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ANNALS OF BOTANY

EDITED BY

V. H. BLACKMAN, Sc.D., F.R.S.

PROFESSOR OF PLANT PHYSIOLOGY AND PATHOLOGY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

AND

R. THAXTER, M.A., PH.D.

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

ASSISTED BY

D. H. SCOTT, M.A., LL.D., D.Sc., F.R.S.

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

J. B. FARMER, M.A., LL.D., D.Sc., F.R.S.

PROFESSOR OF BOTANY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

F. W. OLIVER, M.A., D.Sc., F.R.S.

QUAIN PROFESSOR OF BOTANY, UNIVERSITY COLLEGE, LONDON

AND OTHER BOTANISTS

VOLUME XXXVII

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„ 423, Fig. 23, *for* solution *read* solutions.
„ 451, l. 8, *for* sixty *read* sixty-two.

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EDITED BY

V. H. BLACKMAN, Sc.D., F.R.S.

PROFESSOR OF PLANT PHYSIOLOGY AND PATHOLOGY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

AND

R. THAXTER, M.A., PH.D.

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

ASSISTED BY

D. H. SCOTT, M.A., LL.D., D.Sc., F.R.S.

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

J. B. FARMER, M.A., LL.D., D.Sc., F.R.S.

PROFESSOR OF BOTANY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

F. W. OLIVER, M.A., D.Sc., F.R.S.

QUAIN PROFESSOR OF BOTANY, UNIVERSITY COLLEGE, LONDON

AND OTHER BOTANISTS

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The Distribution of Plants.

BY

H. N. RIDLEY, C.M.G., F.R.S.

IN examining the constituents of the flora of any country one is struck by the great variety of species, genera, and orders represented in it, and secondly, one notices that the species are not altogether those occurring in the nearest lands, though in most countries the greater number of the species have affinities or are identical with those of adjacent countries, even if these countries are separated now by considerable stretches of sea; there are besides a certain number—usually very local and generally consisting of one, or perhaps two, species of a genus—which have no affinity at present with anything else in the area, but with plants of far distant regions, and which are the relics of a long-lost flora. Besides these we have now in all parts of the world, wherever man has trod, a larger or smaller number of plants introduced accidentally or intentionally by man.

Ground bare of plants fills up very rapidly with vegetation brought to it in the form of seeds from the nearest land by various means of dispersal, and eventually becomes so densely covered that there is no room for additional species, and it remains in a state of equilibrium until one of the great factors of change comes again into play.

As a rule, the greatest variation is to be found where several distinct floras are, or have been in the past, sufficiently near to supply any given country with its flora.

In the British Isles we have a central European element, an Arctic element, a Portuguese element, and a North American element. In the Malay Peninsula a large percentage of the species is common to Sumatra or has close affinities with those of Sumatra; a number are represented in Borneo only; others are Javanese, Burmese, Indian, Cochin Chinese, and Siamese; while both in the British Isles and in the Malay Peninsula we have a number of established weeds, in the latter country chiefly from South America and the West Indies, which go to make the mixed floras as we find them to-day.

This mixture of plants in a country is due to the three great factors of change, which are—(1) change of climate, (2) change of the land surface, (3) change due to human agency.

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These three factors have all caused the destruction of large numbers of species, which have been replaced by invasions of others usually from the nearest lands; but in almost every case of which we have records of any of these catastrophes in the plant-world, there have remained survivors of the lost floras for a long time in suitable spots. Plants of these which have been sometimes modified so as to adapt themselves to the change of condition may form a distinct portion of the new flora.

The first two factors have been in action in one or other part of the world more or less continuously, probably from the foundation of the globe, and no part of the world has been free from their action, which has been repeated usually many times.

The third factor, human agency, almost certainly commenced to play its part in altering the vegetation of the world when the human race began its first migrations. The earliest record I know of dates from Neolithic times, when the immigrants from the East brought into western Europe many of our weeds mixed with their cereals and Flax seeds. But the greater effects began to be seen later, about four thousand years ago, and very much more extensively and rapidly within the last two hundred years.

Change of Climate.

This factor does not seem to have come into play in any part of the world, at least to any great extent, in historic times, but we have plenty of evidence of its having played a most important part in the past. We have had in England, since the first appearance of flowering plants, an extensive series of great changes of temperature, from the tropical or subtropical climates of the Eocene period to the milder one of the Miocene, to a temperate climate broken into by one or more Arctic periods and restored again later. Each change was accompanied by a corresponding change in the flora, the destruction of the old flora and the invasion of a fresh one.

In the equatorial regions we have no record of any Ice Age later than the Permian period, nor indeed, so far as I have been able to detect, any period of a temperate climate, but we have had fluctuations of humidity and dryness, alternations of a xerophytic period and of a hot, wet period, the latter causing the disappearance of the xerophytic flora except in a few still more or less dry spots on the higher mountains and on the sea-shore. Such island refuges of an otherwise lost xerophytic flora in the Malay Peninsula are the plateau of Gunong Tahan, 5,000 to 7,000 feet alt., where nearly all the plants are endemic, and the curious sandstone dyke, 1,400 feet high and only a few feet across in parts, which traverses the valley of the Klang river and is known as Klang Gates. Here, surrounded by mountains even higher and lowland jungle of entirely rain-forest species, I found several xerophytic endemic plants mixed with characteristic xerophytic plants occurring also on the dry mountain areas, very many miles away.

The name *endemic* has been used for two distinct classes of plants, and this has led to some confusion. It is used to cover any species confined to a given limited area. These may be either the relics of a lost flora just surviving in one spot, or species evolved in one locality which have spread no farther. It would be advisable to have different words to express these two utterly distinct classes of plants.

It is not at all difficult to distinguish to which class an endemic plant (in the double sense) belongs, for while the relic of a past flora has usually no affinity with any other in its area, the endemics of the second class evolved on the spot have affinities, often close, with abundance of other species in their locality.

Both of these classes are well represented in the Malay Peninsula, as they are in most countries.

A good example of the first class of endemic plants is that of the Gesneraceae of Europe, *Ramondia* and *Haberlea* occurring respectively in the Pyrenees and Balkan mountains. The nearest species of the order are the few occurring in the African mountains, but they have no affinities with the European species, nor have the species of northern India, but they are related to some Chinese and Japanese species; *Ramondia* is most closely allied to *Conandron* of Japan, not only in habit but in having four and sometimes five stamens, with lanceolate acuminate anthers, and also in the form of the fruit. *Haberlea* seems to be most nearly allied to *Ramondia* and *Oreocharis* of China.

Now Clement and Mrs. Reid have shown (Pliocene Floras of the Dutch-Prussian Borders, 'Mededeel. van de Rijksopsp. van Delfstoffen', and 'Quart. Journ. Geol.', lxxvi. 149) that South Europe in the Lower Pliocene period contained a number of forms of Chinese and Japanese affinities, i. e. plants now confined to these regions. It seems impossible to doubt that these European Gesneraceae are relics of this period, or to suggest any other cause for their occurrence here.

The second class of what are included popularly under the term 'endemics' may be exampled in the large numbers of species of Didymocarpi, Sonerilas, Argostemmas, and such-like big genera in the Malay Peninsula, evidently evolved on the spot and not spread farther than that area. I do not intend to offer suggestions to account for this evolution here, as it would lead away from the subject of distribution.

The Changes in Land-surface.

These are at present and most probably always have been slow, though very distinct. We know that in Europe, since the appearance of flowering plants, there have been great changes of land and sea, and we have distinct traces of a former land connexion between the British Islands and North America in the peculiar distribution of such plants as *Sisyrinchium*

bermudianum, Linn., *Eriocaulon septangulare*, With, and two species of *Spiranthes*, while a later land connexion between Portugal and south-west England and Ireland have given us *Pinguicula grandiflora*, Linn., *Erythraea Massoni*, Sweet., *Arbutus unedo*, Linn., *Saxifraga umbrosa*, Linn., *S. Geum*, Linn., and some other species.

The same fluctuation of land and sea has been going on in the Malay Peninsula. This area has been connected, I gather from its flora, with Borneo at one time, and then separated by sea; with Sumatra; with the Tenasserim region, and here, as I hope to show later, then broken off and joined again. Besides these land and sea changes, all of which altered to some extent the constituents of the flora, we have the constant and continuous denudation of the mountains, causing the disappearance or scarcity of many high mountain plants and the formation of plains of sand or mud, according to the constituents of the mountains denuded, and producing a lowland area soon invaded and covered by a flora quite distinct from that of the mountain area.

I give an instance of this factor in distribution from the Malay Peninsula. This region is now connected with Tenasserim by the Isthmus of Kra, and I have shown in a paper on the Flora of Lower Siam ('Journ. Roy. As. Soc. S. Br.' 59, p. 17) that the Isthmus of Kra contains a totally distinct flora from that of the Malay Peninsula, no less than forty genera occurring there which are absent from the Malay Peninsula, while sixty genera of the Malay Peninsula are missing altogether. The Isthmus of Kra is a sandy, flat area in which are scattered large islands of limestone, which, from the occurrence of sea-bird guano in their caves, shows that they were at no very distant period surrounded by sea. The climate is less wet than farther south, and there is a dry period in which most of the herbaceous plants wither.

The mountains of the centre and west of the Malay Peninsula are of granite, and by denudation form mud alluvium, and on the west coast none of this flora occurs, but on the east coast the mountains are of sandstone and have been washed down to form large areas of sandy heaths running down to the east corner of Singapore. All along this coast you may find plants of the Isthmus of Kra flora, and the farther north you go the more you will find.

Here we have a sample of the formation of a new sand-hill area being gradually invaded by a northern flora, since the land connexion was formed by the silting up of a shallow sea between the Burmese and Siamese regions and the Malay Peninsula, which was previously an island.

Changes due to Human Agency.

The alteration of a flora by human agency consists of the destruction of species, and the introduction of other species accidentally or intentionally.

This has probably been going on to some extent from the date of the first extensive migrations of man all over the world, but it was when the first great civilizations began that these changes commenced to seriously affect the floras. As long as the population is small, and the family system prevails, the wandering savage effects little alteration in the flora. It is when the families collect into tribes, settle on an extensive area, and cultivate on a large scale, that the great changes in the flora commence.

In England we do not find any species exterminated by the early immigrants, unless possibly *Trapa natans*, a valued food product, but in India, Ceylon, Java, and probably some other countries we may reckon that a very large portion, especially of the arboreous vegetation, was destroyed from two thousand to four thousand years ago. I have already called attention to this in the case of Ceylon in a paper on Endemism ('Ann. of Bot.', xxxv. 566). The civilization period in India probably dates from an earlier era, and it has been a much more persistently over-populated country. The great plains, nearly treeless, over which one travels in almost any part of peninsular India must, before the advent of man, have borne a totally different and much richer flora, but the great population required forest trees for the timber of their houses and temples, and for shipping and firewood, and the ground denuded of these trees was afterwards cleared for cultivation of materials for food and clothing. The original flora practically only persists now in a few mountainous tracts which were not suited for towns or cultivation areas. Apart from deductions made from the distribution of plants, I think that the fact of the plains of India being formerly afforested is at least strongly suggested by the occurrence all over them of the ape *Semnopithecus*, a genus of monkeys elsewhere exclusively occurring in the heart of tropical forests, and the peacock, a bird which elsewhere occurs only on the borders of heavily wooded country, though it is not, like the monkey, an inhabitant of the interior of lofty forests. Both of these animals have been preserved by man from religious motives, and seem now to have adapted themselves to the life of the villages of the plains.

Java was thickly populated in the twelfth century, and probably earlier. The Dutch commenced to trade there in the seventeenth century, and began to develop the country agriculturally early in the nineteenth century. This development entailed the destruction of the forests to such an extent that one may travel for days through much of the north part of the island and see nothing but rice and sugar plantations, so cleanly maintained that there is hardly a weed to be seen among the rice-plants.

We read in Raffles's 'History of Java' that in the eastern districts fifty to sixty thousand beams a year were delivered to the coast districts in about 1800, the timber of the coast area having been exhausted by then, and, besides this, large quantities of timber were used in local boat-building.

Thus in Java the original flora has almost entirely disappeared over large areas, and in the Tosari district there is hardly an indigenous tree to be seen. I found here but one native tree (*Helicia obovata*, Benn.), and the most conspicuous plants are the Radish of Europe and *Datura arborea*, Linn., of South America. The roadside banks are covered with a mixture of European weeds and indigenous Javanese herbs, which have been able to survive in these spots.

In the less accessible southern parts of Java, I am told, the original forest flora persists, as well as the larger mammalia which have long disappeared from northern Java. There are also some good forests left on the bigger volcanic mountains.

In dealing with questions of distribution and origin of floras, this destruction of the original vegetation must be taken into account, and it must be remembered that in such cases we are restricted to the investigation of the plants which occupy the ground at the present time, and not dealing with the original flora of a few centuries ago.

The fate of a forest flora when timber-felling is started is soon decided. Not only do the big trees with their epiphytic flora disappear, but, owing to the admission of light and heat into the jungle, all herbaceous, shade-loving plants for a long way through the still standing forest perish. Over the cleared land, if not put under cultivation, grows the Lalang Grass, *Imperata cylindrica*, Cyr., in the eastern tropics and other inflammable herbaceous plants elsewhere. These plants are often fired, accidentally or intentionally, and all the rest of the indigenous flora, herbs, and small shrubs, constantly burnt, soon disappear and all that remain are the few species of plants which can survive constant burning; such plants are those with subterranean rhizomes, like the *Imperata*, leguminous shrublets, and herbs whose seeds are uninjured by the passing fire, Macarangas (Euphorbiaceae), whose buds are protected from injury by resin, and trees like *Cinnamomum iners*, the resinous leaves of which burn with so great rapidity that the main buds and trunk are unharmed, as the fire passes too quickly by to really injure the tree.

Where land has got into this state after a few years, either by cultivation or by being cleared and overgrown with *Imperata* or such inflammable plants, it may be hundreds of years before any of the original vegetation comes back, and then only if big areas of original unhurt forest remain in the vicinity. In Province Wellesley, at Tasek Gelugur, was a flat, sandy plain covered with a dense growth of *Imperata*; possibly, as its name (Tasek, a lake) denotes, it was originally a swampy lake district: it had not been cultivated within the memory of man, and natives assured me it was in exactly the same state fifty years previously. Besides the grass, hardly any plants occur except a few shrublet Leguminosae. If the country becomes regularly settled by a large population or is extensively cultivated, all the

indigenous flora is destroyed. Big areas which in 1888 were covered with a trackless dense forest, such as the country lying along the railway between Klang and Kwala Lumpur, are now entirely covered with Pará rubber trees for many miles, and nothing at all is left of the indigenous flora, which has completely vanished, never to return. Already many local species collected by myself in 1899 to 1900 in such areas are now apparently quite extinct.

Where countries are thinly populated, as in the interior of the Malay Peninsula, tenanted only by the scattered wild tribes known as the Sakai, very little alteration in the flora is made by the people; they may fell the jungle round their huts, but they do not occupy one spot long; a death in the family or the shortage of game sooner or later causes them to leave, and the surrounding forest soon closes over these clearings again.

But where a village springs up and permanent cultivation is made, the indigenous flora soon vanishes. Sometimes plants of special economic value, such as rattans, rubber, gutta-percha, or wood-oil trees, are so extensively sought that they become rare, if not extinct.

I have visited an old Malay settlement on the Pahang river where I could only find one species of rattan, a valueless *Daemonorops*. As the rattan stems are cut before fruiting, the other species, valued for tying in house-building and for export, had been exterminated by not being allowed to fruit. Again, the wood-oil trees, *Dipterocarpus grandiflora*, Blanco, in all accessible parts round Malacca, were nearly exterminated (until more or less protected by Government), in the process of extracting the oil, by making large holes in the trees; and near Kuching, in Borneo, one could see hundreds of trees of *Dyera Lowii*, Hook. fil., standing dead from having been tapped to death for the Jelutong rubber. While in cases of complete destruction of the flora the trees, lianes, jungle herbs, and epiphytes disappear, there comes an invasion of open-country herbs and bushes, partly from surrounding areas, should there be any open country, heaths, or sandy plains to supply them, and partly plants introduced accidentally and occasionally intentionally by man. Curiously, plants introduced intentionally for use or ornament comparatively seldom establish themselves as part of the new flora—that is to say, reproduce themselves naturally and spread so as to form an all-important feature; but in the East Indies we may cite, as ornamental plants introduced and run wild, such examples as *Oxalis rosea*, Jacq., *Lantana mixta*, Linn., *Mimosa pudica*, Linn., *Lochnera rosea*, Rchb. fil., two species of *Turnera*, *Anacardium occidentale*, Linn., and the aquatics *Eichornia speciosa*, Kth., and *Limnocharis emarginata*, H. B. K., and in Africa Opuntias and *Argemone mexicana*, Linn. All these are tropical American plants mostly introduced originally for ornament, and now forming a conspicuous part of the flora. The larger number of weeds, however, are inconspicuous herbs: Grasses, Compositae, herbaceous Rubiaceae,

Amarantaceae, and such like introduced accidentally. All plants thus imported by man and run wild I class as weeds.

Weeds.

Weeds are usually identified by their being confined in their habitats to cultivated ground, or roads, or paths, as such classes of habitats did not occur previously to the advent of man, and it is clear that plants now confined to such spots in any given country must have been introduced by man. Some plants, however, like *Lochnera rosea*, the West Indian periwinkle, grow exclusively on sea-sand; others, like *Capsicum minimum*, Linn., establish themselves on limestone cliffs, or, like the Wallflower, on ruined walls, &c. In such cases their original habitat and place of origin can only be adduced by their history, if known, and otherwise by their affinities with other species allied to them.

All weeds must have been wild in some country, but may have been so long diffused over the surface of the globe that it is very difficult now to identify their original home.

A certain number of species of plants are only known in cultivation; of these some are derived from well-known wild forms, especially those of western Europe, whose history we know. In other cases we can only guess the origin by knowing where allied species are still actually to be met with in a wild state.

The greater number of the Malay fruit trees are not known in a wild state anywhere. They must have been wild somewhere in the Malay area, as they nearly all belong to local genera, but are specifically distinct from any species occurring in a wild state. The cause of this is, I think, that in very early days when a native found any of these trees in a forest he went and regularly gathered the fruit, taking it to his village, where the seeds thrown away germinated and formed an orchard. Often too, if a Malay finds an abandoned fruit tree in ripe fruit he fells it to save the trouble of climbing it. By persistently taking the fruit he prevents the tree reproducing itself by seed in its native haunts, so that the species eventually dies out. Or again, if he finds a number of these trees together, he will start a village round them and so bring them into cultivation. This, I believe, accounts for the absence in a wild state of the Durian (*Durio zibethinus*, Murr.), Mangosteen (*Garcinia mangostana*, Linn.), Rambutan (*Nephelium lappaceum*, Linn.), and Pulasan (*Nephelium mutabile*, Bl.), Betel-nut (*Areca catechu*, Linn.), and many other species which have never, so far as I have been able to ascertain, been found in a wild state.

We have two difficulties in tracing the migration of weeds; one is that our earliest herbaria are of so modern a date that we have little clue as to when the weeds first appeared, and secondly that, when botanists did begin

to form herbaria, they gave no information as to whether the specimens were obtained in altered or cultivated ground or not.

Weeds commonly follow the tracks of the largest migrations of man. The greater number of weeds in the Malay Peninsula are South American or more probably West Indian in origin.

In the sixteenth century the Jesuits brought useful plants from South America to Manila, their most important station in the East Indies. They brought the pineapple, Capsicums, Papaya, *Achras sapota*, Cashew-nut, and, apparently as a curiosity, the Sensitive plant. Most of these plants, the useful ones at least, were conveyed to Goa, and thence to Malacca, where Linschoten records them in 1583. It must have been in those days that most of our Malayan weeds came to Asia, but they appear to have come mainly to the Malay Peninsula through Java from the Philippines at a later date, for nearly all are abundant in Java and there are still a number of common weeds there which have not yet reached the Malay Peninsula, the stream of human migration to which has been strongest from Java.

We possess fewer weeds from India because, until the extensive introduction of Tamil labour, there was but slight migration from India. A few, chiefly medicinal plants, *Eryngium foetidum*, Linn., and *Leonurus sibiricus*, Linn., have been brought by the Chinese. Once in the country the area which one of these plants occupies depends on its adaptability to various classes of position and soil, or perhaps, more strictly speaking, to the extent of the area with suitable soils and conditions, and secondly to its means of dispersal.

Plants which thrive in made soil, or cultivated ground, will spread as far as there is any such ground; those to which the sandy and gravelly drier roadsides are suitable will spread as far as such roads go.

Plants with adhesive fruits or seeds seem to travel fastest and farthest; such plants are *Paspalum conjugatum*, Berg., *Ageratum conyzoides*, Linn., and *Bidens*, and it is interesting to note that most of the tropical weed Compositae, and the most abundant, are those with adhesive fruits, not those with plumed achenes.

Weeds with berries, bird-dispersed, such as *Solanum oleraceum* and *Passiflora foetida*, Linn., also travel fast, and many small herbs with minute seeds, such as the herbaceous Rubiaceae, *Oldenlandia* and *Borreria*, the small Euphorbias like *E. thymifolia*, Linn., and very many Grasses; mostly roadside weeds are quickly distributed by rainfall.

Some of these plants are now among the most widely distributed species in the world, and it can be readily shown that the duration of time in which the plant has been in its extended area plays practically no part in the wideness of its distribution. In other words, age has little or nothing to do with area.

It is unnecessary here to give a list of the weeds introduced into the

Malay Peninsula, but it may be interesting to point out that the greater part of the Compositae occurring here, twenty-six out of thirty-five species, and a large proportion of the Labiatae, Amarantaceae, and Grasses in the southern part of the peninsula have been undoubtedly introduced within a comparatively few years. Indeed, exclusive of the Bamboos, the forest region covering the greater part of the peninsula, where unaltered, contains only single species of *Leptaspis*, *Lophatherum*, and *Centotheca*, with one or two species of *Panicum* and *Isachne*.

The mountain Gunong Tahan, never visited by man until Mr. H. C. Robinson reached it in 1905, bore only a few Grasses, viz. one of these Panicums and a couple of rare Isachnes. A few indigenous Grasses grow on the tops of some of the other mountains and some on the sea-shores, but far the greater number are clearly recent introductions. The number of weeds in our area is still increasing, and is likely to continue so doing for very many years.

Methods of introduction of weeds.

Weeds are introduced into new countries by a variety of ways, and I give here some account of the chief ways in which they are introduced. Undoubtedly very many seeds of aliens are brought accidentally or intentionally into countries where they fail to make good or establish themselves. A study of Dunn's 'Alien Flora of Great Britain' illustrates this very well.

Weeds introduced in pot-plants.

A certain number of plants have been introduced casually in soil in pots of plants sent from other countries, and have been able to establish themselves in their new homes. Conspicuous among them are *Pilea muscosa*, Lindl. (Urticaceae), and *Peperomia exigua*, Miq. (Piperaceae), South American plants now established in the East Indies, and *Cardamine hirsuta*, Linn., probably of European origin but now spread over large areas of temperate or sub-tropical lands, though it does not seem to thrive in the tropics. The only time it appeared in Singapore it grew on rubbish heaps in the gardens, where the soil of pots of plants, sent, I believe, from Kew, had been emptied out. It failed to establish itself. *Drymaria cordata*, Willd. (Caryophyllaceae), a plant of unknown origin, is spreading all over the tropics in the same way, though it seems to confine itself to the highlands, about 5,000 ft. alt., in hot and wet regions. It appeared abundantly in the roadsides and gardens of the Semangkok Pass, Selangor, 4,000 ft. alt., in 1921, probably introduced with rose-trees from Java.

Weeds introduced in cereal or other seeds.

A very large number of plants have been introduced into alien

countries in seeds of rice plants in the tropics, and vegetable and corn seed in temperate climates. Clement Reid has shown that a considerable number, including the Fumitories, *Matricaria inodora*, Linn., *Centaurea cyanus*, Linn., *Euphorbia helioscopia*, Linn., &c., occur with the Flax introduced by Neolithic man, and Poppies, *Stachys arvensis*, Linn., &c., first appear in Roman times. The Neolithic weeds are the earliest we have any record of. Many European weeds occur in the vegetable grounds of Tosari in Java, obviously introduced in the vegetable seeds from Europe.

The rice-fields of the tropics contain also many species disseminated with rice-seed from other countries; such plants are *Scirpus grossus*, Linn. fil., and a number of other Cyperaceae and small Scrophularineae.

By transport of cattle.

Another method of introduction of weeds is by the transport of cattle. Large numbers of seeds of Grasses and other herbaceous plants are brought in the fodder, which with the dung is cleared out often on the foreshore on arrival at the port, and the seeds frequently germinate and soon establish themselves. Amaranthi, *Cleome viscosa*, Linn., and *Gynandropsis pentaphylla*, DC. (Capparidaceae), *Herpestes monniera*, H. B. K., and *Scoparia dulcis*, Linn. (Scrophulariaceae), *Panicum colonum*, *Paspalum conjugatum*, and *Imperata cylindrica* owe their wide distribution largely to this cause. An interesting case of a cattle-dispersed plant is that of *Clitoria cajanaefolia*, Benth. (Leguminosae), a shrub with showy, pale violet flowers, which is a native of eastern Brazil, but is now abundant in Singapore, south Johor, and Sarawak, in Borneo. The pods contain a number of very viscid seeds which become readily attached to the hair of cattle browsing among the bushes, and are so transported from place to place. It is often to be found along cart-tracks where cattle pass constantly, but more usually where they are grazed. The plant seems to have been first introduced into Java, probably as an ornamental shrub, and then, borne by cattle, imported thence to Singapore, and later to Johor. Mr. Larkin, a planter, told me that it did not appear on his estate on the Tebrau river in Johor until cattle were brought there from Singapore.

Another method of introduction is in the form of packing material, or by the attachment of the seeds or fruits to cargo, or in ballast on ships. An interesting example of this is the grass, *Chloris barbata*, Sw., probably indigenous in Africa, but now abundant in India, Ceylon, and South America. In Java and the Malay Peninsula it is quite confined to the regions of the docks and harbours, being abundant in these places in Singapore, Johor, and Province Wellesley, but it has not spread, so far as I have seen, 500 yards from these spots. The spikelets adhere by their awns to cloth. It occurs on sea-shores in Ceylon, and there seems no reason that it should not do so in the Straits Settlements, except that the soil in the neighbourhood of the

docks is clay, which apparently does not suit it, and the sandy area, though not far off, is barred by buildings and river-clay deposits.

Imperata cylindrica is also used for packing material, and is no doubt conveyed considerable distances in this way. I have picked up in the streets of Pernambuco, in Brazil, a fruiting spike of one of the African Pennisetums which had obviously been brought in packing for some goods from Africa.

Besides these ways in which plants get introduced there are many other cases of casual introduction, and, as some of the weeds are now among the most widely dispersed plants in the world, it is essential, in making any theories on 'Wides', as they are sometimes called, to know the history of each species included under this term. This requires a complete study of the ecology and past history of every species, so far as it is obtainable.

I give a few illustrations of the stories of weed distribution which I think are very instructive. A curious history attaches to *Glycosmis citrifolia*, Lindl. (Rutaceae), which is now apparently thoroughly established in St. Vincent, the Bahamas, Cuba, and French Guiana, where it appears to be abundant on the sandy sea-coasts. There is no other species of the genus occurring in the New World, all the others being confined to India, Malaya, and China. Griesbach, finding it apparently wild in the West Indies, actually described it as a new species under the name of *Glycosmis americana*.

The plant is an inconspicuous shrub with very small white flowers and small flesh-coloured pulpy berries, and it is neither attractive nor useful in any way. Its presence in the American region could never have been accounted for had it not been for a note on a specimen in Kew Herbarium, which states that, according to Dr. Broughton, it was introduced from England in 1788 to Jamaica under the name of the Mandarin Orange, by Henton East, Esq. The plant is a native of the Malay Peninsula, Java, and Hongkong, and the American plant agrees in all respects with the local form from Hongkong.

Cissampelos Parcira, Linn. (Menispermaceae), has a rather puzzling distribution. At one time a number of species had been made of the plant, but Diels, in his 'Monograph of Menispermaceae', has reduced them to one widely distributed and variable species. The plant occurs all over the tropics except apparently West Africa and Polynesia, is common in India, East Africa, and the Philippine Islands, scarce in the Malay Peninsula, and absent from Java. It is probably indigenous to South America.

It was mistaken at one time for the source of the true *Pareira brava* of South America (*Chondrodendron tomentosum*, Ruiz and Pavon), a drug highly valued by the Portuguese, and it seems very probable that the plant was introduced by them into the Philippines and India, as so many American plants were in the sixteenth century, and that it has run wild in Asia and Africa since. Its drupes are red and probably bird-dispersed, but that will not account for its wide distribution.

Scoparia dulcis, Linn. (Scrophulariaceae), is a bushy herb or shrublet

with very small white flowers, and small capsules of very small seeds. It was described by Linné from Jamaica specimens in 1753, and is undoubtedly a plant of South American origin. It is now abundant all over Africa, South America, and the Malay Peninsula and Archipelago. A note by J. Rotheram in a copy of Linné's 'Species Plantarum', ed. 2, 1762, states that it was used as a remedy for venereal disease in Africa. It is recorded for the Congo in Tuckey's voyage in 1816 by Christopher Smith. Loureiro met with it in Cochin China in 1773, and Robert Brown found it in Australia in 1802. It first appeared in India at Serampore in 1845, but it is very scarce there to the present day and has never been met with in Ceylon.

My earliest record in the Malay Peninsula is 1884, but it was probably there before. It has since travelled farther in Pahang and other parts than any South American weed except perhaps the grass, *Paspalum conjugatum*. Wherever buffaloes or cattle go, this plant follows, and it is frequently to be seen springing up from the dung of these animals, which readily feeds on it.

Besides a reputation as a drug in venereal disease, it is also considered beneficial in consumption. The Malaya call it Te'Macao (Macao Tea), which implies that they consider it as having been derived from China. I would suggest its having been carried about at first as a drug, probably dried whole, capsules and all, and by this means got from the West Indies to Africa and to the Philippines. There is nothing to show it was ever cultivated. From these places it has spread, mainly in cattle fodder, to the Malay Islands and Peninsula as far north as Siam, in fact wherever the Malay buffalo and cattle are sent. Its absence from Ceylon, and largely from India and Polynesia, is due to the fact that there has never been a cattle trade between these countries and the Malay or African regions.

This is a good sample of the wide distribution of a plant, extremely abundant in its area, which has obtained marked extension in a very short period of time, viz. about 200 years.

By way of comparison as to the difference in rapidity and wideness of distribution of species, I will give an illustration from two English weeds, both Compositae, *Galinsoga parviflora*, Cav., and *Matricaria discoidea*, DC. They are both herbs of American origin.

Galinsoga parviflora, Cav., occurred in Spain as early as 1794, and has since appeared in Holland, Germany (Berlin, 1812), Italy, and Austria, more or less sporadically and apparently chiefly as an escape from botanic gardens. In England the earliest record is at Twickenham, where it was collected by Rudge before 1809. It is now abundant in vegetable fields round Kew, being first reported in 1861. It has appeared at Guildford, and there is a specimen from Hertfordshire in the Natural History Museum, and in 1912 at the Tweedside with other aliens brought in wool. It has not spread very far from the Kew locality, though very

abundant there. Sowerby reports it as having been introduced into England as an ornamental annual in 1796, and I have seen a specimen, probably cultivated, from Chelsea in 1802. In other parts of the world the earliest dates I have procured are Peru, 1806; North America, apparently wild, 1893; India, 1845; Java, 1899; New Zealand, 1894; Africa, 1912.

Galinsoga possesses fruits with broad scales forming a pappus, and thus has a superior method of dispersal to those of the *Matricaria*, which possesses no scales or plumes at all on the achenes.

Matricaria discoidea, DC. (*M. suaveolens*, Buch.), is a much smaller, inconspicuous weed with small and light fruits quite unprovided with any particular means of dispersal. When ripe they become detached from the receptacle and are partially covered by the involucral bracts which curl over them. The plant is only about four to six inches tall. Holding the achenes on my hand, about two feet from the ground, I find that in a strong wind they are blown three or four yards before falling to the ground. At the normal height of the plant they would not, of course, travel so far.

The plant's first record in England that I can find is 1878, Kew (Druce), but I find specimens from Berlin, 1853; Königsberg, 1862; Dorpat, 1869. It now occurs on roadsides and paths all over England, from Surrey and Berkshire to Aberdeen, and probably farther, and is abundant everywhere.

Now here are two weeds, one of which (*Galinsoga*) has been over a century in the country, and though very abundant locally has not yet migrated more than a few miles from the spot in which it was first introduced, and another, the *Matricaria*, with poorer facilities for seed-dispersal, has in less than half a century spread over the whole of England and Scotland.

It is quite clear that in these cases the age of duration or time in which the two plants have been in the country is not commensurate with the extent of their distribution. The spread of the later introduction has been more rapid and wide than that of the earlier one.

Widely distributed Plants.

I intend in this section to deal mainly with Phanerogamous plants, but will make a few remarks about Cryptogams first, chiefly illustrating by Malayan species.

We know little at present about the cellular plants of the Malay Peninsula, as they have been little collected and still less worked out. They are, on the whole, much more widely distributed than flowering plants, or rather there are more widely distributed kinds than there are of flowering plants. This would give some colour to Dr. Willis's theory

of age and wide dispersal, as we may assume that the cellular plants were the earliest evolved, but we know that the spores of these plants are lighter and more easily dispersed than those of any Phanerogams, and that the plants are much less exigent in their requirements of suitable soils and climates. They are, too, the first vegetation to appear on exposed surfaces. Treub's investigations on Krakatau Island in 1886, three years after the total destruction of all vegetation by volcanic action, shows that the blue-green algae were the first colonists on the bare pumice and volcanic ash and exposed blocks of rock (Ernst, 'New Flora of Krakatau', p. 64). The same phenomenon appears on other exposed surfaces, such as a newly-built wall, as I have often observed in Singapore. First appears a coat of algae, then mosses, then ferns, and lastly, when these have made sufficient soil, come flowering plants.

A few Myxomycetes have been collected in Singapore, of which may be mentioned as of wide distribution :

Physarum nutans, Pers.: Europe, Australia, New Zealand, North America.

Physarum compressum, All.: with the same distribution, and the West Indies and South America.

Stemonitis fusca, Roth.: Europe, Ceylon, Java, Australasia, North and South America.

Lycogala miniatum, Pers.: Europe, Tropical Africa, North and South America.

Other Fungi are more local, especially of course the parasitic ones. We have in the Malay Peninsula :

Clavaria fusiformis, Sowerb.: also Europe and North America.

Agaricus campestris, Linn.: whole world.

Hygrophorus puniceus, Fries, as plentiful in Singapore as in Europe, and many others.

Of Lichens *Cladonia rangiferina*, Linn., is as abundant on the Malay mountains as it is in palaearctic regions.

Vascular Cryptogams.

Rhizocarpeae. We have an *Azolla* widely distributed over Africa and Asia, and undoubtedly carried about by man; and a *Marsilea* which occurs in rice-fields and roadside ditches in Penang and Province Wellesley, and is probably introduced also.

Selaginella.

We have thirty-seven species, of which twenty-one are endemic. *S. flabellata* occurs in all the tropics except Africa; and one or two other species go as far as China and one into Polynesia.

Of Lycopodiaceae, *Lycopodium* and *Psilotum*, there are no endemic

species. The epiphytic species have a smaller distribution than the terrestrial species, as the epiphytic area in the world is smaller than the non-epiphytic area. Of *Lycopodium*, three species, all terrestrial, occur in Asia, Africa, and America, and one, *L. complanatum*, over the palaearctic zone and into the tropic mountain regions, but our variety *thujoides* looks very different from palaearctic *complanatum* and may be specifically distinct. The remaining species range over India or Africa to Polynesia, except three confined to the Malay Isles.

Psilotum. Two species very readily propagated by bulbils as well as spores, growing on trees, rocks, and ruins freely, have a wide distribution all over the tropics and to Japan and New Zealand.

It will be noticed there is a very marked difference between the distribution of Selaginellaceae and Lycopodiaceae. According to the age and area hypothesis this would show that the Selaginellaceae were a very modern group and the Lycopodiaceae an ancient group, for which there is no further evidence.

The real cause, I think, is this : the Selaginellas are low-growing, often creeping, plants, producing comparatively few spores, and, growing in dense forests, have a comparatively slow dispersal, while the Lycopodiaceae are either high-borne epiphytes on the top of lofty trees or grow in dry open places, and produce great abundance of spores which are readily dispersed by wind. It is interesting to note that one of the earliest plants to reappear on Krakatau after the destruction of its flora was *Lycopodium cernuum*, of world-wide distribution, showing how rapidly this plant is dispersed by its light and abundant spores and its open-country habitat.

Ferns.

In the Malay Peninsula we have about four hundred species. A certain number, but not very many, are endemic, most extend over the Asiatic tropics, and many to Madagascar and Africa. Only about twenty also occur in America, and one or two of these may be escapes from cultivation. Only three species occur also in Europe, viz. *Pteris aquilina*, Linn., *Trichomanes radicans*, Sw., and *Lastraea thelypteris*, Desv.

Pteris aquilina, Linn., occurs nearly all over the world in temperate and tropical regions. It is quite absent from oceanic islands, and its earliest record is from late glacial or Neolithic deposits in Sweden (Gunnar Anderson, in Clement Reid, 'Origin of British Flora', p. 168). I have some evidence that it is occasionally, at least, transported by man, the spores attaching themselves to cloth, gunny-bags, &c. It is abundant in sandy soil in the Malay Peninsula, but was quite absent from the plateau of Gunong Tahan when the locality, hitherto unvisited by man, was explored by Mr. Robinson in 1906. In 1910 I visited the mountain, and beneath the floor of Robinson's old hut, and beneath one occupied by a surveyor a year or two

later than 1906, I found two or three plants of the Bracken, but no more occurred on the whole plateau. It is usual in camping here to put the rice-bags and such baggage under the raised floor of the huts, and there can be little doubt that the spores were brought in the baggage to these spots. Bracken is often used for packing and litter for animals, and it has probably largely at least made its way about the world in this way.

Trichomanes radicans, Sw., occurs over a wide area, but seems always to be scarce. It seems to be very variable, if all the plants included under its name are specifically identical, and is a plant of warm temperate regions which seems to have descended into the tropics along mountain chains. It is not known fossil, and is absent from oceanic islands.

Lastraea thelypteris, Desv., is widely distributed, though absent from America, but I have some doubt as to all the specimens recorded being of the same species.

Some others of the more widely distributed ferns are plants of great adaptability, such as *Litobrochia incisa*, Thunb., abundant in the dark, wet hill forests from 2,000 feet altitude and upwards, which I have found established in culverts by the roadside in the lowlands of Singapore, doubtless an escape from the Botanic Gardens, and as the highest ascending plant on the bare volcanic rocks of Sibayak Mountain in Sumatra with a low temperature and a full sun exposure.

Ceratopteris thalictroides, Linn., is an aquatic in ditches in the tropics, the spores perhaps borne about by water-fowl, and *Acrostichum aureum*, Linn., is a tidal-mud plant widely spread along the tidal rivers. Both these plants, by virtue of their peculiar habitats, have no fern competitors to contend with.

Ferns like some of the Adiantums and *Cheilanthes farinosa*, Kaulf., which have wide distributions, are popular garden plants, which have established themselves in many localities.

As ferns have an exceptionally favourable dispersal method in their minute spores and much less exigence in the matters of fertilization and habitats than flowering plants, it is extraordinary that species of an order of such undoubted antiquity should have such limited area of distribution as they do at present, if age was any qualification for extent of area.

The most widely distributed Flowering Plants.

I now deal with the most widely distributed Phanerogams exclusive of sea-borne species and weeds.

The most extensively distributed flowering plant in the world is, I believe, the Common Reed, *Phragmites communis*, Trin. I include under it *P. Karka* (*P. Roxburghii*), as I fail to see any real distinction between the plant of the temperate region and that of the tropics. The Reed ranges all over Europe, Asia, Africa, America, and Australia, but appears

to be absent from New Zealand and Polynesia. It is able to adapt itself to cold climates as far north as Finland, 69·40° N., and to the hot, wet lowlands of the Equator. It is known to reach an altitude of 10,000 feet in Tibet. It grows in wet, open spots, swamps, river banks, and watercourses, as well as on the sea-shore, on clay or sandy deposits with apparently equal facility. It is found fossil in the Cromer forest bed of the Preglacial Pliocene period.

The Reed is absent from oceanic islands such as Cocos, Christmas Island, and Fernando de Noronha, but there is hardly any suitable ground for it in any of these islands, which are over 200 miles from the nearest mainland, though it was one of the first plants to appear in Krakatau after the destruction of the flora by the eruption in 1883, Dr. Treub having found it there in 1886, where it was one of the first fourteen flowering plants to appear. Sumatra and Java, the nearest land from which it could come, are twenty-three to twenty-five miles away, and there is no doubt that the seeds were blown from there by the wind. Besides its dispersal by wind, the plumed fruits may perhaps be borne about by adhesion to the feathers of water-fowl or small birds nesting in the reed-brakes, many of which fly long distances. Like most grasses, the Reed is wind-fertilized and does not require the use of insect pollinators.

Here we have a plant possessing the greatest adaptability to soil and climate—only requiring sufficient moisture for its growth—and a good *dispersal mechanism*, though apparently for comparatively short distances, the two most important qualifications for wide dispersal. It does not appear as a fossil earlier than the Pliocene, though of course it may be older, and it is not by any means a primitive form of grass, but in the matter of dispersal throughout the world it has far outdistanced any of its contemporaries of the Pliocene beds.

Cynodon dactylon, Linn., is another grass of remarkably wide distribution. It ranges from Studland Bay, in Dorset, and Marazion, in Cornwall, all through southern Europe, as far north as North Germany, all over Africa and the warmer parts of Asia, Australasia and Polynesia, and North and South America. It has been suggested that it has been introduced to England in ship-ballast, but I see no evidence of this, as it does not occur with ballast plants in any other localities. I have seen it in both of its English habitats, one of which, Studland Bay, has never been a port and contains other Mediterranean plants, e. g. *Polypogon monspeliensis*, which do not grow on the beach. It may be to some extent a sea-dispersed plant, but it is difficult to see why it is confined to those two localities, and does not occur occasionally in other maritime spots. It prefers sandy ground, but is not a beach plant, and if planted in a clay soil soon disappears. In some localities it may have been introduced by man, possibly in foreign grass seed, as it has occasionally occurred temporarily as an

alien, e. g. on Kew Green. Against its being sea-dispersed is the fact that it is absent from the oceanic islands, Cocos, Christmas, Fernando de Noronha, &c., and did not appear on Krakatau after the eruption.

It is not known as a fossil at all, and its origin seems to have been Africa, as there are other species of the genus there.

Sanicula europaea, Linn. (Umbelliferae), is a plant of open woods in temperate regions, and the cooler parts of mountain forests in the tropics. It occurs in central and northern but not arctic Europe, the whole of Africa to the Cape, India, Ceylon, China, Japan, and in the mountains of the Malay Peninsula, Java, Sumatra, and Celebes. There are allied species apparently distinct in North America, but none in South America. The Malayan form has been considered distinct by some botanists, but it seems merely a warm country form. It does not appear to have been found fossil.

The fruit is armed with hooked bristles by which it can adhere to the fur of animals, and be so dispersed. Though the area covered by this plant is very large, it is mainly continental, and its occurrence in the islands mentioned shows a former land connexion with the mainland of Asia, corroborated by the presence of other palaearctic plants with it. It is absent from Borneo together with these palaearctic plants.

Anacardium occidentale, Linn., the Cashew-nut. This tree in the Malay Peninsula is commonly to be found in sandy spots along the coast and also on heaths, e.g. at Setul. It appears to be quite wild, and, though the natives do occasionally eat the kernels, I have never known them plant it, nor have I seen it near their houses. The form here appears to be the wild form which occurs in similar localities in Brazil, with a small, usually green, thickened peduncle to the fruits, not the cultivated form with a thick, reddish, fleshy peduncle. Seeds of it, apparently quite sound, occur in the sea, and I have little doubt that it is to some extent sea-borne. It is undoubtedly a native of Brazil, whence it was introduced, probably by the Jesuit Fathers, to Manila. Linschoten mentions it as occurring in Malacca in 1583, together with the Papaya, Chillis, and the pineapple, but it is only mentioned by Garcia in 1593 ('Historia Aromatum', ed. iv) as occurring in Brazil. There are several other species of the genus in tropical America, and it occurs now in the Seychelles and Madagascar and Ceylon, as it does in Singapore, on the sandy coasts. It still retains its original Brazilian name, Cashew, in the form of Gajus in Malay. It seems to be absent from Australia and Polynesia, and, except the cultivated form, inland from Africa. It seems certain that the original form introduced was the cultivated one with the large pear-like peduncle, and that it has established itself on our sea-shores, reverting to the small-fruited wild form, and been spread along our coasts by the sea.

Brasenia peltata, Pursh. This little water-lily was apparently common in Pliocene times in Russia, Germany, and Switzerland, but has entirely disappeared from Europe. It now persists in Manchuria, Khasiya, Japan, Australia, Angola, and North America. It is obvious that at one time it was very widely diffused over the world, but, like so many of the earlier plants, it has died out except in the palaearctic regions and in a few other isolated spots. The drying up or, what is more common, the silting up of lakes may account for the disappearance and isolation of these aquatics.

Naias. These water-weeds seem to be very easily dispersed, largely, I believe, by water-fowl, and some of them have a remarkably wide distribution; one may compare them in the matter of distribution and dispersal with the Characeae.

Naias marina, Linn., a brackish water and marine plant, practically occurs all over the world except tropical Africa and Malaya, but chiefly in temperate regions. It occurs in the Cromer forest bed.

N. minor, All., which occurs with it in the Cromer forest bed, is confined to Europe and temperate and tropical Asia as far east as Manchuria.

N. graminea, Del., occurs earliest in Britain in the interglacial period. It is widely spread over tropical and subtropical Asia. Its occurrence in a hot-water canal at Manchester shows how easily these plants get about the world.

N. flexilis, Rostk., known from interglacial deposits in Sweden and Germany and confined to northern Europe and North America.

Here we see that for four species of *Naias*, all of approximately the same geological antiquity and with very suitable dispersal mechanisms, we have very different areas of distribution.

The marine species, which can adapt itself apparently to sea, brackish, and fresh water, and can stand considerable variations of temperature, is the most widely distributed. The continuity of the sea largely ensures this, and is the reason why maritime plants, as will be shown, are more widely distributed than freshwater plants of streams and rivers which are not continuous.

N. minor, All., a freshwater species, has not reached America and does not seem to stand heat well.

N. flexilis, Rostk., is palaearctic and nearctic only, being a cold-climate species.

N. graminea, Del., is a warm-water species and cannot stand cold, so its area is limited.

It is quite clear that the area of distribution of *Naias* depends not on the age of the species at all, but on adaptability to climate and environment, the species with the largest continuous area, the sea, being the most widely distributed.

There are a few species of plants which occur in tropical Asia and South America which do not appear to be sea-borne, and as far as can be judged are not weeds. These are chiefly small-seeded Cyperaceae such as *Cyperus Haspan*, Linn., *Heleocharis capitata*, Br., and *H. chaetaria*, R. and S., *Fuirena umbellata*, Rothb., and *Rhynchospora aurea*, Vahl., all inhabiting damp, swampy, open spots or marshes, and *Scleria lithosperma*, Sw., which is an open-forest plant. All these plants occur all over Asia and Africa as well as South America (except the *Scleria* not recorded from Africa), but they are absent from oceanic islands. The swamp species are perhaps carried about by wading birds, as they have the habit of appearing very soon on the edges of artificial ponds where sandpipers constantly come. More research is required into this form of dispersal, but I cannot otherwise account for the appearance of these plants and some other swamp and aquatic plants in artificial lakes such as those in the Singapore Botanic Gardens. Wide as their distribution is, none are nearly as widely distributed as the Reed and *Cynodon dactylon*, as they are exclusively tropical, and cannot stand even a temperate climate. *Polygonum hydropiper*, Linn., and *P. minus*, Huds., are similarly widely distributed, though absent from South America, and probably dispersed by wading birds. They range from Europe, through India, the Malay Peninsula, and Java, to Australia, and *P. hydropiper* to North America. *P. minus* is absent from America and New Zealand. *P. hydropiper* first appears fossil in the Neolithic period, *P. minus* is found in the Tegham beds.

