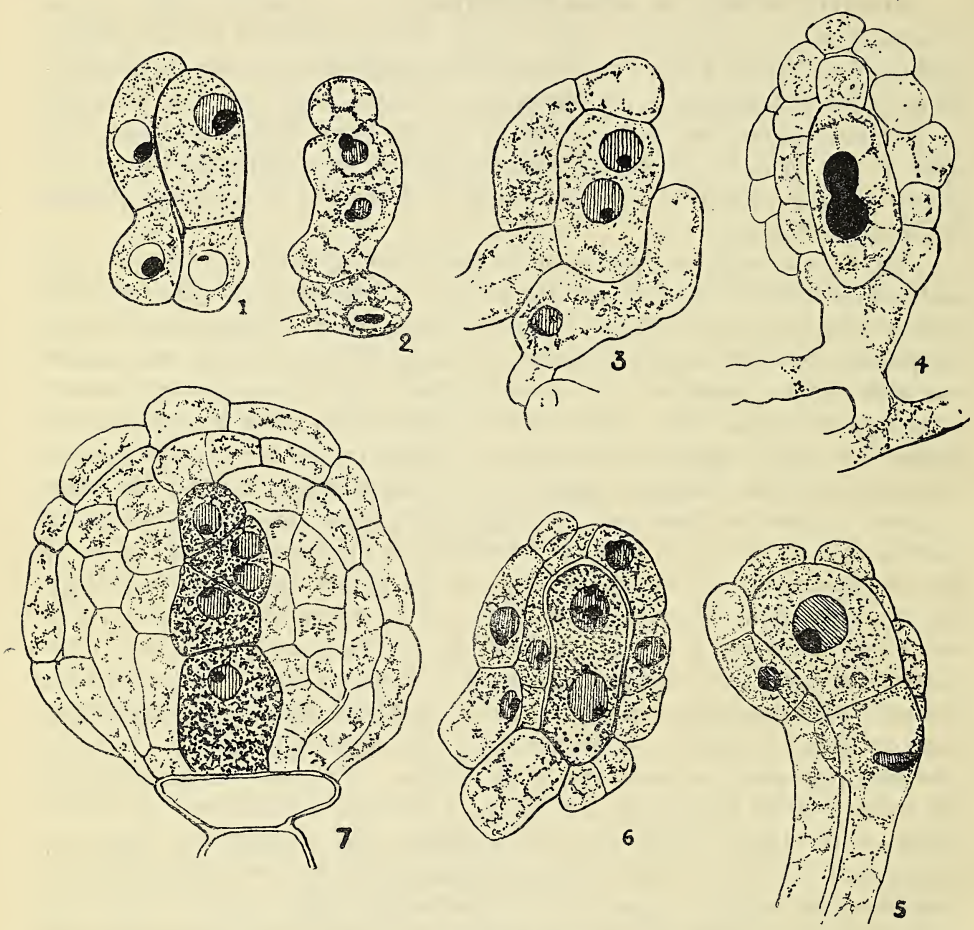


Fig. 17. × 1700.

different from that observed by Harper and ourselves, we can only conclude, with Harper[1], that the methods used by that worker were hardly adequate for the elucidation of the question at issue.

The material used was fixed in Flemming's weak fluid diluted with an equal volume of water, and cut 4–6 μ in thickness. The fixation was rather erratic, a certain number of the young stages and all the older perithecia being badly fixed—probably owing to the weakness of the fluid.

[1] Annals of Botany, xiv, 1900, p. 330.

It may be suggested here that the most satisfactory homology of the parts of the perithecium in *Sphaerotheca* is to regard the oogonium as a uninucleate ascogonium, which, after fertilization, develops directly by division into a row of cells, i. e. into a *single ascogenous hypha*, of which the usual penultimate cell becomes the ascus. This row of cells cannot satisfactorily be compared with the whole 'scolecite' of *Ascobolus*, for that is certainly not, itself, a product of fertilization.

V. H. BLACKMAN.

H. C. I. FRASER.

LONDON.

THE POSITION OF MAXIMUM GEOTROPIC STIMULATION.—In a recent paper in which he discusses the position of maximum geotropic stimulation, Fitting[1] refers to a note by me published in this Journal in 1899[2]. I then obtained results with apogeotropic organs which seemed to prove that the optimum position lay at 45° below the horizontal, but experiments of the same kind and others which are still more conclusive, carried out by Fitting, all indicate that the horizontal is the optimum position. Fitting suggests that the difference in my results is possibly due to a slight deviation from the horizontal in the position of the axis of my klinostat, which error would, as he proves experimentally, be quite sufficient to account for my results.

Although Fitting's experiments are so convincing as to leave little doubt that the error must lie with me, it yet seemed desirable to repeat my experiments.

I again made use of grass-haulms (those of *Lolium perenne*) and fixed them on an intermittent klinostat at an angle of 45° to the horizontal axis (the position of which was most carefully adjusted) so that they were for periods of 25 minutes alternately 45° above and below the horizontal. The results, unlike those of my earlier experiments, quite agreed with those of Fitting, for there was no indication of any difference in the amount of stimulation in the two positions. Of twenty-eight grass-haulms ten remained straight; eleven curved towards the horizontal with an average curve of 6·1°, and seven curved in the opposite direction with an average curve of 11·7°. The experiments, five in number, were all carried on for about twenty-three hours.

In order to obtain more positive results I then employed a method suggested and carried out by Fitting. Inclining the axis of the klinostat 22·5° from the horizontal, I so arranged the haulms that they were alternately horizontal and 45° below the horizontal. Almost without exception they curved decidedly away from the side which was stimulated whilst horizontal, showing that the stimulus in that position is greater than it is when inclined 45° below the horizontal. Of

[1] Untersuchungen über den geotropischen Reizvorgang, Teil I. Jahrb. für wiss. Bot. xli. 2. 1905. See also F. C. Newcombe, Geotropic responses at various angles of inclination. Ann. of Botany, 1905, p. 319.

[2] On the gravitation stimulus in relation to position. Ann. of Botany, 1899, p. 620.

thirty-seven haulms, thirty-three curved from the horizontal with an average curve of 14°, and only four curved in the opposite direction with an average curve of 4°. There were five experiments, each carried on for about twenty-three hours.

My experiments thus fully confirm Fitting's conclusion that the horizontal is the position of maximum stimulation.

D. F. M. PERTZ.

Botany School, Cambridge,
July, 1905.

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