In a paper on the Cyperaceae of the Welwitsch Herbarium ('Trans. Linn. Soc.', ii, p. 122), I called attention to the extraordinarily large proportion of Cyperaceae common to Africa, chiefly the West Coast, and South America and the West Indies, and showed that these plants were largely forest plants. The proportion of species of other flowering plants common to the two continents is small, but I would add *Pothomorphe peltata*, Miq. (Piperaceae), and *Lophiocarpus guyanensis*, Mich. (Alismaceae), both of which range from South America, through Africa, into the Malay Islands; neither of these species is likely to have been dispersed by man, nor could they be dispersed by birds. The *Pothomorphe* is not cultivated nor, so far as I am aware, used by man, and it does not produce drupaceous fruit like the Pipers. *Lophiocarpus* occurs in the Malay Peninsula in rice-fields only, and I had thought it might have been carried about in rice-seed, but the large size of its seeds makes this improbable, and prevents its being carried on the feet of birds.

Arldt, in 'Die Entwicklung der Kontinenten und ihre Lebenswelt', gives a series of maps showing the distribution of land and water from early periods. Assuming that these are approximately correct geologically, a land connexion between Africa and Brazil appears in Silurian times and continues through the Chalk period to the Neocomian. This land area includes

eastern South America, Africa, the Mascarene Islands up to the western Himalayas—the Malay Peninsula, Ceylon, and Sumatra being submerged. This area, Sud-Atlantis, gets smaller in early Tertiary eras, but there is still a connexion between Guiana and North Brazil up to Cape Verde and south to Angola. In the Miocene period Sud-Atlantis is broken through and Africa and America are quite separated, and never reconnect.

If this disposition of sea and land is endorsed by other geologists, it would account for the large number of genera and some species common to both sides of the Atlantic, and especially would account for the forest Cyperaceae of Angola and Madagascar occurring in South America but being absent from tropical Asia, and the date of this flora would be before the Miocene period.

It might be suggested that the plants common to Africa and America had been transported by sea-currents or by birds, but against this there is the fact that hardly one of the African maritime or sea-shore plants dispersed by sea occurs in the New World, nor any of the New World species in the Old World, and, besides the fact that it does not appear that birds cross from the Old World into the New, the plants referred to are not such as are bird-borne.

Sea-dispersed Plants.

In deciding whether a plant comes into this class or not we have to take into account any special modification of the fruit or seed for dispersal by sea, such as the thick corky pericarp of *Barringtonia speciosa*, Forst., or the fibrous woody pericarp of *Cerbera*, or the enlarged bladder-like calyx of *Hernandia*, and it is further essential that the plant may be able to grow on the sandy beach or in tidal mud, as the case may be. A great deal has been written by Schimper, Hemsley, and others on the strand flora and its dispersal, and Guppy, 'Observations of a Naturalist in the Pacific', has summed up most of this work, and added so much that comparatively little has to be added. I cannot agree with many of the latter's deductions from the facts, but I do not intend to criticize them in this paper; I will merely content myself with a few remarks bearing on the strand flora of the Malay Peninsula which are not treated of by Schimper or others.

Mr. Guppy writes a good deal about the distribution of plants of the strand flora, i.e. sea-dispersed plants found inland, especially in the Polynesian Islands. This occurs, as is well known, in many parts of the world, the strand flora being met with often on the tops of mountains. I think it will be found that in all cases the strand flora in such spots is due to the sea having formerly reached these altitudes and left its flora there stranded. There are no such examples in the Malay Peninsula, so far as has yet been seen, as there is indeed no evidence of the sea having been

over or up to the ranges of hills in the interior, at least later than Mesozoic times, but there are a few cases of strand plants being found inland a long way from the present sea-coast. Thus at Kanga, in Perlis, at the base of the huge limestone islands now far off the sea in a great sandy plain, I found the little *Boerhaavia repanda*, Willd. (Nyctagineae), a typical sea-sand plant, while in the damper spots of the plains grew *Dolichandrone Rheedii*, Seem., a typical tidal-mud plant. Here the whole area, at no great distance of time submerged by the sea, had gradually silted up with the sand and gradually pushed the sea-coast far away, while these plants still remain and thrive stranded as they were by the departure of the sea. I have met with *Boerhaavia repens*, Linn., too, growing between the railway lines far inland in Java at Muntilan. Here I imagine it was brought in the ballast for the line. On a cart-track in Bukit Tangga, Negri Sembilan, thirty-six miles from the sea, and on railway banks in Kota Bharu, Kelantan, I have seen in sandy spots the sea-shore Convolvulus, *Ipomoea biloba*, well established though far away from its ordinary sea-sand habitat almost within the splash of the waves. Here again I have little doubt that it was brought in ballast from the sea-coast to which the railway ran, and contrived to establish itself on the sandy fields near the railway. But, except for these cases, it is remarkable to what a short distance the strand flora goes inland, even in such apparently favourable localities as the sandy heaths of Pekan, in Pahang, where the sandy country runs continuously to the strand-flora region.

As a rule, sea-shore plants and tidal-mud plants disappear altogether when, by deposit of silt or shifting of the tidal river, the ground they grow on ceases to be suitable for the strand flora. The Singapore river at Tanglin, from road-making, town-building, &c., has long ceased at this point, about four miles from the sea, to be tidal, and all the waste ground in the economic gardens near it was a low swampy patch covered with a wood of *Cinnamomum iners*, Bl., *Premna foetida*, Reinw., Macarangas, *Ficus*, &c., but when it was cleared a large clump of the tidal-mud fern *Acrostichum aureum* was found still growing there, and the ground was full of Nipa palm fruits, which last a very long time underground, showing that this must have been at one time a tidal-mud river. I once came across in Johor, near Gunong Pantai, a long way from any tidal mud and surrounded by dense forest, a large patch of the tidal-mud fern which must have marked a long-disappeared tidal river filled up and covered with heavy hill and lowland forest. These stranded sea-shore plants do not seem to spread at all, but remain, for the most part at least, in the same spot where they were left when abandoned by the salt water.

Dolichandrone Rheedii, Seem. (*D. spathacea*, Schum.), mentioned above, is rather an interesting plant from another point of view, as shown by Sprague's account of the genus in 'Kew Bulletin', 1919, p. 304. The genus,

belonging to the order Bignoniaceae, contains nine species, of which three are endemic in North Australia, one in Portuguese East Africa, three in the forests of southern India, one in the Irawaddy district, Burma, and one in Lower Siam. All these appear to be quite local plants in distribution. They are middle-sized trees with long-tubed, fragrant white flowers opening in the dusk and falling in early dawn, with long pods of winged seeds, the wings of the seed being thin and hyaline and longer than the body of the seed, so that they are easily dispersed by wind, as is the case in most plants of the order. But *D. Rheedii* is an inhabitant of tidal-river mud. Closely resembling the other Indian species in habit, foliage, and flowers, and most closely allied to the Burmese *D. serrulata*; it differs most remarkably in its seeds. Instead of having thin hyaline wings longer than the body of the seed at each end of it, the seed has at either end a short, oblong, corky prolongation, quite unsuited for wind dispersal and quite unlike any other Bignoniaceous seed. When dropped from a height it falls straight to the ground, while a winged seed of *D. serrulata* flutters, rotating as it goes, to a considerable distance. By the shortening and thickening of the wing of the seed it has been adapted for sea-dispersal.

As has been mentioned, the other species of the genus are confined to limited areas, but this species occurs in mangrove swamps and tidal rivers all round the Bay of Bengal as far as Ceylon, and to Travancore and Malabar on the west coast of the peninsula along the coasts of Burma, the Andamans and Nicobars, the Malay Peninsula and the Malay Islands from Sumatra to New Guinea, and the Philippines to New Caledonia and the Solomon Islands.

The distribution of this plant shows clearly the superiority of sea-dispersal, both in time and distance, over dispersal of winged seeds by wind, and, further, as all the other plants in the order have the peculiar thin-winged seeds possessed by the other species of *Dolichandrone*, we may certainly assume that *D. Rheedii* is derived from one of the thin-seeded species, probably *D. serrulata*, Seem., of the banks of the Irawaddy, as it so closely resembles it that specimens of the two plants have often been taken for each other. The seed-wings in *D. Rheedii* persist, but have been converted into shorter, thick corky floats. This species is therefore younger in time than the other winged-seed species, yet its distribution is far wider than that of any other species: it is in fact another case which militates strongly against the age and area hypothesis.

Most of the large orders of plants contain one or more species whose fruit is adapted especially for sea-dispersal; usually one species only occurs in a large order, the rest being inland plants having no such adaptation. In these cases it will be almost invariably found that the distribution of the sea-dispersed plant is very much wider than that of the inland species. Thus one may instance *Calophyllum* (Guttiferae), of which we have twenty-

four species in the Malay Peninsula, all inland plants and endemic, except one reaching to Cochin China, and one of rather dubious distribution to the Malay Islands, and the maritime *C. inophyllum*, Linn., ranging from Africa, through India, Ceylon, and the Malay region, including Christmas Island, to Australia and Polynesia.

Heritiera elata, Ridl. (Sterculiaceae), is a rare tree in Singapore forests only. *H. littoralis*, Dryand., inhabits tidal swamps and sea-shores, common over the whole of tropical Asia. *H. elata* has no means of dispersal except rolling of the fruits. A tree which fruited heavily was surrounded the following year with hundreds of seedlings, next year the number had largely diminished, till in three or four years hardly one had survived. *H. littoralis* has seeds modified for floating in the sea, and I have never seen it so heavily fruiting, yet it is far more widely distributed than the jungle tree.

Sophora (Leguminosae) is a genus of about fifty inland species ranging over the tropics and subtropics, all species local and of limited distribution, except *S. tomentosa*, Linn., a maritime species with sea-borne seeds, with a distribution over Florida, West Indies, Brazil, and the whole of Asia and Africa.

Scaevola (Goodenoviae) contains about fifty species confined to Australia, a few in New Zealand and Polynesia, one in China, and a few in the eastern Malay Archipelago. These are not sea-dispersed plants and are all very local. *S. Koenigii*, Vahl., a fleshy sea-shore shrub with fruits adapted for sea-dispersal, is common on the coasts of Mauritius, India, Ceylon, Siam, China, Formosa, Malay Peninsula and Archipelago, including Christmas Island, and Polynesia, a far wider distribution than all the rest of the genus put together.

Derris, Linn., is a genus of Leguminosae widely spread over Asia, but more or less local. Most of the species have thin, indehiscent, one-sided pods, drifted to a short distance by wind, and inhabit inland forests and plains.

Derris thyrsiflora, Benth., is found in low, open country; its pods are blown to about sixty yards from the plant by wind. It is confined to the Malay Peninsula as far north as Mergui, and Sumatra and Java. Closely allied to it is *D. sinuata*, Thw., a tidal-river plant with pods which bear several seeds and break up into joints and are sea-dispersed. It is distributed over Ceylon, Burma, the Malay Peninsula, and Borneo, a wider area than that of *D. thyrsiflora*. *D. uliginosa*, Benth., is a sea-shore species with pods specially adapted for sea-dispersal. It occurs from East Africa and the Mascarene Islands on all the Asiatic coasts to China and Japan, Formosa, Australia, and Polynesia. No other species of the genus has anything like this distribution, most being quite local. *D. scandens*, Benth., however, a sea-shore and river plant, occurs on most of the Asiatic coasts.

Pemphis acidula, Forst. (Lythrarieae), a monotypic genus, and *Tournefortia argentea*, Linn. fil. (Borageineae), have a distribution from the

Mascarene Islands along the coasts of India (*Tournefortia*, Ceylon and Andamans only), through the Malay region to Polynesia. Both are very scarce in the Malay Peninsula, though abundant in the Malay Islands, because they both grow on raised coral-reefs and there is practically no raised coral-reef in the peninsula.

The following maritime plants, besides the above mentioned, occur all over the tropics in both hemispheres: *Caesalpinia Bonduc*, Roxb., and *C. Bonducella*, Flem. (Leguminosae), *Ipomoea biloba*, Linn. (Convolvulaceae), *Hibiscus tiliaceus*, Linn., *Sida cordifolia*, Linn., *S. rhombifolia*, Linn., *S. carpinifolia*, Linn. (Malvaceae) (these Sidas are also weeds of cultivation), *Cassytha filiformis*, Linn. (Laurineae), *Fimbristylis spathacea*, Roth., *Remirea maritima*, Aubl. (monotypic Cyperaceae), *Paspalum vaginatum*, Sw. (Gramineae). All except *Remirea* and *Cassytha* belong to genera of many species of inland plants, none of which has anything like their distribution. Many other maritime plants have an area of the Mascarene Islands and all Asiatic coasts to Polynesia, but have not reached or settled in America. In all cases the story is the same; they are far more widely distributed than their inland non-maritime allies.

It is in the highest degree improbable that the inland species of these plants, numerous as they often are, can be derived from the few or solitary maritime species, but on the contrary the maritime species, retaining the general form of the fruit modified for sea-dispersal, must be derived from one or other species of the inland ones; consequently the widest distributed species must be later in time of evolution than the local and often endemic species.

Bird-carried Seeds.

There are a number of widely distributed plants which inhabit swampy ground and edges of ponds and open streams, but which do not appear to be weeds or to have been helped in any way by man. They chiefly consist of Cyperaceae. Such are *Rhynchospora aurea*, Vahl., *R. glauca*, Vahl., *Cyperus Haspan*, Linn., *C. radiatus*, Vahl., *C. digitatus*, Roxb., *Eleocharis fistulosa*, Schult., *E. variegata*, Kunth., *E. capitata*, Br., and *Polygonum hydropiper*, Linn. From the way in which most of these plants appear on the edges of artificial ponds, where the sandpipers often alight after their long migrant flights, I should suggest that these waders bring them in their feathers or possibly attached to their feet. Most have quite small seeds which could be easily carried in this way, but Guppy has shown that Cyperaceous seeds can be successfully carried by ducks in their intestines, and *Polygonum* seeds by various other birds in the same way. The Jussiaeas (Onagraceae) seem to be carried about largely by water-fowl, as they have the same habitat, but the American species, though closely allied to the Asiatic ones, appear to be specifically distinct.

The large artificial lake in the Botanic Gardens in Singapore contained a number of aquatic plants which were certainly not planted there, and which did not, as far as I know, occur in any spot whence they could have been drifted into the lake. They were *Enhydrias angustipetala*, Ridl., *Blyxa malayana*, Ridl. (Hydrocharideae), *Naias graminea*, Del., a *Chara*, and two Utricularias. I have little doubt that these were brought from considerable distances by wading birds or ducks. A lake like this in a country where ponds and pools are extremely scarce is always very attractive to birds on migration, and ducks, jacanas, sandpipers, and even cormorants have appeared from time to time on this pond.

It will be noticed that a considerable proportion of the most widely distributed species of plants are Cyperaceae and Gramineae, wind-fertilized plants which do not require pollination by insects, and I would suggest that this has a considerable bearing on the rapid distribution and the large number of plants of one species occurring together, often over considerable areas such as the extensive tracts of *Imperata arundinacea*, Cyr., *Chrysopogon aciculatus*, Trin., the dense masses of *Paspalum conjugatum*, Berg., along mountain paths, the large swamps almost entirely of *Eleocharis equisetina*, Presl., in Setul. I have never seen in the Malay Peninsula large areas of any single species of insect-fertilized plant, except the single-tree forests of *Dryobalanops* and of *Avicennia*, with which I will deal elsewhere.

Of the Wide Distribution of Orders of Plants.

In 'Journ. Linn. Soc.', xliv, p. 439, Mr. Guppy, in a paper entitled 'Plant Distribution from the Standpoint of an Idealist', suggested that orders were evolved first, then genera, then species. It is difficult to see how an order, an accumulation of species, could in the first instance be evolved before the species. The larger orders seem, from what we know of Eocene plants and from the story of distribution, to have appeared at an early date, such orders, that is, as Anonaceae, Laurineae, Leguminosae, and Myrtaceae, but that all orders, even large ones, were evolved before the genera is very easily disproved.

We have in tropical Asia a number of genera which are wanting in Australia and Polynesia, but which extend to Africa and are again well represented in South America. They have received no assistance from man, and are not widely dispersed by birds or by sea. Such genera are *Tetracera* (Dilleniaceae) and *Xylopia* (Anonaceae), and we have even one species, *Pothomorphe peltata*. Now the only way these plants could have crossed the ocean from Africa to South America is by a former land connexion such as is shown in Arldt's maps. This land connexion is believed to have broken down before the Pliocene period, and these plants must have crossed before that. Now in South America we have two big, besides several small, orders peculiar to that country which have even better means of dispersal

than these plants, viz. Bromeliaceae and Cactaceae.[1] Some at least of the species of these orders, notably the Opuntias, introduced into India and Africa have thriven remarkably, so that there is no reason to suppose that if they ever got across naturally we should not find traces of them, but there are none; the only possible deduction is that these orders were not evolved till after the connecting land had disappeared and the Tetraceras, Xylopias, &c., and *Pothomorphe peltata* had been evolved.

In the same way we may fairly decide that the Pandanaceae absent from the New World, though thriving when planted there, were evolved in Africa and Asia after the connective bridge was broken, and this is more remarkable in that structurally *Pandanus* appears to have been a very primitive plant. It has always struck me as remarkable that this genus of maritime and marsh habit, with leaves and fruits that preserve remarkably well, has not been found fossil in the European deposits at all.

Summary of widely distributed Plants.

I have dealt herein mainly with such widely distributed plants as occur in the Malay Peninsula, but this covers really the most widely distributed plants in the world of flowering plants. Those that are to be found over the large area of the world's surface fall into four groups:

1. The weeds, plants which have been accidentally or intentionally carried by man to various countries and there, finding suitable soil and climatic conditions, have spread themselves widely from the position to which they were first introduced. They belong to many different orders, but are chiefly herbaceous, and their area of distribution on arriving at their new position chiefly depends on their means of dispersion—adhesiveness of seeds or fruits, and wind dispersion, being the two most successful methods.

2. Plants dispersed by sea-currents. These all naturally grow on the sea-shore, either in sandy beaches or on tidal mud. The greater number of those of the Malay Peninsula cover an area from the Mascarene Islands over the Indian Ocean to North Australia and the Polynesian Islands in the Pacific Ocean; a smaller number occur also in South America and West Africa.

3. A small number of swamp plants, chiefly Cyperaceae, which appear to be dispersed by water-fowl, occurring in both hemispheres.

4. A few which are capable of thriving in temperate and tropical regions, such as *Phragmites communis* and *Cynodon dactylon*, with a few palaearctic forms which have descended along the mountain chains as far south as the equator. These latter, illustrated by *Sanicula europaea*, are very scanty in the Malay Peninsula and do not occur in America. They are more abundant in Sumatra and Java.

[1] *Rhipsalis Cassytha*, Gaertn. (Cactaceae), epiphyte, occurs in Ceylon as well as tropical Africa and tropical America. It is the only Cactacea wild in the Old World.

There is no evidence to show that all these various widely dispersed species are of specially great antiquity, or that the area occupied by them depends primarily on their age, as suggested by Dr. Willis. It is certainly not the case in the plants which I have called weeds, where we often know the date of the introduction of the plant into a given area. Their rapidity of dispersal depends on the area of suitable conditions for growth and their means of dispersal. On the other hand, we know that the Cycadeae are a very old group of plants now reduced to about eighty species, all remarkably local and confined to very limited areas, not one of which can be compared in extensive area-dispersal to the Reed, *Ipomoea biloba*, or *Paspalum conjugatum*, nor even to the Sanicle; while the Nipa palm inhabiting England in the Eocene period (for *Nipadites Burtini* is really hardly distinguishable from the Nipa of the present day) is now confined to the Indian Ocean from Ceylon and Bengal, down the Malay Peninsula to North Australia, and the Caroline and Solomon Islands, not having got so far as the Mascarene Islands, peninsular India, or Polynesia, in spite of its abundance as a drift plant both by seed and the large clumps of rhizome always to be seen drifting in Malayan seas. It is true it requires tidal mud for its growth, but there must be tidal-mud rivers in Samoa, Africa, and America quite suitable for this plant; we find, however, that, old as it is, it has a distribution now no wider than many of the doubtless more modern species which frequent the same area.

An examination of what is known of the early floras of the Eocene and Miocene periods serves to show how local now are many of the genera existing in those times; such instances are the genera *Sequoia*, *Thujopsis*, *Salisburia*, *Taxodium*, *Andromeda*, *Cinnamomum* (absent from America and Africa), *Liquidambar*, *Platanus*, *Hakea*, and many others.

Some of the older genera persist widely dispersed, as one might expect, occurring in both hemispheres, but many more have gradually disappeared and only persist now in a few isolated spots.

It would be quite natural to imagine that plants of great age would be more widely dispersed throughout the world than more modernly evolved species, as having had more time for their dispersal, but the changes which the world has undergone since their evolution have been accompanied by extensive changes in the flora, the old species disappearing or persisting as endemics or very local plants in different corners, where they have held their own in spite of the fluctuations of climate and changes in the earth's surface. The ecology, and especially the habitats and methods of dispersal, of each plant must be studied in the field before we can formulate any idea as to its history or the study of plant distribution in general.

On the 'Squamulae Intravaginales' of the Helobieae.

BY

AGNES ARBER, D.Sc., F.L.S.

(*Keddey Fletcher-Warr Student of the University of London*).

With five Figures in the Text.

(i) Introduction.

IT has long been known that certain scale-like structures, to which the name *squamulae intravaginales*, or *squamulae intraaxillares*, was given by Irmisch, are found among the leaf-bases of the Helobieae (Potamogetonaceae, Naiadaceae, Aponogetonaceae, Scheuchzeriaceae, Alismataceae, Butomaceae, Hydrocharitaceae) and of certain members of the related family, Araceae. The earliest published record of the existence of these squamules seems to be that of Nolte (16), who, nearly a century ago, observed them in *Stratiotes*. But we owe the first comprehensive treatment of the subject to Irmisch (10), who published a paper in 1858, which, though brief and unillustrated, laid the foundation of our knowledge of the structures in question; he also carried his observations further in other papers (11, 12, 13, 14). Caspary (5), Prillieux (17), Sanio (18), Bornet (2), Bayley Balfour (1), Buchenau (3 and 4), T. G. Hill (9), Fauth (7), Harvey Gibson (8), Serguéeff (21), Cunnington (6), and Solereder (22) have added further information about the morphology and distribution of the squamules. There are many good illustrations of their external appearance, both in the papers just cited—especially that of Bornet (2)—and in Kirchner, Loew, and Schröter's 'Lebensgeschichte der Blütenpflanzen Mitteleuropas' (15). Schilling (20), who has studied the squamules from the point of view of function, regards them as organs which secrete mucilage, but, according to Solereder (22), this does not hold good universally.

The number of squamules found in association with each leaf may range in different species from two to many; each of the squamules may either consist of a single plate of cells, or it may be a comparatively solid body, in which the upper and lower epidermis are separated by several

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elements. There is great variety in shape, and the appearance may be modified by a fringe of marginal hairs. But, despite this astonishing range of variation as regards number, form, and structure, the squamules all agree in the lack of vascular tissue, and in being apparently of a trichome nature.

Although we possess such a large amount of information about these squamules, the literature often reveals a certain vagueness on the practical question of their exact point of origin, and on the theoretical question of their morphological interpretation. They have generally been spoken of as 'intravaginal' or 'intra-axillary', or as occurring 'at the leaf-base', except in certain cases in which they have been observed to take their origin from the axis. Caspary (5), in 1858, called them *stipulae intrafoliaceae*, while Prillieux (17), in 1864, described them as very small stipules, and in more recent times Buchenau (4) has suggested that in the Alismataceae they should be regarded as 'Ligulargebilde'. Bornet (2) treated them as appendages of the leaf *above* them on the axis, while Irmisch and apparently all the other writers who have considered the squamules hold them to belong to the leaf *below* them, in regard to which they occupy a more or less axillary position. That some obscurity and uncertainty should exist on these points is not, however, surprising, since the greater part of the work on the subject predates the general employment of the microtome in botany; and, without serial sections, one could scarcely hope to arrive at a complete understanding of the relations between these minute and delicate squamules and the rest of the shoot. The object of the present paper is, with the help of the microtome, to trace the history of the development of the *squamulae intravaginales* in a small number of cases from among the Helobieae, in order to see if it be possible in this way to obtain a firmer basis for the interpretation of these structures.

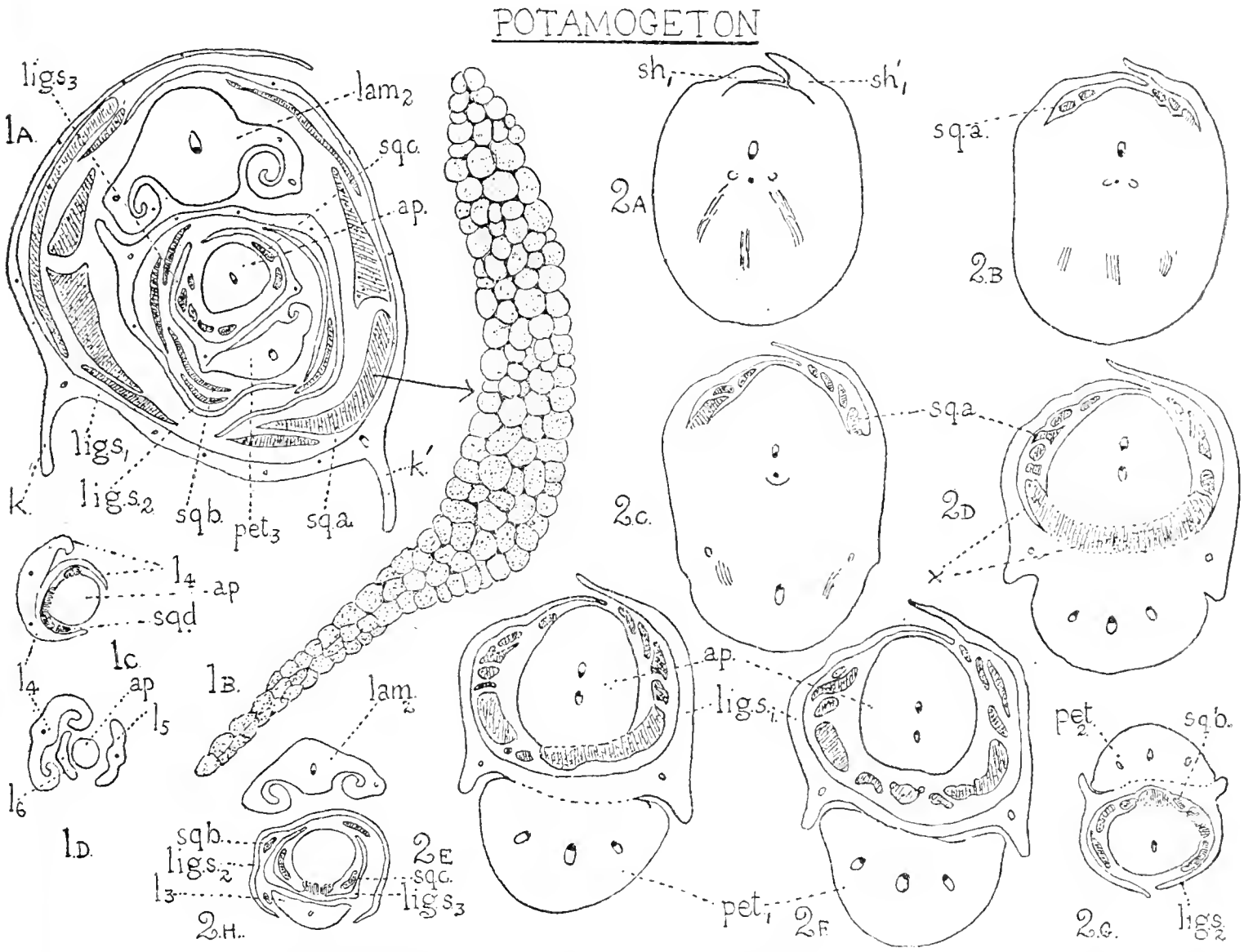
I am indebted to Professor Ostenfeld, of Copenhagen, for material of *Cymodocea isoëtifolia*, Asch.

(ii) Description of Observations.

Potamogetonaceae—*Potamogeton*.

Fig. 1 A gives a general idea of the appearance of the squamules among the leaves, as they are seen in a transverse section of the inner part of an apical bud belonging to a broad-leaved, submerged species of *Potamogeton* (unidentified). In the case of the outermost leaf represented, only the ligular sheath (*lig.* $s._1$) is included; a large number of squamules (*sq. a*) are seen between it and the next leaf. One of these squamules—that indicated by an arrow in Fig. 1 A—is shown on a larger scale in Fig. 1 B. The leaf inside this set of squamules is cut through the base of its limb (*lam.*$_2$) and ligular sheath (*lig.* $s._2$), on the inner side of which another set of squamules (*sq. b*)

are seen. A still younger leaf (*pet.*$_3$ and *lig. s.*$_3$) has further squamules (*sq. c*) within it, surrounding the developing apex (*ap.*). In Figs. 1 C and D, the changes which are passed through by this growing-point, between the level of Fig. 1 A and the tip of the shoot, can be followed. Fig. 1 C shows another leaf (*l.*$_4$) and a further set of squamules (*sq. d*), while in Fig. 1 D



FIGS. 1 and 2. *Potamogeton* sp. (broad-leaved, submerged form). Fig. 1 A, transverse section of inner part of apical bud (× 47); in case of outermost leaf represented, only the ligular sheath (*lig. s.*$_1$) with its keels (*k.* and *k'.*) are shown; limb (*lam.*$_2$) and ligular sheath (*lig. s.*$_2$) of second leaf; petiole or base of limb (*pet.*$_3$) and sheath (*lig. s.*$_3$) of third leaf; *ap.*, shoot-apex. Three sets of squamules (shaded), *sq. a*, *sq. b*, *sq. c*, are seen outside second leaf, third leaf, and apex respectively. Fig. 1 B, transverse section of squamule marked with arrow in Fig. 1 A (× 193). Figs. 1 C and D, changes in shoot apex (*ap.* of Fig. 1 A) as tip is approached (× 47). Fig. 1 C, fourth leaf (*l.*$_4$) detaching itself from apex, and squamules (*sq. d*) occurring between it and apex. Fig. 1 D is close to tip of shoot, and shows highest leaves (*l.*$_5$ and *l.*$_6$) on either side of apex (*ap.*). Figs. 2 A–H, series of transverse sections from below upwards through three successive young leaves and growing-point of another bud (× 47); sections broken on side towards petiole of leaf 1, so this region reconstructed; lettering as in Fig. 1; *sh*$_1$ and *sh'*$_1$, margins of sheathing leaf-base.

the extreme apex of the axis is reached (*ap.*), lying between the two youngest leaves (*l.*$_5$ and *l.*$_6$). The set of squamules within leaf 4 in Fig. 1 C are the youngest to be found in this shoot; I cannot with certainty detect any inside leaf 5 or leaf 6.

The sections drawn in Figs. 1 A–D are too far above the bases of most of the leaves to give much enlightenment on the origin of the squamules: for this we must turn to Figs. 2 A–H, which represent sections from a series

passing from below upwards through the basal regions of three successive leaves belonging to another apical bud. In Fig. 2 A the first of these leaves is just beginning to detach itself from the growing apex; the earliest sign of this detachment is the appearance of the two free overlapping margins of the sheathing leaf-base (*sh.*$_1$ and *sh.*$'_1$). In Fig. 2 B the free flaps of leaf tissue have increased in length, and the first *squamulae intravaginales* (*sq. a*) have made their appearance. Figs. 2 C and D show further stages. It will be seen that the earlier squamules are budded off successively in two series—one on either side—from the boundary zone between the shoot apex and the free part of the leaf. The points of origin of these two series travel farther and farther apart as the sheath detaches itself. Opposite the median region of the sheath, however, the squamules become free in the form of a continuous zone (marked with a cross in Fig. 2 D) which separates almost immediately into constituent individuals (Figs. 2 E and F). In Figs. 2 E and F the squamules form a wreath surrounding the growing apex, and interrupted only opposite the margins of the ligular sheath (*lig. s.*$_1$) of the first leaf, which is now almost detached from its petiole (*pet.*$_1$); this leaf is thus cut slightly nearer its base than such a leaf as that marked *lam.*$_2$ and *lig. s.*$_2$ in Fig. 1 A. Figs. 2 G and H carry on the history to a higher level. In Fig. 2 G the apex (*ap.*) of Fig. 2 F is represented by a second leaf (*pet.*$_2$ and *lig. s.*$_2$), enclosing the shoot apex, which is surrounded by a series of squamules (*sq. b*). In Fig. 2 H the limb (*lam.*$_2$) and ligular sheath (*lig. s.*$_2$) of *l.*$_2$ are entirely separate, and another leaf (*l.*$_3$) with its ligular sheath (*lig. s.*$_3$) is detaching itself, while a third series of squamulae (*sq. c*) appear on its inner side.

A series of sections, similar to those drawn in Figs. 2 A–H, but cut from two successive leaves of another species, *Potamogeton natans*, L., are seen in Figs. 3 A–G. The history of the origin of the squamules closely recalls that in the species already described. When the development of the first of these leaves is followed, however, it is found that there is a slight separation between the leaf and the shoot-axis in the median plane, at a level at which the flaps of the leaf-sheath are still attached to the flanks of the axis (Fig. 3 C). A band of squamular tissue (marked with a cross in Fig. 3 C) is thus left temporarily attached to the upper median region of the young leaf; but almost at once it becomes free on that side also (Fig. 3 D). In Fig. 3 E the ligular sheath (*lig. s.*$_1$) is completely detached from the petiole (*pet.*$_1$). In this figure, also, we see the first indication of the separation of the leaf-sheath of the next leaf (*sh.*$_2$) from the shoot-apex. Figs. 3 F and G show further stages in the development of this second leaf and the squamules within it (*sq. b*). These squamules remain attached to the growing apex—in the median region marked with a cross in Fig. 3 F—even after the leaf has become completely free.

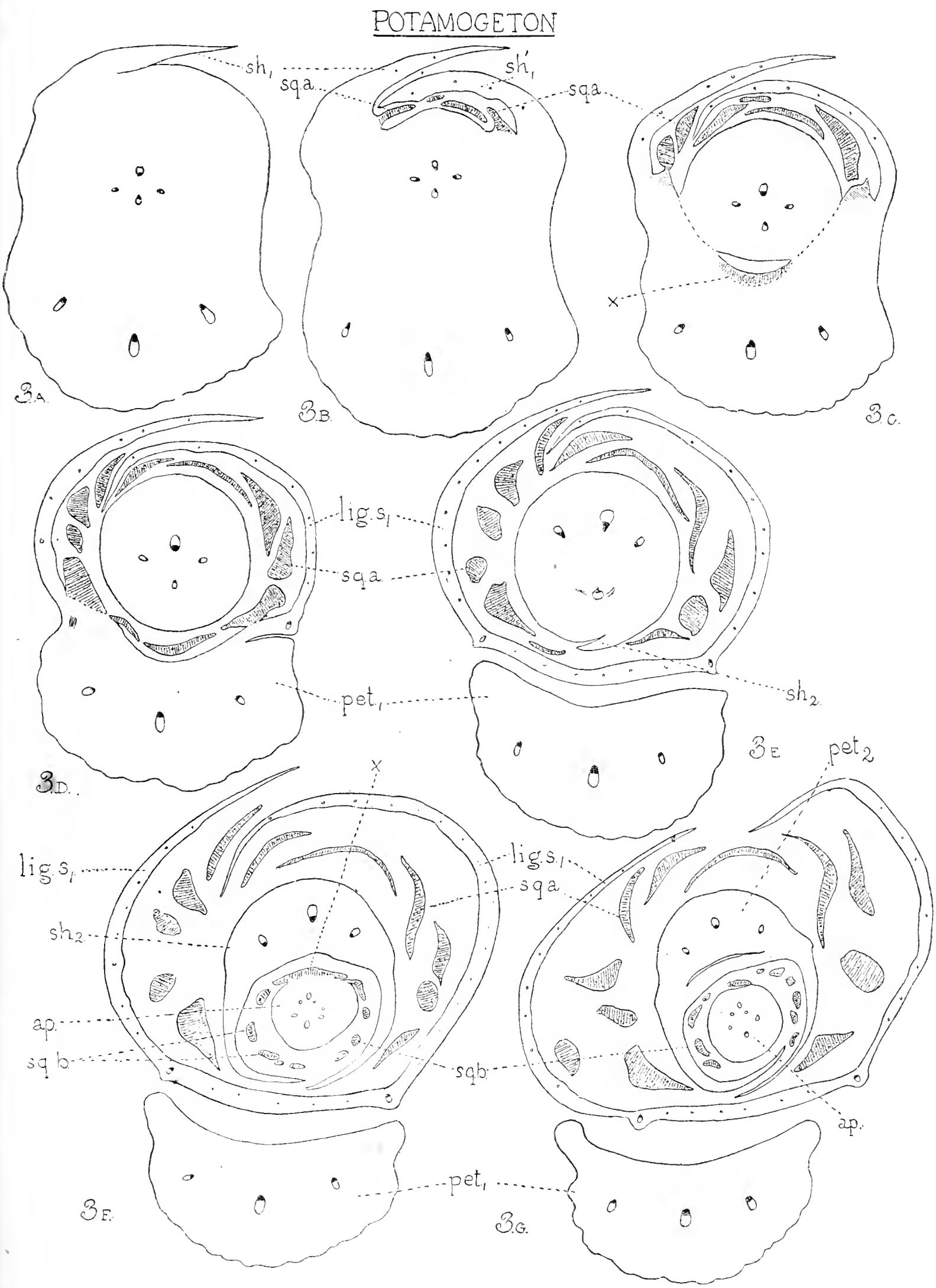


FIG. 3. *Potamogeton natans*, L. Figs. 3 A–E, series of transverse sections from below upwards through apical bud including two successive leaves and the squamules within them (× 47); lettering as in Fig. 1, see also explanation in text. Figs. 3 F and G show further stages in the development of the second leaf and the squamules. Fig. 3 F, from a broken section, somewhat reconstructed as regards *lig. s.*$_1$ and *sq. a* on right.

Potamogetonaceae—*Cymodocea.*

I have not made any thorough study of this genus, but I include it here because, in serial sections through an inflorescence of *Cymodocea isoëtifolia*, Asch., I noticed the development of *squamulae intravaginales* in connexion with the prophyll of a lateral branch; their origin is illustrated in Figs. 4 A–E. Figs. 4 A and B show the main axis, with a bulge on the side on which a lateral bud (*b.*) is about to develop; the margins of its prophyll (*sh.* and *sh.'*) are beginning to detach themselves. In Fig. 4 C we reach the level at which the lateral bud is completely free, but the prophyll (*pr.*) is still attached to the bud on the adaxial side. Squamules (*sq.*) are seen developing from the angle between the prophyll and the axis of the bud. When they are

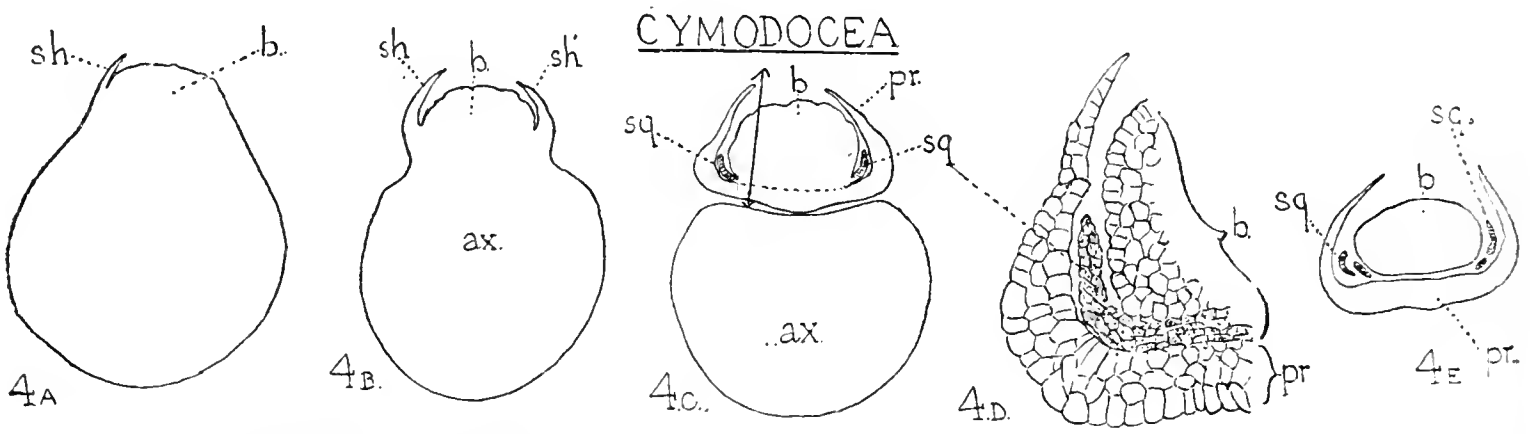


FIG. 4. *Cymodocea isoëtifolia*, Asch. Figs. 4 A, B, C, E, series of transverse sections (× 23) passing upwards from below through an axillary bud, *b.* (whose further history has not been followed), and its prophyll, *pr.*, borne laterally on an axis, *ax.* Figs. 4 A and B show detachment of margins of prophyll, *sh.* and *sh.'* Fig. 4 C, complete detachment of lateral bud, from which the prophyll is at this level only partly free; dotted line indicates plane of separation between prophyll and axis of bud; *sq.*, squamule. Fig. 4 D, region to left of arrow in Fig. 4 C (× 77). Fig. 4 E, stage at which bud, squamules, prophyll, and parent axis have become free from one another.

examined in detail, it is found that they arise from the tissues of the bud-axis, and not from those of the prophyll; this point is illustrated in Fig. 4 D, which represents the part of Fig. 4 C to the left of the arrow, on a larger scale.

Scheuchzeriaceae—*Triglochin.*

Figs. 5 A, C, F, G, H, from serial sections of a bud of *Triglochin maritima*, L., show, at different levels, the relations of the young leaves and the associated squamules. Fig. 5 A is a slightly oblique transverse section in which the sheath of the outermost leaf ($l._1$) has detached itself on the left-hand side, but is still one with the axis on the right. Only a few of the squamules external to it are included in the section. The further history of the leaf, up to the point of separation of the ligular sheath ($lig.\ s._1$) and the petiolar limb ($pet._1$) can be followed in Figs. 5 C, F, G, H. But from our present point of view it will be simplest to concentrate attention on the *second* leaf and the squamules immediately outside it. In Fig. 5 C this leaf ($l._2$) is free on the left-hand side, but still fused with the young axis on the right. The leaf is surrounded by squamules, of which those

on the left are free, whereas those on the right are attached to the external surface of the leaf, just below the level at which it divides from the axis. Of the three squamules marked with a cross in Fig. 5 C. the two right-hand ones are seen fused with one another and with the axis in Fig. 5 B, which is cut at a level between Figs. 5 A and 5 C. The structure of the young squamules, at the levels of freedom and of attachment, is shown in Figs.

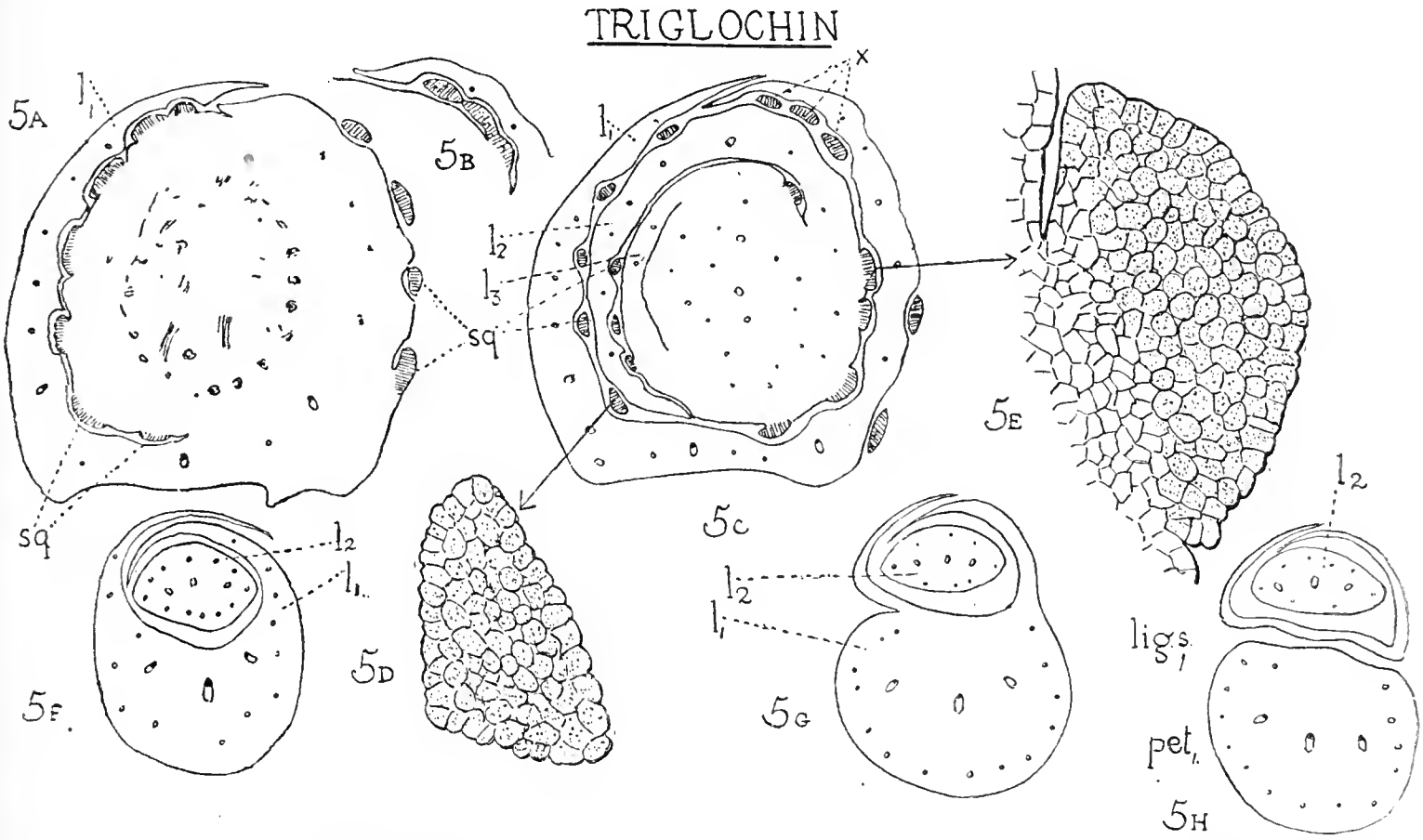


FIG. 5. *Triglochin maritima*, L. Figs. 5 A, B, C, F, G, H, serial transverse sections from below upwards through young leaf, l_1, and the apical bud with younger leaves, l_2 and l_3, which it encloses; squamules, *sq.*, shaded (× 23). Fig. 5 A, margin of leaf-sheath of l_1 free on left side, but, owing to slight obliquity of section, cut at a lower level on right-hand side, and there fused with axis. Fig. 5 C, section at a slightly higher level, showing three sets of squamules, external respectively to l_1, l_2, and l_3. Fig. 5 B shows the three squamules marked with a cross in Fig. 5 C, cut at a slightly lower level. Fig. 5 D, detached squamule marked with arrow to left of Fig. 5 C (× 193). Fig. 5 E, attached squamule marked with arrow to right of Fig. 5 C (× 193). Figs. 5 F, G, H, sections through l_1 and l_2 at higher levels, to show development up to point of separation of ligular sheath and petiole of l_1 (× 23).

5 D and E. A third set of squamules, external to l_3, are visible on the left-hand side in Fig. 5 C.

(iii) The Origin of the Squamules.

Of the plants in which we have described the ontogeny of the squamules, *Triglochin maritima*, L., is the one in which the interpretation of the facts observed is open to least doubt. Irmisch (10) regarded the squamules in this genus, not as outgrowths from the leaves, but as originating independently from the axis, but more recent writers (3, 4, 9) merely speak of them as arising in the leaf-axils. I think that the sections which I have drawn in Figs. 5 A, B, C show conclusively that it was Irmisch who correctly seized the relation of the parts. But in the light of the 'Leaf-skin Theory', recently put forward by E. R. Saunders (19), I think that Irmisch's concep-

tion needs restatement. If we interpret Fig. 5 A, p. 37, on Saunders's theory—which I adopt here because it appears to me to clarify our ideas of the spermophyte shoot—we should say that the surface of the 'axis' on the left-hand side (inside l_1), which is seen giving rise to squamules, is, in reality, the 'leaf-skin' belonging to leaf 2; this leaf-skin is regarded as extending downwards from the exsertion of leaf 2, so that it clothes the axis throughout the abbreviated internode separating leaf 1 and leaf 2. I should, thus, describe the squamules of *Triglochin*, not as belonging to the leaf *beneath* them, but as appendages of the dorsal (lower) surface of the leaf next *above* them; they are developed near the extreme base of its downward extension—that is to say, in the region in which it has no free existence, but forms the leaf-skin for the internode. The only case, in which an explanation, more or less of this type, has been already offered, is that of *Cymodocea aequorea*, Kon. (Potamogetonaceae), described by Bornet (2) under its old name *Phucagrostis major*, Cavol. Bornet's work predates the appearance of the Leaf-skin Theory by many years, so he naturally did not express his conclusions in these terms, but in describing the squamules he definitely related them to the leaf *above* their place of origin, and spoke of them as left behind by the elongation of the internode. Irmisch (14) discussed Bornet's view, and attempted to refute it. His chief argument was that squamules occur in certain positions (e. g. above the highest leaf of an inflorescence axis) in which there is no foliage leaf above them to account for their presence. But it seems to me that the squamules in such cases may possibly be related to bracts or sepals occurring at some distance above them, for Saunders has brought forward evidence that modified leaves of this type may provide a downward prolongation of leaf-skin as effectively as if they were foliage leaves. Bornet's conclusions were based on a close and delicate examination of the squamules of *Cymodocea aequorea*, Kon., as solid objects. In the case of another species of the same genus (*C. isoëtifolia*, Asch.) I have shown (p. 36), by the radically different method of serial sections, that it is possible to convince oneself that the squamules do not belong to the leaf in whose axil they are found, but arise from the apparent axis above this leaf (Fig. 4 D, p. 36); this observation is thus confirmatory of Bornet's view.

In the other Potamogetonaceae which I have examined (*Potamogeton natans* and *P.* sp.) the squamules arise from a zone of tissue forming the boundary between the sheathing leaf-base and the 'axis' which it encloses. Whether this boundary tissue should be treated as belonging to this leaf or to the axis is, in this case, rather a subtle question. In the broad-leaved, submerged *Potamogeton* represented in Figs. 1 and 2, p. 33, there are clear indications that the squamules, on the side remote from the opening of the leaf-sheath, are still fused with the growing apex, after the leaf becomes detached from it. Examples of this attachment are seen in the squamules

(*sq. d*) inside the leaf $l._4$ in Fig. 1 C, and in the squamules inside the ligular sheaths of two successive leaves from another bud in Figs. 2 E and G. In *Potamogeton natans* the same connexion with the 'axis' is seen in the case of the squamules marked with a cross inside the inner leaf ($sh._2$) in Fig. 3 F. An exception, however, is illustrated in Fig. 3 C, for here the median band of tissue marked with a cross, which is destined to form a squamule, becomes detached from the growing apex before freeing itself on the side on which it adheres to the leaf. Despite this one exception, we may, I think, claim that the indications in *Potamogeton* are favourable to the view that the squamules, like those of *Cymodocea*, are derived from the surface of the growing apex immediately above the leaf in whose axil they appear to be located.

Leaving the cases which I have myself examined, and turning to the records in the literature relating to other Families, we find that the Hydrocharitaceae are the group about which we have most information on the question of the origin of the squamules. In the case of *Stratiotes aloides*, L., Nolte (16, p. 3, Plate I, Fig. 5) observed that, if the leaves were carefully removed, the squamules remained adhering to the axis. Again, although Solereder (22), in describing the squamules of *Elodea canadensis*, Michx., speaks of them as situated 'an der Blattbasis', Sanio (18), nearly half a century earlier, had pointed out that in this plant the development indicates that the squamules arise, without connexion with the leaves, from the external cell-layers of the stem apex. Furthermore, Bayley Balfour, in his beautiful memoir on *Halophila* (1), explicitly states that, in this genus, the squamules 'arise from the axis, and have no organic connection with the leaves'.

In the case of the Aponogetonaceae, the only knowledge we possess of the origin of the squamules is Serguéeff's statement (21) that they arise from the stem rather than the leaf. And in the Araceae, which, though not members of the Helobieae, show relationship with the Aponogetonaceae, Irmisch (14) found squamules occurring on the axis above the level of exsertion of each foliage leaf.

It seems to me that the indications met with in the literature, as well as the observations recorded in the present paper, agree in pointing to the conclusion that in the Scheuchzeriaceae, Potamogetonaceae, Aponogetonaceae, Hydrocharitaceae, and Araceae—that is to say, in all those Families in which we have any definite information about the origin of these structures—the *squamulae intravaginales* originate immediately above the leaf in whose axil they are found, and arise from the surface of the internode—that is to say, from the 'leaf-skin' belonging to the leaf whose point of exsertion lies next above them. It may be objected that, if this interpretation be correct, in seedlings of the Helobieae we ought to find squamules belonging to the cotyledon situated on the outer surface of the hypocotyl in its basal

region. I am not aware that the existence of squamules in such a position has ever been recorded, but though their discovery at the junction of hypocotyl and radicle would be a strong confirmation of the views expressed in this paper, I do not think that these views are necessarily discredited by their absence. For in the exposed position which the 'collar' presents—unprotected by the leaf-sheaths which enclose the base of every other internode—the tendency to the formation of squamules may well be held in abeyance by the absence of the requisite physiological conditions.

If the squamules throughout the Helobieae are, as seems probable, dorsal basal appendages of the leaves, it becomes unnecessary to discuss the idea that they are *stipulae intrafoliaceae* (Caspary, 5), or the more recent suggestion that in the case of the Alismataceae they are ligular structures (Buchenau, 4). For neither stipules nor ligules are ever found on the *dorsal* surface of the leaves to which they belong. The case of the Alismataceae must, however, be treated as an open one for the present, since we have no exact information as to the origin of the squamules in this Family; it is, of course, probable that they arise in the same way as in the other Helobieae, but this cannot be assumed without proof. I have examined serial sections of buds of *Sagittaria sagittifolia*, L., from this point of view, but the thinness of the squamules, the extreme abbreviation of the internodes, and the crowding of the young leaves have, so far, prevented my arriving at any certainty as to the way in which the squamules originate.

(iv) Summary.

From a study of the origin of the *squamulae intravaginales* in *Potamogeton* and *Cymodocea* (Potamogetonaceae) and *Triglochin* (Scheuchzeriaceae), it is concluded that these structures are not appendages of the leaf in whose axil they are found—as has often been assumed—but that they originate from the surface of the internode separating this leaf from the next leaf above. The records in the literature indicate that this is also true for the Hydrocharitaceae, Aponogetonaceae, and those Araceae which possess squamules. In terms of the 'Leaf-skin Theory' of E. R. Saunders (19), the squamules should therefore be described as outgrowths from the downward, axis-clothing prolongation of that leaf whose level of exsertion comes next above their place of origin.

Balfour Laboratory,
Cambridge.

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The Conduction of Geotropic Excitation in Roots.

BY

R. SNOW

(*Research Fellow of Magdalen College, Oxford*).

With four Figures in the Text.

CASES have long been known in which stimulus striking on one part of a plant organ leads to a responsive movement in another part: we have to suppose that the excitation set up in the perceptive region is conducted along the plant organ to the region of response. Such conduction seems to offer special opportunities for investigating what is the nature of the excitation conducted. But further interest attaches to those cases where the responsive curvature is carried out in a direction determined by the direction of the stimulus. For in these the intercalated phase of conduction seems to make it possible to examine in what way the direction of stimulus thus determines the direction of response.

Amongst the best-known plant organs which show such conduction are roots and the so-called cotyledon of grass seedlings. In these, as shown by Ciesielski for the former and Rothert (1896) for the latter, excitation can be conducted back from the tip to produce a curvature in the elongating region.

A great advance was made when it was discovered by Boysen-Jensen (1913) that in the case of the etiolated cotyledon of the Oat, *Avena sativa*, such conduction can take place through a layer of gelatine. For if the tip is cut off and stuck on again with gelatine, and then excited by stimulus of light, while the lower zones are kept darkened, responsive curvature towards the light will follow in the darkened lower region. This has been confirmed for various other grasses by Pál (1918), and further shown to hold for traumatic stimulus in many other seedlings (Stark, 1921).

It therefore occurred to the writer to investigate by similar methods the old problem of the conduction, from root-tip to elongating region, of the excitation set up in the tip by the stimulus of gravity.

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Methods.

The experiments were made on roots of *Vicia Faba*, the Broad Bean. The beans were germinated in moist sawdust. The tips were cut off at 2 mm. from the vegetative point. While being cut, they were held horizontal for a few seconds, but in a plane at right angles to that in which they were aftewards pinned. For success in the experiment, certain small details must next be attended to. A 10 to 15 per cent. solution of gelatine was found the best strength. This was sterilized by boiling and a very small drop of it applied warm to the end of the stump with a sterilized brush: excess of gelatine spoils the result. The tip can then be picked up with another slightly moistened brush and replaced. The surface tension of the gelatine pulls it back into position and ensures a good fit. The parts next to the cut must not be covered with excess moisture, or the gelatine will diffuse away: nor must they be quite dry, or the joint may crack open at the edges. The gelatine should not flow over on to the sides of the stump. Roots 20 or 25 mm. long were found to serve the best. They were placed horizontal after operation, in moist boxes in such a way that their geotropic curvatures should be at right angles to the plane of the cotyledons, for, as shown by Sachs (1871), in this plane the root is steady, but in the plane of the cotyledons it nutates strongly. The gelatine solidifies after a varying time: in several roots that had responded well, it was examined in section under the microscope after twenty-four or forty-eight hours and found to form a uniform layer about 50 μ thick. There had been no growth of the cells to form contact through the layer.

Most of the experiments were made with beans harvested nearly a year previously. Beans harvested in August and used in August and September were found less suitable, as they excrete so much water from the end of the stump that the gelatine is apt to be washed away. The various operations were made with the help of a watchmaker's lens.

The results of all experiments were recorded by drawing.

Section 1. Conduction of Geo-excitation through a Gelatine Layer.

In the following experiments, only curvatures in the elongating region are considered. The various displacements that often take place at the base of the root are here omitted as due to other causes.

(*a*) *Controls.*

1. Thirty-two roots were decapitated and the ends of the stumps painted with gelatine, but the tips not replaced. Roots laid horizontal. After fifteen to twenty-four hours, 27 had not curved at all, 4 had curved down very slightly, 1 had curved down strongly. Even after decapitation, therefore, a few roots curve down.

2. Five roots were decapitated, the tips killed by boiling, and stuck on again with gelatine. Roots laid horizontal. After twenty-four hours none had curved. Replacement of a dead tip has no effect.

(*b*) *Tips replaced with Gelatine.*

3. In all 76 roots were decapitated at 2 mm. from the vegetative apex, and the tips stuck on again with gelatine. They were then placed horizontal. After fifteen to twenty-four hours, 45 curved downwards, 31 remained straight.

4. In certain earlier experiments, 13 roots were decapitated at 2 mm.

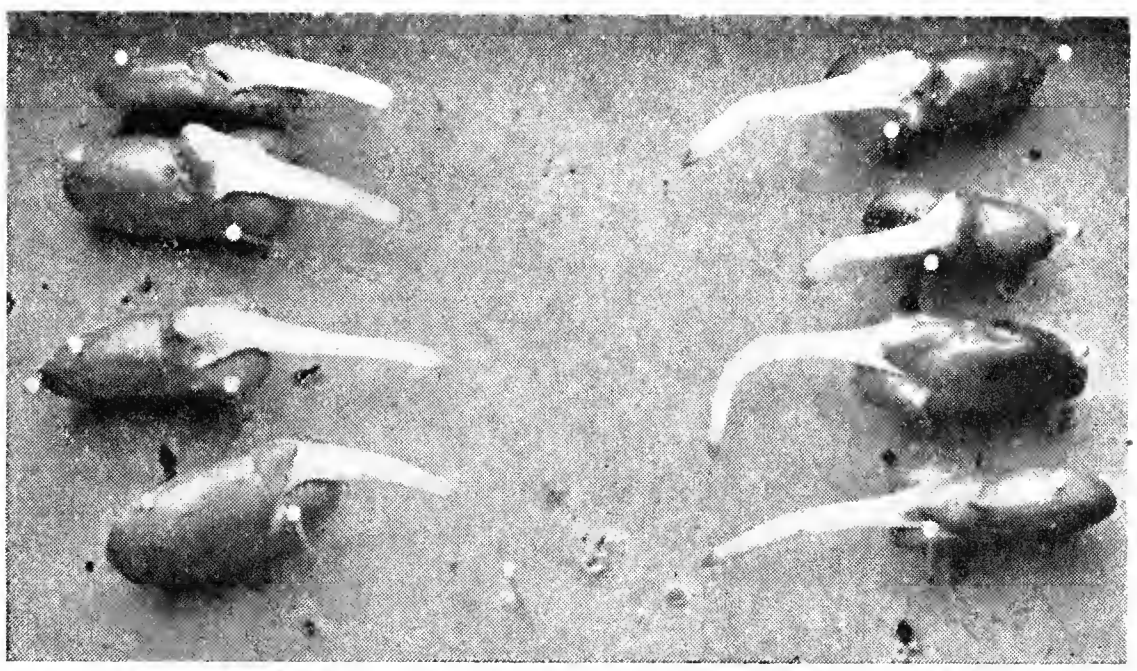

FIG. 1.

from the end of the root cap, and the tips replaced with gelatine. They were placed horizontal. After fifteen to twenty-four hours, 9 curved down, 4 remained straight.

The extent of curvature in Experiments 3 and 4 varied from a slight curve to one of 90°. Curves of about 30° were the commonest. Intact roots laid horizontal for comparison were often found not to curve through more than 45°.

Those roots which failed to curve often showed an enlargement at the end of the stump: this enlargement is also shown by decapitated roots without the tips replaced. But those that curved down usually did not show this enlargement, or showed it to a less extent. This suggests that in them the gelatine had made proper connexion between stump and tip, but that in those which failed to curve it had not made such connexion.

To show the extent of curvature obtained, a favourable experiment is illustrated (Fig. 1). This represents a complete set of roots, operated on at one time. The four roots on the left were simply decapitated, and have been slightly displaced downwards from the base, but have scarcely curved

in the elongating region. The four on the right, with tips replaced, have curved down to different extents.

The above results should leave no doubt that replacement of the tip does lead to geotropic curvature.

Attempts to produce geotropic curvatures by cutting off the tip, and attaching it in a horizontal position to a glass slide while the stump remained vertical and then replacing the tip, were not successful. This is, perhaps, not surprising, since the tips must have been under very abnormal conditions. Attempts were also made to present the tip only to gravity, without presenting the stump, by cutting off the tip obliquely and then rotating it through 180°, as shown in Fig. 2. But the results, though favourable, were not pronounced enough to be convincing. Reasons will be given later for considering that the results of this section can scarcely be explained except as due to conduction of excitation from tip to elongating region.

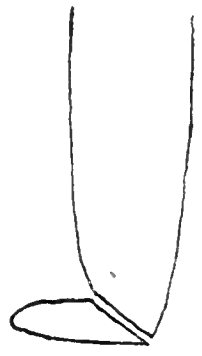

FIG. 2.

Section 2. Replacement of Tips in Anomalous Positions.

5. Nine roots were decapitated and the tips stuck on again with gelatine in a position covering half the stump, as shown in Fig. 3. They were then kept vertical. After twenty-four hours, 5 were still straight and 4 slightly curved towards the side covered by the tip.

FIG. 3.

6. Six roots were decapitated, the tips killed by boiling and then stuck back, covering half the stump, as above. After twenty-four hours, 2 were still straight, 3 were curved slightly towards the side covered by the tip, and 1 strongly so curved.

It would seem natural to explain this result as due to some substance diffusing from the dead tip, passing straight up the side of the stump covered by the tip, and retarding its growth.

7. The results of experiments to be referred to later will show that geotropic response can be brought about by conduction of excitation along either the upper or the lower half of the root alone. In the case of the lower half, if such excitation travels back by a straight path from the tip (as we shall find reason to believe to be the case), then it must be of such a kind as to cause retardation of growth in the lower half of the responding zone, which is affected by it.

If, then, the tip is stuck back so as to cover half only of the end of the stump (as in the last two experiments), and the root is then laid horizontally with that side of the stump that is covered by the tip uppermost (Fig. 4), we should expect a similar retarding influence to be transmitted now along the upper side of the stump, and so cause it to bend up. Unfortunately, it

is difficult to know just how much overlap to allow in order to ensure the best connexion of the conducting tissues. In all, 20 roots were used for this experiment. Of these, after twenty-four hours, 12 remained straight, 2 bent well up, 4 slightly up, and 2 slightly down. But since such roots, even if kept vertical, tend to bend slightly towards the side on which the tip is placed (cf. Experiment 5 above), such results are insufficient, even after allowance has been made for the slight sensitivity of the decapitated stump.

FIG. 4.

Section 3. The Paths of Conduction.

If excitations leading to tropic responses can be conducted through gelatine, this would seem, as pointed out by Páal (1918), to exclude any theory by which they consist in the passing on of any kind of induced protoplasmic polarity. Such a theory has been put forward by Fitting (1907) chiefly to explain certain experiments which seemed to show that in the cotyledon of *Avena*, after incisions had been made more than half-way through from opposite sides, photo-excitation could still pass down by a sinuous path from the tip to the responding region and there bring about phototropic response.

A simpler view of the relation between conduction and response would be that curvature in a certain direction is due not to any polarized nature of the conducted excitation, but to the fact that unequal intensities of excitation are conducted along the two sides of the organ, and so set up differences in rate of growth in the responding zone. These unequal intensities of excitation would in turn have been due to the unequal effects of the stimulus on the two sides of the perceptive region.

If we adopt this view, it will clearly be necessary to suppose also that the excitation is conducted only in straight lines: for otherwise the difference set up between the two sides of the perceptive region would be obliterated before the excitation could reach the responding region. Thus any evidence of conduction along a sinuous path would seem to make it necessary to reject any explanation along these lines, and to adopt one similar to Fitting's: for no other would seem possible.

Some, however, of his results with *Avena* have been doubted by later investigators (cf. Páal, 1918, p. 444 seq.).

In the case of roots, also, Pollock (1900) and Fitting (1907, p. 231 seq.) have carried out experiments suggesting that traumatic excitation could be conducted tortuously around incisions. But apparently nothing was done to exclude conduction by diffusion straight across the cuts instead of round their margins. The question, therefore, seemed to need re-investigating. The case of geotropism was taken first for investigation, as the traumatic curvatures obtained appeared to be generally less vigorous.

Mica Slips inserted from one Side.

8. Twenty-two beans were taken, and a single transverse cut made just half-way through, at 2 mm. behind the vegetative apex. A slip of mica was slid into the cut. They were then pinned with the roots vertical. After twenty-four hours, eleven remained straight, one bent slightly away from the cut, nine bent slightly towards the cut, one bent strongly towards the cut.

From this it is clear that the traumatic stimulus tends to produce a slight positive curvature.

9. Six beans were taken, treated as in the last experiment, and then pinned with the root horizontal and the cut and mica slip on the upper side. All curved clearly down in the responding zone.

10. Nine beans were taken at one time and treated in the same way, but pinned with the root horizontal and the cut and mica slip on the lower side. Two remained straight, one curved slightly down, six curved strongly down, making sharp curves of 60° or 70° in the responding region. These curves were obviously altogether different from those in Experiment 8.

11. Fourteen beans were taken, treated in the same way, and pinned so that the mica slips were inserted laterally in the horizontal root. Ten remained straight, two curved slightly down, two curved strongly down.

From the results of Experiments 9 and 10 it is clear that after connexion between tip and responding zone has been broken to a depth of half-way through the root, either from above or from below, the geotropic excitation can be conducted back along the remaining intact half. This seems to happen more easily when the lower half is left intact, since with other less vigorous roots curvature often did not occur when it was the upper side that was intact, though it did when the lower side was intact. The case of the intact lateral half (Experiment 11) is more doubtful: possibly the excitation is transmitted successfully, but response becomes difficult if only a lateral half of the responding zone is affected.

Mica Slips inserted from two Opposite Sides.

12. Twenty-one beans were taken, and cuts made just half-way through the roots from opposite sides. One was at 2 mm. behind the vegetative apex, and one at 2·75 mm. behind. Mica slips were inserted. The beans were pinned with the roots horizontal, and the cuts one on the upper and one on the lower side. Only curvatures occurring above the second cut were considered. Sixteen remained straight, one bent well down, three bent slightly down, and one bent slightly up.

13. Twenty-four beans were treated as in Experiment 12, but pinned so that the two cuts and mica slips were lateral on the horizontally placed root. Twenty-one remained straight, one bent down, two bent slightly down.

If the results of these two experiments are compared with those for decapitated roots in Experiment 1, it will be seen that there is no significant difference.

It thus appears that the geotropic excitation cannot be conducted in a sinuous path so as to pass round two cuts made from opposite sides and filled with mica slips. There is indeed no reason to suppose that it travels otherwise than in straight lines. Further, since, as shown in Experiments 9 and 10, geo-excitation can be effectively conducted back along either an upper or a lower half-root alone, it appears that these excitations, if they travel in straight lines, must be of two different kinds, one in the upper half leading to relative increase of growth, and one in the lower half leading to relative decrease. A similar state of things appears to hold for the responses to light and gravity of the *Avena* cotyledon. For there, according to Purdy (1921), photo-excitation can travel down from the tip either by way of the side farthest from the light alone, or, less easily, by the side towards the light alone, and in either case can still bring about positive curvature. Similarly, geo-excitation can travel back from the cotyledon tip by either the lower side or, less easily, the upper side alone. She reports, however, no experiments on the critical question whether in *Avena* conduction can or cannot take place by a sinuous path.

Discussion.

A decapitated root, as is well known, does not curve down in response to gravity, or only seldom. Yet this is not because it is unable to respond, for if the root is first exposed to gravity while intact, and then decapitated, it curves down normally. The simplest explanation of this, as suggested by Charles Darwin, is that the decapitated root is unable to perceive the stimulus of gravity, since perception takes place in the tip. In conformity with this, we have found that if, after decapitation, the tip is stuck on again with gelatine, the reconstituted root is able to perceive the stimulus of gravity and respond to it. It is, then, natural to suppose that the tip perceives the stimulus normally and transmits back excitation through the gelatine layer.

And nothing now stands in the way of this explanation. For, firstly, it is beyond doubt that excitation can be conducted through a gelatine layer, as in the case of the *Avena* cotyledon; and, secondly, it can hardly be doubted now that in the intact root excitation due to stimulus of gravity is transmitted back from the tip. For, as further evidence of this, besides

Czapek's well-known 'glass-boot' experiments (1895, p. 255 seq.) there is now Haberlandt's research (1908) by Piccard's rotation method. In this, various seedlings, including *Vicia Faba*, were fixed to a turn-table that could be rotated rapidly about a vertical axis. They were placed so that the roots pointed obliquely downwards at 45° to the vertical, and so that the imaginary prolongation of the axis of rotation intersected the roots at a short distance behind the tip. In this way, the tip of the root on one side of the axis and the responding region on the other were exposed to transverse centrifugal forces in opposite directions. It was found that the responding region possessed a certain sensitivity, which was, however, marked by the greater effect transmitted from the tip, when the point of intersection was 1·5 mm. or more behind the tip.

But though it is thus probable that geo-excitation in the root can be conducted through gelatine, it is not easy to get such definite evidence of this as can be obtained when the stimulus is that of light or of wounding. For in these cases it is easy, after the operation, to stimulate the tip of the organ without stimulating the responding region; but with gravity this cannot easily be done, and the attempts to do so described earlier were scarcely successful. There remains, therefore, another possible explanation. The responding region of the root, as has been shown by Haberlandt, is itself slightly sensitive to gravity. It might be, then, that the effect of decapitation was to make perception impossible, not only by removing the highly sensitive tip, but also by destroying the sensitivity of the responding region by some effect of wound shock or correlative disturbance. When, therefore, the tip was replaced, it might bring about response, not by transmitting back geo-excitation through the gelatine, but by transmitting some influence that restored the sensitivity of the responding region.

A process similar to this seems indeed to occur in the *Avena* seedling. For in this and in other grass seedlings, so long as they are intact, the lower zones of the cotyledon, as shown by Rothert (1896), are to some extent sensitive to light. But after decapitation they become quite insensitive. It has now been shown by Brauner (1922, p. 540) that in *Avena*, if the cotyledon tip is cut off and kept in the dark, and the *lower* zones exposed to stimulus of light, and if the seedling is then put in the dark again and the tip replaced, a positive curvature follows. The replacement of the tip must, then, have restored the sensitivity of the lower zones.

It is remarkable, however, that in the grass seedlings it is only complete removal of the tip that makes the lower zones insensitive; deep wounds made in it do not have this effect. If, then, similar relations held for the root, we should expect it to bend when laid horizontal even after the infliction of a wound on the tip. But, on the contrary, it is well known that any deep cut made in the tip makes the root insensitive to gravity. It does not seem, then, that there is anything in the root similar to this sensitivity of

the lower zones of the grass cotyledons when connected with their tips, and loss of sensitivity in their absence. It seems rather that when the root tip is replaced with gelatine it must bring about response of perceiving the stimulus of gravity and transmit back the true geo-excitation through the gelatine, though a definite proof of this cannot yet be given.

Incidentally, this result of Brauner's shows, as he points out, that what is lacking in the decapitated cotyledon is neither the power of perceiving nor that of responding, but some intermediate phase of the tropistic processes. To call it insensitive is therefore perhaps misleading.

As to the light which the gelatine experiments throw on the nature of conduction, the processes most naturally suggested as capable of passing through the gelatine are, as pointed out by Páal, the diffusion of soluble substances and the electric current. The latter he considers excluded in the case of *Avena*, as conduction was not found to take place through a platinum plate. But this conclusion seems hardly convincing, firstly because metals in contact with living tissues polarize very rapidly, and secondly, because short-circuiting would occur in the metal plate so that no current production in the tissues on one side of it could have any effect on those on the other side.

Still, it certainly seems more likely that such conduction through non-living media is due to diffusion, especially since Ricca (1916), working with *Mimosa*, and Stark (1921, p. 110), with *Avena*, appear to have extracted such soluble stimulating substances.

Naturally, it does not at all follow that throughout all its course the conduction is effected by any such purely physical process. Indeed, it is most unlikely that it should be, in view of Fitting's experiments (1907, p. 219 seq.) on the effects of the local application of warmth and anaesthetics to the conducting region in *Avena*.

These agents were often found to prevent conduction, although not killing the tissue. In this respect *Avena* differs strikingly from *Mimosa*, in which conduction, now known to take place in the wood, can pass through stretches of anaesthetized or even dead stem.

It seems, then, that in conduction of the *Avena* type the living tissues must in some way be actively concerned; and accordingly it has been suggested (Páal, 1918, p. 432) that the conduction may be a composite process, in which excitation at one point leads to the production of soluble stimulating substances, which diffuse away to neighbouring points of the tissue and excite them in turn, with production of more of the substances, and so on. The purely physical part of this process might be able to pass through a layer of gelatine, but yet not be able to travel far through the living tissue unless aided by renewal of the excitation.

But if, as the experiments on the paths of conduction have led us to suppose, there are two kinds of conducted excitation, one on the concave

side leading to decrease of growth, and one on the convex side leading to increase, then clearly we need first to determine whether both of these can pass through the gelatine, or one of them only. It is hoped shortly to investigate this question, for, until it is settled, suggestions as to the relation between conduction in the gelatine and conduction in the living tissue are perhaps premature. Meanwhile, in *Avena* the feeble response when conduction is only possible by the near side, and the stronger response after decapitation and replacement of the tip with gelatine, seem to indicate that at least the excitation conducted by the far side can pass through the gelatine.

It may, however, be noted that Brauner (1922) has observed in the *Avena* cotyledon a vigorous protoplasmic streaming, which might enable soluble substances to pass down it much faster than they could by simple diffusion.

Summary.

1. If the tips of decapitated roots of *Vicia Faba* are stuck on again with gelatine, the roots in most cases become again capable of bending down in response to gravity. It appears that excitation set up in the tip must be conducted back through the gelatine.

2. If a cut is made half-way through the root from one side, at a point 2 mm. from the vegetative apex, and a mica slip is inserted into the cut, to prevent diffusion across it, the root can still respond to gravity by bending down in the elongating zone when placed horizontal, whether the cut be on the upper or the lower side. Hence either the upper or lower side alone can conduct back excitation from the tip.

3. If two mica slips are inserted half-way through from any two opposite sides at 2 mm. and 2·75 mm. from the apex, and the root placed horizontal, it does not curve down, or no more frequently than a decapitated root. Only curvatures above the second cut are here referred to. The excitation cannot therefore be effectively conducted back from the tip by a sinuous path.

These results are briefly discussed.

I wish to thank Professor Sir Frederick Keeble for encouragement during this investigation.

The Department of Botany,
Oxford,
October 1922.

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On the Presentation Time and Latent Time for Reaction to Gravity in Fronds of Asplenium bulbiferum.

BY

F. M. O. WAIGHT

(*Associate of University College, Reading*).

With one Diagram in the Text.

IT has already been shown [1] that fronds of *Asplenium bulbiferum* are negatively geotropic, and further that the specific irritability is not constant throughout their development.

In 1921 Miss Prankerd suggested to me that some measure of the intensity of this irritability might be gained by ascertaining the presentation time at different stages of development, and this I have attempted to do under her direction.

The presentation time as defined by Czapek [2] is the shortest period of stimulation which will produce any response on the klinostat, and the latent time is the period elapsing between the beginning of stimulation and the first indication of response. In this case, therefore, the presentation time is the period of horizontality necessary to secure the slightest movement upwards of the frond, and the latent time is the period from the beginning of horizontality to the moment when the movement is just visible. The first two stages in the development of the frond previously noted,[3] namely, Infant, when the leaflets are in the apical coil, and Adolescent, when the leaflets are unfolding, have been further subdivided into Early Infant, fronds 0·8–3·0 cm.; Middle Infant, fronds 3·0–5·5 cm.; Late Infant, fronds over 5·5 cm. with no leaflets unfolded; and Adolescent 1, 2, 3, &c., according to the number of pairs of leaflets unfolded from the apical coil.

The ferns were grown in a greenhouse, and although it was impossible

[1] Prankerd, T. L. (1922): On the Irritability of the Fronds of *Asplenium bulbiferum*, with Special Reference to Graviperception. Proc. Roy. Soc., B., xciii.

[2] Czapek (1898): Jahrb. f. wiss. Bot., xxxii.

[3] Prankerd: loc. cit., p. 144.

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to keep the temperature constant, readings were taken morning and evening on a maximum and minimum thermometer. Readings of the relative humidity, which could be kept fairly constant at 80–90 per cent., were also recorded daily.

For stimulation the fronds were placed horizontally on their sides, and adaxially to the incident light.[1] In order to see if any movement had occurred, the outline of the frond was traced on glass, almost in contact with it, unless this was impracticable on account of epinastic curvature, when the frond was placed against a scale and its position read as exactly as possible through a pin-hole. At the end of the period of stimulation, the plant was either rotated on the klinostat, or, when this was impossible owing to the size or weight of the pot, it was placed so that the frond was quite vertical. The following tables, made from actual experiments, typical of a large number, show that any difference in the results obtained by the two methods is inappreciable:

Length of frond in cm.	*Period of stimulation in hours.*	*Angle of curvature.*	*Latent time in hours.*	*Position of plant after stimulation.*
		Stage: *Late Infant.*		
7·2	2	10°	6	On klinostat
8·5	2	10°	6	On klinostat
6·5	2	5°	6	Upright
		Stage: *Adolescent* 3.		
10·6	¾	5°	5½	On klinostat
20·0	¾	7°	5½	Upright
10·0	¾	7°	5½	Upright
		Stage: *Adolescent* 4.		
12·0	¾	10°	5	On klinostat
12·5	¾	10°	5	Upright

When upwards of four hundred experiments had been carried out in this way, an approximate idea was gained of the presentation time at the various stages above mentioned. Several critical experiments for each stage were then made when the temperature was as nearly as possible 20° C. (never above 21° C. or below 19° C.). The angle recorded was in every case the maximum reached, and the results of these experiments were checked by those where no curvature took place with the same or somewhat lower periods of stimulation. This is important, as exposures much in excess of the presentation time often produce movements of little or no greater amplitude.

Column 1 in the tables shows that considerable difference in the length of the frond does not affect the presentation time and latent time. It has been found that these periods depend on the *stage*, and only indirectly on the age or length of the frond.

[1] The reasons for this position have already been given. Prankerd: loc. cit., p. 145.

After about eight pairs of leaflets are unfolded, the presentation time is much more difficult to measure, owing to the fact that all the varied types of movement previously[1] described may, and often do, take place—some to an increased degree—until maturity is reached. Nutation is probably at its maximum, and, although varying greatly in amount, may be as much as 20° right and left of the vertical. Growth in the dark of course avoids heliotropism, and control experiments have been made in a dark room; but here constant conditions, particularly the important one of temperature, could not usually be obtained.

Physiological variability of individual fronds finds expression in the different angular heights attained; for however morphologically similar several fern fronds may be, they will almost certainly not behave identically under precisely similar conditions. Hence the presentation times deduced should be regarded as those periods which, under the given conditions, will generally produce movements of about 5° and only very rarely exceeding 10°.

Tables showing results of experiments at various stages of the frond's development.

Temperature 20° C. (approx.); humidity 85 % (approx.).
P. T. = presentation time.
L. T. = latent time.

Stage: *Early Infant* (apical coil just above soil level).

Length of frond in cm.	*Period of stimulation in hours.*	*Angle of curvature.*	*Latent time in hours.*
1·5	$8\frac{1}{4}$	4°	16
0·8	8	5°	16
1·6	$8\frac{1}{4}$	—	—
1·2	$7\frac{3}{4}$	—	—

P. T. = 8 hours; L. T. = 16 hours.

Stage: *Early Infant* (apical coil about a centimetre above the soil).

1·5	4	10°	10
1·5	4	5°	10
1·5	4	4°	10
2·3	4	—	—
2·7	$3\frac{1}{2}$	—	—
2·6	3	—	—

P. T. = 4 hours; L. T. = 10 hours.

Stage: *Middle Infant.*

5·2	3	11°	$7\frac{1}{2}$
5·0	3	10°	8
3·0	3	7°	9
3·4	3	5°	9
3·8	3	5°	$7\frac{1}{2}$
3·0	3	—	—
3·0	3	—	—

P. T. = 3 hours; L. T. = 8 hours.

[1] Prankerd: loc. cit., p. 144.

Stage: *Late Infant.*

Length of frond in cm.	*Period of stimulation in hours.*	*Angle of curvature.*	*Latent time in hours.*
6·5	2	15°	$5\frac{1}{2}$
6·5	2	10°	6
7·2	2	10°	5
8·5	2	10°	6
6·5	2	5°	7
7·0	2	5°	7
5·5	2	—	—

P. T. = 2 hours; L. T. = 6 hours.

Stage: *Adolescent* 1.

7·5	$1\frac{1}{2}$	10°	$6\frac{1}{2}$
6·5	$1\frac{1}{2}$	8°	5
7·5	$1\frac{1}{2}$	7°	$5\frac{1}{2}$
5·0	$1\frac{1}{2}$	—	—
7·7	$1\frac{1}{2}$	—	—

P. T. = $1\frac{1}{2}$ hours; L. T. = $5\frac{1}{2}$ hours.

Stage: *Adolescent* 2.

8·5	1	13°	5
11·2	1	10°	5
8·0	1	9°	$6\frac{1}{2}$
8·0	1	7°	$5\frac{1}{2}$

P. T. = 1 hour; L. T. = $5\frac{1}{2}$ hours.

Stage: *Adolescent* 3 *and* 4. (Number of pairs of leaflets unfolded placed in brackets after length in this and the next table.)

12·0 (4)	$\frac{3}{4}$	10°	5
7·0 (3)	$\frac{3}{4}$	10°	$5\frac{1}{2}$
10·0 (3)	$\frac{3}{4}$	8°	$5\frac{1}{2}$
8·5 (3)	$\frac{3}{4}$	7°	$5\frac{1}{2}$
10·6 (3)	$\frac{3}{4}$	5°	6
9·5 (4)	$\frac{3}{4}$	—	—
11·5 (4)	$\frac{3}{4}$	—	—

P. T. = $\frac{3}{4}$ hour; L. T. = $5\frac{1}{2}$ hours.

Stage: *Adolescent* 5, 6, *and* 7.

16·0 (7)	$\frac{3}{4}$	12°	$5\frac{1}{2}$
14·5 (5)	$\frac{2}{3}$	7°	5
21·5 (5)	$\frac{1}{2}$	7°	$4\frac{1}{2}$
13·2 (6)	$\frac{1}{2}$	8°	$5\frac{1}{2}$
24·5 (7)	$\frac{1}{2}$	—	—
17·7 (5)	$\frac{1}{3}$	—	—
12·0 (6)	$\frac{1}{3}$	—	—

P. T. = $\frac{1}{2}$ hour; L. T. = 5 hours.

Stage: *Adolescent* 8.

20·2	2	10°	5
13·0	1	5°	$5\frac{1}{2}$
13·7	$\frac{5}{6}$	—	—
11·7	$\frac{3}{4}$	—	—
20·0	$\frac{3}{4}$	—	—

P. T. = 1 hour; L. T. = $5\frac{1}{2}$ hours.

Stage: *Adolescent* $\frac{2}{3}$ (*approx.*).

18·0	3	10°	6
15·7	3	10°	7
21·2	$2\frac{1}{2}$	5°	5
18·5	$2\frac{1}{2}$	5°	$5\frac{1}{2}$
19·5	$2\frac{1}{2}$	—	—
18·3	$2\frac{1}{4}$	—	—
14·0	2	—	—
28·5	2	—	—

P. T. = $2\frac{1}{2}$ hours; L. T. = $5\frac{3}{4}$ hours.

Stage: *Adolescent* $\frac{3}{4}$ (*approx.*).

Length of frond in cm.	*Period of stimulation in hours.*	*Angle of curvature.*	*Latent time in hours.*
17·5	$3\frac{3}{4}$	10°	6
16·2	$3\frac{1}{2}$	7°	$6\frac{1}{2}$
17·0	$3\frac{1}{2}$	7°	$6\frac{1}{2}$
16·5	3	—	—
18·7	$2\frac{1}{2}$	—	—
19·5	$2\frac{1}{2}$	—	—
	P. T. = $3\frac{1}{2}$ hours; L. T. = $6\frac{1}{4}$ hours.		
	Stage: *Adolescent* $\frac{4}{5}$ (*approx.*).		
30·0	$4\frac{1}{2}$	5°	7
	P. T. = $4\frac{1}{2}$ hours; L. T. = 7 hours.		
	Stage: *Adolescent* $\frac{7}{8}$ (*approx.*).		
13·5	6	5°	8
32·0	$5\frac{1}{2}$	—	—
	P. T. = 6 hours; L. T. = 8 hours.		

From the above tables it will be seen that the presentation time is eight hours at a very early stage in the life of a frond, and decreases during its development until it reaches a minimum of half an hour, when the fifth to seventh pairs of leaflets are unfolding, rising again until all irritability to gravity ceases, when only the rudiments of the last two or three pairs of leaflets remain in the apical coil. Towards the end of its existence geotropic response is expressed by a twist of the rachis in its own plane, since the strong epinastic curvature taking place at this time seems to prevent any upward movement of the frond.

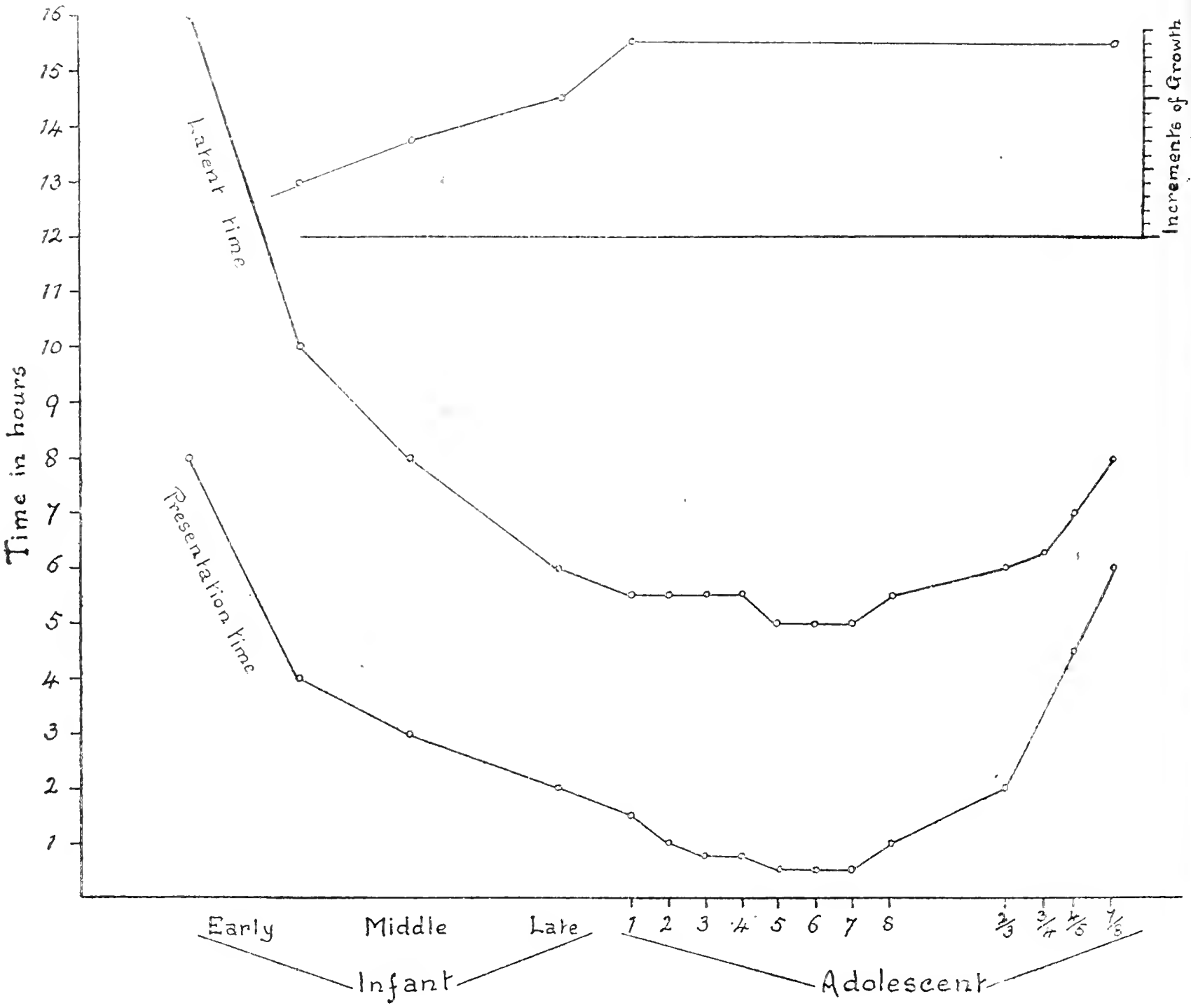
The above facts are shown graphically (p. 60) by plotting the presentation times and latent times as ordinates, and the stages of the frond as abscissae. The latter must of necessity be somewhat arbitrary, and can only correspond approximately with periods of time, as the rate of growth of a frond depends largely on temperature, which could not be kept constant through the six weeks or so of its development.

In the course of the work it was found that the presentation time in the Late Adolescent stages bore a more constant relation to the proportion of leaflets unfolded than to the actual number; hence the later stages are represented as fractions which express approximately the ratio of the number of leaflets unfolded to the total number possessed by the frond.

The curve for latent time has been plotted from the average of the figures in column 4 of the tables. It follows in general the curve for presentation time, being similarly affected by the stage of development of the frond, but not nearly to so great an extent, since its range is only sixteen to five hours as against eight hours to half an hour. This seems to be in accordance with the view expressed by Jost,[1] that distinct sets of phenomena are involved in perception and reaction.

[1] Jost, L. (1907): Lectures on Plant Physiology, p. 441.

As previously described,[1] the frond of *Asplenium bulbiferum* passes through a grand period of growth whose maximum under favourable conditions is 1–1·8 cm. a day at adolescent stages.[2] The increments are shown separately in the graph as ordinates corresponding with the same stages



of frond development. This brings out the fact that though response to gravity ceases while the frond is still growing, the presentation time is least when growth is most rapid. Thus maximum growth is associated with minimum presentation time, and hence with maximum geotropic irritability, in so far as the one is a true measure of the other.

[1] Prankerd: loc. cit., p. 144.

[2] One cm. is also approximately the interval between each pair of leaflets at the time of their unfolding, hence about one pair a day are produced at 15°–20° C.

Summary.

1. The fronds of *Asplenium bulbiferum* exhibit a grand period of irritability to gravity as measured by presentation time. This corresponds with the stage of development of the frond and not directly with its age or length.

2. At 20° C. and 85 per cent. humidity the presentation time decreases from eight hours at a very early stage of development to a minimum of half an hour when the fifth to seventh pairs of leaflets are unfolding. It then rises to about six hours, when response to gravity ceases, a little before the frond is mature.

3. The latent time shows a range of sixteen to five hours and is also affected by the stage of the frond, but to a much less extent than the presentation time. The ratio between the two periods increases until both are at their minimum value, when it again decreases.

It is hoped to extend the present work to other ferns, and to ascertain the effect of temperature and other factors upon the time periods studied.

My thanks are due to Mr. Lacey, of the South-Western Polytechnic, and Prof. R. C. Maclean, of Cardiff University, for the loan of excellent klinostats, and to Prof. W. Stiles for affording me the opportunity of carrying out this research in his laboratory. I also wish to thank Miss Prankerd for all her help and kindness, without which it would have been quite impossible for me to have done this work.

On Direct Nuclear Divisions in the Vegetative Mycelium of Saprolegnia.

BY

FRANCIS E. V. SMITH.

With twelve Figures in the Text.

SINCE de Bary began his well-known researches on the cytology of the Saprolegniaceae, this subject has been studied by many investigators, the majority of whom have been mainly concerned with the sexual organs. Those who have studied the cytology of the vegetative organs have investigated the phenomena associated with the formation of zoospores (Hartog, 1887, 1895 ; Rothert, 1890 ; Trow, 1895).

Trow and Hartog both described nuclear divisions in the vegetative portions of the mycelium, but while Hartog saw a form of mitotic division, in which four granular chromosomes appeared and spindles were absent, Trow was unable to observe anything comparable to this, finding only amitosis.

The following investigation was therefore undertaken with the object of ascertaining the actual process of nuclear division.

Isolation of the Fungus.

The fungus was found growing on flies in the pond of the Bristol University Botanic Garden. From the mixture of fungi present a well-developed sporangium of *Saprolegnia* was teased out with fine forceps. After being washed several times in distilled water it was shown on microscopical examination to be free from zoospores and stray pieces of mycelium. It was then transferred to a Petri dish containing beef gelatine and the dish was tilted at an angle of 30°, and thus the infusoria and bacteria were drawn away from the fungus (Lechmere, 1910), which was finally transferred to sterilized flies floating on well-aerated, distilled water. To reduce bacterial growth to a minimum the water was changed every two days.

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Identification of the Species.

The species does not correspond exactly with any of those described in Rabenhorst's 'Flora', and it is considered to be a hybrid which shows affinities to at least three recognized species, but resembles most closely *S. dioica*, of which it is considered to be a variety possessing stouter hyphae and fewer antheridia. The antheridia are produced on separate branches, thus indicating that it is very closely allied to that species. The relations of its characteristics to various species are given in the following table:

Size of hyphae	17–30 μ	*S. torulosa* and Lechmere's sp.
Form of oogonium	Spherical to cylindrical, no spines or warts	*S. torulosa*, *S. dioica*, and Lechmere's sp.
Size of oospheres	18–20 μ	Any species.
Number of oospheres	8–26	*S. dioica* and *S. torulosa*.
Antheridia	Not very numerous, always diclinous	*S. dioica*.

Technique.

The discovery of a good fixative was a matter of some difficulty. The outer walls of the hyphae are very thin, and unless fixation was instantaneous plasmolysis resulted, with the consequent shrinkage of the cytoplasm. The most satisfactory liquids were Merkel's solution and a modification containing acetic acid:

Acetic acid (5 per cent.)	100 parts.
Platinic chloride (1 per cent.) . . .	5 parts.
Chromic acid (1 per cent.)	10 parts.

This gave excellent results, both in quickness of killing and in preservation of the nuclei, especially when used at 70° C.

During the fixing and washing processes it was most convenient to handle the material while still attached to the fly. After the flies had been washed in 50 per cent. alcohol for six hours they were placed in water and the mycelium scraped off with needles. At this stage the hyphae were fixed to the slide, by using a thin film of Meyer's fixative; very little was required, as an excess absorbed the stain and thus lowered the value of the preparations. The hyphae were placed in a drop of water on the prepared slide and dried in an incubator. Care had to be taken to prevent all the water from evaporating, though if too much water were left all the hyphae floated off again. At the right moment the slides were placed in 70 per cent. alcohol for two minutes, thus permanently fixing the material to the glass. From the 70 per cent. alcohol they were placed in water and were then ready for staining. By this method the long delay experienced by Hartog was obviated.

Those who studied the sexual organs used microtome sections, but

it was found that in the case of the vegetative hyphae many sections were lost through imperfect adhesion to the slide. A much better idea of the state of the hyphae was obtained by mounting them whole, and no difficulty was found in examining them. Three methods of staining were used: Flemming's triple stain, iron-haematoxylin, and Delafield's haematoxylin, though it was found necessary to alter the usual times recommended for the first of these. The slides were removed from water to a saturated solution of aniline-water-safranin, where they were left for three to six hours. They were then treated thus: water three minutes, gentian violet (aqueous) thirty minutes, water one minute, orange G (aqueous) one minute, 90 per cent. alcohol one minute, 100 per cent. alcohol one minute, clove oil five minutes, benzol, Canada balsam. Although the process of taking the material from water to 90 per cent. alcohol may seem very drastic, in only a few cases did shrinkage of the cytoplasm take place to any appreciable extent, and, since the stain was only used for the nuclear figures, this did not matter. Xylol as a clearing agent seemed to cause too great a shrinkage of the cytoplasm, and so benzol was substituted.

Cytological Observations.

In the early stages the floating hyphae are slender and contain a mass of dense cytoplasm with small vacuoles. They grow rapidly in breadth until they have reached the normal thickness, by which time the cytoplasm has been reduced to a thin peripheral layer. The intramatrical hyphae, on the other hand, are very poor in cytoplasm.

Protein granules constitute the main portion of the cytoplasm, and show very distinctly in stained preparations. In living material they are visible under high-power objectives, and seem to be constantly moving, both in streaming and in Brownian movement. Cytoplasmic streaming is continuous, chiefly in the direction of the apex, though slight back currents occur. It appears that the tendency is to form zoosporangia, for, when the accumulation of cytoplasm at the apex has become sufficiently dense, a septum is formed and a zoosporangium cut off. As soon as the cytoplasmic continuity is thus broken, fresh cytoplasm accumulates below the septum, so that, by the time the zoospores have formed and escaped, sufficient cytoplasm and nuclei have accumulated below the septum to fill the empty zoosporangium case completely. All that remains is for the septum to grow upwards into the old sporangium and the cytoplasm immediately below follows.

In one instance a count of nuclei was made in a preparation treated with Flemming's stain, in which the zoospores were about to escape from the sporangium. The cytoplasm for a distance below, of about the length of a sporangium, was practically as dense as that in the zoospores. The

number of nuclei in this part was between 270 and 280, while the number of uninucleate zoospores was about 280; this shows that no nuclear divisions take place in the sporangium.

The cytoplasm in the upper parts often appears frothy. Throughout the plant are found occasional granules of the cellulose-like substance to which Pringsheim gave the name of cellulin, since it gives most of the reactions of cellulose.

The nuclei are very numerous in the hyphae, but always occur in much

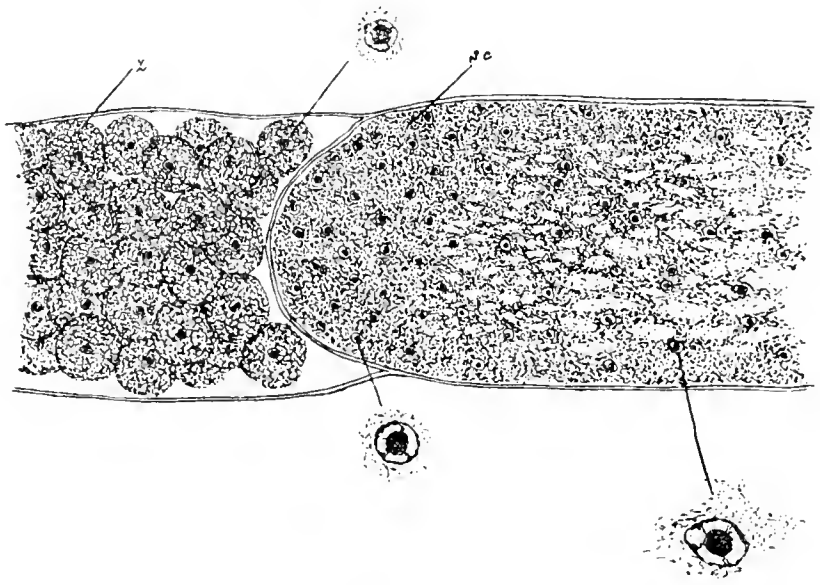

FIG. 1. The apical sporangial region of a hyphal filament, showing the dense cytoplasm, *s.c.*, and spherical nuclei. *z*, zoospore. × 500 and × 1,500.

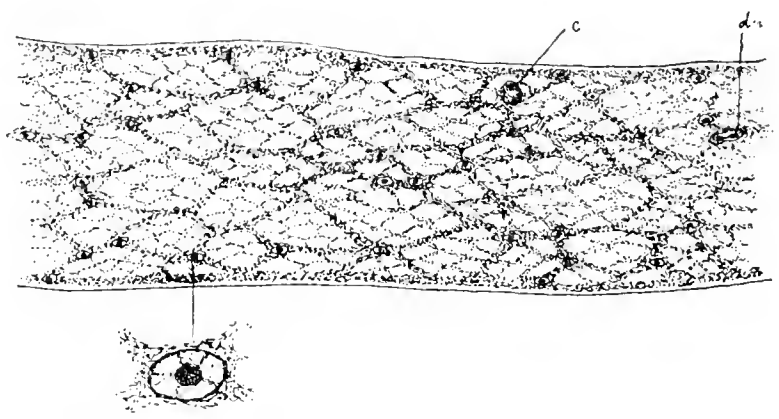

FIG. 2. The middle portion of a hypha, showing two kinds of cytoplasmic strands and elongated nuclei. Most of the nuclear divisions occur in this region. *d.n.*, dividing nucleus; *c.*, cellulin body. × 500 and × 1,500.

greater numbers towards the tip, whither they are carried by the rapidly streaming cytoplasm. The typical nucleus is spherical in shape, but both shape and size vary according to the position in the plant (Figs. 1–3).

The main portion of the nucleus consists of a central body which stains very deeply with all nuclear stains. Surrounding this is a layer of nucleohyaloplasm which is bounded externally by a distinct nuclear membrane, which in all good preparations is found to be of uneven thickness, due to the presence of granules of chromatin; these granules are not evenly distributed, but usually appear as occasional mounds upon the nuclear membrane (Figs. 4 and 5). The central body is suspended within the nucleus by fine linin threads arising in most cases from the chromatin granules on the

nuclear membrane. This central mass consists mostly of chromatin, and appears at first to be a nucleolar body. It is comparable with a similar body found in the vegetative nuclei of *Penicillium*, to which Guéguen (1899) applied the indefinite term 'chromoblast', as the function of this body is not yet realized. Its shape is usually spherical or ovoid, but in a few cases it is slightly flattened to a disc-like form. In some lightly stained preparations it appears to be fluid within, with a more deeply stained outer coat.

In examining a long hypha of the fungus, it was noticed that the nuclei are neither uniform in shape nor size, but that there is a gradual transition in shape from a spherical form in the zoosporangium to a long torpedo-like form in the lower parts of the thallus.

In the zoospore the cytoplasm is dense and fairly evenly distributed,

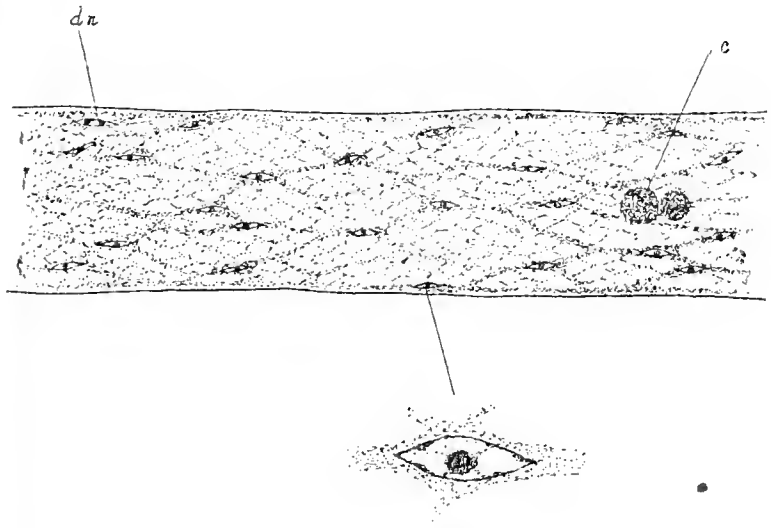

FIG. 3. The base of a hypha, where the cytoplasm is much less dense. The nuclei are long and pointed at the ends. × 500 and × 1,500. *d.n.*, dividing nucleus; *c.*, cellulin body.

FIG. 4. A typical resting nucleus, spherical in shape, with pronounced linin threads and granules on the membrane. × 3,000.

FIG. 5. An elongated form showing chromatin on the membrane. × 3,000.

and the nucleus spherical, the central body of which constitutes the main portion. The hyaloplasm is not so broad as in the other parts and less chromatin is present on the outer membrane. The size is about 3·5 μ in this condition, which is considered to be the resting condition. A similar state of affairs is found immediately below the septum, where the cytoplasm is also quite dense; in these nuclei the hyaloplasmic layer is slightly deeper (Fig. 1). A short distance below this region the cytoplasm becomes less dense, and there is a rapid transition from the homogeneous condition just described to a state in which strands appear in the cytoplasm. These at first are quite close together, consisting of dense cytoplasm with nuclei distributed within them. Between the main strands, which, because of the rapid streaming, run chiefly longitudinally and diagonally, is a network of finer strands. The latter form connexions between the neighbouring main strands, and some cross the lumen and connect the cytoplasm on opposite sides of the hypha. The quantity of cytoplasm gradually becomes less in the lower parts, so that

the strands are fewer in number, although the density of each is not greatly lessened (Fig. 3).

The constant upward streaming seems to cause a tension or strain within the semi-liquid cytoplasm. This strain is apparently transmitted throughout the length of the hypha, with the result that in the less dense portions at the base the strain is the same as in the apex. As, however, the cytoplasm is denser in the apex, the effect on each individual strand is not so great here. In the basal portions the strands are fewer, with the result that each is in a state of greater tension. The nuclear membranes are in close contact with the cytoplasm, so that the tension in the cytoplasm is transmitted to the nuclei, which, being of a plastic nature, respond to the strain by becoming elongated. In the hyphal tips where the cytoplasm is evenly distributed, the tension is equal on all sides, with the result that the nucleus is spherical. Where the cytoplasm is in a state of greater longitudinal strain the nuclei respond by assuming an elongated form, the extent of the elongation being proportional to the tension, and in some cases a length of 6 μ with a breadth of 2 μ is attained.

The central nuclein body responds in a similar way, assuming a shape varying from spherical to oval according to the strain on the outer membrane; but the change is not so marked, and in some cases the nuclein retains its spherical shape even in elongated nuclei. It is probable that the alteration in the shape of the chromoblast is caused by the linin threads which connect it to the outer membrane, so that in cases where the linin is not well developed, or where it is concentrated towards the middle, the spherical form is retained.

Amitosis.

No nuclear divisions take place in the zoosporangium, nor in the region just below the septum, but they are found chiefly in the region of the middle of the hypha, and below this.

In every case the nuclei in the vegetative hyphae were found to divide by a direct division. In the oogonium, mitotic divisions have been observed by Hartog, Trow, Davis, and Claussen, but the writer has not discovered anything comparable to spindle fibres or chromosomes in the vegetative nuclei.

The division begins with an elongation of the chromoblast, the membrane remaining intact until the end of the division. The chromatin granules on the membrane and the linin threads are visible throughout the whole process. After the chromoblast has elongated slightly the nuclear membrane becomes larger. A median constriction then appears in the chromoblast, and after a short time a similar constriction begins in the membrane (Fig. 6). Elongation of both parts continues throughout the whole process, with the result that the constrictions become gradually more

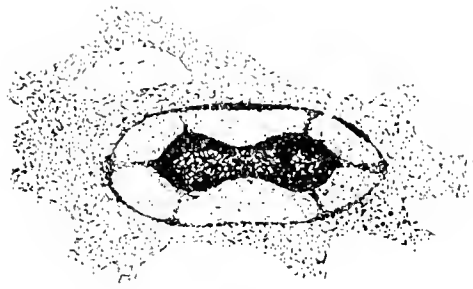

FIG. 6.

FIG. 7.

FIG. 8.

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

FIG. 6. The first stage in the amitotic elongation of the chromoblast. × 3,000.

FIG. 7. The second stage in the division. × 3,000.

FIG. 8. The third stage. The chromoblast is constricted in the middle and is still elongating. × 3,000.

FIG. 9. The fourth stage. The two daughter chromoblasts are now joined only by a thread. × 3,000.

FIG. 10. The fifth stage. The two daughter chromoblasts have separated and have assumed the spherical form once more. × 3,000.

FIG. 11. The sixth stage. The nuclear membrane has become more constricted and the whole nucleus longer. The chromatin on the membrane has regained its original density. × 3,000.

FIG. 12. The two daughter nuclei are about to separate. × 3,000.

marked. The parts of the nucleus farther away from the constrictions remain about the normal width, but the centre becomes gradually thinner until the two portions of the chromoblast are finally joined only by a thread (Fig. 9), at which stage the outer membrane appears hour-glass-shaped. For a short time the remnants of the thread joining the two daughter chromoblasts remain, but gradually they are withdrawn into the new bodies, which round off into the typical spherical form (Figs. 10 and 11). The constriction of the membrane becomes more acute until the two sides touch. Finally, the pull of the cytoplasm appears to draw the two new nuclei apart. The linin threads are irregularly distributed at the two ends, where they seem to hold the two daughter chromoblasts to the rapidly elongating membrane.

The chromatin on the membrane does not seem to increase in quantity until the final stages, so that when the nuclear membrane first elongates these chromatin masses become stretched and therefore thinner. It is only after the daughter nuclei start to round off again that these slowly regain their original density (Fig. 11).

The time at which nuclear division takes place is fairly constant, though there is reason to believe that a few divisions take place at all times of the day and night. From the results obtained from a series of preparations which were fixed at every thirty minutes throughout the twenty-four hours, it can be stated that the majority of the vegetative nuclear divisions take place between the hours of 10 p.m. and 2 a.m., a maximum being reached at midnight. All the nuclei in a hypha do not divide at the same time, and in any case are not all found in the same stages as is often the case in oogonia.

This division about the time of midnight, together with other observations made, indicate that, in spite of many exceptions, there is a regular cycle of events, causing the zoospores and sporangia to be found in a similar condition at a given time of the day. A brief outline of this cycle is given below, in which a variation of two hours either way may be allowed on the times given (see opposite page).

Observations were made on living material, but in the hyphae themselves no results were obtained, owing to the difficulty of seeing the nuclei. Even methylene blue was of no assistance, since it did not stain evenly, and the cytoplasmic granules absorbed most of the stain. In the zoospore better results were obtained, and in a germinating specimen a number of divisions were seen during the night. No nuclear structure was visible, but constrictions in the nuclei were observed, and when the material was stained with iodine some eight or nine nuclei appeared. The cytoplasm streamed all the time, and with it the nuclei moved rapidly. The germ-tube had a dense mass of cytoplasm in the tip, but the nuclei remained in the zoospore for some time. When branching was about to take place in the young hypha, a nucleus was found in the neighbourhood of the branch origin.

Time.	*Zoosporangium.*	*Zoospore.*
12 noon	Spores germinate.	Liberated.
4 p.m.	Septum growing upward.	Second motile stage.
4.30 p.m.	Ditto.	Reaches host and germinates.
10 p.m. to 3 a.m.	Septum still growing, nuclei lower in the hypha divide, to be carried upward for the next sporangium on the next day.	Movement of cytoplasm rapid, germination advancing, nuclei divide sufficiently to serve the hypha next day.
5 a.m.	Old sporangium filled by outgrowth of septum.	
6 a.m.	Cytoplasm ceases to flow into new sporangium.	Germ-tube lengthens, branches formed, no more nuclear divisions.
6.30 to 7 a.m.	Septum formed and cytoplasmic continuity broken.	
7 to 11 a.m.	Zoospore origins formed and zoospores round off.	
11 a.m. to 12 noon.	Zoospores escape.	

The vegetative nuclei can be stimulated to division at any time of day. A culture is placed in a very cold greenhouse for two days, where the water can be kept at about 4° C. The flies are then suddenly removed to water at 22° C., and are left at that temperature, which is a little above the optimum, for from ten to twenty minutes. The material is then fixed with hot acetic Merkel's solution. Nuclear figures can usually be produced in this way; they are always amitotic.

Discussion.

Hartog and Trow, who have both studied the nuclei of the vegetative hyphae of *Saprolegnia*, disagree on the method of division. The latter states that a direct division takes place in the zoospore, but in his figures this is not well shown. Hartog describes a rudimentary mitosis which he calls a transition between a direct and an indirect division. He saw four small granular chromosomes, which split longitudinally and then separated, no spindles being present. His figures are not distinct, and it is difficult to see how he could have observed such small granules splitting longitudinally without staining them; in only one or two figures is it possible to distinguish the four chromosomes. No similar figures were seen during this investigation.

If the division is by the indirect method, the chromatin granules should have a part in chromosome formation. Here, however, the membrane remains intact during the division of the chromoblast, and only separates in the last stages when the chromatin has divided. The fact that the linin threads remain attached to the chromoblast throughout the

division is further evidence in favour of direct division. It is possible that both forms occur, for in *Valonia* Fairchild (1894) describes both mitotic and amitotic divisions.

Amitosis has been found in the vegetative mycelia of several of the fungi, notably in *Penicillium* (Guéguen, 1899) and the yeasts (Wager, 1898, and Guilliermond, 1903). Guilliermond also figured amitosis in *Mucor*, and this compares very favourably with that described above. The nucleus in yeast has a very different construction from that in *Saprolegnia*, and consequently cannot be compared, but the division described for *Penicillium* appears to be very similar to that described in this paper.

The type of amitosis in *Saprolegnia* is not a mere fragmentation, but it is an ordered division somewhat allied to that described for the nucleus of *Ancylistes* by Dangeard (1903). This nucleus, however, has a true nucleolus which passes out of the membrane as division commences. The chromoblast in *Saprolegnia* has not a true nucleolar function, since it takes an active part in the division.

Summary.

1. The fungus investigated appears to be a variety of *Saprolegnia dioica*. The vegetative nuclei are typically spherical, but vary in size and shape according to the concentration of the cytoplasmic strands and the consequent tension exerted by them.

2. Only amitotic divisions of the vegetative nuclei have been observed during the course of this investigation.

3. As a rule the nuclei divide between 10 p.m. and 2 a.m., but they can at any time be stimulated to division by removing the fungus from very cold water to water at 20° to 22° C.

The writer wishes to express his thanks to Professor O. V. Darbishire for suggesting the subject of this investigation and giving much helpful advice and criticism while it was being carried out.

Cryptogamic Research Laboratory,
Department of Botany, University of Bristol,
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The Effect on Certain Plants of altering the Daily Period of Light.

BY

J. ADAMS,

Central Experimental Farm, Ottawa, Canada.

PLANTS growing within the Arctic Circle complete their development during the summer months at a time when there is continuous illumination during the whole twenty-four hours. From this it may be inferred that a daily alternation of light and darkness is not essential for the growth of certain plants.

On the other hand, plants growing at places between the Equator and the Arctic Circle are subject to an alternation of light and darkness during each day, the relative length of which varies according to the latitude and the time of year. At a spot half-way between the Equator and the pole—that is, in latitude 45°—during the summer months the period of daylight is much in excess of the daily period of darkness. In such localities, assuming that the rainfall and soil are suitable, there is an abundant development of natural vegetation. The question then arises—Is this alternation of light and darkness necessary for the growth of such vegetation, and what would be the result if the relative length of day and night were artificially altered, other factors being left as far as possible the same? Supposing the periods of light and darkness were made approximately equal during each 24 hours, what would be the effect on plants as regards (1) the height of the stem, (2) the total weight of the plant, (3) the time of coming into flower? It was to obtain some further information on these subjects that the experiments detailed below were undertaken.

Before describing these experiments in some detail it will be advisable to review briefly some of the conclusions arrived at by previous investigators.

Kjellman (3) experimented with *Lepidium sativum* in the Arctic regions, allowing some plants to remain exposed to continuous illumination for 24 hours, while other plants were kept dark from 8 p.m. to 8 a.m. After two months he found that the illuminated plants were both taller and heavier than those which had been kept in the dark for 12 hours daily. Similar differences, but even more pronounced, were found by him in his experiments with Arctic plants such as *Cochlearia fenestrata* and *Catabrosa algida*.

Curtel's experiments (5) in Norway during a period of continuous

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sunlight proved that assimilation proceeded uninterruptedly, the minimum occurring at midnight, at the time of least illumination.

He also concluded (10), from observations made upon the influence of diffuse light on flowers, that the flowers were less brilliant in colour and fewer in number, and the fruits were smaller in diffuse light than in direct illumination, while very diffuse light made the formation of flowers impossible.

Bailey (6), using a protected electric arc light during half the night, was able to hasten the development of lettuce in a greenhouse by two weeks.

Vöchting (7) proved that under a weak illumination the formation of flowers in numerous phanerogams was either entirely prevented or only incompletely achieved. He found that the formation of flowers was closely connected with the activity of the leaves.

Bonnier (9) found, as the result of exposing plants to continuous electric light, that there was a much greater development of chlorophyll as well as other internal differences.

Pfeffer (12) states that 'within certain limits, a decrease in the illumination produces an accelerated rate of growth in a phototonic plant and an increase a diminished rate'.

On the other hand, Macdougal (13) appears to take a different view. He says: 'The same results have been attained in another form by the exposure of growing plants to continuous exposure to electrical illumination, or to an illumination in which daylight was supplemented by nocturnal illumination from electric arcs, or flames. In all such instances the amount of growth, as indicated by the length of the shoots and of the separate members, was greater than under ordinary conditions of alternating daylight and darkness. If light exerted a direct retarding, or paratonic, influence upon the processes of growth, such results would be impossible.'

Schimper (14), speaking of the action of continuous light during summer, states that it has a retarding action on growth, but furthers assimilation and the formation of pigments and other substances. He further states that growth in length of stems and roots is at its optimum when light is totally excluded.

Regarding the action of light on the development of flowers, Schimper continues: 'The minimum of light for the formation of flowers is lower for shade plants than for sun plants; yet the former generally produce fewer flowers than do the latter. The interior of a forest is poorer in flowers than a meadow, and certain regions with intense or prolonged illumination, such as the higher regions of vegetation in mountains, polar countries, and many deserts, are characterized by a great abundance of flowers. In such cases, however, other factors co-operate.'

Osterhout (15), commenting on the stunted stems of plants growing at high altitudes, states that 'the plants in question are comparatively warm

during the day when the sun is shining, but cool off very rapidly after sunset and remain cold during the night. Normally the greatest growth takes place at night, hence chilling them at this time explains, in part at least, their stunted growth.'

Warming (18) says: 'The development of plants depends not only upon the intensity but also upon the duration of the light to which they are exposed. For instance, in Finland or the north of Norway barley ripens its grain in eighty-nine days from the day of sowing, but in Schonen (in Sweden, 55–7° N.) it requires 100 days, despite the higher temperature and the more intense light; and the explanation of this must in part be that in the former places prolonged illumination promotes anabolism.'

He further states that 'intense light retards the growth of the shoot; consequently heliophytes are compact and have short internodes, but sciophytes have elongated internodes; species clothing the forest soil are mainly tall and long-stemmed'.

By the use (19) of an acetylene light Craig was able to force the development of flowers within a shorter time in the case of Easter lilies.

Hayden and Steinmetz's experiments (20) on the ripening of String Beans under electric lamps proved that they ripened in about half the time required for those grown under natural conditions with daylight only.

Palladin (22) says that 'plants grow more slowly by day than by night, so that it appears that light exerts a retarding influence upon growth'.

De Besteiro and Durand (23, 24), experimenting with Garden Pea under light of intensities of $\frac{1}{9}$, $\frac{1}{3}$, $\frac{1}{2}$, and full sunlight, found that the dry weight of the entire plants increased with the intensity of light and in the proportions of 2, 6, 7, and 11.

Wiessmann (25) found as the result of growing Oat plants on a roof and in a court that those on the roof, which were more fully exposed to the light, not only stooled better, but also flowered and ripened seed earlier and had a greater yield of both straw and grain than the plants grown in the court.

Garner and Allard (27, 32) experimented with a large number of plants, some of which were kept in the dark for a certain number of hours daily. Different species behaved in different ways under this treatment, but they found that some species so darkened came into flower much earlier than other members of the same species which were exposed to the full period of daylight. Another conclusion reached was that 'in all species thus far studied the rate of growth is directly proportional to the length of the daily exposure to light'.

Adams (28), experimenting with Flax, found that the plants exposed to the full period of daylight were taller and heavier and reached the flowering stage sooner than those darkened for a number of hours each day.

By employing continuous illumination (31) by means of an Osram

lamp Klebs was able to make a small beech-tree develop leafy shoots continuously for eight months and an oak-tree for seven months, three of which were in winter. He also states that humidity promotes growth and at the same time hinders the formation of flowers. He further adds that the essential condition for flower-production is the accumulation of carbohydrates in the plant, a result which is directly dependent on the amount of photosynthesis. Hence, by using artificial illumination from an Osram lamp, he was able to make *Sempervivum* flower at any time of the year.

It is evident from the above summary that considerable difference of opinion exists among observers as to (1) the effect of light on growth, that is, extension in length, and (2) the effect of light on the production of flowers. In order to throw some further light on this problem two series of tests with different plants were carried out during the summers of 1920 and 1921. The experiments made in 1920 were carried out in the greenhouse, while those of 1921 were conducted on plants growing in open ground for the most part.

Greenhouse Experiments, 1920.

Four species of plants were used for the experiments, namely, Flax, Wheat, Sunflower, and White Mustard. The seeds were sown in six-inch pots. Six pots were planted with each species of seed, three being exposed to the light, while the other three were kept dark for a short time each day while the experiments lasted. The experimental pots were darkened by being covered with large flower-pots inverted, in which the hole in the bottom had been tightly closed with a cork. At first pots of $9\frac{1}{2}$ inches in diameter were employed, and towards the end of the experiments, when the Sunflowers were too tall to be covered by pots, they were placed in a wooden cupboard from which light was excluded.

In experiments with environmental factors it is extremely difficult so to arrange the conditions that only one factor will vary at the same time. The pots were not watered during the time of darkening, so that both the pots exposed and those covered received the same amount of water each day. It is conceivable, however, that slightly more water would pass into the air from the soil of the pots left exposed than in the case of the pots covered. It is also possible that the temperature inside the cover might differ slightly from that of the rest of the greenhouse. It is not probable, however, that the difference in either case would be so appreciable as to have much effect on the final result. Some observations on the temperature inside the cover were made in the case of the experiments carried out in 1921.

To indicate the exact procedure followed, a more extended account of the operations in the case of Flax is given, while for other species only a summary of results is given in each case.

Flax, 1920.

The three pots exposed to light were labelled L 1, L 2, and L 3, while the three darkened periodically were marked D 1, D 2, and D 3. The seeds were sown on 31 May, and after they had germinated and grown considerably several of the seedlings were pulled up in order to give the remainder more room to grow. The final thinning out took place on 8 June, ten seedlings of nearly equal vigour being left in each pot. The heights of these seedlings were carefully measured before any of the plants were darkened; the heights were again taken at the end of the experiment, as well as the total weight of the plants after they had been pulled up by the roots and the earth shaken off.

The dates, duration of darkening, and weather conditions were as follows:

9	June	Darkened	10.30	to	11.30	Sunny
10	,,	,,	9.30	,,	10.30	Cloudy, then sunny
10	,,	,,	3.20	,,	4.20	Sunny
11	,,	,,	10.20	,,	11·20	Cloudy
12	,,	,,	10.35	,,	11.35	Cloudy
14	,,	,,	10.30	,,	11.30	Mostly sunny
15	,,	,,	10.30	,,	11.30	Mostly cloudy
16	,,	,,	10.25	,,	11.25	Sunny
17	,,	,,	10.10	,,	11·40	Mostly sunny
18	,,	,,	10.15	,,	11.15	Cloudy
19	,,	,,	10.05	,,	11·35	Sunny
21	,,	,,	10.15	,,	11.15	Cloudy
23	,,	,,	10.00	,,	12.00	Sunny and cloudy
24	,,	,,	10.30	,,	11.30	Sunny
25	,,	,,	10.00	,,	11.00	Mostly sunny
25	,,	,,	2.30	,,	3.30	Sunny and cloudy
26	,,	,,	11.40	,,	12.40	Sunny and cloudy
28	,,	,,	9·35	,,	10.40	Cloudy
29	,,	,,	10.15	,,	11.10	Cloudy
30	,,	,,	9·35	,,	11.35	Cloudy
2	July	,,	10.25	,,	11.25	Cloudy
3	,,	,,	10.00	,,	12.00	Sunny
5	,,	,,	10.00	,,	11.00	Sunny
6	,,	,,	10.05	,,	11.05	Sunny

It will be seen, therefore, that the period of darkening extended from 9 June to 6 July. The plants were darkened on twenty-two days. The total period of darkening amounted to twenty-eight hours, namely, nineteen times for one hour, two times for one and a half hours, and three times for two hours. All the occasions of darkening were a.m., except three which were partly or wholly p.m. About half of the times of darkening were sunny and the other half cloudy.

The results obtained from each set of plants were as follows:

The first column gives the average height of each plant on 9 June; the second column the average height on 7 July; the third column the height of the tallest plant on 7 July; and the fourth column the total weight (including roots) on 7 July.

	Average height on 9 June.	*Average height on 7 July.*	*Tallest on 7 July.*	*Total weight.*
	mm.	mm.	mm.	grm.
L 1	26·9	341·3	380	13·720
L 2	26·4	417·7	482	16·470
L 3	27·6	451·2	500	15·400
Average	27·0	403·4	454	15·197
D 1	24·8	307·9	355	15·670
D 2	27·1	313·9	365	13·700
D 3	25·8	321·2	355	13·710
Average	25·9	314·3	358	14·360

While the unshaded plants were at the beginning of the experiment on 9 June 4·2 per cent. taller than the darkened set, at the end of the experiment on 7 July the unshaded lot were 28·3 per cent. taller than the other set.

Wheat, 1920.

The period of darkening was similar to that of Flax from 9 June to 5 July and covered altogether twenty-seven hours.

The seeds were sown on 31 May. On 8 June the number of seedlings was reduced to ten in each pot. The heights of the seedlings were measured on 9 June, while on 6 July the total weight of the stem and leaves was determined for each set. The figures were:

	Average height on 9 June.	*Weight on 6 July.*
	mm.	grm.
L 1	112·0	28·370
L 2	104·2	25·800
L 3	118·5	30·250
Average	111·6	28·140
D 1	114·9	26·000
D 2	106·6	24·150
D 3	102·5	28·070
Average	108·0	26·070

The average height of the unshaded plants on 9 June was 3·3 per cent. above that of the darkened set. The weights were presumably in the same proportion. On 6 July the average weight of the unshaded plants was 7·9 per cent. above that of the others.

Sunflower, 1920.

The periods of darkening were similar to those of Flax from 9 June to 6 July. On 7 July the plants were darkened for one hour, making the total time of covering altogether twenty-nine hours.

The seeds were sown on 31 May. On 7 June the seedlings were reduced to ten in each pot. On 9 June the heights of the seedlings were

measured, and on 8 July the heights were again measured, after which the plants were pulled up and weighed after the earth adhering to the roots had been removed as far as possible. The figures were:

	Average height on 9 June.	*Average height on 8 July.*	*Tallest on 8 July.*	*Total weight.*
	mm.	mm.	mm.	grm.
L 1	36·3	554·5	607	124·820
L 2	29·8	563·5	635	135·380
L 3	30·3	537·5	580	121·130
Average	32·1	551·8	607	127·110
D 1	37·1	548·7	650	119·020
D 2	36·5	585·7	625	132·120
D 3	25·9	484·4	540	109·650
Average	33·2	539·6	605	120·260

While the average height of the unshaded plants on 9 June was 3·3 per cent. less than that of those subsequently darkened, the height of the unshaded plants on 8 July was 2·2 per cent. more than that of the other set.

White Mustard, 1920.

The seeds of this species were sown on the same date as those previously mentioned, and the period of darkening was similar, but towards the end of the experiments all the plants became so badly affected with green fly that no satisfactory results could be deduced from them. Accordingly another set of experiments was started.

The plants were darkened on sixteen days between the 12th and 29th of July. They were covered altogether eighteen hours, the sky during one half of the time being sunny and during the other half cloudy. The heights and final weights of the plants were determined as in the foregoing experiments. In addition, the number of flower-buds present in each pot was observed before the plants were pulled up in order to be weighed.

The seeds were sown on 8 July. On 12 July the seedlings were thinned, ten being left in each pot, the heights of which were determined. The heights were again measured on 23 July. The complete figures were as follows:

	Average height on 12 July.	*Average height on 23 July.*	*Average height on 30 July.*	*Tallest on 30 July.*	*No. of flower buds.*	*Total weight.*
	mm.	mm.	mm.	mm.		grm.
L 1	13·0	73·9	146·3	173	7	20·000
L 2	13·2	72·5	146·0	194	10	17·950
L 3	12·1	78·9	176·6	215	9	22·750
Average	12·8	75·1	156·3	194	8·7	20·230
D 1	12·1	55·0	114·6	145	5	13·570
D 2	10·6	58·3	126·2	158	4	16·000
D 3	12·0	67·5	134·6	169	7	14·050
Average	11·6	60·3	125·1	157	5·3	14·540

The average height on 12 July of the unshaded plants over those shaded was 10·3 per cent.; on 23 July this had increased to 24·5 per cent., while on 30 July it was 24·9 per cent.

Experiments in Frame, 1921.

The first set of experiments during 1921 was made with seeds planted in the soil within the frame. The under surface of the windows used as a roof was covered with sheets of brown paper firmly secured to exclude the light. The check lot of plants in a frame immediately adjoining the first was left uncovered throughout the experimental test.

It was found in practice that this method was less satisfactory than the tests carried out in the greenhouse or the open ground, as the factors other than light were more difficult to control. More moisture was evaporated on sunny days from the soil of the uncovered frame than from the one that was darkened. This was remedied to some extent by watering the uncovered plot occasionally. The temperature inside the covered frame as well as the relative humidity were higher than in the adjacent frame left uncovered. On 10 June the temperature at 3·25 p.m. was 101° F. inside the frame, while it was 95° F. outside it. The relative humidity at the same time was 57 inside the frame and 51 outside. Notwithstanding these difficulties it seems advisable to give the results obtained, as they serve to corroborate the figures obtained by other methods.

The darkening of the frame extended from the 2nd to the 25th of June and took place on nineteen days in all. The total duration of darkening amounted to eighty-five and a half hours, or an average of three hours thirty-four minutes for each of the twenty-four days during which the experiment lasted. The shortest period of darkening the frame on any one day was two hours, and the longest six hours, while the commonest was five hours. The average length of day between the 2nd and 25th of June, measured from sunrise to sunset, amounted to fifteen hours thirty-four minutes. The effect of covering the frame was to make the daily amounts of daylight and darkness equal during the twenty-four days of the experiment. The weather during fifteen of these days was bright and sunny. Care was taken to ensure that the frame was not covered when rain was falling.

The plants experimented with were Flax, Wheat, and Soy Bean.

Flax in Frame, 1921.

The seeds were sown in two rows on 23 May, one row in each frame. On 2 June seventy-eight plants were left in each row, and on 21 June the number was still further reduced, thirty-two being left in the uncovered frame and thirty-one in the one darkened.

In the frame which was constantly exposed to light the first flower opened on 5 July, on 11 July there were fifteen plants in flower, and on 12 July there were sixteen plants in flower.

In the darkened frame the first flower did not open until 11 July, while on 12 July two plants were in flower.

Wheat in Frame, 1921.

One row of seeds was sown in each frame on 23 May, and on 2 June twenty-six plants were left in each row.

In the uncovered row five plants were in ear on 7 July, and on 11 July there were twenty-two in ear. The first ears, three in number, in the covered row did not make their appearance until 11 July.

The plants were pulled up on 13 July and gave the following results:

	Average height.	*Tallest plant.*	*Total weight.*	*No. of plants in ear.*	*Total No. of ears.*
	mm.	mm.	grm.		
Plants exposed to light	497·5	780	127·57	25	49
Plants covered	316·8	479	99·22	11	11

Soy Bean in Frame, 1921.

One row of seeds was sown in each frame on 23 May, and twenty-five plants were left in each row on 2 June. This number was reduced to twenty-three on 13 June.

In the uncovered row two came into flower on 5 July, and on 7 July three were in flower. In the covered row the first plant came into flower on 7 July.

The plants were pulled up on 13 July and were measured and weighed with the following results:

	Average height.	*Tallest plant.*	*Total weight.*	*No. of plants in flower.*
	mm.	mm.	grm.	
Plants exposed to light	258·2	394	255·15	23
Plants covered	340·3	475	283·50	22

The plants in the covered row were growing in slightly better soil. The figures should be compared with those obtained from the plants grown in the open ground, which are given farther on.

Experiments in Open Ground, 1921.

In order to keep all the factors except the amount of light as nearly as possible the same, it was found desirable to use wooden boxes for the purpose of excluding light from each set of experimental plants. The use of wooden coverings tended to lessen the difference in temperature and relative

humidity between the air surrounding the shaded plants and that around the plants left uncovered. For example, on 18 June at 10.55 a.m. the temperature of the air inside one of the wooden boxes was 76° F., while outside the box it was 72° F. On 20 June at 3.25 p.m. the temperature was 88° F. inside and 91° F. outside the box. At no time were the plants covered while rain was falling. During dry weather both sets of plants were watered alike.

In the case of Flax, Wheat, White Mustard, and Soy Bean the seeds on the darkened plot were sown in two rows, the distance between the rows being nine inches. The seeds on the exposed plot were sown in exactly the same manner, and an interval of eighteen inches separated the darkened from the exposed plot. There were thus four parallel rows of the same length in which the soil was apparently quite uniform. The Sunflower seeds were sown in two parallel rows with an interval of twenty-four inches between the exposed and the darkened row.

Flax in Open Ground, 1921.

The seeds were sown on 23 May. On 6 June sixty-three plants were left on each plot, the number being gradually narrowed down to forty, at which figure they remained to the close of the experiment. The plot was darkened for seventy hours extending from 6 June to 25 June, and on sixteen days the weather was either sunny or partly sunny at the time. In the exposed plot seven plants came into flower on 3 July, and on 6 July thirty-nine were in flower. On the latter date the first plant came into flower on the darkened plot.

The heights and weights of the plants when they were pulled up on 12 July were as follows:

	Average height of stem.	*Tallest plant.*	*Total weight.*	*No. in flower.*
	mm.	mm.	grm.	
Exposed plot	566·3	780	170·100	40
Darkened plot	553·5	697	106·310	35

Wheat in Open Ground, 1921.

The seeds were sown on 23 May. On 2 June twenty-two plants were left in each plot, the number being afterwards reduced to nineteen. The plot was darkened for seventy hours from 2 June to 21 June, the weather being sunny for the greater part of the time. In the exposed plot one plant came into ear on 7 July and on 12 July, fourteen were in ear. In the darkened plot the first three plants came into ear on the latter date. The plants were pulled up on 16 July with the following results:

	Average height of stem.	*Tallest plant.*	*Total weight.*	*No. of plants in ear.*	*Total No. of ears.*
	mm.	mm.	grm.		
Exposed plot	585·5	800	283·500	19	49
Darkened plot	448·7	662	191·360	15	18

White Mustard in Open Ground, 1921.

Seeds were sown on 23 May, and on 2 June forty-two plants were left in each plot, the number being afterwards reduced to twenty-seven. The period of darkening was exactly the same as for wheat. In the exposed plot six plants came into flower on 27 June, and on 30 June eighteen were in flower. On the latter date the first plant in the darkened plot came into flower. The plants were pulled up on 5 July with these results:

	Average height of stem.	*Tallest plant.*	*Total weight.*	*No. of plants in flower.*
	mm.	mm.	grm.	
Exposed plot	616·0	790·0	1006·425	25
Darkened plot	460·7	647·0	453·590	17

Soy Bean No. 1 in Open Ground, 1921.

The seeds were sown on 23 May, and on 2 June eighteen plants were left in each plot, the number being reduced later to seventeen. The period of darkening was exactly the same as for wheat. In the exposed plot two came into flower on 4 July, and on 6 July three were in flower. The first flower in the darkened plot opened on the latter date. The plants were pulled up on 12 July with the following results:

	Average height of stem.	*Tallest plant.*	*Total weight.*	*No. of plants in flower.*
	mm.	mm.	grm.	
Exposed plot	452·7	528	517·380	17
Darkened plot	316·3	399	226·800	17

Soy Bean No. 2 in Open Ground, 1921.

The seeds were sown on 23 May, and on 2 June sixteen plants were left in each plot, the number remaining the same to the end of the experiment. The period of darkening extended from 2 June to 25 June and comprised eighty-four hours. In both the exposed and darkened plots the first two flowers appeared on 2 July. The plants were pulled up on 11 July with these results:

	Average height of stem.	*Tallest plant.*	*Total weight.*	*No. of plants in flower.*
	mm.	mm.	grm.	
Exposed plot	440·4	555	467·760	15
Darkened plot	358·1	446	262·240	16

Sunflower No. 1 in Open Ground, 1921.

The seeds were sown on 23 May, and on 2 June twelve plants were left in each plot, which number remained to the end of the experiment. The time of darkening extended from 2 June to 21 June and comprised seventy hours. In the exposed plot five plants were in flower on 23 August and ten on 31 August. The first flower in the covered plot opened on the latter date. On 6 September the stems were cut off at the ground level, the roots being left in the ground. The results were:

	Average height of stem.	*Tallest plant.*	*Average weight of plant.*	*Heaviest plant.*	*No. of plants in flower.*
	cm.	cm.	grm.	grm.	
Exposed plot	257·39	294·64	1808·460	2863·290	12
Darkened plot	197·27	251·46	444·14	1417·470	5

Sunflower No. 2 in Open Ground, 1921.

The seeds were sown on 23 May, and on 2 June fourteen plants were left in each plot, the number being afterwards reduced to twelve. The period of darkening was exactly the same as in the case of Wheat. In the exposed plot the first plant came into flower on 25 August, and on 2 September four were in flower. The first flower in the darkened plot opened on the latter date. The stems were cut at the ground level on 7 September. The results were:

	Average height of stem.	*Tallest plant.*	*Average weight of plant.*	*Heaviest plant.*	*No. of plants in flower.*
	cm.	cm.	grm.	grm.	
Exposed plot	273·05	294·64	1434·000	5145·410	12
Darkened plot	237·49	274·32	682·750	1233·200	5

Experiments with Indian Corn and Wax Bean in Open Ground, 1921.

In this series there were twelve rows, six being Indian Corn and six Wax Bean. Each pair of rows was of approximately equal length, and one row of each pair was darkened, while the other was exposed to daylight. The seeds in all the rows were sown on 17 June and the period of darkening extended from 27 June to 13 July, the sunny days during the time of darkening being slightly in excess of the cloudy days. One row of Indian Corn was darkened on the average for two hours daily, another for three and a half hours daily, and the third for five hours daily, the procedure in the case of the rows of Wax Bean being similar. The average length of daylight between 27 June and 13 July was about fifteen and a half hours, reckoned from sunrise to sunset.

Indian Corn No. 1.

There were six plants in each row. One row was darkened for thirty-four hours, or an average of two hours daily, the actual time of darkening varying from thirty-five minutes to six hours on any one day.

On 29 July the exposed plot had three plants with staminate inflorescences protruding, while on the darkened plot only one was protruding.

Indian Corn No. 2.

There were nine plants in each row. The period of darkening amounted to fifty-nine and a half hours, or an average of three and a half hours daily, the actual time of darkening varying from one hour to seven hours on any one day.

On the exposed plot five plants had staminate inflorescences protruding on 29 July, while on the darkened plot there were four with staminate inflorescences.

Indian Corn No. 3.

There were thirteen plants in each row, which were afterwards reduced to twelve. The period of darkening amounted to eighty-five hours, or an average of five hours daily, the actual time of darkening varying from one and a half hours to eight hours on any one day.

On the exposed plot on 29 July eleven plants had staminate inflorescences protruding, while only one was visible on the darkened plot.

On 23 August twelve plants on the exposed plot bore pistillate flowers, while on the darkened plot there was only one with pistillate flowers.

The plants were pulled up on 8 September with the following results:

	Average height of stem.	*Tallest plant.*	*Total weight.*
	cm.	cm.	grm.
Exposed plot	124·35	153·67	2353·000
Darkened plot	81·23	121·92	1247·380

Wax Bean No. 1.

There were seven plants in each row. The period of darkening was the same exactly as in Indian Corn No. 1, and averaged two hours daily.

On 20 July three plants were in flower on the exposed plot, while there was only one on the darkened plot.

The plants were pulled up on 23 July with these results:

	Average height of stem.	*Tallest plant.*	*Total weight.*
	mm.	mm.	grm.
Exposed plot	393·7	505	141·750
Darkened plot	382.6	452	134·660

Wax Bean No. 2.

There were ten plants in each row. The period of darkening was the same as in Indian Corn No. 2, and averaged three and a half hours daily.

On 20 July on the exposed plot five plants were in flower, while on the darkened plot four were in flower on the same date.

The plants were pulled up on 23 July with these results :

	Average height of stem.	*Tallest plant.*	*Total weight.*
	mm.	mm.	grm.
Exposed plot	366·9	434	219·710
Darkened plot	335·6	422	170·100

Wax Bean No. 3.

There were sixteen plants in each row, which were afterwards reduced to fourteen. The period of darkening was the same as in the case of Indian Corn No. 3, namely, five hours daily on the average.

On the exposed plot on 20 July eight plants were in flower, while on the darkened plot there were none. The first flower on the darkened plot opened on 22 July, on which date eleven were in flower on the exposed plot.

The plants were pulled up on 25 July with the following results :

	Average height of stem.	*Tallest plant.*	*Total weight.*
	mm.	mm.	grm.
Exposed plot	531·0	815	411·080
Darkened plot	317·5	425	219·710

Experiments with Tomato in Greenhouse, 1921.

There were twelve pots with one plant in each. Four of these were left uncovered, and the remainder were darkened in sets of four each, the period of darkening being the same in each four. Wooden boxes were used as covers. The seeds were planted on 23 May. The period of darkening extended from 6 June to 30 June and comprised altogether ninety-two and a half hours, or an average of three hours forty-two minutes per day. This made the daily periods of light and darkness approximately equal. The actual time of covering on any one day varied from two to six hours. The different pots received approximately the same amount of water.

In the exposed pots the first plant came into flower on 4 July, and on 7 July all four were in flower.

In the first series of darkened pots the first two plants came into flower together on 11 July, while in the second series of darkened pots the first plant came into flower on 9 July.

The plants were cut at the ground level on 18 July with the following results :

	Average height of stem.	*Tallest plant.*	*Total weight.*
	mm.	mm.	grm.
Exposed pots	295·5	310	77·970
Darkened pots, Ser. 1	255·0	283	63·790
,, ,, ,, 2	294·5	330	77·970

Experiments with Dandelion in Greenhouse, 1921–2.

Seeds were obtained from plants which had flowered in 1921 and on which the seeds were ripe in the month of May. Two pots were sown on 28 May 1921, and subsequently five plants were left in each pot. One of the pots was darkened periodically, a wooden box being used for the purpose, while the other pot was exposed to daylight. The period of darkening extended from 16 June to 30 July and included altogether 157½ hours, about two-thirds of the time being sunny.

On 31 August one flower head in the darkened pot opened, but there was none on that exposed to light. The five plants in the latter case were larger than those in the darkened pot. No further flowers were produced during the year 1921. On 29 March 1922 the first head in the pot exposed to light opened, while the first head in the darkened pot did not open until 3 April.

The total number of flower heads produced by the five plants in the pot exposed to light was twenty-two, while in the darkened pot the total number of heads was nine. In the darkened pot both plants and heads were smaller than in the case of the pot exposed to light.

Experiments with Liver-leaf (*Hepatica acutiloba*, DC.) *in Greenhouse.*

In order to test the effect of a shortened period of light on plants which grow naturally in the shade of a forest, two species were experimented with, namely Liver-leaf (*Hepatica acutiloba*, DC.) and Coolwort (*Tiarella cordifolia*, L.). It might naturally be expected that too much light in the case of such plants would have an injurious effect.

Six plants of Liver-leaf were dug up from the floor of a wood and placed in pots on 20 June 1921. The plants were approximately of the same size. After they had become properly established in the pots, three were darkened from 4 July to 30 July. The total time of darkening amounted to ninety-four and a half hours. The weather during about two-thirds of the time of covering was sunny.

The dates of opening of the first flower in the three pots exposed to light were respectively 20 February 1922, 28 February 1922, and 8 March 1922.

The corresponding dates in the three darkened pots were 1 March 1922, 6 March 1922, and 10 March 1922.

The total number of flowers produced in each of the three pots exposed to light was sixteen, twenty-two, and twenty, while in the darkened pots the numbers were twenty-four, fifteen, and thirteen.

Experiments with Coolwort (Tiarella cordifolia, L.) *in Greenhouse.*

The time of planting and the dates and duration of covering were exactly the same as for Liver-leaf.

The first flower in the three pots exposed to light opened on 25 March 1922, 29 March 1922, and 2 April 1922, respectively. In the darkened pots the corresponding dates were 27 March 1922, 29 March 1922, and 1 April 1922.

The total number of flowering scapes in the pots exposed to light was fourteen, seventeen, and twenty-one respectively, while in the darkened pots the numbers were eighteen, seventeen, and twenty.

Discussion of Results.

From the above experiments the following conclusions may be drawn:

1. The plants exposed longest to the action of light (*a*) attained the greatest weight, (*b*) attained the greatest average height, (*c*) commenced to flower earlier than those which were darkened for a number of hours each day.

2. The above results were not so marked in the case of Soy Bean and Tomato.

3. In latitude $45\frac{1}{2}°$, where the experiments were carried out, the other plants were able to make use of the prolonged daily period of light during the summer months with beneficial results.

Some further comment on these results seems desirable in view of the conflicting conclusions arrived at by the other investigators whose views were briefly summarized above. Apparently some restatement of the action of light on plants is necessary.

1 *a*. As light of a certain intensity is necessary for photosynthesis, it necessarily follows that, if other conditions remain the same, the plants exposed longest to light will attain the greatest weight, and this was confirmed by the experiments.

1 *b*. As growth in length normally takes place as the result of cell-division and cell-elongation at or near the tip of the stem, and as organic material is required for such cell-division, it necessarily follows that plants provided with a larger supply of such material as the result of prolonged photosynthesis are in a better position for making increased growth than those not so well supplied. Hence plants with a longer period of illumination should attain a greater height than those illuminated for a shorter time (other conditions remaining the same), which again is borne out by the experiments.

A better understanding of the situation will be arrived at if attention

is concentrated on what takes place in a single cell of the spongy parenchyma of the leaf during daylight.

At the points where such a cell is in contact with neighbouring cells, water and certain inorganic substances in solution, which have been absorbed by the roots and have travelled up the vessels of the stem, are passing into the cell in question.

Where the cell-wall abuts on an air-space, water is passing through the cell-wall and is being evaporated, eventually diffusing through the open stoma into the atmosphere.

Certain organic substances in the cell are being broken down, with the formation of carbon dioxide as a result.

Carbon dioxide is being used by the chlorophyll corpuscles for the manufacture of starch grains or other carbohydrate.

Enzymes are at work within the cell, transforming starch into sugar and effecting various other changes.

An exosmosis of elaborated products is taking place through the points of contact of the cell with other cells, which elaborated products eventually find their way into the sieve-tubes and are conducted to the growing regions or places of storage within the plant.

At night or during darkness the stomata close, there is no diffusion of water vapour or other gases from the interior of the plant to the outer atmosphere, photosynthesis ceases, and the flow of water into the cell from the vessels stops or is reduced to a minimum. The conditions are therefore most favourable for the action of enzymes on the accumulated products of photosynthesis and the translocation of materials to the growing tissues of the plant. If the night temperature is sufficiently high, an extension in length of the stem would be the natural result.

It is not claimed here that every relation of a plant to light is capable of explanation in this way, but only its growth during darkness.

1 *c*. A plant normally flowers after it has attained a certain height and has stored up a quantity of reserve materials within its tissues. This is illustrated by biennial plants, bulbs, &c. A plant growing in dull light may have such a struggle for existence that it has no reserve material worth mentioning. As a result it will either not flower or will produce flowers very sparingly. Some trees in certain seasons produce such an abundance of flowers and fruits that it requires an interval of one or sometimes two years before the tree can accumulate sufficient reserve material for the formation of flowers again.

As has been proved by the experiments, those plants subjected to a longer period of illumination grew faster and attained a greater weight as the result of prolonged photosynthesis. They would therefore naturally be in a position to bear flowers before those plants subjected to a shorter period of daylight.

2 and 3. The behaviour of Soy Bean and Tomato as compared with the other plants tested would tend to indicate that there is a certain optimum relation between the daily amounts of light and darkness within which a plant will attain its best development. But probably further experiments with these two species are necessary before any broad conclusions can be drawn.

In the case of the other species experimented with, which grow naturally farther north, they were able to utilize the full period of daylight during the summer months with good effect. Kjellman's experiments showed that continuous illumination for twenty-four hours in the arctic regions produced better results than twelve hours of light and twelve of darkness. Until, however, some experimenter tests the result of exposure to eighteen hours of light and six of darkness or twenty-one of light and three of darkness daily, it would probably be rash to assume that in the case of plants growing in temperate climates there is no upper limit to the daily optimum amount of light.

Summary.

The effect on certain plants was noted of excluding light for a number of hours during the months of June and July, when the normal period of daylight in latitude $45\frac{1}{2}°$ is greatly in excess of the period of darkness. Some of the plants were covered for a period of three and a half hours, thus equalizing the duration of light and darkness; in other cases the plants were darkened for one, two, or five hours, on the average, for each day.

Some of the experimental plants were grown in pots in a greenhouse, others were planted inside a frame, while still others were planted in open ground. To exclude the light, large inverted flower-pots were used, also sheets of brown paper fastened to frames, and in other cases large wooden boxes were employed for the purpose. Care was taken to keep all the other conditions as uniform as possible.

The plants experimented with were Wheat, Indian Corn, Liver-leaf, White Mustard, Soy Bean, Wax Bean, Flax, Coolwort, Tomato, Sunflower, and Dandelion.

In almost all cases the plants exposed longest to the action of light gave the following results:

(*a*) Greatest average weight, (*b*) greatest average height, (*c*) earliest flowers.

The conclusion is drawn that growth or extension in length can take place both in light and in darkness, and that in both cases the amount of growth within a definite period of time is largely determined by the supply of available reserve material and the readiness with which this can be drawn upon by the growing parts.

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A Criticism of Beutner's Theory of the Electromotive Force of Diphasic Liquid Systems and their Relation to Bio-electrical Phenomena.

BY

DOROTHY HAYNES.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

THE development of the theory of concentration cells and its extension to two-phase systems has placed the study of bioelectrical phenomena upon an entirely new footing, and there are few physiologists who still doubt that it is within the scope of physical chemistry to furnish a complete explanation of such phenomena without the intervention of any specialized hypothesis. Nevertheless, much remains obscure, owing to the complexity of physical structure and chemical composition which is characteristic of the living organism, and none of the various theories put forward affords a complete explanation of the facts. It must be remembered that the generalizations of physical chemistry have been developed almost entirely from the study of very simple chemical compounds, and that in applying them to mixtures of complex organic substances there is some danger that insufficient attention may be paid to the essential differences between such mixtures and those of simple inorganic salts. It is by the careful study of these more complex systems that we may hope to attain to further comprehension of the electrical behaviour of living tissue.

Physiologists must therefore welcome so comprehensive a series of experiments as those described by Beutner in his recent work,[1] although they may be unable to follow him in all his theoretical conclusions. The experiments in question were carried out on two-phase systems consisting of water and 'oil', i.e. a liquid immiscible with water. It is in the properties of such systems that Beutner seeks a clue to those of living tissues, and it is as a result of their investigation that he reaches the conclusion that it is the salt content rather than the acidity of the cell which determines its electrical behaviour. Such a view, if substantiated, is of fundamental impor-

[1] R. Beutner: Die Entstehung elektrischer Ströme in lebenden Geweben. 1920.

tance for physiology, and no further justification will be needed for a careful review of the evidence upon which it is based.

The systems investigated by Beutner are of two general types, represented by the following schemes :

System I.

	D		A	C	B		E	
Hg KCl N/1	|	M_1X_1 water [c]	|	 oil	|	M_2X_2 water [c]	|	Hg KCl N/1

System II.

	D		A	C	B		E	
Hg KCl N/1	|	MX water [c_1]	|	 oil	|	MX water [c_2]	|	Hg KCl N/1

In these systems the potassium chloride in contact with the electrodes is frequently replaced by sodium chloride ; MX represents a salt of which M is the cation and X the anion, and c, c_1, c_2 in square brackets represent concentrations. It will be seen that System I consists of different salts of the same concentration in contact with 'oil'; System II of the same salt at different concentrations. In experimenting with systems of the type of System I, Beutner's general practice was to use salts having a common cation or a common anion. In this case M_1X_1, M_2X_2 will be replaced by M_1X, M_2X, or MX_1, MX_2. The 'oil' is a liquid which may be of very various chemical composition, ranging from acidic substances such as salicyclic aldehyde or benzaldehyde to definitely basic substances such as *o*.-toluidine.

In both systems potential differences may arise at the boundaries A, B, and C. Those at D and E Beutner considers to be obliterated by the more concentrated salt solutions in contact with the electrodes.

Beutner follows Haber's method in deriving the theory of such systems from a consideration of the following :

	A		C		B	
metal	|	aqueous solution of salt	|	solution in oil of the same salt in equilibrium with the aqueous solution.	|	metal

The e.m.f. of this system is zero, since work cannot be done by transference of salt across the boundary C ; hence the potential at C is equal and opposite to the sum of the potentials at A and B. If, therefore, the hypothesis of solution pressure is extended to oils, the boundary potential can be expressed as :

$$Ec = RT \log \frac{\text{C water}}{\text{C 'oil'}} \times \text{const} \quad . \quad . \quad . \quad (1)$$

C water, C 'oil', representing the concentrations of the cation in the two liquids.

In both the above systems two such interphasic potentials exist at A and B, the difference of which is equal to

$$RT \log \frac{C_1 \text{ 'oil'}}{C_2 \text{ 'oil'}}, \quad . \quad . \quad . \quad . \quad . \quad . \quad (2),$$

since the other factors cancel out ; and in Beutner's opinion this expression represents the total e.m.f., which is therefore determined by the relative concentrations of the common ion M (or X) in the layers of oil adjacent to the aqueous solutions. There are, however, considerable difficulties in accepting the conclusion that the total e.m.f. can be expressed by a logarithmic formula of this type.

The derivation of expression (1) is not entirely satisfactory, since little is known of the 'solution pressure' of metals in liquids other than water. The same result can, however, be obtained by the use of Nernst's partition formula :[1]

$$E = \frac{RT}{m_1} \log \frac{k^+ y^+}{c^+} \text{ (for positive ions)} = \frac{-RT}{m_2} \log \frac{k^- y^-}{c^-} \text{ (for negative ions)}$$

where m = valency, k partition coefficient between phases, c and y the concentration of the ions in the two phases.

More serious difficulties arise in its practical application. It should be remarked that the 'oil' in Beutner's experiment was not saturated with salt and was in equilibrium only at the surface layer. In most cases so little salt penetrated the oil that the salt produced no permanent effect upon its properties, and if a series of salts, of which the first and last members were the same, were brought into contact with the 'oil' the difference of potential at the beginning of the experiment was found to be identical with that at the end. The conductivity of the 'oil' must therefore have been mainly due to other ions, and since most of these 'oils' were acid it may be assumed that hydrogen ions played the principal part. Beutner's argument rests on the assumption that this hydrogen ion concentration is unaffected or is equally affected by the various salt solutions in contact with the 'oil'; which, however, can hardly be the case. Haber and Klemensiewicz[2] draw attention to this point in connexion with Riesenfeld's experiments on phenol, and it seems probable that the discrepancy between theory and experimental results which the latter observes may be referred to this cause. In this case the discrepancy is of no great magnitude, but where free acid is present, as in many of Beutner's experiments, the effect becomes of far greater importance. To take the following example, Beutner draws an analogy between the '*oil*' *system* :

[1] See Riesenfeld : Ann. d. Physiol., viii. 616 (1902).
[2] Zeitsch. f. physik. Chem., lxvii. 385 (1909).

	A		B	
+ Na in water		Na conc. – Na dil. 'oil' solution		Na in water –
	(Na oleate)		(NaCl)	

and the *Nernst system*:

+ Ag metal | Ag° conc. – Ag^{1} dil. | Ag metal –

and regards the former as a system reversible in respect to sodium ions, while he limits the effect of free acid in the 'oil' phase to that which it produces by increasing the solubility of the sodium salt. This is to ignore the fact that unless the ions of an electrolyte are completely insoluble in one of the two phases—an impossible assumption here—the distribution of these ions must affect the potential at the boundary, and this more especially where organic acids are concerned, since the partition coefficient between organic liquid and water is likely to be far higher for organic anions than for hydrogen ions.

In Beutner's systems the matter is further complicated by the nature of the salts in contact with the acid oil. Not only must the distribution of these salt ions be taken into account, but also the possibility of reaction.

We may consider the case of an 'oil' very frequently used by Beutner—a solution of salicylic acid in salicylic aldehyde. As salicylic acid is not insoluble in water as he supposes, it will react with the salts with which it is in contact, and double decomposition will take place at both boundaries. At A salicylic acid, oleic acid, sodium salicylate, and sodium oleate will be present; at B salicylic acid, hydrochloric acid, sodium salicylate, and sodium chloride. At A, therefore, there will be a decrease of hydrogen ion concentration; at B an increase. The effect of free hydrochloric acid is entirely ignored by Beutner, and his reasons for neglecting so potent a factor are discussed in the sequel. Here it will merely be pointed out that a solution of salicyclic acid will increase in acidity by admixture with sodium chloride, since salicylate ions will be removed as undissociated sodium salicylate and the concentration of hydrogen ions will consequently increase. When considerable quantities of undissociated salt are present this effect becomes important, and this has been shown by the writer [1] to be the case in buffer solutions. An 'oil' in which undissociated salt is soluble will evidently produce a very similar effect, and in the system under discussion there will therefore be a difference of concentration of acid on the two sides; work will be done by the transference of hydrogen ions from a region of greater to one of less concentration, and on this account a difference of potential will arise.

Before going farther into this matter consideration must be given to the effect of diffusion potential. Beutner discusses this in connexion with the work of Haber and Klemensiewicz [2] and of Cremer [3] on diphasic

[1] Biochem. Journ. xv. 440 (1921). [2] loc. cit. [3] Zeitsch. f. Biologie, xlvii. 1 (1906).

systems. By a curious misinterpretation he regards their theories as mutually exclusive, and attempts to decide between them; whereas both recognize the possibility of potential differences arising at all three boundaries, and both attempt, by an appropriate adjustment of the conditions of experiment, to eliminate certain of these—Haber the diffusion potential at C by the interpolation of a phase of constant composition: Cremer the interphase potentials at A and B by similarity of composition in the two aqueous solutions. How far these devices are successful is of course a matter for criticism, and Beutner justly points out that the results of measurements of potential in Cremer's nitrobenzol-picric acid system do not bear out his theory, as is recognized by Cremer himself.[1]

Beutner denies the existence of a diffusion potential in the oil systems with which he deals on the ground that the addition of such a substance as salicyclic acid to the oil phase increases the e.m.f., whereas on the hypothesis of a diffusion potential the presence of excess of electrolyte on both sides should tend to obliterate it. In the light of the considerations discussed above, it will be seen that this argument has little force, and on theoretical grounds Beutner's general proposition, that no diffusion potential exists between solutions of different concentrations, is entirely inadmissible.

The total e.m.f. of 'oil' systems such as the above must therefore be the algebraic sum of the following:

1. The interphase potentials at A and B. These will be determined by the relative concentrations of electrolytes in the layers adjacent to each boundary, and in certain cases their sum may be expressed by the formula $RT \log \frac{C_1}{C_2}$, where C_1, C_2 represent the concentration in 'oil' of salts present in equivalent concentration in the two aqueous solutions.

2. The diffusion potential due to difference of concentration and to difference in the nature of the ions on each side. Where the same electrolyte is present in different concentrations this potential can be calculated by

[1] Cremer found the system

A		C		B
M/δ NaCl sat. with nitrobenzene and picric acid	Nitrobenzene sat. with NaCl and picric acid	Nitrobenzene sat. with NaCl		M/δ NaCl sat with nitrobenzene

to be positive on the acid side. He regards the interphase potentials at A and B as equal and opposite, and ascribes the effect to diffusion potential at C in spite of the difficulty of maintaining this explanation. Beutner ascribes it to a difference of interphase potential at A and B arising from the greater solubility of sodium chloride in nitrobenzene containing acid, and quotes conductivity measurements to show that the solubility of sodium chloride in nitrobenzene is increased by the presence of picric acid. This argument is entirely inconclusive, for hydrochloric acid must be formed by the reaction of sodium chloride and picric acid, and a great part of the increased conductivity must be due to this cause. In the opinion of the writer, the effect is in all probability due to the fact that while picric acid is only slightly soluble in water, hydrogen ions are highly soluble. On this account an excess of hydrogen ions may easily be present in the aqueous phase even when the nitrobenzene phase contains only a very small quantity of dissociated acid.

the well-known formula $RT\frac{u-v}{u+v}\log\frac{C_1}{C_2}$, where u and v are the velocities of cation and anion in the 'oil' phase.

In certain cases one or other of these potentials may become negligible. For example, in the system

+ NaCl M/10	guaiacol	KCl M/10 (or aniline HCl M/10 or $MgCl_2$ M/10)

the measured e.m.f. agrees very closely with the logarithmic formula. In this case chemical reaction with guaiacol is probably negligible and diffusion potential very small, as would be the case if the various ions move with no great difference of velocity in the 'oil' phase. If so, Beutner's very precarious method of estimating the ratio of concentrations by changes of conductivity would also give valid results. In other cases diffusion potential must be very important. This is most probable where such a salt as sodium oleate is in question, and will account for the divergencies from the logarithmic formula observed by Beutner even where free acid is absent.

There is one group of experiments—expressed above by the scheme of System II—in which interphasic potentials play a part in somewhat different circumstances from those described above. Beutner has found that the production of an e.m.f. by difference of concentration is very largely dependent upon the presence of free acid and is greater with strong acids than with weak, but because the effect can be obtained with acids such as oleic acid, which are only slightly dissociated in water, he maintains that this effect is no 'measure' of the acidity. He looks upon it as due to 'non-proportional ionic distribution', i.e. to double decomposition. To make Beutner's outlook upon this matter clear, a statement will be quoted relating to the effect of potassium chloride on salicyclic aldehyde containing salicylic acid :

'Soviel ist aber sicher, dass KCl nicht nur als solches in dem säurehaltigen Aldehyd zugegen ist, sondern dass die freie Base sich mit der überschüssigen Säure vereinigt. Wie sollte sonst auch die Beobachtung zu erklären sein, dass pikrinsäurehaltiges Nitrobenzol mehr Salz bei Schütteln aufnimmt als säurefreies Nitrobenzol? Auch in diesem Falle muss eine solche Umsetzung mitspielen. Das durch Umsetzung gebildete Kalisalz unterliegt einer elektrolytischen Dissoziation; es entstehen also in dem säurehaltigen Aldehyd Kaliumionen, die nicht vom KCl herstammen. Um die Nichtproportionalität der Ionenverteilung zu erklären, ist ferner anzunehmen, dass die Konzentration des umgesetzten Kaliumsalzes im Aldehyd der wässrigen KCl-Konzentration *nicht* proportional ist. Wie dies möglich ist, kann allerdings theoretisch noch nicht begründet werden.'[1]

[1] loc. cit., p. 97.

It has been shown above that a simple explanation of the reaction exists, and that it is a necessary consequence of the establishment of equilibrium between dissociated and undissociated salt. How the writer of the above paragraph can have failed to realize that such a reaction connotes a change of hydrogen ion concentration is very hard to understand. He appears to have been led, by his experiments on the neutralization of acids by alkalies, to the conclusion that in solution in 'oils' all acids behave as weak acids, and that in consequence their dissociation is negligible compared with that of salts. Beutner's strongest acid however appears to have been salicylic acid, which, though highly dissociated in water, obeys the dilution law. Not only is it impossible for a chemist to regard the ionization of salicylic acid as throwing light upon that of hydrochloric acid, but very rigorous proof will be needed before chemists will be prepared to accept the proposition that the presence of varying quantities of any acid is without effect upon potential in such a system as that under discussion. In the case under consideration hydrochloric acid would diffuse at the boundary and would be present also in aqueous solution, and we have therefore the system exhaustively studied by Haber and Klemensiewicz in which solutions of different hydrogen ion concentration are separated by a phase in which the concentration of the ions is constant, the constancy in the present instance being due to the presence of a large excess of very slightly dissociated acid. There can be no reasonable doubt that the e.m.f. of systems of the type of System II is due to the same cause as that of the system studied by these two workers. Loeb[1] calls attention to the fact that the differences of potential which Beutner would attribute to the presence of lipoid substances can also be obtained with proteins, and he suggests that the difference of potential may be due to the establishment of the Donnan equilibrium. This explanation, however, would hardly seem to meet the case, since the Donnan equilibrium depends upon the concentration on one side of a membrane of some ion to which it is impermeable, and no such ion is available in Beutner's systems.

It is with these systems, whose potential depends upon difference of salt concentration, that Beutner has been able to show the most striking analogies between 'oils' and the substances which he entitles 'physiological objects'. Uninjured apple-skin, for example, shows a concentration effect markedly similar to that of an 'oil' containing free acid. The parallelism of the two experiments is very striking, and the discovery may well be of value as a method of investigating differences in various kinds of cuticle, but it cannot take us very far in the interpretation of the cellular mechanism of the cell. Apple-skin, with its coating of wax and specialized nature, is very far removed from the plasma membrane of the living cell, and Beutner finds that tissues without cuticle do not give very definite

[1] Journ. Gen. Physiol., iv. 351 (1922).

indications. It is hardly necessary to point out at the present time that no membrane which acts merely by restricting solubility can serve to explain the differences of permeability which the living cell exhibits. Such a membrane could not maintain differences of concentration[1] on either side of it, and if it were permeable to a dilute solution, a concentrated solution would penetrate very rapidly. It is therefore no analogue of the plasma membrane, and Beutner's claim to have rendered a service to electrophysiology similar to that rendered by Nernst to electrochemistry would seem to be somewhat insecurely founded, even if the physical basis of his theory were satisfactory.

The Current of Injury.

Beutner concludes his work by an attempt to explain the 'current of injury' in apples. He regards this as a consequence of the system

conducting solution	membrane containing acid (cuticle)	membrane containing a small amount of acid (flesh of fruit)	conducting solution

and he compares this with Cremer's nitrobenzene system, which is positive on the acid side. Beutner states that the outer skin of an apple consists of a mixture of fatty acids and higher alcohols, and that the tissue itself contains far less acid, but he does not state whence his information is derived; and on the basis of experiments on uncut apples, in which he found that the expressed juice showed no difference of potential against M/50 KCl, he considers himself justified in regarding the hydrogen ion content of the juice as without electrochemical effect. This latter proposition can hardly be maintained. What Beutner really shows is that the sum of the potentials at A, B, and C is zero in the system

		A	B	C	
KCl M/1	KCl M/50	apple-skin	apple-juice		KCl M/1

When the 'current of injury' is in question a different set of phase boundaries arises—among them the junction of the waxy layer with the cell-walls, which in the writer's opinion may play an important part, since the cell-wall is likely to contain free acid in aqueous solution. Further experiments are needed before the phenomenon of the 'current of injury' in apples can usefully be made the subject of detailed discussion; it is most certainly valueless to attempt to interpret it in terms of a system which postulates for the fruit a homogeneous 'flesh'. Beutner has rendered a real service to physiology by laying stress on the wide range of electrical phenomena which is shown by simple diphasic systems. It is unfortunate that the value of his work should be diminished by faulty interpretation and its significance minimized by an attempt to make it the basis of a general

[1] Cf. Osterhout: Journ. Gen. Physiol., v. 225, 1922

explanation of bio-electrical phenomena. On the physical side he fails altogether to substantiate his statement that salts rather than acids play the predominating rôle in the systems he investigates. A careful review of the experimental evidence shows that the differences of potential which he obtains can be correlated with a difference of hydrogen ion concentration whenever free acid is present in the 'oil', and that the theory of salt action is derived from a misinterpretation of the complex chemical systems with which he deals.

On the biological side Beutner lays himself open to serious criticism. He claims to have superseded such unproved theories as that of differential permeability by a theory resting on clearly ascertained facts; but on examination these 'facts' prove to be little more than a reintroduction of the lipoid theory without consideration of any of the difficulties which this theory has already encountered, and in his attempt to provide a solution for particular problems—in his theory of the current of injury and in the analogy which he appears to seek between cuticle such as apple-skin and the plasma membrane of the cell—Beutner assumes a simplicity and uniformity of structure for the living organism to which no physiologist can give countenance.

Experiments on the Growth of Fungi on Culture Media.

BY

WILLIAM BROWN, M.A., D.Sc.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

With seven Figures in the Text.

THE experiments to be described in the present paper had their origin in certain unexplained results which were met with in the course of an investigation of the growth of fungi in atmospheres of different composition.[1] It was there found that the effect of moderate concentrations of carbon dioxide (10–20 per cent.) consisted in general in a reduction of growth as measured in terms of the diameter of the fungal colony. This result was obtained, for example, with cultures of *Botrytis cinerea* and *Alternaria grossulariae* on a variety of media. Here the cultures in air were in advance of those in 10 per cent. carbon dioxide throughout the whole period of examination. On the other hand, certain clearly marked exceptions to this rule came to light. Thus it was found that with cultures of *Sphaeropsis malorum* on certain media the colony growing in 10 per cent. carbon dioxide at first lagged behind the one growing in air, but later on very distinctly surpassed it. It was with the view to a better understanding of such behaviour that the present study was undertaken.

In the course of this study it was found that the method of experiment adopted (which consisted chiefly in the systematic measurement of the rate of spread of the fungal colony) appeared to offer a promising basis for physiological work on fungi, on which account the scope of the investigation has been considerably extended. Briefly expressed, the aim of the investigation has developed into the study of the *form* of fungal cultures, chiefly in its relation to external factors. In how far this can be accomplished with the methods at present available, or how far these can be developed to meet the new problems that arise, only subsequent work can show. Since the completion of the work of which the present paper is an account, considerable progress has been made in a number of directions, and the writer believes that it will be possible by work along these lines to attack two large

[1] Ann. Bot., xxxvi, p. 257, 1922.

problems of fungal physiology. One of these is the problem of strains—i.e. accepting the existence of several strains of the same organism, what physiological differences can one establish between them, and can one correlate with these any of the distinct appearances of the particular strains? Such an investigation is much required in these days, when numerous strains are being discovered in many organisms. If in these cases, as is generally conceded, the morphological criteria are insufficient, and therefore recourse has to be had to physiological means of differentiation, it is obviously important to gain some idea as to what physiological characters will be of value in this connexion, i.e. what physiological characters are sufficiently constant and determinable with sufficient accuracy to be of use from the systematic standpoint.

The second problem may be described as that of the 'suitability' of a particular nutrient for a particular fungus. On this basis one may attempt to interpret the different growth appearances of an organism on one medium as compared with those on another, and in particular to explain the fact of an organism growing well on one medium and not at all on another. This latter problem is of the greatest interest in pathology, as its solution would form a vantage ground for the study of immunity in so far as the latter is based on nutritional factors.

The present paper deals with the so-called 'staling' of fungal cultures. The term 'stale' and its derivatives are in common laboratory use, and as some such term will be required in the present account, if much circumlocution is to be avoided, it is proposed to adopt the term throughout. By a 'stale culture' is understood one which has ceased or practically ceased growing; by a 'stale medium' one understands a medium which, through the growth in it of an organism, has been made useless, or nearly so, for further growth of the same or other organism. By 'staling substances or products' one means those metabolic products of the organism which are responsible for slowing down or stopping its growth. The use of this last term tacitly assumes that the cause of the slowing down of growth in a culture is the presence of products due to the organism and which are deleterious to growth, and that the staling is not to be ascribed merely to the removal from the medium of certain food substances: for many fungi, at any rate, this assumption appears to be fully justified. It is interesting to note that the phrase commonly used in German literature in this connexion is 'gebrauchte Nährlösung', a phrase which refers simply to the history of the cultural solution and not to any properties which it may have developed in consequence of that history.

In defining a particular word for use in this connexion one must take into account the present state of knowledge on the subject. Without going into details, one may state it as follows: The earlier workers, e.g. Duclaux,[1]

[1] Duclaux: Traité de Microbiologie, 1900. Ref. in Lafar's Handbuch, vol. i, p. 504, 1907.

believed that a 'used solution' is necessarily a stale solution—that is, that the growth in a medium of a particular organism rendered the medium less fit for further growth, at any rate of the same organism. Even to-day this rule will probably be found to cover the great majority of cases. But exceptions have been shown to exist, e.g. in a more recent work of Nikitinsky,[1] according to which a certain amount of growth of a fungus in a medium may render the latter more suitable for the further growth of the same fungus. A special and very important illustration of the same thing is the so-called 'Bios' effect described by Wildiers.[2] Here it was found that the growth of an organism in a fresh cultural solution was enormously accelerated by the transference with the inoculum of some of the metabolic products from the older culture, and in some cases this transference was indispensable. Thus it is clear that a 'used solution' is not necessarily a stale solution in the common acceptation of the term stale. It is of course possible that the accelerating substances which must be postulated to explain Nikitinsky's and Wildiers's results are the same as the staling substances, the difference in effect being due simply to difference in concentration. In that case the extension of the term 'staling' to include the effects of stimulation could be defended on grounds of analogy with, for example, toxic substances. Thus it is usual and justifiable to describe copper sulphate as toxic to fungi even though it has been demonstrated that it stimulates fungal growth when present in very minute quantity. However, the identity of the active growth-affecting substances in the case of fungi has not been demonstrated, and any extension of the term 'stale' along the lines indicated above is not at present justified. Further, any definition of staling cannot, in the present state of our knowledge, be based on the 'staling substances' themselves, which are in the main substances of unknown chemical nature, the existence of which is postulated to explain observed effects. The definition must be made on the basis of the observed effects.

The question now remains as to what are the effects observed. These will appear in the sequel, but it may be said here that the general rule is that the rate of spread of a fungal colony increases to a maximum at which it remains steady or from which it subsequently declines. Evidence will be brought forward to show that this decline from the maximum rate of growth can be controlled and modified in a variety of ways, all of which indicate that certain products of the metabolism of the fungus are affecting the medium at the growing margin in such a way as to cause a retardation of growth, and further, that if these products could be removed or otherwise rendered inoperative, the colony would continue growing at the maximum rate. A colony, therefore, which, though kept under the same external conditions, is not growing in diameter at the same rate as formerly, will be

[1] Nikitinsky: Jahrb. f. wiss. Bot., 1904, xl. 1.
[2] Wildiers: Koch's Jahresber., 1901, xii. 133.

described as showing staling at its growing margin, and in accordance with the amount of falling off will be described as more stale or less stale as the case may be. Further, as the basis of measurement is that of increase in diameter, the term staling will in the present paper have reference to growth at the margin of the colony only.

Experimental Method.

The general method of experiment was the same as that described in the earlier paper dealing with the growth of fungal colonies (loc. cit., p. 270), so that it is unnecessary to repeat the details here. Certain further particulars will be given below in describing particular experiments.

The fungi mainly dealt with were *Sphaeropsis malorum* and a species of *Fusarium*,[1] both of which show well-defined staling phenomena. Both have also advantages in this respect, that there is no trouble in either case from air-borne spores, the former only producing spores, if at all, in very old cultures, the latter forming its spores in moist masses. Fungi which possess air-borne spores are troublesome in this kind of work, as the disturbance incidental to measurement is liable to scatter spores on to the uninvaded portion of the plate, thereby producing colonies which interfere with the free growth of the mother colony. The removal of the lids of the Petri dishes for purposes of measurement brings in the risk of contamination from outside, and this has to be guarded against. This trouble has been met in two ways—by disinfecting the laboratory at fairly frequent intervals and by cutting out contaminating organisms when they are first noticed. By carrying out these precautions and by avoiding any work with such fungi as *Penicillium glaucum* and *Rhizopus nigricans* during the course of these experiments, no appreciable trouble was met with in the way of contamination of the plates from outside.

The stock cultures from which the inocula are taken are of course kept pure in the usual way in sterile tubes.

The actual measurements were made in each case in two directions at right angles to each other, each measurement being made to the nearest half-millimetre. In cases where the colony for some reason or other had not grown circularly, measurements were made along the long and the short diameter, and the average taken. When the greatest accuracy was required, successive measurements were made along marked directions.

When the object was to compare successive daily growths with each other, the cultures were kept at uniform temperature in an incubator. In a large number of experiments, however, where it was only sought to draw comparisons between different cultures under the same temperature condi-

[1] This is the same fungus as was dealt with in the earlier paper already cited. Its systematic position will be set out in a subsequent publication.

tions, the cultures were grown side by side in diffuse light at the general laboratory temperature.

The method of studying growth by increase of diameter offers certain contrasts with that of dry-weight measurements. A comparison of the two methods may conveniently be given here.

As regards simplicity the former has obviously great advantages. Thus it is possible to carry out experiments on a large scale, a circumstance which allows of abundant repetition of experiment and effective control in each case. There is, further, the very great advantage that the growth of any one colony can be followed throughout all its stages, whereas the dry-weight method involves the destruction of a culture for each measurement taken.

A second point of comparison is that the method of linear measurement is suited to growths on solid media (agar, gelatine, &c.), while the method of weight measurement can only be carried out effectively in the case of cultures in liquid media. It is certainly possible to determine the dry weight of a culture on gelatine media by dissolving away the latter with gentle heat, a process which can be carried out without killing the fungal mycelium, and thus without appreciable loss of cell contents. A similar method is not available for the case of agar media, and if it could be shown that reliable figures were obtainable by taking the dry weight of the killed (and presumably extracted) fungal mycelium after removal of the agar by high temperature, the fact remains that that method would be extremely laborious. This circumstance is unfortunate, as agar is in general a much more suitable solid medium than gelatine, partly by reason of convenience, but especially on account of the fact that gelatine is broken down by many fungi, and for that reason cannot be used in experiments where relations between growth and nutrition are being tested. Thus the method of dry-weight measurement, though well suited to the study of, for example, yeasts and such fungi as normally live in a liquid substrate, is not well adapted to the conditions under which fungi are generally grown and studied in the laboratory, viz. on solid media. Moreover, it should be noted that the method of studying the growth in terms of the spread of a colony from its point of inoculation is more calculated to throw light on some important problems, such as the spread of a parasitic organism from its point of infection over the host tissue, than is likely from studies by the dry-weight method. In the latter case the conditions are essentially different, involving as they do the simultaneous development of a large number of colonies in the medium with mutual interference from the start.

The greatest objection to the method of linear measurement is that it affords in many cases no indication whatever of the amount of mycelium in the fungal colony. Thus two colonies of the same diameter may possess vastly different amounts of mycelium, a fact which may be readily

visible to the eye though an exact measurement of the difference may be difficult to obtain. In fact in many cases there is a direct correlation between vigorous marginal growth and scantiness of mycelium within the culture.

But while the method of linear measurement gives in general no indication of the amount of growth made, it is well to remember that the alternative method is not free from objections in this respect. Numerous contradictions have arisen in the literature of fungal nutrition from misinterpretation of the results of dry-weight experiments. An example will suffice to show how errors have arisen. Suppose the object is to compare the nutritive value of two substances or two cultural media, A and B, for a particular fungus. The simple method is to start the fungus on the two media and at the end of an arbitrary time, say a fortnight, determine the dry weights of the mycelia produced in each case. Working in this way different investigators have come to the most contradictory results. The source of the apparent contradiction lies in the fact that the same fungus may have a growth-time relationship widely different on different media. In one case the increment of growth may be rapid at first and then reach a moderately low limiting value: in the other it may slowly increase to a high maximum. Again, the curve of dry weight of a fungus does not remain at a steady value but again diminishes through degenerative changes. Thus the dry-weight method cannot be safely used unless one is prepared to follow the growth at short intervals throughout its whole history. Short of that, the results will have no value. These considerations greatly increase the laboriousness of the dry-weight method.

All things considered, one may safely say that the method of linear measurement is the best to apply in exploratory work. By this means one can indicate problems of limited dimensions to which the more searching method of dry-weight determination can profitably be applied.

There remains the question of the relative accuracy of the two methods. The advantages, quite apart from the number of controls that can be employed, appear to rest with the method of linear measurement. Experience in this laboratory has shown that it is difficult to obtain an even curve of growth as determined by dry-weight measurement, as irregularities tend to appear under apparently identical conditions among the various cultures. In linear measurements of growth, the degree of uniformity obtainable depends on a variety of factors. With the same fungus, it may vary with the medium; thus it is difficult on some media to get uniform starting of the different controls while it is quite easy on others. Again, the degree of uniformity varies with the phase of growth of the colony, and it will be shown subsequently that the tendency to lack of uniformity among the various controls is an indication that staling is taking place.

As regards the two fungi mainly dealt with in this paper, *Sphaeropsis*

and *Fusarium*, the experience of the writer is as follows: Uniformity of starting is somewhat greater with the latter; thus it is possible to place very similar masses of *Fusarium* spores on the plates, and, within wide limits of size of inoculum, the initial growth of the resultant colony is the same in all cases. With *Sphaeropsis*, the inocula are small portions of mycelium and it is not so easy to ensure uniformity of inoculation. The resultant young colonies vary more among themselves than is the case with *Fusarium*, but it has been found that if the first two days' growth be subtracted from the following readings, much better agreement is shown. Under suitable conditions the degree of uniformity observed is surprising, and it is a common result to find the various controls agreeing among each other to within a few millimetres over a diameter of 10 centimetres.

The following graphs (Figs. 1–6) will serve to illustrate the different types of growth met with in different fungi, and with the same fungus under different conditions, and they will indicate the nature of the problems which arise in a study of this description.

In the experiment from which Figs. 1–3 were constructed, the various fungi were grown in the usual way on somewhat deep (1 cm.) layers of potato agar in closed Petri dishes. The medium was the same in all cases, and the growth tests were run concurrently. The experiment was done in triplicate, and each measurement is the average six readings, i.e. of two from each culture in the set. The ordinate corresponding to each plotted point on the curves represents the increment in diameter in the preceding twenty-four hours.

An examination of the curves in Fig. 1 brings out the following particulars:

In the early stages, the growth-rate increases day by day, so that the curve of total growth is convex to the time-axis.

In the 15° C. curve, the growth-rate had not reached a steady value at the end of the experiment, by which time the culture had reached a total diameter of 10 cm. and had almost covered the whole surface of the medium. The 20° curve, on the other hand, showed signs of reaching a steady growth-rate: and in the curve of growth at 25° this limiting value had been reached, and a subsequent slight falling off recorded. This falling off is very pronounced in the 30° curve, where growth ceased entirely after the first few days.

The general statement of these results is that the growth-rate increases to a maximum which is sooner reached the higher the temperature. At the two higher temperatures a decline in growth-rate sets in, which is more pronounced the higher the temperature. Whether such a falling off would appear at the two lower temperatures if the cultures were grown on a larger surface of medium was not determined. It was sufficient for the present purpose to show that this fungus would grow to a diameter of 10 cm. at

ordinary temperatures without showing any signs of marginal staling. Staling effects, however, do appear at 25°, and especially at 30°, but it will be noted that both these are supra-optimal temperatures. Whether these effects were due to actual staling substances appearing in the medium, or to direct injury to the fungal hyphae from prolonged exposure to the high temperature, was not investigated.[1]

Fig. 2 gives the corresponding results for *Alternaria grossulariae*.

The curve for growth at 30° is not drawn in, as it is practically identical with that at 25°. This fungus shows somewhat different growth features from those of *Botrytis*. It will be noticed that we have here a more slowly growing fungus with higher cardinal points for temperature. A striking

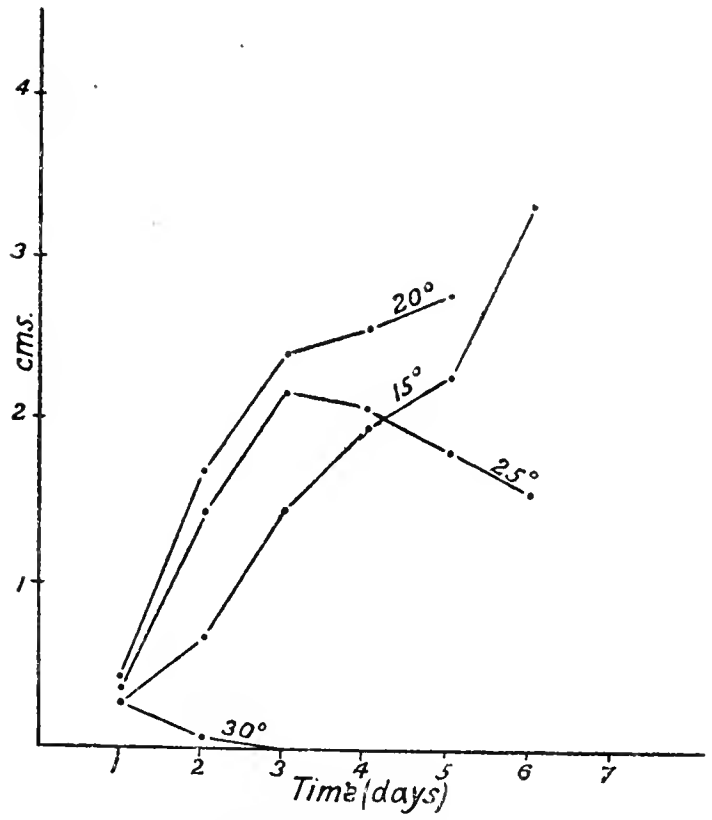


FIG. 1. *Botrytis cinerea* on potato agar.

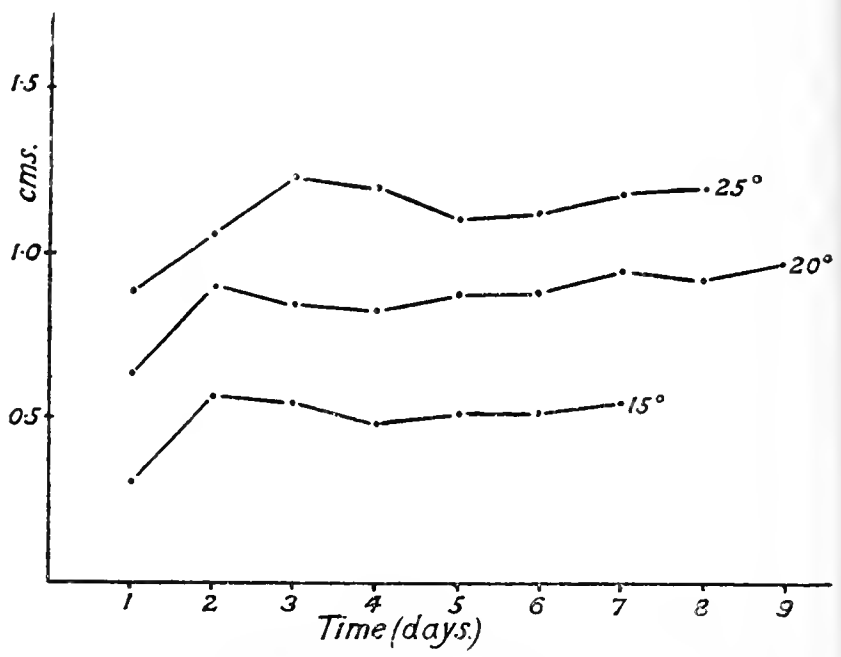


FIG. 2. *Alternaria grossulariae* on potato agar.

difference also is the shortening of the initial period of accelerated growth, so that all the cultures have reached their limiting growth-rate by about the second day. From that time onwards, all the curves are roughly parallel to the time-axis, so that the growth-rate in each case on the eighth day is not sensibly different from that on the second. There is no indication of staling in any case.

Both the preceding fungi may be cited as illustrations of the non-staling type, i.e. putting aside the case of the 30° curve for *Botrytis cinerea* which brings in considerations of supra-optimal temperature and is outside the scope of the present paper.

Fig. 3 illustrates the staling type of growth, with other features similar to those described in Fig. 1 and which need not be repeated here. The essential point to notice is that, in contrast with the first two cases, there is distinct staling of the fungal colonies at all the temperatures. In

[1] Compare, on this point, Balls, Ann. Bot., 1908, xxii. 557.

all cases the rate of growth at the seventh day is much reduced from what it was on the second and third days.

One can obtain all gradations from those fungi which would, as far as can be seen, grow on indefinitely at the same rate to those which form small colonies and then cease growing entirely. Fungi of the latter type are sometimes referred to as showing 'limited growth', but there are little grounds for thinking that there is anything absolute in their limitation. They are simply to be looked upon as fungi which have very strong 'staling tendencies', and one could anticipate that, if means were devised for removing or reducing in intensity the staling products, the amount of growth would cease to be limited.

A simple and effective way of demonstrating that the slowing down of the marginal rate of growth is not due to anything inherent in the growing

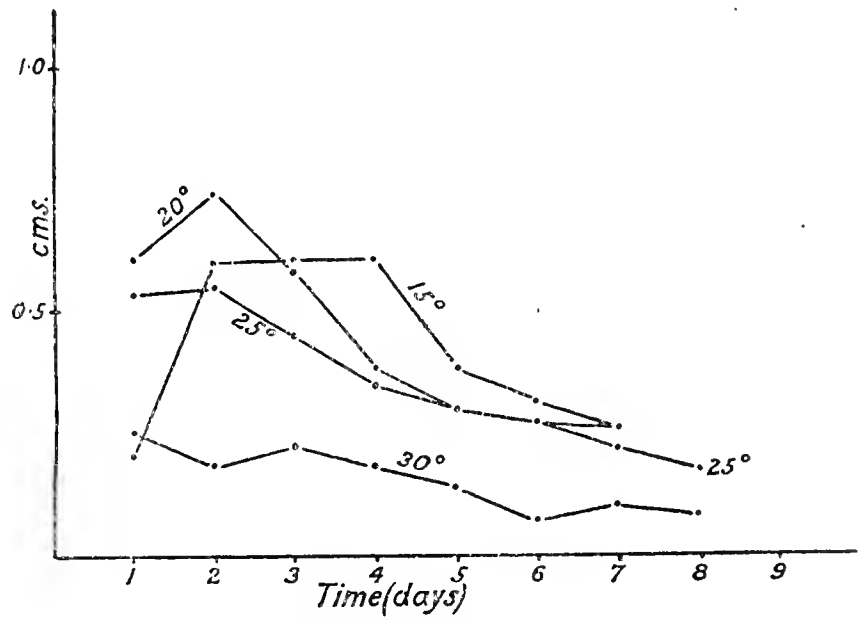


FIG. 3 *Fusarium* sp. on potato agar.

hyphae, but is caused by staling products diffusing outwards from the mycelial mass of the colony, is to cut away day by day the central portion of the colony, leaving only a fine fringe of marginal mycelium. Experiments of this type were carried out with the present and with another more strongly staling *Fusarium*. It was seen that the difference due to cutting out the central region of the colony was imperceptible when the fungi were grown on a medium on which no marginal staling took place (e.g. a dilute potato extract with agar), whereas when the medium was one which gave staling (e.g. potato agar of standard strength), the effect of cutting out the central portion of the colony was to postpone the incidence of staling. An illustration of the latter result is given in Table I.

TABLE I.

	Growth during						
	First 2 days.	*3rd day.*	*4th day.*	*5th day.*	*6th day.*	*7th day.*	*8th day.*
Uncut	0·85	0·85	0·9	0·7	0·5	0·3	0·3
Cut	0·85	0·85	0·9	0·78	0·65	0·63	0·68

The amount of staling shown by a particular organism varies from one medium to another and is dependent upon the amount of the particular medium present. Figs. 4 and 5 illustrate this point.

Fig. 4 gives the daily growth-rates of *Sphaeropsis* on apple extract agar (A.A.), and potato extract agar (P.A.). In each case the medium is presented in large or small quantity by varying the depth of pouring. In one set the plates are deep (about 1 cm.) and in the other shallow (about 2 mm.). The ordinate corresponding to each plotted point represents the average daily growth since the preceding reading. The points brought out by this graph are: (1) that there is distinct staling on potato agar, but none on apple agar; (2) that the rate of spread of the cultures on shallow potato agar is much less than on the deep medium, whereas both shallow and deep platings grow at approximately the same rate on apple agar.

A large number of experiments have been performed on the same lines

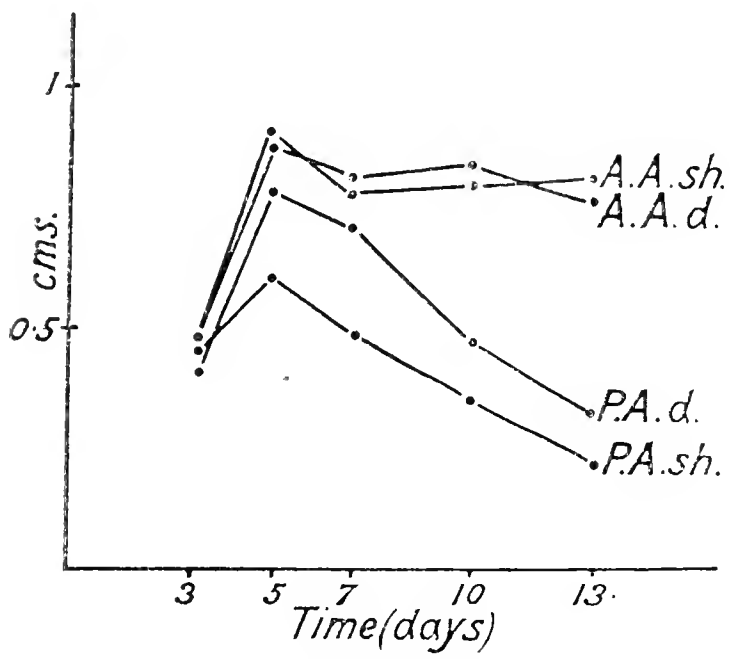


FIG. 4. *Sphaeropsis malorum* on apple agar (deep and shallow) and potato agar (deep and shallow).

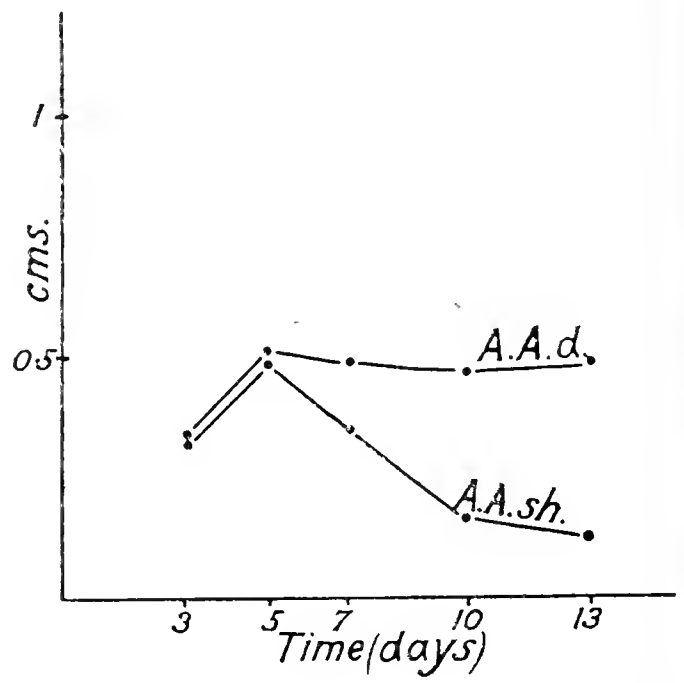


FIG. 5. *Fusarium* sp. on apple agar (deep and shallow).

as those illustrated in Fig. 4, and it is possible to generalize. When the depth of medium is varied, it is found that the rate of growth on the deep and shallow plates is at first identical. If the fungus does not stale on that particular medium, the rates of growth on the deep and shallow plates continue the same. If the fungus stales, then the rate of growth on the shallow plates after some time is different from that on the deep plates. In the present case the growth on the shallow plates lags behind the deep ones, and this is the rule on most ordinary media. But there are interesting exceptions to this rule which will be described in a subsequent paper.

This method of comparing the growth on deep medium with that on shallow has proved to be of great value, as one can obtain a good idea from inspection of the two sets of cultures as to how far staling is taking place.

Fig. 5 gives the growth of *Fusarium* on apple agar. In this case there is obvious staling on the shallow plates, though it had not appeared in a pronounced form in the deep plates. This experiment illustrates the

fact that *Fusarium* stales under conditions in which *Sphaeropsis* does not; this is in accordance with a large body of evidence which indicates that *Fusarium* is a fungus with stronger staling tendencies than *Sphaeropsis*.

The amount of staling shown can also in certain cases be modified by a slight alteration of the conditions under which the experiment is set up. Fig. 6 is an example of this. Both graphs represent the growth of *Sphaeropsis* on shallow layers of potato agar, the difference being that in the one case (the lower graph) growth took place in closed Petri dishes, in the other the Petri dishes were placed with the lids off in large five-litre containers. The difference was simply one of the volume of atmosphere in direct communication with the growing fungus, and it was sufficient to influence distinctly the incidence of staling. This type of experiment has been repeated many times under varying conditions with *Sphaeropsis* on potato agar culture, and the above result has been confirmed in every case. In the case of *Fusarium*, the same result is obtained, but as a rule to a less marked degree.

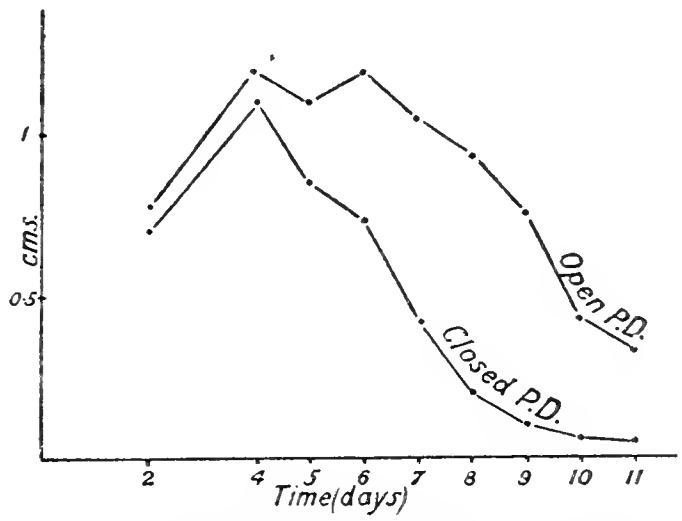


FIG. 6. *Sphaeropsis malorum* on potato agar.

The foregoing result indicates that we are dealing here with an effect produced by differences in the conditions of gaseous exchange in the two cases. The two main possibilities which suggest themselves are (1) that in the closed Petri dishes limitation of oxygen supply is the cause, (2) that a gas which retards growth tends to accumulate in the closed Petri dishes.

Table II, which is a fuller record of the experiments from which Fig. 6 was taken, indicates which of the above alternatives is the likely one. The figures in the table represent the total diameter (in cm.).

TABLE II.

Total growth of Sphaeropsis on Potato agar, T. 14–15°:

	2nd day.	*4th day.*	*5th day.*	*6th day.*	*7th day.*	*8th day.*	*9th day.*
Closed P. D.'s; deep	1·65	3·98	4·97	6·07	7·09	8·0	8·52
,, ,, shallow	1·50	3·76	4·65	5·40	5·85	6·10	6·20
Open ,, deep	1·65	4·12	5·28	6·56	7·71	8·74	9·6
,, ,, shallow	1·64	4·10	5·25	6·54	7·62	8·55	9·33

From this table it is seen that the effect of opening the Petri dishes is to increase the growth both on the deep and shallow plates, but especially on the latter. The shallow closed plates show very strong staling, the corresponding deep plates show definite staling at about the eighth day, while the cultures in the open plates only show slight staling at the end of the experiment, so little in fact that the deep and shallow plates are still

growing at almost the same rate. Now the Petri dishes are of approximately the same volume, and if limitation of oxygen is the factor concerned in staling, one would anticipate that staling would appear more strongly in the closed Petri dishes with deep layer of medium than in the corresponding ones with shallow layer of medium, first because there is greater free air-space in the latter, and secondly because there is throughout greater fungal growth in the former and consequently greater demands on oxygen supply. This, however, is not the case, whence it follows that the supply of oxygen is not the factor responsible. This conclusion is in agreement with former work, where it was shown that the amount of growth made by a fungus is independent of oxygen pressure within very wide limits.[1] The second alternative is therefore the one indicated, i.e. the differences observed are due to the action of gaseous or volatile products of the metabolism of the fungus.

The remaining portion of the experimental part of this paper will be devoted to an account of the nature of this action and how it can be demonstrated. It will aid to clearness to state here the main conclusions arrived at. The two gaseous products concerned in this differential action are carbon dioxide and ammonia. The former arises of course in the ordinary process of respiration; the latter is a product of the break-down of certain nitrogenous compounds (proteins, peptones, amides) which are present in most media, e.g. in potato and in all media containing gelatine. According to the extent to which these products accumulate or are disposed of, the amount of staling is affected. The ammonia, which is produced on some media in an amount greater than the fungus can take up, dissolves in part in the medium and renders it stale, or, more correctly, makes it more stale than it would be if this product were removed. The staling action of ammonia is partly counteracted by the carbon dioxide, and in accordance with the experimental conditions which allow the one or the other to accumulate there will be greater or less staling. From the physico-chemical point of view the principles involved are better stated as follows: When carbon dioxide is in excess, the ammonia goes into solution in the medium in the form of the bicarbonate; when ammonia is in excess, as ammonium carbonate and free ammonia. The latter is the more actively staling combination, probably on account of its higher hydroxyl concentration, but whether the greater activity is entirely due to this is not certain.

Two main lines of experiment were adopted. The first of these consisted in exposing plates of fresh medium to the gases given off by fungal cultures, after which the staling action of the exposed plates was tested by inoculating them with various fungi. The second method consisted in growing fungal colonies in atmospheres which were controlled in respect of their carbon dioxide and ammonia content.

The method of experiment in the first case is very simple. A batch of

[1] Loc. cit., p. 266.

plates, as uniform as possible (i. e. containing the same depth of the same medium), was inoculated with the fungus to be tested. When these cultures had reached a certain age a second batch of plates of the same size was prepared, and one of the second batch, still without inoculation, inverted over each culture of the first batch, the lids being removed. Similar uninoculated plates were kept unexposed as controls. After one to two days' exposure all the plates of the second batch were removed and their lids restored as rapidly as possible, after which they were inoculated with the same or another fungus, and the growth in a given time, usually two days, determined.

As an illustration the following experiment may be quoted. The staling was produced by ten-day-old cultures of *Sphaeropsis* on potato agar. The exposed plates had shallow layers of potato agar. After three days' exposure, the exposed and control plates were inoculated with *Sphaeropsis*. The diameter of the new growths in two days were as follows:

On unexposed plates (eleven colonies) . 1·57 cm. (max. 1·65, min. 1·5).
On exposed plates (twelve colonies) . . 0·42 cm. (max. 0·5, min. 0·3).

This staling effect soon disappears if the exposed plants are allowed to remain for some time with their lids off before inoculation. Also, the growth on the exposed plates tends to recover its normal rate as time goes on, probably by gradual escape of the staling gas. Again, the staling effect is more pronounced if the exposure is continued after inoculation.

A large proportion of these experiments were carried out before the nature of the staling substance was recognized. Cultures on potato agar, where the evolution of ammonia is small and not readily recognizable as such by its smell, were employed, and so a large number of control experiments were made to eliminate other factors. Two of these may be mentioned. A certain amount of drying of the exposed plates takes place during the process, but tests showed that this was of no importance. Also the carbon dioxide evolved by the cultures plays no part. Plates exposed in 40 per cent. carbon dioxide show no signs of being stale after removal from the gas. The absorbed carbon dioxide is apparently dissipated with great rapidity.

Deep plates of a given medium are more slowly staled by this treatment than shallow ones, as is illustrated by Table III.

TABLE III.

Plates of Potato Agar exposed to Cultures of *Sphaeropsis* on Potato Agar.

	Growth of Sphaeropsis in	
	First two days.	*Second two days.*
Unexposed, deep	1·91	2·54
,, shallow	1·94	2·37
Exposed (two days), deep	1·77	2·35
,, ,, shallow	1·10	2·07

These figures also illustrate recovery from staling.

With the same depth of pouring, some media are more readily staled than others, e.g. potato agar is more readily staled than potato gelatine (with the same concentration of potato extract in each), as is shown by the following figures, which represent two days' growth of *Sphaeropsis*:

TABLE IV.

	Unexposed.	*Exposed to Sphaeropsis Culture on Potato Agar.*
Potato agar (agar 1·5 %)	2·08	1·03
Potato gelatine (gelatine 10 %)	1·84	1·83

Any of the usual media (plum, prune, apple, &c.) made up with gelatine behave in the manner indicated.

Under the same conditions some fungi are more easily staled than others. The following table (V) illustrates the behaviour of four fungi in this respect. The cultures used to produce the staling were *Sphaeropsis* on potato agar and potato gelatine, both series being of the same age. The exposed plates were of potato agar. The figures in round brackets represent the percentage growth, that on the unexposed plates being taken as 100.

TABLE V.

Fungus tested.	*Unexposed.*	*Exposed to Sphaeropsis on P. A.*	*Exposed to Sphaeropsis on P. G.*
Botrytis cinerea	1·82 (100)	0·64 (35)	0·0 (0)
Sphaeropsis malorum	1·88 (100)	0·83 (44)	0·0 (0)
Fusarium sp.	1·52 (100)	1·18 (77)	0·74 (48)
Penicillium glaucum	0·94 (100)	0·79 (84)	0·45 (48)

A review of the experiments made on this point shows that *Botrytis cinerea* is more readily staled than *Sphaeropsis*, which again is more sensitive than either *Fusarium* or *Penicillium*. The two last are not definitely distinguishable in this respect.

As to the nature of cultures showing staling, the magnitude of the effect depends on age. For the first few days it is negligible, then it reaches a maximum, and in old cultures it again becomes small. The period of most active evolution of ammonia was not accurately determined, but the effect is usually well seen in cultures of ten days to a month. The effect appears sooner in shallow than in deep cultures, though after a time the latter show it just as strongly.

The effect also depends on the fungus used. With potato agar as medium, *Fusarium* and *Sphaeropsis* produce staling in a marked degree, *Botrytis* only very slightly, and *Monilia fructigena* not at all.

The composition of the medium on which the fungus is grown is of great importance. There is no evolution of staling gas from cultures on Richards' solution with agar.

It is interesting to compare the growth curve of *Fusarium* or *Sphaeropsis* on potato agar with that on potato gelatine (same potato extract in each case) in the light of the results shown in Table V. The difference between the growth curves on potato agar and potato gelatine consists in this—that, other conditions being the same, staling is shown sooner on potato agar, but subsequently it becomes more intense on the gelatine medium. This result is explicable on the grounds of two factors: (1) the greater evolution of ammonia from the gelatine cultures as growth proceeds ; (2) the greater capacity of gelatine media to absorb free ammonia without becoming alkaline. The first of these factors is illustrated in the table just given ; the second has already been illustrated in the experiments dealing with the staling of fresh media when exposed to fungal cultures (Table IV). A more instructive illustration is given in Table VI. To equal volumes of potato agar and gelatine equal quantities of a series of dilutions of ammonia were added, and the P_H of each determined colorimetrically. The concentrations of ammonia added were in the order 25, 5, 1.

TABLE VI.

Medium.	*Ammonia added.*	P_H.
Potato gelatine (10 % gelatine)	0·0	5·4
,, ,, ,,	1·0	5·4
,, ,, ,,	5·0	6·3
,, ,, ,,	25·0	9·2
Potato agar (1·5 % agar)	0·0	6·4
,, ,, ,,	1·0	8·1–8·2
,, ,, ,,	5·0	9·5
,, ,, ,,	25·0	too high to measure

This table shows that the addition of a small amount of ammonia to potato agar has a comparatively greater influence in diminishing its H-ion concentration than is the case with potato gelatine. The earlier incidence of staling in the cultures on potato agar is readily understandable in the light of this result.

Incidentally one may note that agar media are preferable to gelatine media for the purpose of keeping stock cultures, at least in the case of certain organisms. The large evolution of ammonia that may occur on gelatine media may be injurious or even fatal to the organism.[1]

The evolution of an alkaline gas can be shown simply by placing a piece of litmus paper in the lid of the culture. Extended observation has shown that the more rapidly a particular culture changes the colour of red litmus paper placed in the lid, the more vigorously does it produce staling in a plate of fresh medium exposed to it, and in particular where no alkaline gas can be demonstrated by means of litmus, no staling effect on a plate

[1] For an illustration of an effect of this nature see Boas, Ber. d. deut. bot. Gesell., xxxvii, p. 63, 1919.

of medium is produced. Further, a plate of medium on exposure to a culture of *Sphaeropsis* is protected from staling, at any rate in the neighbourhood of the watch-glass, by the insertion between the two of a small watch-glass containing dilute acid.

That the gas was ammonia was shown by its giving Nessler's reaction. It was also identified by isolation as the chloride. Alkylamines might be present in small quantities, but they were not demonstrated.

In order to be certain that the cultures producing the staling were absolutely free from any bacterial contamination (which cannot be guaranteed in Petri dish cultures where the lids have been removed), the experiments were carried out with pure tube-cultures of the staling fungus. The medium to be staled was used in the form of a film on sterilized slides. The results obtained were identical with those already described.

The amount of ammonia present in tube cultures was determined for a number of media. The tubes contained 25 c.c. of the medium, half of them being inoculated with *Sphaeropsis*, the other half left uninoculated. After four weeks' growth (at 18° C.) of the inoculated tubes, the contents of each tube were distilled over magnesia, and the distillate collected in decinormal sulphuric acid. The amount of alkaline gas passing over was then determined by titrating against decinormal alkali. The figures in the table represent cubic centimetres of decinormal solution. For each medium, eight tubes were analysed, i. e. four inoculated and four uninoculated. The results, which showed good agreement among themselves, gave the average values shown in the following table:

TABLE VII.

Agar 1·5 %; gelatine 10 %.

Medium.	*Amount of Alkaline Distillate from Inoculated Tubes.*	*Uninoculated Tubes.*
Potato agar	3·03	0·98
Potato gelatine	11·7	2·03
Richards' solution (KNO_3 1 %) with agar	0·5	0·17
,, ,, (KNO_3 1 %) with gelatine	9·3	0·3
,, ,, (KNO_3 0·1 %) with agar	0·28	0·13
,, ,, (KNO_3 0·1 %) with gelatine	13·55	0·6

We see here that there is a moderate amount of ammonia present in the cultures on potato agar, and much more on all the cultures of gelatine media (including, from other experiments, apple gelatine). The small quantities obtained with the two Richards' solutions plus agar fall within the experimental error limits of the determinations. Though the amount of ammonia present in these gelatine media is considerable, it is not all present in the free state, being neutralized up to a certain limit by the acidity of the nutrient solution and of the gelatine itself. With media such as apple gelatine and Richards' solution gelatine, where the nutrient solution is itself fairly acid, free ammonia is late in appearing in the

culture. As potato extract is practically neutral, free ammonia appears sooner in cultures on potato gelatine.

It now remains to show how far the ammonia given off by a fungus is responsible for slowing down the growth of the fungus itself, and also to demonstrate what part the carbon dioxide of respiration plays in the staling process.

The general method of experiment was to compare the growth of a fungus on a given medium in closed Petri dishes with the simultaneous growth of the same fungus on the same medium in open Petri dishes. The latter were stacked in 5-litre glass containers with an air-space between each. In the bottom of the containers 100 c.c. of a liquid were placed, either water or 1 per cent. sulphuric acid or 1 per cent. caustic soda. The initial atmosphere was either ordinary air or air with its carbon dioxide content made up to 5 per cent. It was not possible to obtain incubator space for all the containers used, so all the experiments were carried out at laboratory temperature. As only the relative degree of staling in the cultures as differently set up was of importance, and as each one had the same temperature conditions, the experiments lost nothing in value on this account.

Fig. 6 illustrates the fact that the growth of *Sphaeropsis* on potato agar is much improved by removing the lids of the Petri dishes and placing the latter in large containers; in addition 100 c.c. water were placed in the bottom of each container. This experiment has been repeated a large number of times and always with a distinct, positive result. The following series of figures is typical of the results obtained, and shows the magnitude of the effect produced. In all the experiments recorded in the table the medium was potato agar, poured deep, with the exception of the ones marked shallow. These experiments were performed at all seasons of the year, and so laboratory temperature varied considerably from one to the other. There is thus no correspondence between the average growth per day in each experiment. Each figure represents the average of four cultures.

TABLE VIII.

Growth of *Sphaeropsis*.

Duration of Experiment.	*Closed Petri Dishes.*	*Open P. d.'s in Container over 100 c.c. Water.*
7 days	7·45	8.62
5 ,,	5·93	6.04
6 ,,	5·56	5·8
10 ,,	8·1	8·7
10 ,, (T. 17° C.)	9·27	10.67
10 ,, (T. 14° C.)	5·48	6·45
13 ,, deep	8·40	10·50
13 ,, shallow	6·05	9·45
6 ,, deep	5·05	5·38
6 ,, shallow	3·81	4·40

All cultures were examined every two or three days, and at each time of measurement each culture was started anew in ordinary air. By following the growth at intervals it was seen that in the early stages all the cultures were growing at the same rate, but by and by staling set in in the closed dishes, whereas it was later in appearing in the open ones. Cultures of *Sphaeropsis* on deep layers of potato agar do not stale appreciably for several days. Thus differences of the type recorded above are not striking in the shorter experiments on deep medium. With shallow layers of medium staling sets in sooner, and thus it is found that more striking differences are obtained with shallow plates, as the above table shows.

In accordance with the results here recorded it is possible to take a culture (especially of *Sphaeropsis*) which has been kept in a closed Petri dish until it shows obvious marginal staling, and then, by removing the lid and placing in a large container in the usual way, to effect a very marked recovery of the rate of growth at the margin. The effect is still more marked if the plates are transferred to an atmosphere containing a moderate concentration (5 or 10 per cent.) of carbon dioxide.

Attempts were made to obtain results similar to those recorded in Table VIII by comparing the growth in closed Petri dishes with that in dishes which simply had the lids raised so that free air exchange could take place, the use of containers being dispensed with. The majority of these experiments failed through contamination at the edges, but even where trustworthy readings could be made the same result was not obtained. The result was unexpected, and an explanation of the apparent discrepancy was sought for in the fact that the cultures thus exposed to laboratory air show a considerable amount of drying up of the medium. Control experiments in which the cultures were inverted over desiccating agents showed that in fact exposure to drying of a plate on which a fungus was growing resulted in slowing down the rate of growth. Plates of various media which had been inoculated with *Sphaeropsis* were inverted over Petri dishes of the same size containing 15 c.c. of various concentrations of sulphuric acid. The following table shows a typical set of results. The figures represent the growth in four days of *Sphaeropsis* on potato agar.

TABLE IX.

Closed Petri Dishes.	*Petri Dishes inverted over*		
	25 % H_2SO_4.	15 % H_2SO_4.	10 % H_2SO_4.
5·54	4·55	5·12	5·29

There was a gradual reduction of growth-rate with increasing desiccation. Exactly similar results were obtained with other media, e.g. plum gelatine. In this connexion a somewhat striking result was met with, in that while desiccation during growth slows down the rate of growth, a

comparable amount of drying of the medium before inoculation has no such effect. Obviously this result can only apply within certain limits. Though this line of experiment was not followed out farther, the suggestion seemed to be either that some transpiration effect was coming into play or desiccation of the growing cultures led to a concentration of the staling substances, especially, perhaps, on the surface-layer of the medium.

It was obvious from these control experiments that it was necessary, in using various solutions as gas absorbents, to have the solutions weak in order to avoid any effects due to drying of the cultures. Hence 1 per cent. sulphuric acid and 1 per cent. caustic soda were used.

The dependence of staling upon carbon dioxide and ammonia was best brought out by experiments such as those recorded in Table X.

Table X.

(*a*) Growth of *Sphaeropsis* on potato agar in open Petri dishes in 5-litre containers:

Control.	*Growth in open P. d.'s in container over*		
(*In closed P. d.'s.*)	100 c.c. H_2O (air).	100 c.c. 1 % NaOH.	100 c.c. H_2O (5 % CO_2).
8·1	8·7	7·7	9·4

(*b*) Growth of *Sphaeropsis* on potato agar in closed Petri dishes with watch-glasses containing a few cubic centimetres of various liquids inside:

P. d.'s with nothing inside.	*P. d.'s with water.*	*P. d.'s with* 1 % NaOH.	*P. d.'s with* 1 % H_2SO_4.
5·69	5·87	5·77	6·52

Summarizing the results of all the experiments made with potato agar as medium on the lines indicated in Table X, one can draw the following general conclusions:

Experiments in containers.—The amounts of growth over water and over 1 per cent. sulphuric acid are not appreciably different, as the staling factor is very much reduced by simple dilution due to the large air-space introduced. Both are distinctly greater than the growth in closed Petri dishes and in the open Petri dishes over soda. Of the last two, one was sometimes the greater, sometimes the other, and no general rule was found. The growth in the containers over water in 5 per cent. carbon dioxide was always the best.

Experiments in closed Petri dishes.—Here the small amount of water introduced is of no value as it rapidly becomes alkaline: the improvement due to the introduction of sulphuric acid is here rendered distinct.

Experiments similar to those described for *Sphaeropsis* were carried out at the same time for *Fusarium*. The results here were of the same type, and all the results established for *Sphaeropsis* found a parallel in the experiments with *Fusarium*. The results were, however, somewhat different in degree. The most striking difference lay in the fact that while

one could always get distinct improvement in *Sphaeropsis* cultures by simply increasing the volume of free atmosphere over the cultures, i. e. by growing the cultures in large containers, the corresponding effect with *Fusarium* was in many cases only slight. This indicates that the ammonia factor is relatively less important in the staling of *Fusarium* than in that of *Sphaeropsis*. On the other hand, the effects produced by a moderate concentration of carbon dioxide are in every respect as marked with *Fusarium* as in the other case.

An experiment with *Colletotrichum Lindemuthianum* indicated that its behaviour was very similar to that of *Fusarium*.

On examining the results given in Table X one sees that the general result can be expressed in the following form :

(*a*) The growth of *Sphaeropsis* (on a medium from which ammonia is evolved) is improved by any treatment which causes dilution of the ammonia or its removal.

(*b*) The growth of the same fungus is improved by allowing the accumulation (within limits) of the carbon dioxide of respiration. Over soda, which keeps the carbon dioxide concentration down to practically zero, growth is least ; over water, where under the experimental conditions a concentration of about 2 per cent. carbon dioxide may be reached, intermediate growth is obtained ; in 5 per cent. carbon dioxide the growth is best of all.

To *Fusarium* statement (*a*) applies to a less degree, statement (*b*) equally well.

The only criterion of a staled culture so far given is based on measurement of the diameter of the mycelium at stated intervals. Staling begins when the daily rate of growth begins to diminish. Staling, however, brings on certain characteristic appearances in a fungal colony, so that a staled culture can be recognized as such at a glance.

If the initial inoculum is very sharply localized, the resulting colonies of *Sphaeropsis* or *Fusarium* on potato agar are in their earlier stages very nearly perfect circles, and as long as there is no staling at the margin—as determined by day-by-day measurements—the colonies remain circular. Also the various controls are extremely uniform in their rate of growth. Once staling at the margin appears, the colonies tend to depart from the circular outline, the margin becoming wavy or indented. Such a sinuous margin is in the writer's experience an invariable indication of staling, at least in the two fungi here dealt with.

The staled cultures further begin to show considerable variation in their rate of spread, so that the variation between the individual cultures becomes much greater. This variability is much greater as a rule in plates where the medium is shallow than where it is deep. Shallowness of medium in many cases accelerates the incidence of staling, and therefore the small differences in depth which occur from plate to plate and from

one part to another of the same plate produce a marked effect on the growth.

Another striking contrast between staled and unstaled cultures of the two fungi in question is illustrated in Fig. 7, which represents diagrammatically a section in elevation of two cultures of either fungus, the first unstaled and the second staled. In the unstaled culture the height of the 'surface' of the aerial mycelium slopes down gradually to the growing margin, which latter may have a more or less broad fringe with practically no aerial mycelium. The margin of a staled culture is as represented in (*b*) of Fig. 7. Here the mycelium drops suddenly at the margin, and this feature gets more pronounced as staling advances; finally one may observe even an overhanging margin.

On plates where the medium is poured deep, a feature of staling is a tendency for the submerged portion of the culture to grow slowly past the aerial portion, and thus the diameter of the submerged growth is greater than that of the aerial growth. Cultures showing this appearance are very stale. This suggests that the deeper layers of the plate are slightly less staled than the surface, though why this should be so is not clear. This peculiar effect is not noticeable with shallow media.

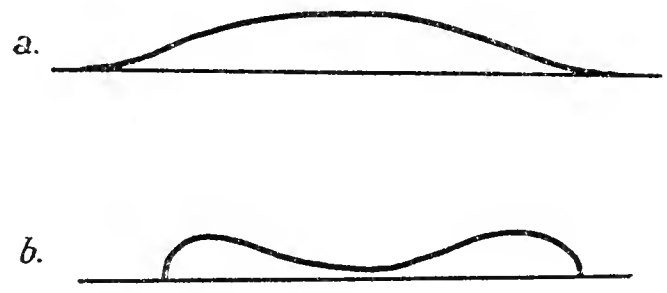


FIG. 7. (Diagrammatic.) Vertical section of unstaled (*a*) and staled (*b*) colony.

A further distinction between a staled and an unstaled colony is to be observed in the nature of the general mycelial growth. In the colonies (*Sphaeropsis*, *Fusarium*) growing in closed Petri dishes the mycelium in the central portion of the culture tends to collapse, giving the appearance as if it had been mown. On this central collapsed region *Fusarium* forms abundant masses of spores, and on the surrounding upstanding aerial mycelium spore masses appear borne aerially, either scattered promiscuously or showing some kind of zonal arrangement. When the culture is grown in 5 per cent. carbon dioxide, where the staling effect is reduced, collapse of the central mycelium is much delayed, and there is everywhere a greatly reduced tendency to sporulation. The exact relationship between sporulation and staling is probably very complex, but there would appear to be every probability that, if the dynamics of the staling process were more completely investigated, considerable light would be thrown on the problem of sporulation.

The same appearances are shown by *Sphaeropsis* cultures, with the exception that no spores appeared in any case, at least during the limited duration of each experiment. Here it is only necessary to compare the type of growth in a closed Petri dish with that in an open one (in a large

container) in order to see in the former all the distinct morphological features associated with staling, while the latter shows none of them.

The question of the intermingling or non-intermingling of two fungal colonies (of the same or of different species) is intimately connected with the question of staling. The only physiological interpretation of the non-intermingling of two colonies is that by the combined action of the two colonies the zone of medium immediately between them becomes so stale to both fungi that neither of them can cross it. The phenomenon is not one of absolute incompatibility, and investigation would probably show that any fungus could be made to intermingle with any other under appropriate conditions. In the case of species which have an intense staling action, it would, of course, be much more difficult to find such conditions than with species which have only a feeble staling activity. The question is obviously one of the chemical nature of the various staling products and of the manner in which they may be rendered ineffective.

That intermingling or the converse is a relative matter can be easily demonstrated. If two colonies fail to intermingle under a given set of circumstances, any alteration of the latter which tends to reduce staling will tend to produce intermingling. Thus, if two colonies planted at a certain distance apart fail to meet, they can be made to intermingle by reducing sufficiently the distance between the two inocula. Also, while two colonies may meet when sown at a certain distance apart on a deep medium, they may fail to do so when grown under like conditions on a shallow layer of the same medium. Again, intermingling or non-intermingling can be determined by simply manipulating some of the staling factors, e.g. the gaseous factors discussed in the present paper. Table XI illustrates an experiment with *Sphaeropsis* along these lines. The top row of figures in the table represents the number of days from the start of the experiment; the figures below represent the shortest distance from the margin of one colony to that of the other; the medium was potato agar in Petri dishes.

TABLE XI.

Closed dishes in air:	0.	5.	7.	8.	9.	12.	15.	17.
Deep medium	7·0	1·7	met	—	—	—	—	—
Shallow medium	7·0	2·3	1·4	—	0·8	0·8	0·8	0·75
Open dishes in container in air:								
Deep medium	7·0	1·25	met	—	—	—	—	—
Shallow medium	7·0	1·85	0·25	met	—	—	—	—
Open dishes in container in 7·5 % CO_2:								
Deep medium	7·0	1·1	met	—	—	—	—	—
Shallow medium	7·0	1·4	met	—	—	—	—	—

General Discussion.

In the earlier portion of this paper the growth-rate curves of a number of fungi were given, from which it appeared that after an initial period of slow growth the rate of growth reached a maximum at which it remained steady, or from which it subsequently declined. This effect of reduced rate of growth is obviously a case of staling of the growing margin of the mycelium by the products formed within its mass, a conclusion with which all the results of the present paper are in agreement. These products either diffuse outwards beyond the limits of the growing margin or, as in the case of ammonia, pass into the atmosphere of the culture and from there are absorbed by the cultural medium. The result of this is that the portion of the medium on which the growing margin of the colony is entering becomes less suitable day by day for the growth of the fungus, and thus staling of the margin begins.

In any case where staling does not occur (e.g. *Botrytis* growing on potato agar), one must assume that the fungus produces no appreciable amount of staling substances or that it is very insensitive to their presence, whereas in the case of a staling culture, e.g. *Fusarium* on potato agar, the assumption must be either that the fungus produces large quantities of staling substances or that it is highly sensitive to their action. Now, though the ultimate proof must be a chemical one, there is little doubt that it is the former alternative that rules. *Botrytis* is a non-staling form simply because it produces relatively little staling substance. In both cases the dominant feature of the staling process from the chemical point of view is the development of an alkaline reaction in the potato medium, and this is very much more pronounced with *Fusarium* than with *Botrytis*. Again, it is easy to show that the sensitiveness of *Botrytis* to alkalinity in the medium is much greater than that of *Fusarium*. (See Table V.)

The views here put forward are strikingly confirmed by a comparison of the behaviour of colonies of the two organisms growing on the same plate, as compared with controls in which each is growing alone. The growth of *Fusarium* in presence or in absence of *Botrytis* is very much the same: that of *Botrytis* is obviously affected by the presence of the *Fusarium*. On such a medium as potato agar where ammonia is evolved by the *Fusarium*, the growth of the *Botrytis* colony is reduced and finally stopped in all directions. On a medium from which ammonia is not evolved, the *Botrytis* colony is strongly staled on the side next the *Fusarium*, so that it appears excentric, while the *Fusarium* colony will preserve its circular outline much longer.

The only view which will thus meet the experimental facts is that *Botrytis* tends to be a fungus of unlimited growth simply because its capacity to form staling products is small, whereas *Fusarium*, though it is compara-

tively tolerant of staling substances, nevertheless produces them in such quantity that its growth tends to be limited.

Though the amount of staling shown by a fungus depends on the experimental conditions, and especially on the nature of the medium on which it is grown, yet it would appear that a fungus which shows staling on one medium is likely to show a tendency in the same direction on any other medium, and thus one could speak in a general way of *Fusarium* as being of the staling, and *Botrytis* as being of the non-staling, type.

In interpreting the growth-curves of the same fungus when grown under different experimental conditions, one has to take account of the amount of mycelium within the body of the colony, and it is in respect of this point that the difficulty of this method of experiment lies. However, certain broad conclusions can be drawn. Take, for instance, the growth of *Sphaeropsis* on potato agar in a closed Petri dish as compared with that in an open one, all other conditions being the same. Here the mycelia under the two conditions are identical in all appearances in the early stages. The differences in amount of staling which appear later cannot be due to different rates of mycelium production, as the amount of mycelium present at the time of incidence of staling is obviously the same in the two cases. In fact, the non-staling culture has probably much more mycelium after a time than the staled one, and thus the unstaled type of growth is maintained in spite of what is probably a greater evolution of the staling substances. The only interpretation which will suit the facts is that the staled growth in the one case results from the accumulation of an active staling substance, an accumulation which is kept in abeyance in the other by the experimental arrangements.

The more staled growth that one finds on certain media when present in a shallow as compared with a deep layer, is to be interpreted somewhat differently. Here it is obvious from inspection that there is less mycelial growth at all stages on the shallow layer of medium. In this case the interpretation must be that though a greater amount of staling substances is formed on the deep layer in accordance with the greater amount of mycelium present, nevertheless it is not so effective in producing staling at first as it is free to diffuse away into a larger volume of medium. The results of growth measurements on deep and shallow layers of medium depend very markedly on the nature, and especially on the concentration, of the nutrient medium, and this will be dealt with more fully in a subsequent paper.

Throughout this paper we have dealt merely with one factor of staling, viz. ammonia. This, however, is not the only factor, or even the only alkaline factor. Thus it is easy to show that a medium on which *Fusarium* or *Sphaeropsis* has been grown with evolution of ammonia is still alkaline, even after all the ammonia has been driven off. There is thus a formation of fixed alkali which is more marked in the case of *Fusarium* than of

Sphaeropsis. In the experiments recorded in this paper it was shown that the improvement of growth resulting from diminution of the ammonia factor was more marked in the case of *Sphaeropsis* than of *Fusarium*. In the latter case mere removal of ammonia did not very greatly improve growth, but raising of the carbon dioxide concentration in the atmosphere of the colony produced a very distinct effect. Thus we may say that in the case of *Sphaeropsis* the volatile alkali is more effective in causing staling than the fixed alkali, whereas with *Fusarium* the converse is true.

It will be observed that the points brought forward in the present paper explain fully the unexpected results mentioned in the introduction. The fact that some fungi (e.g. *Fusarium*, *Sphaeropsis*) show more growth after a time in a moderate concentratian of carbon dioxide than in air is correlated with the production of an alkaline staling reaction in the medium. Thus, though carbon dioxide at the concentrations employed (10 to 20 per cent.) cannot be looked upon in any other sense than as a retarder of growth, it produces the opposite effect in such cases in virtue of its neutralizing action on the staling products. With such fungi as *Botrytis cinerea*, where production of alkali is very slight, the effect described does not appear.

Summary.

Curves of the rate of growth, as measured in terms of the diameter of the colony, are given for a number of fungi. The general feature of these curves is that in the early stages the rate of growth is small, and that it then rises to a maximum which may, or may not, be maintained.

Fungi which keep up this limiting rate of growth are described as being of the non-staling type; those in which the rate of growth falls off from the maximum are described as being of the staling type.

The growth of *Sphaeropsis malorum* and of *Fusarium* sp. on potato agar which is of the staling type is studied in detail, when it is found that the relative amount of staling can be modified by various modifications of the experimental conditions, by varying the depth of the medium, and in particular by the arrangements made for disposing of two volatile products of the metabolism of the fungus, carbon dioxide and ammonia. According as the one or the other is allowed to accumulate, a greater or less degree of staling will result.

The amounts of ammonia formed in *Sphaeropsis* cultures on a number of media are determined.

Correlations between staling and sporulation, and between staling and the phenomena of intermingling, are indicated.

The Attachments of Porphyra umbilicalis, (L.) J. Ag.

BY

VIOLET M. GRUBB, B.Sc.

With Plate I and eight Figures in the Text.

1. Introduction.

BASAL holdfasts are organs which are characteristic of the vast majority of marine algae, and are found exhibiting every degree of complexity from simple rhizoids to massive hapterons.

The attachment in the Rhodophyceae consists generally of a disc or root-like structure composed originally of filaments formed by the outgrowth of the lower cells of the thallus (5). These filaments may lose their individual structure and become welded into a solid parenchymatous mass (5), but whether this takes place or not the adherence of the basal organ to the substratum is surprisingly strong and adequate to withstand the constant strain to which marine algae are subjected, for a longer or shorter period each day, by the movement of the waves. Certain genera are invariably found as epiphytes or parasites, but the details of their attachments have only been worked out in a few cases. In the well-known case of specialized parasitism—that of *Polysiphonia fastigiata* on *Ascophyllum nodosum*—the attaching filaments are recorded as penetrating among the cells of the host but not actually entering them (8).

2. Habit and Growth of the Species of Porphyra.

During certain seasons of the year *Porphyra* is one of the most common of the red algae that inhabit the coasts of the Northern Hemisphere, and considering its frequency it is curious that its interesting method of attachment has never been investigated in detail. The material for this account was obtained mainly from Durlston Bay, near Swanage, where the three varieties of *Porphyra umbilicalis*, (L.) J. Ag., occur—var. *linearis*, Grev., var. *vulgaris*, Ag., and var. *laciniata*, Ag.

During the summer months there is no sign of the purple fronds, but about November the rocks become covered with sheets of the narrow ribbons; this narrow form was originally separated as a different species

under the name of *P. linearis*, Grev., but Harvey (6) proposed that it should be included as a var. *linearis* of *P. vulgaris*, as he considered it to be only a growth-form of that type; this conclusion is fully upheld by the succession of forms under observation at Swanage.

The majority of the very narrow fronds are fertile and remain so throughout the season. On the rocks near high-water level the fronds hardly increase at all in size during the winter and spring, but remain approximately 5 to 15 cm. in length and 0·3 to 1 cm. in breadth. *Porphyra* is by no means confined to the upper rocks, but the purple sheets stretch down over the ridges of the rocks to below mid-tide level, even extending to the exposed *Himanthalia* zone. In the lower reaches a gradual succession from the *linearis* to the typical *vulgaris* state is noted, the latter being fully attained about April, when var. *laciniata* is also found growing on these rocks in small quantities. Both varieties, however, disappear rapidly towards the end of May and the beginning of June, and by the end of June there is hardly a frond to be found. The time at which this disappearance takes place seems to be determined by the weather, for from all accounts *Porphyra* appears to be unable to withstand the heat of the south coast, and in districts where it does exist all the year round, i. e. Clare Island (3) and the Faroes (2), its survival is probably due to the cooler climate and the spray with which it is saturated even at low water.

3. Material and Methods.

The long thin fronds of *Porphyra* hanging down the sides of the rocks are only attached by minute adhesive discs, and these were detached by scraping the rocks with a knife. The fronds and discs were fixed on the spot in weak Flemming's solution in sea-water, allowing the fixative to act for thirty minutes only. In the laboratory the discs were examined and some were embedded in paraffin in the usual way; microtome sections from 3 μ to 5 μ in thickness were cut in a longitudinal direction, and were stained in Heidenhain's iron-alum-haematoxylin (twenty-four hours), counterstaining in safranin in 70 per cent. alcohol (thirty minutes). Hand sections were also examined and preparations made from discs, which in some cases were boiled in caustic potash to separate the tissues. This, however, was not found to be a satisfactory method, as although it fulfilled its object in removing the gelatinous 'cuticle' (1, p. 5) which surrounds the tissues, yet at the same time it causes the hypha-like filament composing the disc to swell up and assume new forms, so that it was impossible to tell whether one were examining an artificial or the natural state of these threads. Instead preparations were made by teasing and pressing the disc structures until the tissues had become separated one from another and then staining in aqueous safranin.

Porphyra is very rarely found epiphytic, but two specimens of *P.* var. *laciniata* growing on *Fucus serratus* were obtained from Shanklin, and these were fixed, embedded, and stained as above. Also some embedded material of var. *laciniata* growing on a post of an old breakwater at Pegwell Bay, Kent, was kindly given me by Dr. Delf, to whom my most grateful thanks are due both for this and some slides which she had previously made from it, and also for her helpful criticism and suggestions throughout. Microtome sections of this material were stained in safranin (thirty minutes) and very dilute methylene blue (three minutes), and it was found that this double stain differentiated clearly the woody tissue and the hypha-like threads of the attaching disc.

4. Description of the Disc.

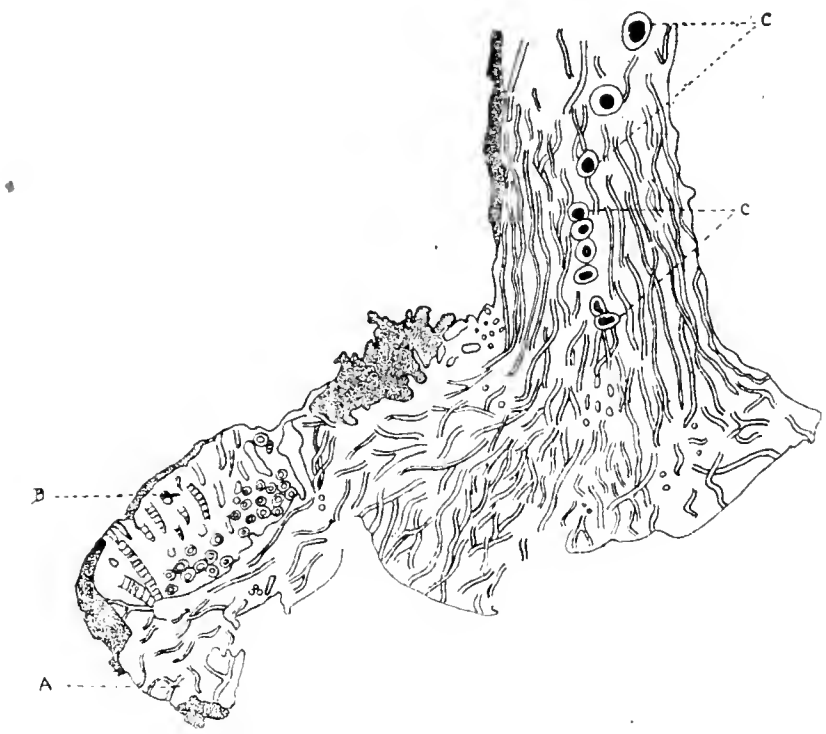
The attaching organs of the British species of *Porphyra* have not previously been described in detail, though they are mentioned by several writers on the Bangiales. Harvey (6) states that the minute disc is always present, and in the species with an upright thallus is accompanied by a short linear stipe which is absent in the horizontal thallus of *P. laciniata*, Ag. The internal structure of this disc has been examined by Thuret (9, p. 59), who notes that in var. *vulgaris* the discs are formed by 'des prolongements radiculaires' of the lower cells of the thallus, and this is corroborated by Berthold (1, p. 3), and Hus (7), who adds that these thread-like projections may swell up in contact with the substratum. The only other fact recorded in regard to these attachments is that Thuret (9) believes that the reproductive cells may be formed from the actual cells taking part in disc formation, but this is denied by later writers.

On examination these minute attaching organs were found to consist typically of discs, circular in outline and from 0·5 to several mm. in diameter, which adhere firmly to the substratum of wood or stone. In longitudinal section it is seen that this tenacious structure is made up of interwoven filaments formed as outgrowths from either surface of the thallus cells. The vegetative thallus is originally one cell thick, and in the upper part of the disc the single layer of cells is continued down the centre of the structure. (Cf. Text-fig. 1, C.)

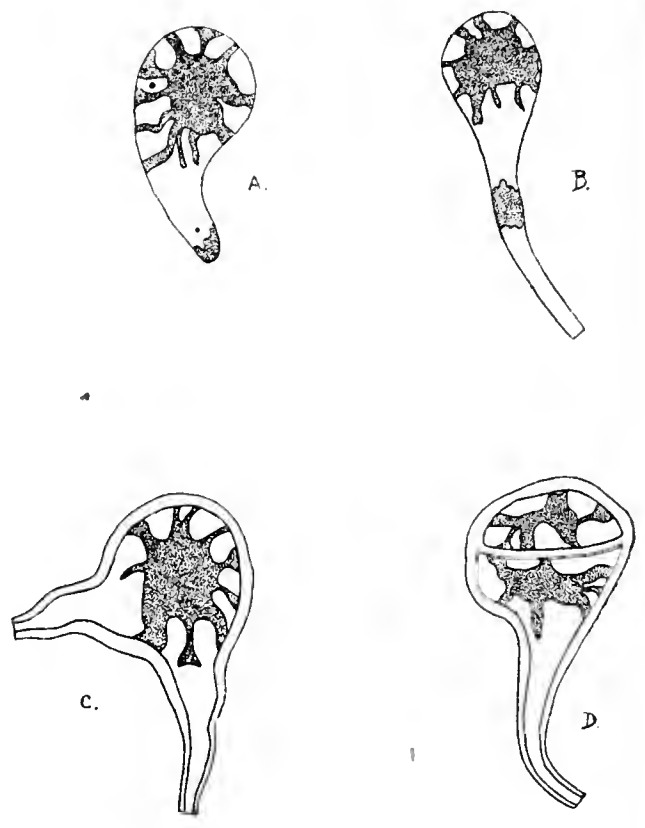
The thallus cells are typically brick-shaped and possess a stellate chromatophore with radiating arms, the ends of which are flattened against the cell-wall, a central pyrenoid and a lateral nucleus lying between the two arms of the chromatophore. On dissection the young filament is seen to arise as a short blunt protuberance (Text-fig. 2, A); when this is about equal in length to the cell itself, a portion of the arm of the chromatophore nearest to the growing tube breaks off and passes down to the tip of the young filament; at the same time the nucleus divides and the daughter nucleus passes into the filament (Text-fig. 2, A); as the latter elongates,

a further portion of the chromatophore passes down, and this is seen causing a bulge in the wall in its journey towards the tip (Text-fig. 2, B).

The full-grown filament, before modifications have taken place, consists of a very long, exceedingly narrow refractive tube, with thick gelatinous walls and no cross-walls. A typical filament with its disc cell attached, which was separated out and measured, was found to be over 11 mm. in length; in discs of *Ulva* with a similar structure Thuret (9) states that the filaments may reach 10 mm. (cp. 4). The staining contents consist of numerous fragments of chromatophore and many highly refractive minute



TEXT-FIG. 1. Longitudinal section through the attaching base of *Porphyra umbilicalis*, showing the central rows of cells (C) and the interwoven branching filaments. At A the proliferating portion is seen, and at B this portion is occupied by a colony of blue-green algae. Drawn with camera lucida. × 150.



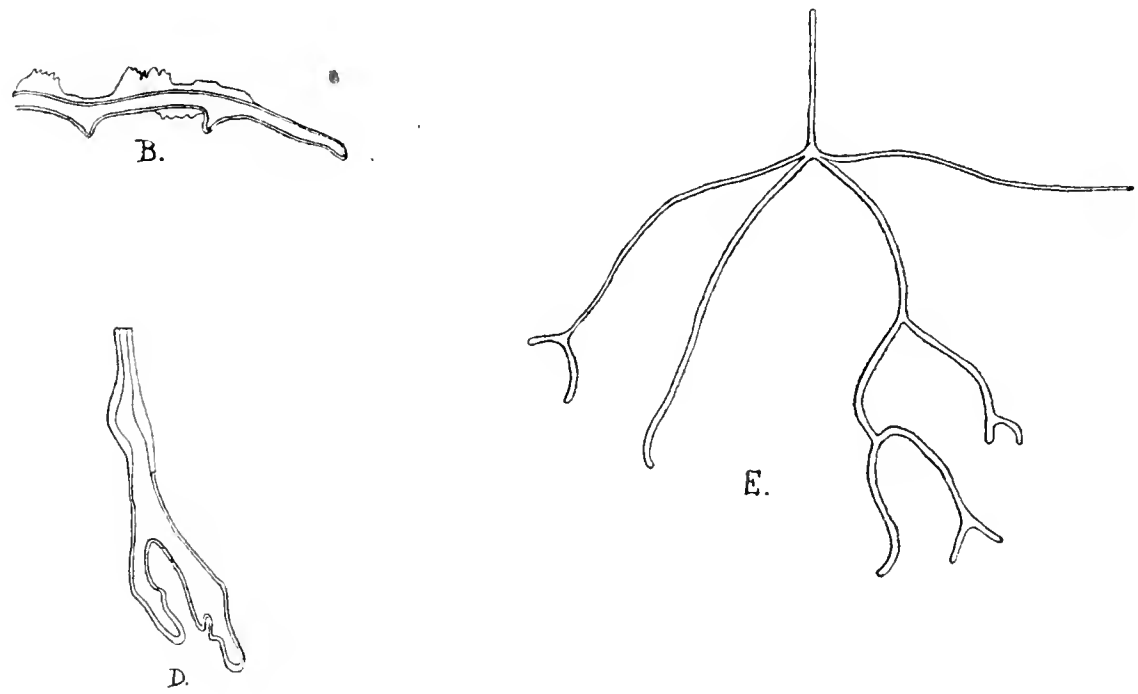
TEXT-FIG. 2. Outline drawings of the cells and filaments composing the disc of *Porphyra*. At A the first stage in the formation of the young filament is seen, showing the chromatophore and nucleus in the tip. B shows a second portion of chromatophore passing down. C, a disc cell producing two filaments. D, a disc cell with a cross-wall. Camera lucida drawings. × 750.

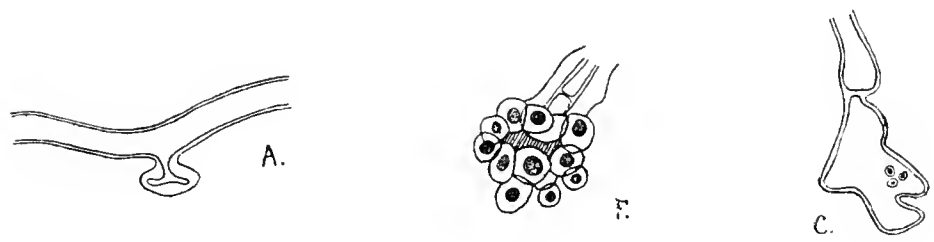
nuclei which are scattered along the length of the tube (Pl. I, Fig. 1). As a rule only one filament is formed from a single cell, though occasionally two may be produced (Text-fig. 2, C); spores, on the other hand, have never been seen forming from a disc cell, and only in one case has the latter been seen with a cross-wall (Text-fig. 2, D).

The disc filaments pursue a winding course towards the base of the attachment, becoming interwoven with one another and forming a resistant tissue several layers in thickness. As they approach the substratum alterations take place in their structure, and the shorter threads, which are formed from cells with thick gelatinous walls in the centre of the tissue, appear to be twice the diameter of the normal filament. The tips of the majority of

the threads now become awl-shaped with the nuclei aggregated at the apex, which is protected by a swollen wall, suggesting that a boring action is necessary if the dense tissue of the disc is to be penetrated.

The disc filaments may be simple or branched, branching as a rule taking place at a swelling and characteristically forming distinct two-armed structures with barbed inner edges having the appearance of clasping organs (Text-fig. 3, D). More rarely a new structure in the shape of a miniature hapteron has been dissected out, and in two cases this consisted of five long arms terminating a filament and themselves branching again at the tips



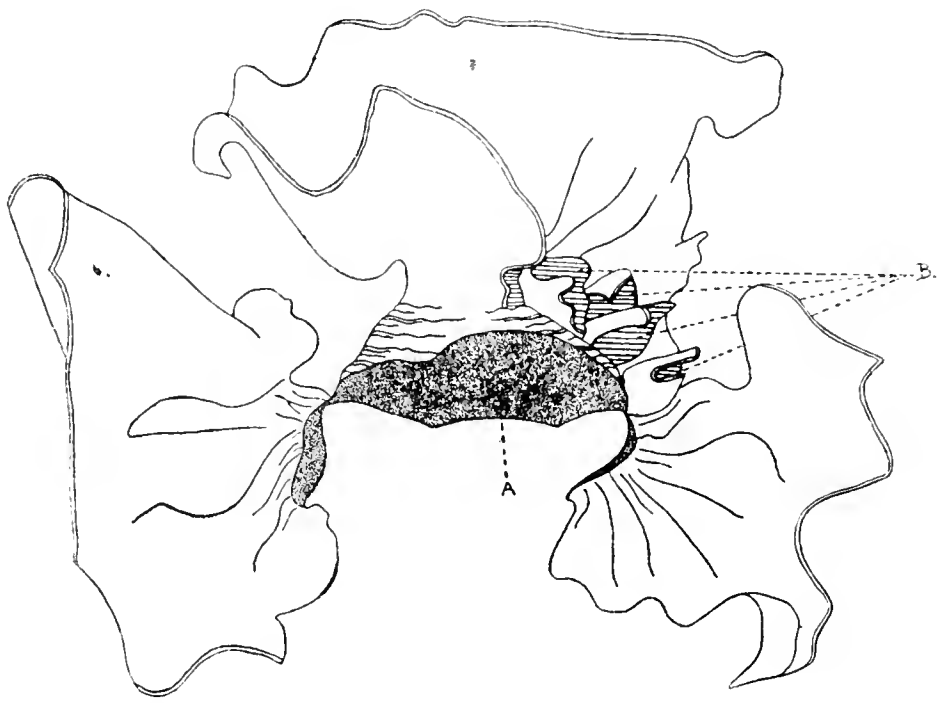


TEXT-FIG. 3. Outline drawings showing the modifications undergone by the filaments of the disc. A and B. Sucker and runner formed as attachment to the rock. C. A swollen multinucleate tip. D. A branching tip. E. Hapteron structure with five long arms. F. A group of cells cut off at the tip of a filament. Camera lucida drawings. × 750.

(Text-fig. 3, E). In other cases the hapteron was in a rudimentary state, a filament ending in short blunt arms which were arranged in more than one plane with regard to the substratum. On finally reaching the substratum other modifications may take place; the filament may creep along at right angles to its former course, here and there putting out suckers which establish a connexion with the rock (Text-fig. 3, A and B). More often the tip or some other portion swells up to give a multinucleate organ (Text-fig. 3, C), which often attains a great size, and may divide up to give cells which are clamped to the ground by their gelatinous walls (Text-fig 3, F). The variety of forms assumed by these modified filaments is endless

and to some extent explains the tenacious adherence to the rocks that is exhibited by the discs of *Porphyra*.

As the filaments forming the disc wind downwards towards the base, many of them turn outwards and form the outer tissue of the structure, the free tips being enclosed in thick gelatinous walls. In older discs cells are sometimes cut off in great numbers from these tips, each with a single nucleus; these cells may be aggregated together and form a compact parenchymatous layer on the outer surface (Text-fig. 7, B). The interest of this differentiation of tissue lies in the fact that this parenchymatous tissue is capable, by cell division, of creeping along the surface of the ground and of putting up new fronds. For example, in Text-fig. 4 a large central disc has given rise laterally to two creeping discs, while other and younger fronds are growing out from the main disc.



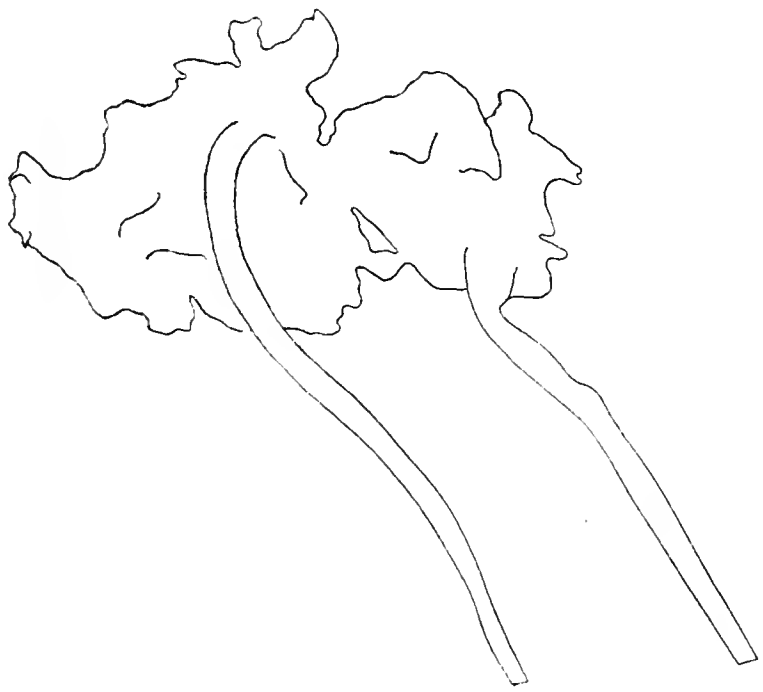
TEXT-FIG. 4. Outline drawing of a proliferating disc of *Porphyra umbilicalis*. The actual attaching surface (A) of the central disc is shown giving rise on either side to lateral attaching surfaces. From the upper surface of the main disc four young fronds have arisen (B) as well as the main frond. Total length of the attaching surface, 5·5 mm.

That vegetative reproduction takes place in this way by a creeping and proliferating disc has been shown by Hus (7) for an epiphytic species of *Porphyra—P. naidum*, And.—from the Pacific coast of North America. This species has a parenchymatous or 'prothallial' base from which new fronds are developed; the base consists of small disc-shaped outgrowths which are one cell in thickness and gradually creep over the blades of *Zostera*; here and there the tissue becomes several layers thick, and from the external layer short blunt protuberances are put out which develop into fronds. I have also noted something similar in *Enteromorpha compressa*, for in a slide lent me by Dr. Delf two young narrow vertical fronds of *Enteromorpha* are clearly connected by a horizontal creeping parenchymatous base, one layer of cells in thickness (Text-fig. 5). This type of vegetative reproduction by a proliferating base seems to be present in

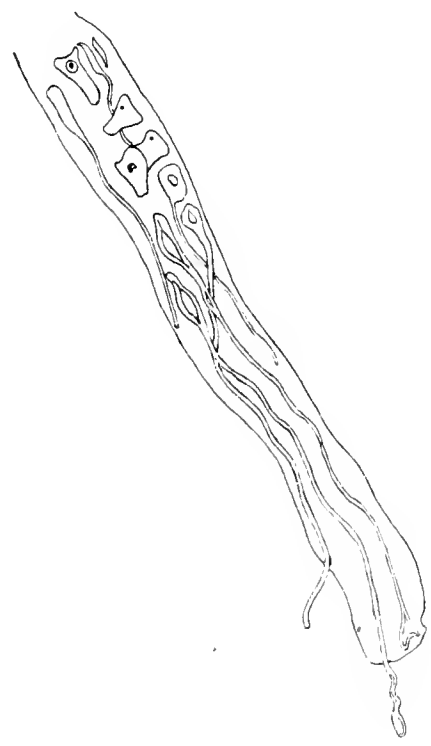
the British species of *Porphyra*, but whether the discs perennate during the summer season when the fronds are absent, and put up new fronds in the autumn, has not yet been ascertained.

5. Sporelings.

It was noted at Swanage that the young fronds of *Porphyra* were usually found growing either in connexion with circular dark patches of the blue-green alga, *Rivularia atra*, or else among a layer of débris on the rocks, composed of mud and filaments of microscopic algae. This is probably not a case of symbiosis, as it does not always occur, but it is



Text-fig. 5. Outline drawing of a young sporeling of *Enteromorpha compressa*, showing the creeping prothallial base (one layer of cells thick) and two linear fronds arising from it. Drawn with camera lucida. × 60.

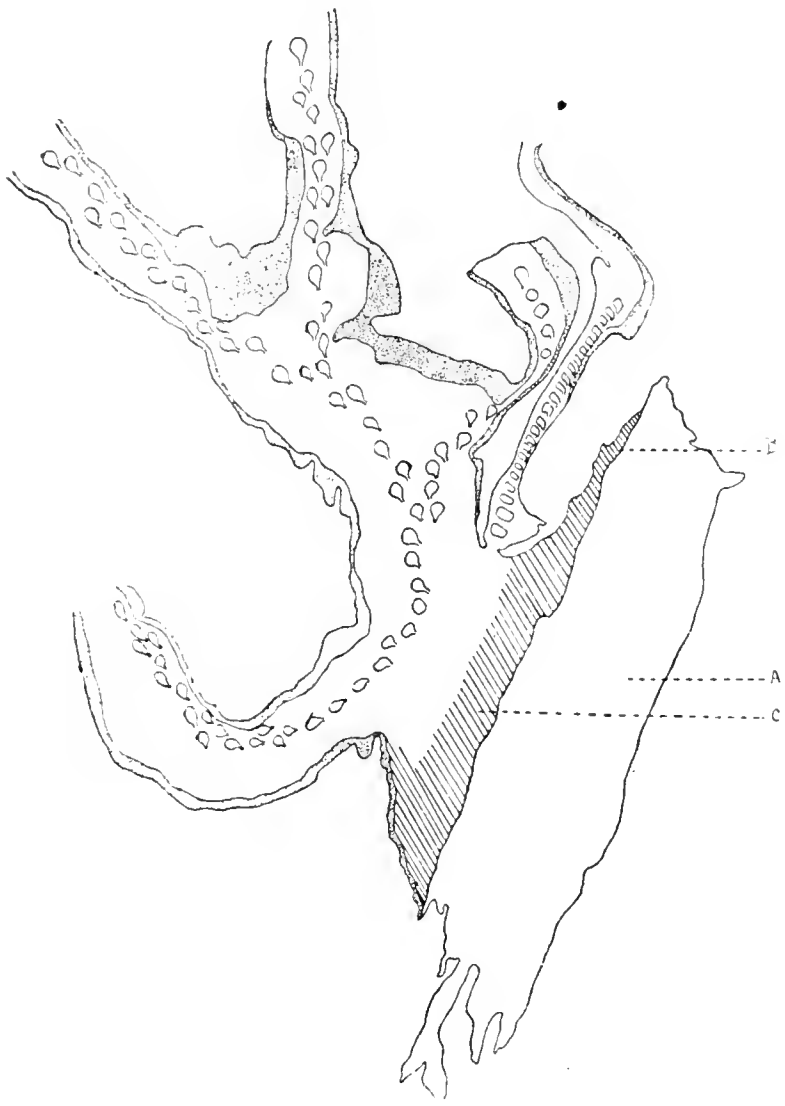


Text-fig. 6. Outline drawing of the base of the sporeling seen in Plate I, Fig. 2, showing the first six rhizoids enclosed partially in a gelatinous sheath. Camera lucida drawing. × 650.

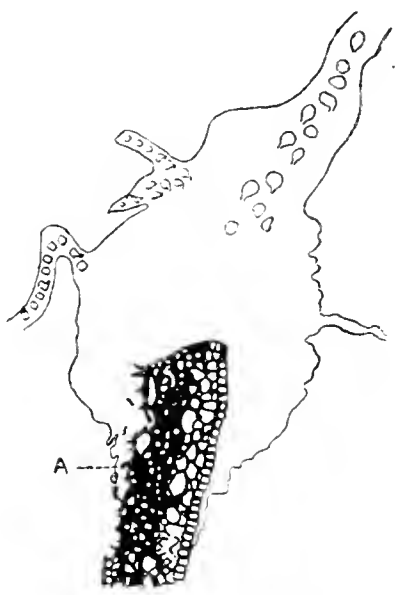
likely that germination takes place more readily in this substratum as the débris serves to protect the sporlings in rough weather. Even when no fronds were visible to the naked eye, dissection and examination of the tangled material would occasionally reveal a sporeling. The youngest plant thus dissected out was only 0·34 mm. in length and 0·03 mm. at its widest point; it consisted of about seventy to eighty cells, a large proportion of which were modified to form rhizoids (Pl. I, Fig. 2). Four of these had elongated greatly, and of these one had a swollen tip and another was branched to form a clasping organ at the base (Text-fig. 6). The formation of filaments which will later combine to build up a disc takes place therefore at a very early stage.

6. Discussion of Cases of Epiphytism.

Although *Porphyra* is typically a rock-dweller, yet many breakwaters stretching out to sea have their vertical sides clothed with the purple fronds. Material taken from such a source showed, in longitudinal sections through the attachment and substratum, the characteristic structure of a normal disc on the exterior of the wood (Text-fig. 7), several fronds with gelatinous walls arising from the upper part of the disc, while the external filaments of



Text-fig. 7. Outline drawing of a longitudinal section of *Porphyra umbilicalis* growing on wood (A), showing the disc attachment with creeping base (B) and thickened tissue formed by branching and division of the filaments (C). Five fronds with thick gelatinous walls (stippled) are being given off from the disc. Cells giving rise to the filaments somewhat diagrammatic. Camera lucida drawing. × 50.



Text-fig. 8. Outline drawing of a longitudinal section of *Porphyra umbilicalis*, var. *laciniata*, on *Fucus serratus*, showing irregular attaching base of *Porphyra* creeping along the host. Fig. 5 of Plate I is taken from the region A, where the cells of the host are disorganized. Cells of host and epiphyte somewhat diagrammatic. Camera lucida outline. × 50.

the latter had undergone modification and were creeping along the surface of the post. The awl-shaped filaments in the interior of the disc had penetrated the xylem to a considerable distance, and the cells of the latter were very disorganized. Where the threads had met with any considerable resistance, they had swollen to an enormous size and formed large circular or irregular multinucleate parenchymatous swellings within the tissues of the xylem (Pl. I, Fig. 3). Where space permitted, the tips of the threads, instead of swelling, had cut off cells, each with a single nucleus and

vacuolated protoplasm, and these cells are seen as circular patches with gelatinous walls within the woody tissues (Pl. I, Fig. 4). The penetration of the host appears to be very complete, for the wood of the post is disorganized and riddled with these parenchymatous masses and tissues of cells.

In the case of the material of *Porphyra umbilicalis* on *Fucus serratus*, the thallus of *Fucus* was attached when found near low-water mark, but the serrated lamina had completely disappeared and only the midrib of the frond was left ; at the top of this a plant of *P. laciniata* was found growing.

Sections showed that it was attached by a large spreading base formed in the usual way from interwoven filaments, and giving rise at the edges to new fronds (Text-fig. 8). As the winding filaments approached the host, they branched frequently and the ends became swollen and gelatinous, but cross-walls were not found so commonly as in the material on the post. The threads had a forcible boring action and penetrated both between and into the cells of the thallus, and could be seen to a great depth traversing the cells of the cortex and branching within the cell cavities (Pl. I, Fig. 5). The cells of the host were full of contents and appear to have been living, but penetration of the filaments of the disc rapidly induced disorganization.

The filaments of the attaching disc of *Porphyra*, therefore, show a capacity for penetrating into as well as between cells of a cellular host, though the alga is typically a rock-dweller. From the fact that the filaments bring about death and disorganization of the cells of the host, it must be inferred that there is no symbiotic relationship present in this case ; on the contrary, in this, the first recorded examination of a British species of *Porphyra* growing on a living host, practically all conditions of true parasitism are fulfilled.

7. Summary.

1. The three varieties of *Porphyra umbilicalis*, var. *linearis*, var. *vulgaris*, and var. *laciniata*, grow commonly in exposed positions and are attached to the rocks, &c., by minute adhesive discs.

2. Observations corroborate the view put forward by Harvey that the narrow form of frond known as var. *linearis* is only a growth-form of var. *vulgaris*.

3. The attaching discs of *Porphyra* are capable of lateral extension and may proliferate into new branches.

4. The disc is made up of numerous interwoven threads of two kinds which are formed as outgrowths from the thallus cells :

(1) long slender filaments ; (2) short stout ones from the lowest cells.

5. Filaments near to, or in contact with, the substratum swell up and branch or produce suckers or hapterons. Filaments on the exterior of the disk may produce a parenchymatous tissue by branching or division.

6. Modifications in the disc filaments are shown to take place very early in the life of the sporeling.

7. *Porphyra* grows usually on a substratum of rock, but in plants occurring on breakwaters the filaments are shown to have the power of penetrating the dead woody tissue. *Porphyra* has hitherto very rarely been found epiphytic, and never parasitic, but in two plants found on *Fucus serratus* the disc filaments had penetrated the living cells of the host. This seems to indicate the capacity for either epiphytism or parasitism if once secure contact is obtained.

WESTFIELD COLLEGE,
LONDON,
July, 1922.

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EXPLANATION OF PLATE I.

Illustrating Miss Grubb's paper on the Attachments of the British Species of *Porphyra*.

All figures drawn with a No. 3 eyepiece (Swift) and D.D. objective (Zeiss), except Fig. 2, which was drawn with a No. 3 eyepiece and A.A. objective (Zeiss), and Fig. 1, which was drawn with a No. 4 compens. ocular (Zeiss) and a $\frac{1}{12}$ apochromatic objective (Swift).

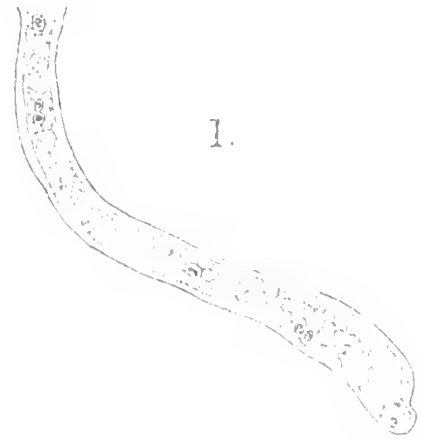
Fig. 1. Apex of a typical disc filament with nuclei ($\frac{1}{12}$ apochromatic).

Fig. 2. Young sporeling. × 250.

Fig. 3. Transverse section of swollen tips of disk filaments (stippled) in tissues of the wood. × 750.

Fig. 4. Transverse section of a group of cells formed from the apex of a disc filament in the wood of the post. × 750.

Fig. 5. Filaments from the base of the disc of *Porphyra* boring into the tissues of *Fucus serratus*. × 750.



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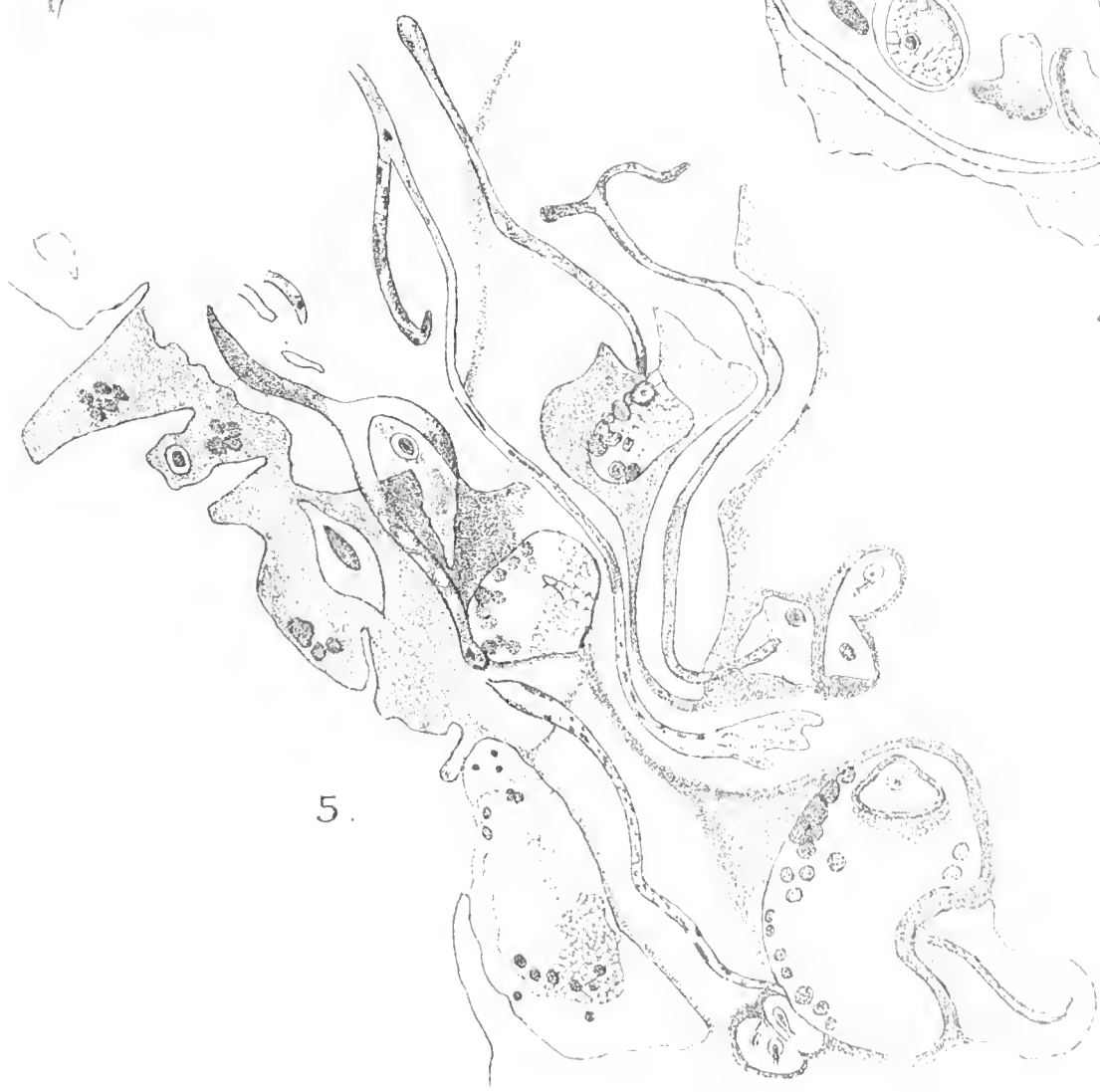

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GRUBB—PORPHYRA.

Dimorphococcus Fritschii, a New Colonial Protophyte from Ceylon.

BY

W. B. CROW.

With one Figure in the Text.

THE genus *Dimorphococcus*, A. Br., has hitherto been known to be represented by two species of colonial Isokontae: *D. lunatus*, A. Br., and *D. cordatus*, Wolle. These organisms are remarkable in their method of colony formation, the four cells that are produced by division in each parent cell remaining attached for some time in groups by the agency of the parent cell membrane, which, at least in the older state, takes the form of connecting threads of mucilage. *Dimorphococcus* shares this type of colony formation with the genera *Westella*, De Wildemann, *Dictyosphaerium*, Ehrenb., and *Radiococcus*, Schmidle. Hence some authorities consider these to form a special family among the unicellular Isokontae: the Dictyosphaericeae (5). But *Dimorphococcus* is distinguished from these related types in a character which gives the genus its name, viz. the dimorphism of its cells.

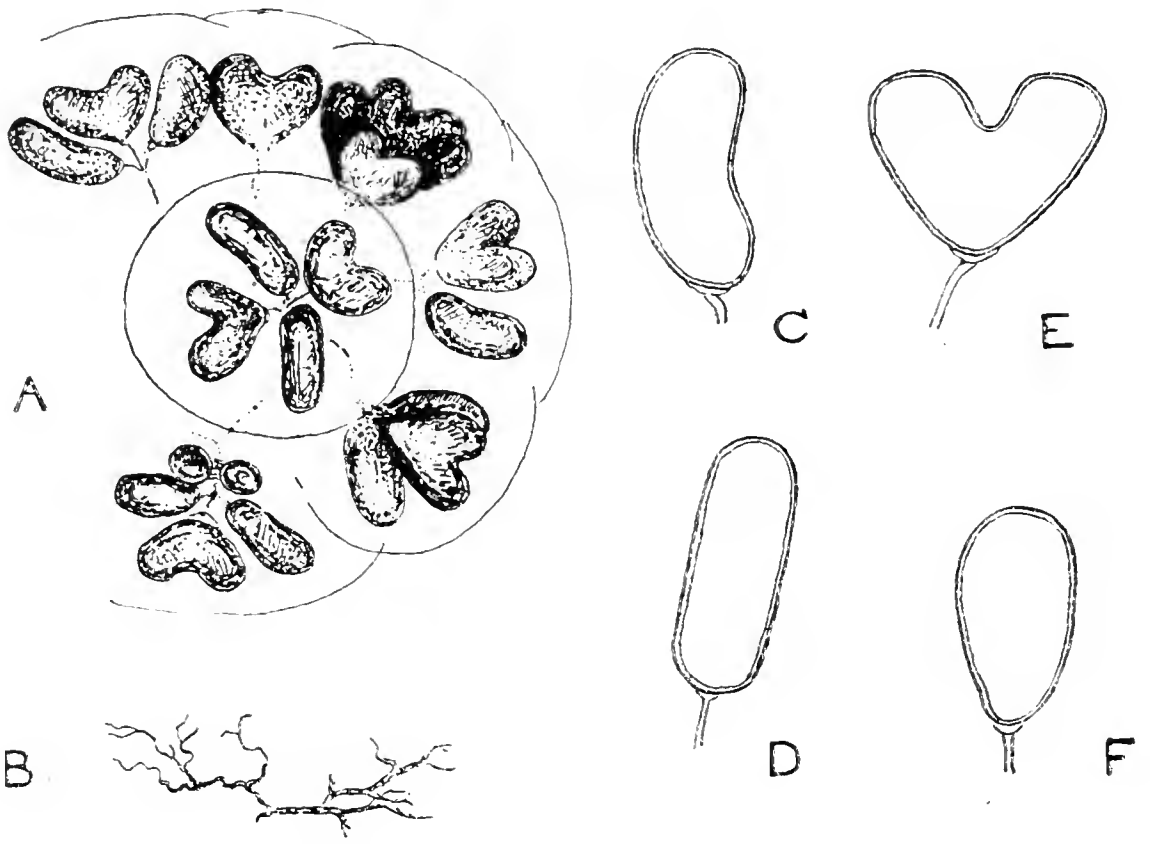
Whilst examining specimens of plankton organisms in a collection from the inland fresh waters of Ceylon, made by Prof. F. E. Fritsch in 1903 and preserved in dilute formalin, I met with some colonies of a colonial protophyte which showed cells with a rich starch content and massive parietal chloroplast. The colony formation, moreover, was of the type mentioned above, and the dimorphism of the cells was very obvious. The organism thus had the essential characters of the genus *Dimorphococcus*, but further investigations showed that it differed from the two species mentioned above.

The colonies occurred in considerable quantities in the following localities: Tank[1] Borlasgama, near Colombo, 8th November; tank at Kekunadure, about five miles from Matara, 8th September; canal leading from river to lake, near Bentottle, 6th September; Lake Madampe at Ambalangodda, near the sea, 13th September; lake at Panadure, 16th September. The organism was not found in collections from several smaller

[1] The 'tanks' in Ceylon are reservoirs of partially artificial origin. In them, however, the micro-organisms live under normal conditions.

ponds that were examined, and thus appears to be chiefly a member of the limnoplankton; in this respect contrasting with *D. lunatus* and *D. cordatus*, which are members of the helioplankton. These two latter species have not yet been recorded from Ceylon.

The colonies of the new species, which may appropriately be named *Dimorphococcus Fritschii*, n. sp., were comparatively large, compact, compound groups. These were ellipsoidal in form, 85–100 μ in length, 70–90 μ in width. Each group was composed of partial colonies of four cells each, embedded in definite colonial mucilage (see A in figure). The cells of each partial colony were clearly dimorphous, the members of one pair being heart-shaped, the members of the alternating pair cylindric and slightly



A. Portion of total colony. B. Portion of skeletal systems of colony dissected out. C. and D. Side and front view of cylindric cell. E. and F. Side and front view of cordate cell. (Specimen from Tank Borlasgama, near Colombo.)

bent inwards. The cells were 12–20 μ in height, the width of the cylindric ones being 5–6 μ. Each cell was borne on a short mucilage stalk and had a small lappet at its point of attachment (see C–F in figure).

All the colonies observed were of regular ellipsoidal form; the dimensions of the colony given above are for well-grown specimens of sixty-four to 128 cells. From actual countings it would appear that the numbers mentioned often actually occur, i. e. the cells of a colony all belong to the same generation. This results from the fact that the cell-divisions are approximately simultaneous in all the cells of the colony, and here *D. Fritschii* may be contrasted with *D. lunatus*, A. Br. The regular form of the colonies in *D. Fritschii* is correlated with this fact.

There was a definite enveloping mucilage as in *Dictyosphaerium*. This can be seen well in glycerine jelly preparations. The presence of enveloping

mucilage has been used by some authors to distinguish between *Dictyosphaerium* on the one hand and *Westella* and *Dimorphococcus* on the other (4). West (5) even divides the Dictyosphaeriaceae into two tribes on the basis of this character. Observations on the mucilage-sheath of *D. Fritschii* show that it has the same nature as that described by Senn for *Dictyosphaerium pulchellum*, Wood, each partial colony having its own mass of mucilage, as will be seen in our figure. The latter should be compared with that given by Senn (3). Incidentally it may be noted that the radial striations shown so prominently in some copies of Senn's figure are delicate structures which can only be seen on staining with Bismarck brown, and are invisible in the unstained condition. It is clear that the absence of a gelatinous sheath cannot be used as a generic distinction for *Dimorphococcus*, the essential feature of the latter genus being, of course, the dimorphism of the cells.

The partial colonies always consist of four cells each. As in the other species of *Dimorphococcus*, the members of each group were arranged in a manner comparable with the arrangement of the petals in a tetramerous corolla. *D. Fritschii* differs from *D. lunatus*, A. Br., in the fact that each cell has its own distinct stalk. In this respect it agrees with *D. cordatus*, Wolle, and to some extent with the species of *Dictyosphaerium*. The mucilage stalks of *D. Fritschii* are much thinner than those of *D. cordatus*, as figured by Chodat (2), but agree with the original of Wolle (6). They are shorter than in any *Dictyosphaerium*. The very peculiar thickening or lappet usually to be observed at the base of each cell, i. e. at the point where the stalk is attached, is a remarkable distinguishing feature of our species. It does not appear to be a mere thickening of the cell membrane, but rather a small segment of the mother-cell wall which has not been used up in the formation of the stalk.

In 1897 Bohlin (1) described the development of the colonies of *Dimorphococcus lunatus*, A. Br. He discovered that the gelatinous threads which hold the cells together are only the remains of the older membranes. Thus the colonies show a certain morphological resemblance to those of *Dictyosphaerium*. In the latter genus, however, the wall of the mother-cell splits into four pieces when the cell divides into four, each piece remaining attached to one of the daughter cells. In *Dimorphococcus lunatus*, A. Br., the daughter cells are adpressed together in tetrads; it is therefore not necessary here for each cell to have its special piece of the parent membrane, and the splitting is suppressed. Further, the walls of the mother and daughter cells do not fuse with one another, and the daughter tetrads are only fixed to the remains of the mother-cell membrane by partial gelatinization.

Bohlin does not believe that the similarity between *Dimorphococcus* and *Dictyosphaerium* indicates a near relationship between them. He points out that Zopf has shown certain morphological similarities to exist between

Dictyosphaerium and *Sciadium*, yet the details of structure of the latter show it to be a genus of the Heterokontae, and the resemblance to *Dictyosphaerium* to rest on analogy only. In view of the differences in details of structure mentioned above, Bohlin thinks that the same applies to the supposed close relation of *Dimorphococcus* and *Dictyosphaerium*, although both, of course, must still be classed in the Isokontae.

The structure of the partial colony *Dimorphococcus Fritschii*, n. sp., and probably also, to a lesser extent, that of *D. cordatus*, Wolle, helps to bridge the gap between the genera *Dimorphococcus* and *Dictyosphaerium*. The fact that each cell possesses a distinct stalk, together with the character of the stalks themselves, as well as the presence of a colonial mucilage envelope in the new species, all recall the morphological features of *Dictyosphaerium*. Finally the presence of the small lappet at the base of each cell, if our interpretation of it is correct, shows that partitioning of the mother-cell wall into four parts is not unrepresented in *Dimorphococcus*, although the greater part of each segment may appear as a mucilage thong at a very early stage.

Bohlin thinks that the partial colonies of *Dimorphococcus* are homologous with the colonies of *Scenedesmus*. This is strongly supported by his description of *D. lunatus*, A. Br. In the latter the cells do not possess individual stalks, the very short strands sometimes connecting adjacent cells being comparable with the intercellular pads of some species of *Scenedesmus*. The four cells of the partial colony, generally closely adpressed (see e. g. 4, Pl. CXXI, Fig. 5), do not develop their corresponding mucilage thongs until they divide. For until the cells of the partial colony divide, they are enclosed in a gelatinous mass formed from the parent wall but not yet organized into thongs. Thus in comparison with *Dimorphococcus Fritschii*, n. sp., and the species of *Dictyosphaerium*, the mucilage threads of *Dimorphococcus lunatus*, A. Br., are late in development to the extent of one cell-generation.

Dimorphococcus Fritschii, n. sp., appears to be closely allied to *D. cordatus*, Wolle.

The latter is not well known, and the figures of Chodat (2) differ remarkably from those of Wolle (6, Pl. CLX, Figs. 30–38). It would appear that *D. cordatus*, Wolle, generally has smaller colonies than *D. Fritschii*, n. sp., is somewhat less regular in colony form, shows much less marked dimorphism in its cells, and above all lacks the colonial mucilage of our species.

In conclusion we give a diagnosis of the new organism and a summary:

Dimorphococcus Fritschii, n. sp.

Coenobio magno, ellipsoido, regulario, denso, coenobiis secondariis quaternarum cellularum compositis uniquoque certa vagina gelatinosa cir-

cumcluso; cellulis cuiusque coenobii secundarii bini et valde dimorphosis, his duobus cordatis, illis duobus inter haec cylindratis et paulo intus curvatis. Cellulis positis in pedunculis brevibus atque tenuibus, lasciniis parvis ad punctum ubi fixatae sunt provectis. Coenobio 85–100 μ longo, 70–90 μ lato. Cellulis 12–20 μ altis, cellulis cylindratis 5–6 μ latis.

Loc. In aquis dulcibus magnis. Insula Ceylonica.

Summary.

1. A new colonial member of the Isokontae, collected in the inland fresh waters of Ceylon by Prof. F. E. Fritsch, is described under the name of *Dimorphococcus Fritschii*, n. sp.

2. The comparative morphology and systematic relationships of the new species are discussed.

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5. West, G. S.: Algae, I. Camb. Bot. Handbooks, Cambridge, 1916.
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NOTES.

TERATOLOGICAL PHENOMENA IN THE INFLORESCENCES OF FAGUS SYLVATICA.—While examining some specimens collected for class purposes, Mr. B. H. Bentley observed on the cupules of several of the female inflorescences of the Beech some small flowers approximating both in size and structure to the male flowers of the same tree. He pointed out the occurrence to the writer and at the same time very kindly suggested that a further examination might reveal some details of interest. Material was therefore collected from various parts of the Sheffield district, and the following observations were made in the course of the examination. It is possible that the phenomena here described have been noted before, but in the limited literature available only one relevant reference was found besides the description in Eichler's ' Blüthendiagramme ', viz. a note describing a hermaphrodite flower of the Beech, by Schnizlein.[1]

The whole of the material was gathered at the end of May and the commencement of June, from trees growing in soil overlying the Coal Measures and Millstone Grit.

The abnormalities observed may be grouped as follows:

1. Inflorescences wholly female, but bearing either a greater or lesser number of flowers than the normal.

2. Inflorescences (with either the normal or abnormal number of female flowers) bearing hermaphrodite and male flowers. (Androgynous.)

3. Inflorescences with no normal female flowers, and showing a marked tendency towards transition to male inflorescence structure. (Transitional.)

From four to six complete foliage leaves are found on the young shoots, whether these be laterally or terminally situated on the branch. The lowest two or three leaves of the shoot usually bear male, the next one or two leaves female, inflorescences in their axils. Higher leaves subtend foliage buds.

The following data may serve to indicate the frequency of the various abnormalities. The last (distal) inflorescence on the shoot is called terminal; the next lower down, lateral.

Of 100 terminal inflorescences gathered indiscriminately from 10 trees at Grindleford, 91 had normal structure, 1 bore only one female flower, 8 were androgynous.

Of 100 lateral inflorescences from the same trees, 75 were normal, 16 androgynous, 9 transitional.

Material from Ecclesall (terminals and laterals not kept separate).

[1] A. Schnizlein: Zwitterblüthen von *Fagus sylvatica*. Bot. Zeit., 1850.

Of 49 inflorescences from one tree, there were 30 normal, 15 with three female flowers per cupule, 2 with four female flowers per cupule, 4 androgynous.

Of 106 inflorescences from another tree, 99 were normal, 1 with only one female flower, 5 androgynous, 1 transitional. So that of 355 inflorescences examined, 17 per cent. were abnormal in some way. It will be noted that in the case where the laterals were kept separate from the terminals, 25 per cent. of the former bore male flowers as compared with 8 per cent. in the latter.

The extra female flowers of the first group are most commonly situated at, or slightly above, the level of the normal pair. They are of exactly the same structure

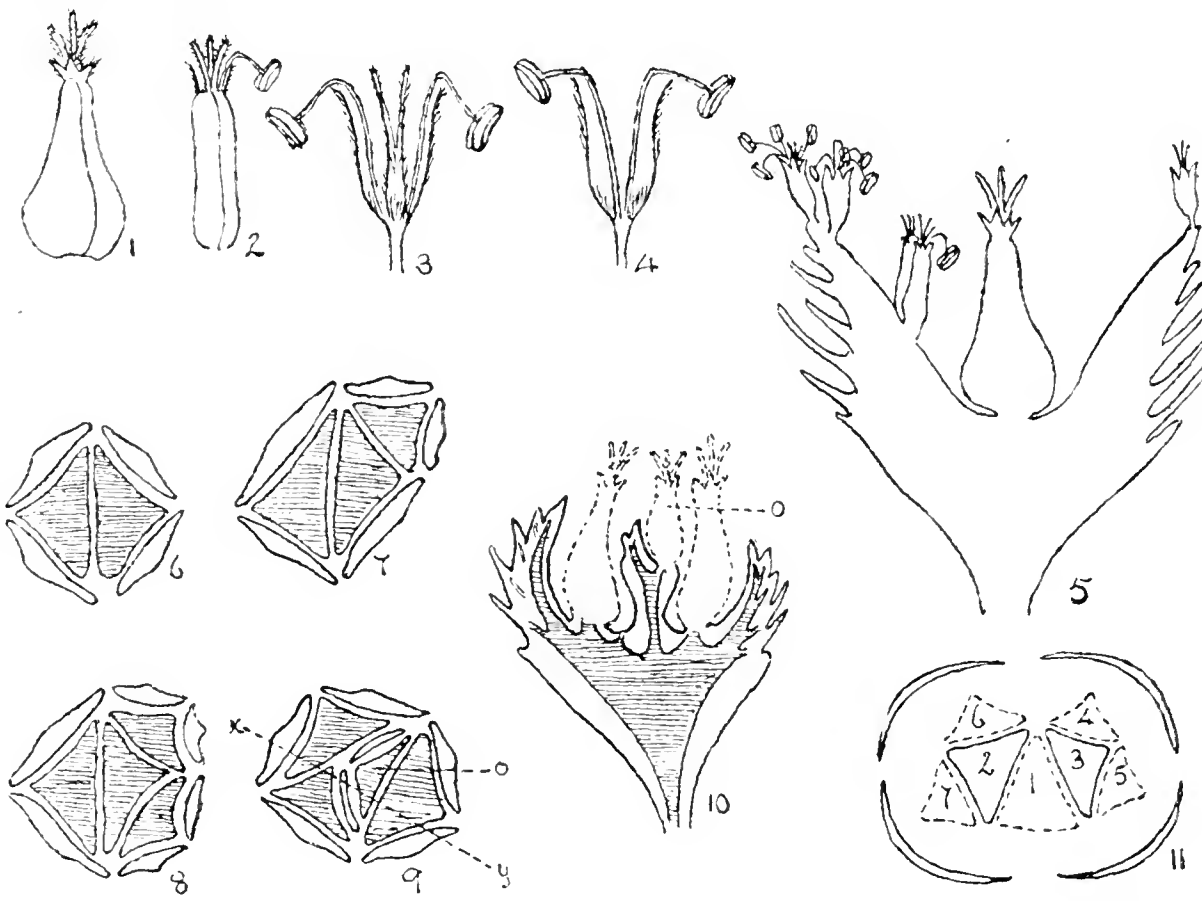

FIG. 1. 1, normal female flower; 2, rudimentary hermaphrodite flower with inferior ovary; 3, male flower with vestigial pistil, the latter having a superior ovary and two stylar arms; 4, male flower with androecium only; 5, vertical section of androgynous inflorescence with rudimentary hermaphrodite flowers on inside lateral walls of cupule and male flowers terminally on same; 6, 7, 8, 9, diagrams of normal and abnormal female inflorescences showing relation of cupule segments to position and number of flowers (shaded); 10, vertical section of inflorescence whose diagram is represented in 9, taken through plane *xy*—vascular system as seen macroscopically, shaded; 11, diagram of theoretical inflorescence with seven possible flowers of dichasium present, see text. *o*. flower on upgrowth from centre of receptacle.

as these, although usually rather smaller in size. Regularly associated with the presence of one or two of such extra flowers is the further segmentation of the cupule. If three flowers are present the cupule is always five-partite, or with four flowers six-partite, as compared with the normal four segments.

From the diagrams 6, 7, 8, 9, Fig. 1, it will be seen that the place of fission of the cupule is to be correlated with the orientation of the flowers. It is usually the case that fission caused by the presence of an extra flower does not proceed so far towards the base of the cupule as do the normal four slits. In the normal inflorescence, however, the four fissures of the cupule do not extend to the same depth; the two situated in the plane of the axis seem invariably to extend the deeper. Whether this extra splitting of the cupule is a result of pressure on the part of the developing flowers, or whether it is due to some process of dialysis, remains obscure.

One isolated case was found, in a cupule with four flowers, in which the most centrally placed of these was situated on an upgrowth from the base of the cupule (see 9 and 10, Fig. 1). Here the distal portion of the upgrowth was continued laterally past the flower, giving rise to two structures of exactly the same nature as the exterior cupule segments. It may be stated here that all the flowers examined in the later stages proved to be abortive, although ovules were present, even in the rudimentary ones.

Androgynous Inflorescences.

In these a floral series may be present leading from normal female flowers on the one hand, through hermaphrodite forms with reduced androecium and a more or less rudimentary inferior ovary, to typical male flowers with or without a vestigial superior ovary and the full complement of stamens on the other (Fig. 1, 1, 2, 3 and 4). As described by Eichler,[1] the male flowers may or may not have such a vestigial pistil present within the perigone, and when it is present may vary in structure from the less reduced type, with three stylar arms and a slightly enlarged base representing the ovary, to the extremely reduced condition with two stylar arms and scarcely any enlargement of the basal ovarian portion. The degree of approximation of the extra flowers to the normal male or female type can be correlated with their position on the cupule. Thus one may say broadly that the higher the insertion of the flower on the cupule segment, the closer does it approach normal male structure. Fig. 1, 5, is a somewhat diagrammatic representation of an androgynous inflorescence with one female flower in the normal position: of the two rudimentary flowers on the lateral walls of the cupule, one is hermaphrodite, while terminally on the segments there are present several male flowers. The one on the right hand, although having a perigone and vestigial pistil, is destitute of stamens. No extra flowers were found on the outside lateral walls of the cupule in any of the material examined. The anthers of these flowers were all in the post-dehiscence stage at the time of examination, but they appeared to be of the normal build.

Transitional Inflorescences.

Fig. 2, 1, is a vertical section of a specimen with eighteen male flowers situated peripherally on the much-reduced capule. The latter was unsegmented and the slight peripheral upgrowth surrounded a shallow depression in which were situated one hermaphrodite and one female flower, both being poorly developed. A curious stage is shown in Fig. 2, 2. The much-attenuated stalk, scarcely thicker than that of a normal male inflorescence, divided distally into two short arms, each of which terminated in a flower with a rather large perigone (*sic*) but entirely destitute of androecium and pistil. Numerous male flowers were clustered on each of the two arms (only a few of these are shown in the drawing).

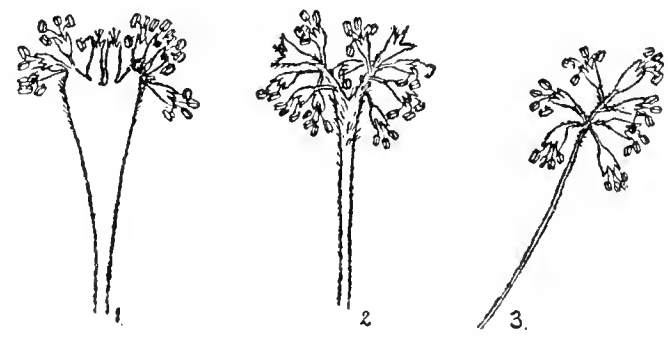

FIG. 2. 1 and 2, transitional inflorescences; 3, weakly developed male do.

[1] Blüthendiagramme.

The chief points of interest resulting from the observations described above seem to be :

1. The frequency of the abnormalities ; whether this is peculiar to a particular year or to the locality in question remains to be seen.

2. The greater frequency of androgynous and transitional inflorescences in the lateral position. This seems to show that although normally the male and female zones of the fertile shoot are sharply delimited, yet there is frequently a tendency for the two to overlap, with, especially in the intermediate region, a consequent reversion of one or more of the inflorescences borne thereon to the probably original androgynous condition.

3. The presence of the flowers on the apices of the cupule segments. Worsdell [1] says that adventitious flowers are rare, and that their occurrence as enations from a leaf surface is entirely unknown. If the cupule segments of the Beech are to be considered as modified bracts or bracteoles, the presence upon them of adventitious flowers offers a parallel to the case of the Nepaul Barley, *Hordeum trifurcatum*, where flower rudiments arise on the inferior or superior paleae of the spikelet.[2]

4. The orientation of some of the extra flowers in the cupule may perhaps be correlated with the position of certain of the flowers of the original dichasium. Fig. 1, 11, shows the possible arrangement of the flowers in a cupule with seven flowers of the dichasium present. Comparing the diagrams 6, 7, 8, and 9 with this, it will be seen that in diagram 7 the position of the extra flower may be correlated with that of flower No. 4, while the two extra ones of diagram 8 may be homologous with Nos. 4 and 5. The interpretation of the arrangement in diagram 9 is doubtful.

[1] Worsdell : Principles of Plant Teratology.

[2] Loc. cit.

L. W. COLE.

DEPT. OF BOTANY,
SHEFFIELD UNIVERSITY.

PRELIMINARY NOTE ON THE REPRODUCTION OF RHODYMENIA PALMATA, Ag.—In spite of the large amount of material of *Rhodymenia palmata*, Ag., available on the shores of the Northern Hemisphere, no account of sexual reproduction in this species has been published. Indeed, as recently as 1919, Dr. Church [1] cited this alga as an example of a member of the Rhodophyceae in which the sexual phase might be regarded as omitted from the life-cycle.

Asexual reproduction. Asexual reproduction is known to take place by means of tetraspores embedded in the outer small-celled tissue of the thallus, 'scattered or in cloudy patches'.[2]

These tetrasporic thalli were found in large numbers, both in the spring and

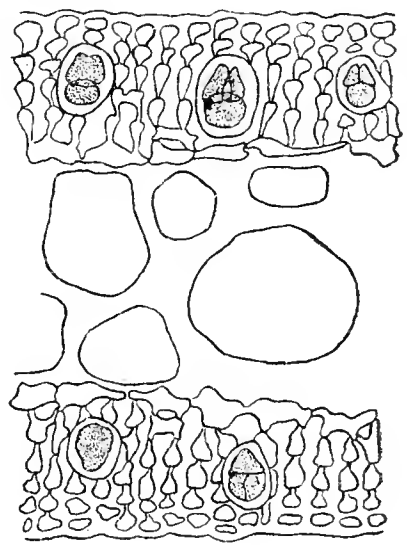

FIG. 1. Transverse section of a tetrasporic thallus of *Rhodymenia palmata*, showing the scattered groups of tetraspores embedded in the tissue forming the outer cortex of the thallus. Camera lucida outline. × 250.

autumn (April to September), at Shanklin, Isle of Wight. The tetrasporangia were either—

1. Scattered singly in the cortical tissue of the thallus (Fig. 1), giving the frond a mottled appearance in surface view; or,
2. Aggregated in sori, which may be marginal or may occupy the centre of the frond.

Sexual reproduction. The procarpial fronds were first found in April 1922, in some material gathered at random from the rocks at Shanklin.

These fronds are similar in form and colour to the vegetative or tetrasporic ones, but on examination the upper part of the frond is seen to be thinner and paler in texture than the rest, and is covered with minute, dark, ill-defined spots which sections show to be groups of procarps. These are developed on both surfaces of the fronds and are not scattered, but are aggregated into small groups situated near each other and arranged in regular acropetal succession.

The procarps are developed from among the smaller cells of the outer thallus tissue; when first differentiated each procarp consists of one or two cells (Fig. 2, A), but later these divide, giving a chain of three or four (Fig. 2, B). The upper cell

[1] Church, A. H.: Historical Review of the Florideae. Journ. Bot., lvii, 1919, p. 329.
[2] Harvey, W. H.: Phycologia Britannica, 1871, vol. iii.

develops a very long hyaline outgrowth—the trichogyne—which first arises as a small blunt protuberance of protoplasm from the carpogonium, covered by the extended gelatinous wall of the thallus (Fig. 2, A). When mature, the trichogyne may be as much as 0·31 mm. in length; the apex is somewhat swollen, the contents staining deeply with Heidenhain's iron-alum-haematoxylin, while the base is constricted at the point of attachment to the carpogonium (Fig. 2, A and B).

The *trichogyne nucleus*, which appears to be characteristic of those Rhodophyceae which include an asexual phase in their life-cycle, is an absolutely constant feature in *R. palmata*. It is a well-marked, spherical body, situated approximately half-way

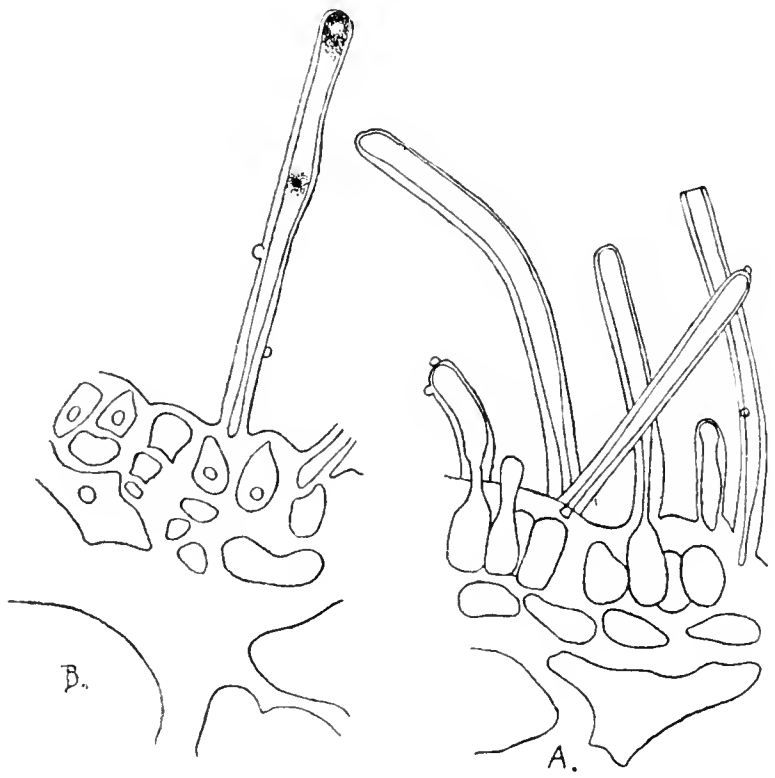

FIG. 2. Outline drawings of transverse sections through procarpial thalli. A. Section through a group of young procarps, showing the trichogyne in various stages of development. B. Section through mature procarps, showing the chains of cells, and an elongated trichogyne with its nucleus. Both drawn with camera lucida. × 750.

down the trichogyne (Fig. 2, B), and it remains visible until the latter shrivels up and disappears.

The *antheridial fronds* have not yet been recognized, but spherical bodies closely resembling spermatia have been found seated in various positions on the majority of trichogynes. More than one of these bodies is usually found on a single trichogyne (Fig. 2, B).

Further investigation is taking place into the differentiation of auxiliary cells, the fusions following fertilization, and the production of carpospores.

SUMMARY.

1. The discovery of the female fronds of *Rhodymenia palmata*, Ag., is recorded, the procarps being developed in groups in acropetal succession.

2. Each procarp bears a long trichogyne, with a distinct trichogyne nucleus. Bodies resembling spermatia have been seen seated on the trichogynes.

3. Further work on the development of the carpospores is expected to confirm the systematic position of *Rhodymenia palmata* as a member of the Rhodymeniales.

V. M. GRUBB.

WESTFIELD COLLEGE, LONDON,
October 1922.

ON A DISEASE OF COCOA AND COFFEE FRUITS CAUSED BY A FUNGUS HITHERTO UNDESCRIBED.—Liberian coffee cultivated in the Gold Coast Colony is subject to a disease which attacks the fruit, and which may be so prevalent in wet seasons as to endanger the whole crop.

The first indication of the disease is a dark purplish-brown discoloration of the berry, which later becomes covered with a white or pinkish-brown mealy incrustation formed by the conidia of a fungus always associated with the disease.

Fruits of all ages may be attacked, but the disease is most serious in the case of young berries, which are arrested in development and eventually become shrivelled and hard.

The same fungus is also responsible for a disease of cocoa fruits. Cross inoculations from Liberian coffee to cocoa fruits, and from cocoa to the fruits of Liberian coffee, have been effected and have given the characteristic symptoms of the disease, from which in both cases the fungus herein described has been reclaimed in cultures.

In natural infections of cocoa the symptoms of the disease—locally known as Mealy Pod—are similar to those caused by *Phytophthora Faberi*, Maub; the point of infection becomes brown, the area of discoloration darkens and increases rapidly until, given suitable conditions of moisture, the whole pod is involved; the mealy masses of conidia, at first white but later tending to become pinkish-brown, which have originally emerged as small pustules, form masses so dense that the pod becomes encrusted, and the pericarp of the fruit is decomposed. The encrusted masses of conidia are the most obvious of the symptoms which distinguish this disease from that caused by *Phytophthora*.

Such infection experiments as have been conducted have not given conclusive results as to the parasitology of the fungus; they tend, however, to indicate that it develops much more readily on wounded or moribund fruits than on healthy ones. It has never been found on the vegetative parts of its hosts. From an economic point of view its occurrence is important, because a large number of pods on a cocoa farm are normally wounded by various natural agents, and are thus liable to infection. The effect of the disease is particularly serious when young pods are infected, as the fungus is able to reach and to destroy the seeds in those cases where the sclerotic tissues, found in the fully developed pod, have not been formed.

The fungus responsible for the disease produces in the tissues of the host a non-septate mycelium of comparatively coarse hyphae, which spreads rapidly through the intercellular spaces. No haustoria have been observed, but, from the intercellular hyphae, branches arise of smaller diameter which penetrate the walls of the host cells, branch freely, and pass from cell to cell. The cells thus attacked are killed and their contents become discoloured.

When the mycelium is well established in the host tissues it proceeds to the formation of conidia. Hyphae collect beneath the epidermis and form a subiculum on which arises a dense mass of conidiophores, and this leads to the rupture of the overlying epidermis. The conidiophores are very variable in form. The simplest ones are upright hyphae terminating in a single conidium, but more usually the conidiophore bears a terminal vesicle to which a whorl of pedicellate conidia is attached. The more complex types may show a series of such enlargements, each with a whorl of conidia or with lateral fertile branches replacing some of the latter.

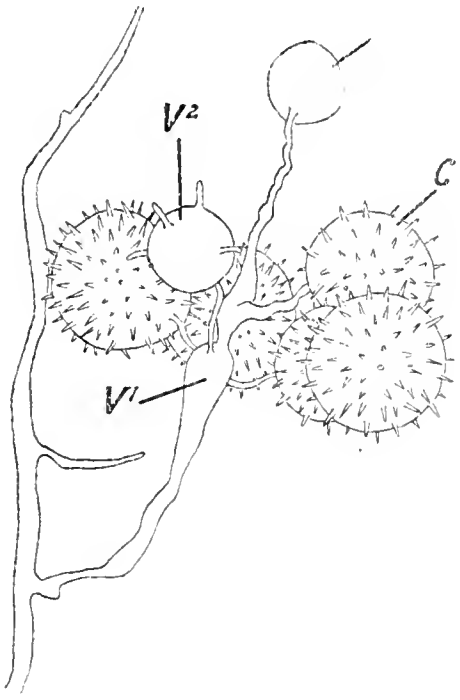

FIG. 1. Conidiophore showing swollen vesicle, v^1, with whorl of 5 conidia, c, and a secondary vesicle, v^2, on which conidia are beginning to develop. ×750.

The conidia are spherical in form, strongly echinulate, with an average diameter of 35 μ borne on pedicels which vary in length up to 30 μ.

Not only are conidia produced on the outer surface of the fruit, but in *Theobroma* they are found on the inner surface of the ovary wall, in mucilage sacs or even in the wider intercellular spaces.

The conidia found in the internal cavities of the fruit are often of larger diameter than the normal type and invariably have much thicker walls; they are perhaps better regarded as chlamydospores. Their germination has not been observed.

The conidia germinate readily both in water and in nutrient solutions. In all cases so far observed they produce a germ tube, which under suitable conditions gives rise to a mycelium on which the characteristic conidia are developed. The fungus is thus readily grown on artificial media, and pure cultures have been established and used in infection experiments.

Sexual Reproduction. The tissues of diseased cocoa pods bearing the characteristic conidia show also an abundance of sexual organs of Peronosporineae type. These are almost invariably found inside the host cells, singly or in groups; very rarely they may occur in the mucilage cavities of the pericarp. They arise from the intracellular branches of the mycelium, and may have associated with them groups of rounded vesicles. The oogonia are small, averaging 40 × 24 μ, rather thick-walled, and characterized by the presence of irregular sac-like outgrowths. These latter structures vary considerably in size and shape, from short rounded bosses to long finger-like processes, often curved and sometimes even faintly forked. The antheridia are amphigynous, completely surrounding the stalk of the oogonium in the manner hitherto only described for species of *Phytophthora* by Pethybridge[1] and Dastur.[2]

[1] Pethybridge, G. H.: On the Rotting of Potato Tubers by a New Species of *Phytophthora* having a Method of Sexual Reproduction hitherto undescribed. Sci. Proc. Roy. Dublin Soc., N. S., xiii, No. 35, 1913, p. 529.

Lafferty, H. A., and Pethybridge, G. H.: On a *Phytophthora* parasitic on Apples which has both Amphigynous and Paragynous Antheridia; and on Allied Species which show the same Phenomenon. Ibid., xvii, No. 4, 1922, p. 29.

[2] Dastur, J. F.: On *Phytophthora parasitica*, nov. spec. Mem. Dept. Agr. India, v, No. 4, 1913, pp. 177–231.

The general relations between the sexual organs is similar to that described by these authors. The antheridium, which is usually terminal, but may be intercalary, is penetrated by the oogonial branch, which then enlarges above to form the oogonium.

It is of interest to record that in ripe oogonia, obtained from old desiccated pods, the antheridia become readily detached, and then show that their walls are intact, and that a definite antheridial membrane surrounds that of the stalk of the oogonium. This might be expected from a study of the early stages of penetration, but it is impossible to distinguish the double membrane in sections of the mature oogonium.

The material so far available has not been suitable for cytological work, but

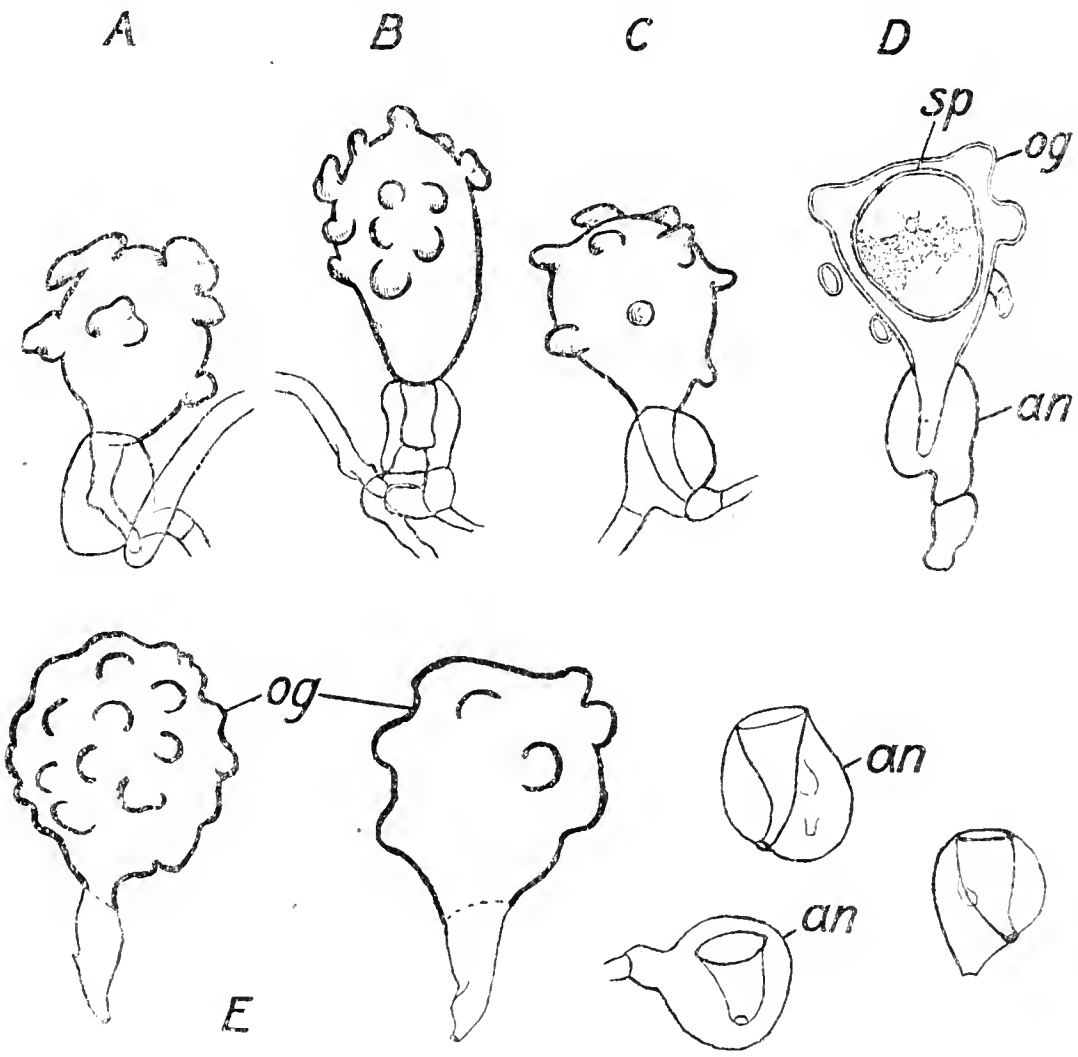

FIG. 2. A, B, C, oogonia from cells of mesocarp of cocoa pod, showing the numerous sacculae and amphigynous antheridia; D, section of oogonium with ripe oospore; E, oogonia with detached antheridia found in desiccated material three and a half months old. *an.*, antheridium; *og.*, oogonium; *sp.*, oospore. × 600.

preliminary observations show that the young oogonium is multinucleate, that little or no periplasm remains after the delimitation of the oosphere, and that the oosphere and ripe oospore are uninucleate. The oospore has a comparatively thin wall with little or no epispore. All attempts to germinate the oospores have so far failed of results.

That a definite relation existed between these sexual organs and the conidial-bearing mycelium, was evident from their constant association and from the fact that healthy pods, infected with the conidia, showed subsequently the presence of oogonia. Any doubts existing have, however, been set at rest by tracing the continuity of hyphae bearing conidia with those bearing oogonia, in mycelia from artificial cultures. These were tube cultures in which both sterilized cocoa pod and a cocoa pod agar were employed.

Up to the present no sexual organs have been found either in the tissues of the coffee pericarp or in artificial media prepared from coffee fruits.

The facts detailed above make it clear that we are concerned with a member of the Peronosporineae which cannot readily be referred to any of the existing genera, though certain of the facts detailed above indicate a close relation to the genus *Phytophthora*. The amphigynous antheridium has hitherto been found only in certain species of this genus, and Lafferty and Pethybridge (loc. cit.) in a recent communication have given a list of fourteen species in which this type of antheridium has been found, either alone or associated with the paragynous form, whereas in five species the latter has alone been recorded.

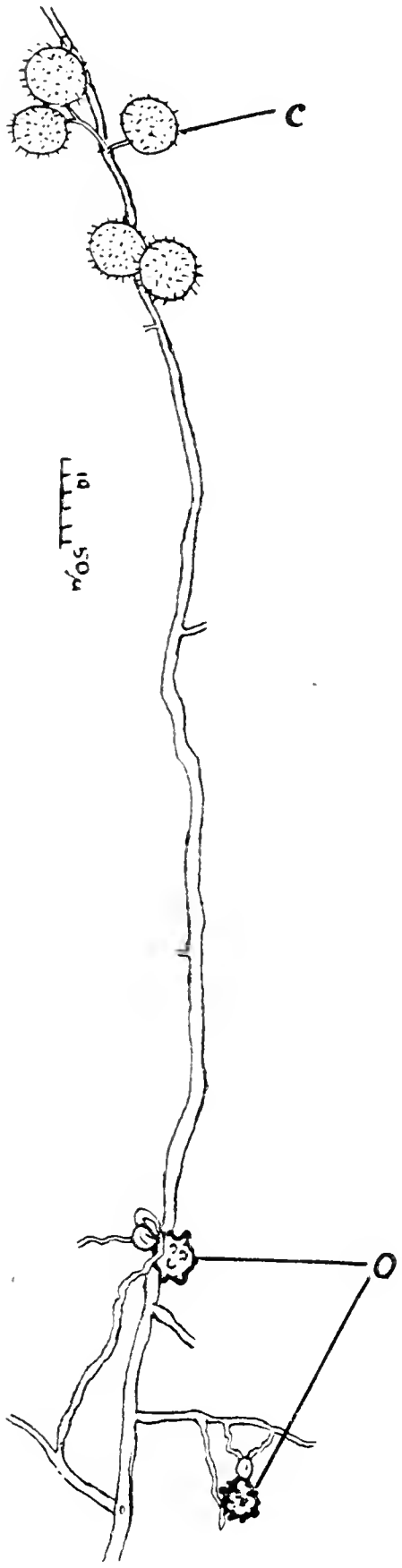

FIG. 3. Portion of mycelium from artificial culture showing conidia, *c*, and oogonia, *o*. × 140.

If the character of the antheridium be regarded as indicating some affinity to *Phytophthora*, the sacculate oogonia mark it off from the species of this genus, though somewhat similar forms have been described in members of both the Ancylistineae and Saprolegnineae. Moreover, the conidiophores are quite unlike those of *Phytophthora* or indeed of any other genus of the family, and the same is true of the large echinulate conidia.

If the conidial fructification be considered alone, it might be possible to associate it very closely with the genus *Muratella*, described by Bainier and Sartory,[1] which these authors are inclined to consider a member of the Mortierellaceae. In both, the conidia are echinulate and borne on globular enlargements of the conidiophores, though in *Muratella* these are usually terminal. The uncertain position of *Muratella*, however, and the fact that in the fungus under consideration the sexual mode of reproduction is known, has inclined the authors to place it in a new genus of the Peronosporineae, based on the characters of the conidiophores and the sacculate oogonia, under the name of *Trachysphaera fructigena*.

Trachysphaera, nov. gen. (τραχύς = rough, σφαῖρα = globe).

Conidiophorum simplex vel ramosum vesiculis terminalibus vel intercalaribus conidia pedicellata gerentibus praeditum.

Antheridia amphigynosa. Oogonia pyriformia e bulloso alte tuberculosa, oosporas episporio tenui vestitas liberas continentia.

Trachysphaera fructigena, nov. sp.

Mycelium intercellulare plerumque continuum non-septatum e hyphis crassiusculis compositum; haustoriis nullis.

[1] Bainier, G., et A. Sartory: Étude morphologique et biologique du *Muratella elegans*. Bull. Soc. Mycol. France, xxix, 1913, pp. 129–36.

Conidiophorae erectae plerumque vesiculo terminali conidiorum pedicellatorum (1–6) verticillium gerente praeditae, interdum evesiculiferae conidio singulo apicali ornatae, interdum vesiculorum (cuiusque vel conidiorum vel conidiorum cum ramulo verticillium gerentis) catenam praebentes.

Conidia sphaerica echinulata 13–48 μ (plerumque *c.* 35) leptodermia hyalina, pedicillis 10–30 μ longis. Chlamydosporae intercellulares echinulatae pachydermes.

Antheridia amphigynosa plerumque terminalia. Oogonia pyriformia parva (plerumque *c.* 40 × 24 μ) satis pachydermia, tuberculis sacculiformibus irregularibus multiformibus tum brevibus obtusis tum longis dactyliformibus rectis vel curvatis raro subfurcatis insigniter exornata.

Hab. in fructu Coffeae libericae et Theobromatis Cacao.

The further investigation of problems connected with the parasitism of the fungus is being actively carried on by one of the authors in the Mycological Laboratory of the Department of Agriculture of the Gold Coast Colony at Aburi.

The authors desire to record their grateful appreciation of the help rendered by Mr. J. Ramsbottom in the consideration of the systematic position of the fungus and in drawing up the diagnosis.

R. J. TABOR.
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EDITED FOR THE

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By A. G. TANSLEY

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EDITED BY

V. H. BLACKMAN, Sc.D., F.R.S.

PROFESSOR OF PLANT PHYSIOLOGY AND PATHOLOGY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

AND

R. THAXTER, M.A., PH.D.

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

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F. W. OLIVER, M.A., D.Sc., F.R.S.

QUAIN PROFESSOR OF BOTANY, UNIVERSITY COLLEGE, LONDON

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A Pathological Anatomical Study of Crystal Cyst Formation in Parenchymatous Tissue in the Genus Anthurium.

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BY

J. A. SAMUELS.

With Plate II and five Figures in the Text.

I. Introduction.

UP to the present time, cell and nuclear fusions in somatic cells have been regarded from an anatomical standpoint as independent phenomena. A recent study of the processes in *Anthurium scandens*, however, has afforded the writer an opportunity to observe a relation between them which complicates the theoretical conceptions of the subject (46).

It is well known that in the last decades many investigators have added greatly to our knowledge of nuclear and protoplasm fusions in vegetative cells, and that their investigations have led to interesting conclusions. One need only mention the multiple nuclear fusions in the endosperm of many Angiosperms (7), the fusions of the tapetal nuclei in the anthers of certain plants (55, 58, 63), the fusions of certain cells in the ovule of *Tropaeolum* (63), the multinucleate cells in the procambium and the plerome of certain plants, and the formation of a symplast after infection by *Synchytrium* and some Heteroderas (25, 37).

It would appear that, in vegetative tissue, most of these cases of cell and nuclear fusions are generally due directly to the influence of the high acidity of the cell or to a certain cell complex. The direct cause, however, may be of a different nature; for example, abnormal physiological conditions.

None of the cases at present known in which the acidity affects a certain part of the tissue are so pronounced as is *Anthurium*, where large cell complexes dissolve, i.e. cell fusion takes place with the formation of a symplast and the appearance of a certain number of raphid crystal colonies.

JUN 19 1923

The fusion of two neighbouring cells representing the origin of crystal cysts in young tissue cannot be discovered without difficulty. The present publication may therefore serve to illustrate such a case in *Anthurium scandens* and *A. Scherzerianum*.

[Annals of Botany, Vol. XXXVII. No. CXLVI. April, 1923.]

The first part of the publication will deal only with actual observations, while in the second part an effort will be made to discuss the different phenomena regarding the presence of several nuclei in a single cell, the cell fusions, the nuclear fusions, the formation of the crystal colonies, and, finally, the end of living conditions in such hypertrophied cells.

II. Technique.

The material for the investigation was collected in the Botanical Garden, Paris. A portion was fixed in a solution of chromo-acetic acid and another portion in 96 per cent. alcohol. It has been found that better results could be obtained from the material treated with alcohol. Furthermore, it may be stated that the best results were secured with sections stained by Heidenhain's haematoxylin. Triple staining, according to Flemming, has also been applied with good results for the early stages, but not so successfully with the later stages.

Young leaves are better for the study of cell and nuclear fusion than leaves advanced in growth.

After the use of the Heidenhain method of staining, where the sections were left in a solution of ferric ammonium sulphate during the night, the raphid needles were dissolved so that thereafter the crystals themselves could not be seen even with the polarization apparatus. There remained but an empty space bounded by the plasma membrane which surrounded the needles. The crystals in the cells of sections stained by the triple staining method of Flemming, however, remained intact.

The sections were cut 12 microns thick and the figures were made with the Abbe drawing apparatus.

III. The Formation of a Cyst.

The cysts were first observed and most thoroughly studied in the perianth of *Anthurium scandens* and *A. Scherzerianum*. Later they were found also in other floral organs, in the stems and roots, and the embryo of both specimens.

The development of the cysts is indicated by the increase of the protoplasm masses and the enlargement of the nuclei of two neighbouring cells. For the time being an attempt to examine this process microscopically has yielded no results.

The cause of the hypertrophy of one of the two isolated cells can probably be attributed to physico-chemical causes, because large cell complexes in the parenchymatous tissue mentioned above are involved. Since the parenchymatous cells of a given region are anatomically and functionally similar, we would perhaps again attribute such a phenomenon to bacterial origin, but thus far no bacteria could be discovered in the cells.

The difficulty of observing bacteria in cells filled with a dense mass of protoplasm is well known to histologists. Since the investigations on this subject are not yet concluded, at present no final judgement may be given.

In the further development of the cysts the nuclei of the two cells assume positions on opposite sides of the common cell wall. Later the wall is ruptured and a fusion or mingling of the cell contents and the nuclei occurs. Pl. II, Fig. 1, shows the large cell which probably arose by the fusion of two other cells and which lies in the centre of the parenchymatous tissue. The hypertrophied cell thus produced is about twice as large as the neighbouring cells. The protoplasm containing numerous small and large vacuoles is strikingly dense and displays an embryonic character. The nucleus gives the impression of having recently arisen by the fusion of two other nuclei.

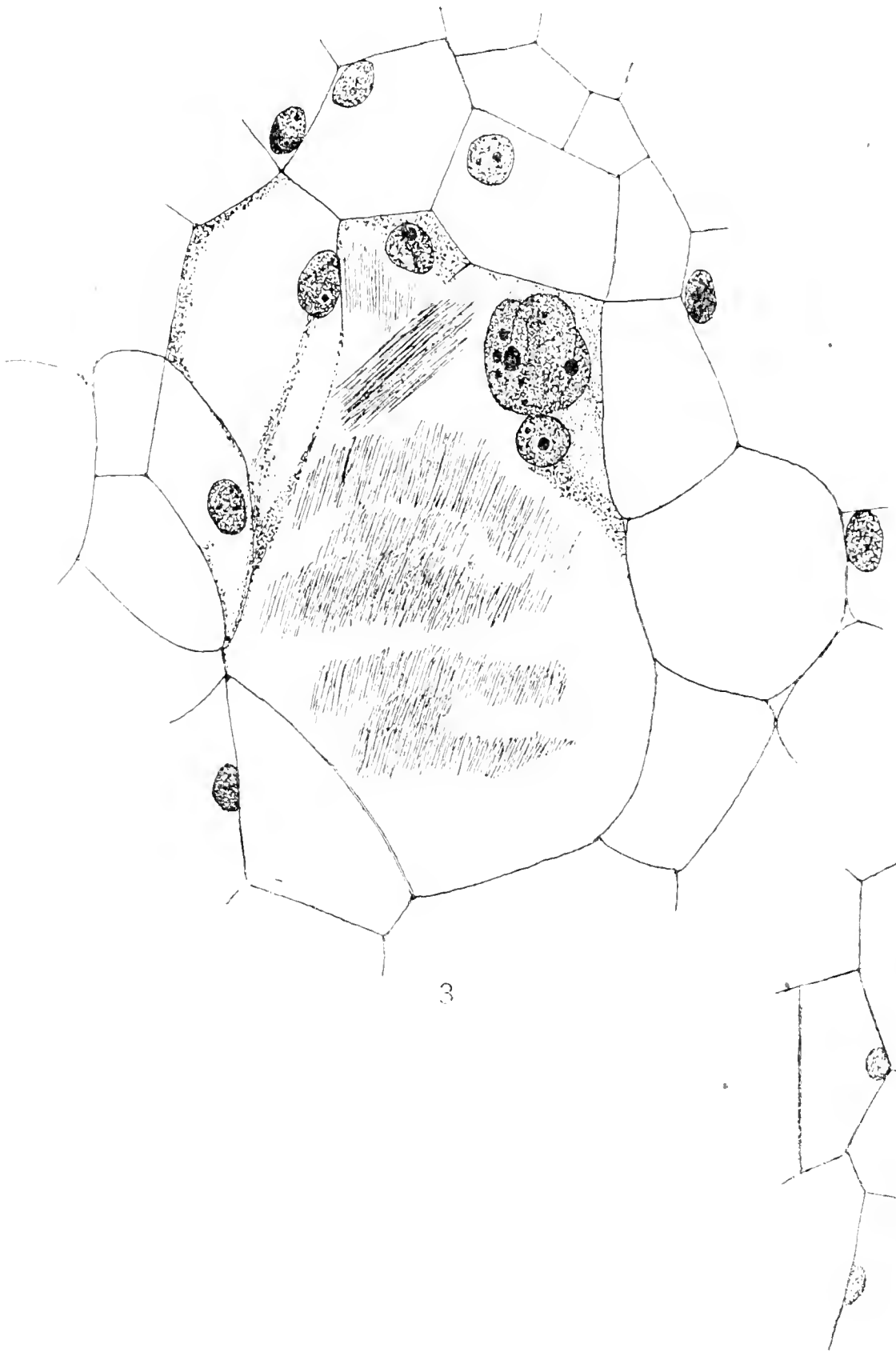
The nuclei of the neighbouring parenchymatous cells generally are not far distant from the hypertrophied nucleus. Pl. II, Fig. 1, shows the nucleus of the cyst lying in one corner of the enlarged cell, while the nuclei of the neighbouring cells lie near the cell walls in the immediate vicinity of the large nucleus. The fusion nucleus may or may not retain its normal spherical form. In some cases a further fusion of cells with the cyst takes place and their nuclei fuse with that of the cyst. In other cases the nucleus of the cyst becomes deformed and may assume a spiral shape. This phenomenon usually occurs in old organs. In immature organs a further fusion of the cyst with the surrounding cells takes place. Pl. II, Figs. 2, 5, 11, and Text-figs. 1, 3, 4, and 5, show fusions or pairing of nuclei. In Pl. II, Figs. 3 and 4, both nuclei lie within the plasma ring that was formed by the union of the two cells.

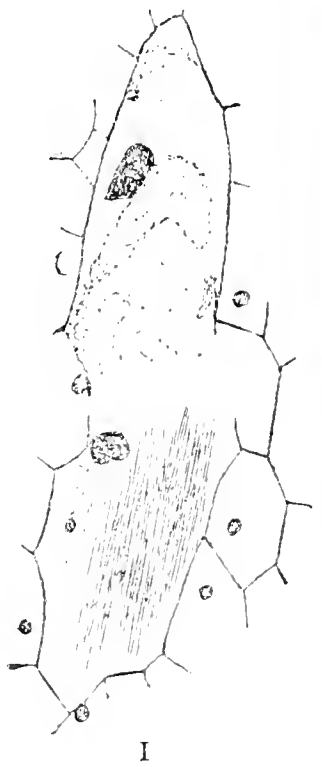
Further enlargement of the cyst at the expense of the neighbouring cells causes a breaking down of the common cell wall and the dissolving of the remnants. This process results in the formation of a large symplast. Pl. II, Figs. 8, 11, and Text-figs. 1 and 3, show clearly how the walls are pierced and the cell nuclei are enabled to fuse with the principal nucleus. Fragments of cell walls undergoing solution are plainly visible.

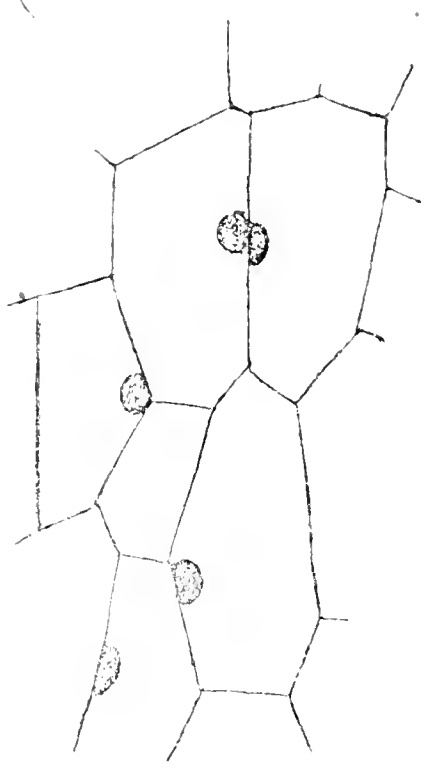
It happens very often that two young neighbouring symplasts fuse together. Text-fig. 1 demonstrates such a case which was observed in the third cell row from the epidermis in the perianth of *Anthurium Scherzerianum*. In one part of this fused cyst we find some nuclei in the act of fusing, while the nucleus of the other part has more or less an elongated form, without, however, any crystal formation in that particular part. The nuclei of the surrounding parenchyma cells maintain their round form. Within the cell, during the process of cell fusion, the protoplasm layer of the cyst is increased in thickness and an extensive crystallization of calcium oxalate takes place.

The first colony of raphids is formed in the immediate vicinity of the

nucleus, which is embedded in an unusually large mass of protoplasm to which it is connected by means of protoplasmic strands. See Pl. II, Figs. 5, 7,







TEXT-FIG. 1. Fusion of the two cyst cells and neighbouring cells in the parenchymatous issue of a perianth leaf of *Anthurium Scherzerianum*, third cell row from the epidermis. The two large cyst nuclei can be distinguished from one another. Formation of a crystal colony in one of the cysts. Obj. 4, ocular 4.

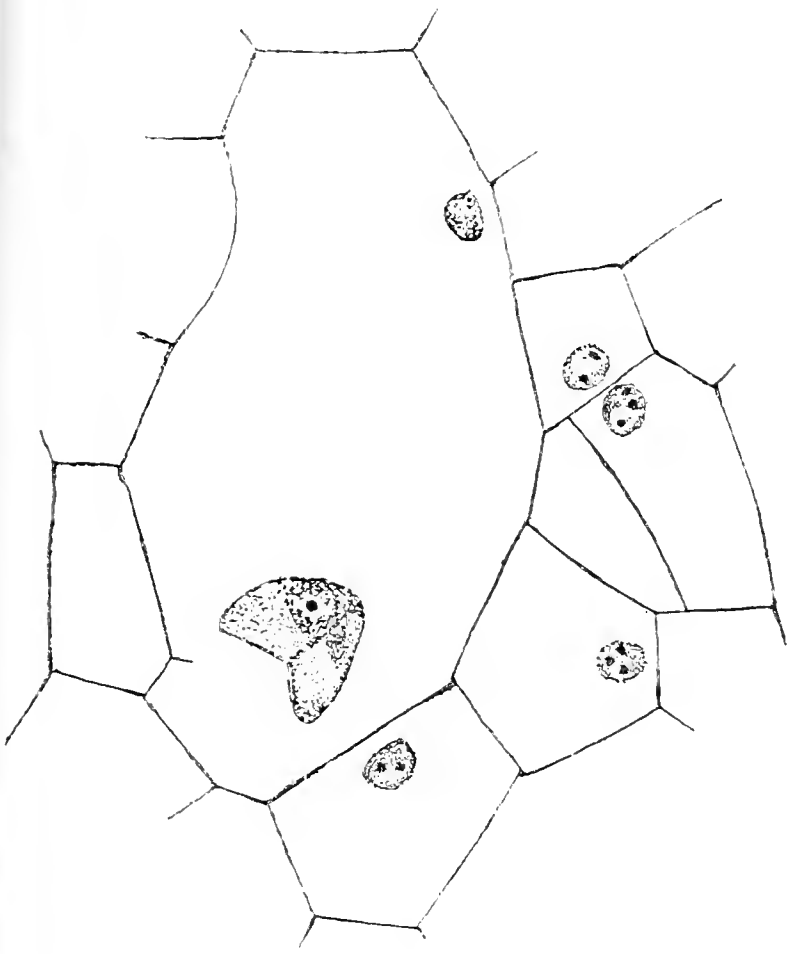
TEXT-FIG. 2. The section following that of Text-fig. 1, showing the nuclei lying against the cell wall. Oil immersion, ocular 2.

TEXT-FIG. 3. Large cyst cell from the parenchymatous tissue of the perianth leaf of *Anthurium Scherzerianum*, demonstrating the large cyst nucleus after the fusion of a number of nuclei. Another nucleus of a neighbouring cell, united, and ready to fuse with the main nucleus. Fusion of the main symplast cell with other neighbouring cells. Many crystal colonies formed in different directions. Nuclei of the neighbouring cells lying more or less against the cell wall. Some of these nuclei becoming homogeneous. Oil immersion, ocular 4.

and 11. If the cyst is conspicuously elongated the nucleus is usually situated at one end of the cell. After the formation of the first raphid colony, others

are formed with the component needles either parallel to the first or pointing in various directions (Pl. II, Figs. 8 and 11; Text-fig. 3).

The position of the groups of crystals indicates clearly that masses of oxalate move in various directions within the cell. This fact shows that various abnormal processes occur. It could not be determined whether the number of raphid colonies correspond to the number of fused nuclei or not. The crystals do not lie within the vacuole, as is usually the case (20, 42, 59),



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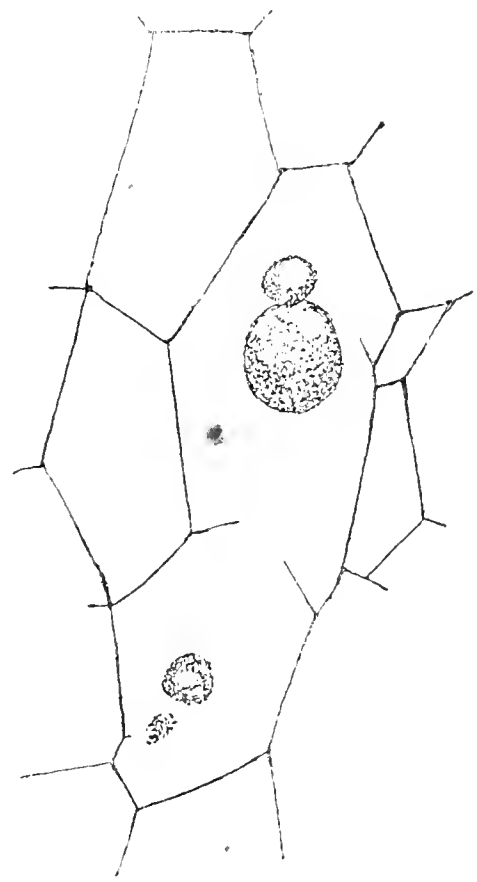

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TEXT-FIG. 4. Part of the tissue of a perianth leaf of *Anthurium Scherzerianum*, third cell row from the epidermis. Fusion of a large cyst cell with a neighbouring cell. Fusion of the cyst nucleus with the nucleus of the latter. At the other end of the cyst another nucleus ready to unite with the large cyst nucleus. Nuclei of the neighbouring cells with a normal form showing a tendency of lying along the cell wall. Oil immersion, ocular 8.

TEXT-FIG. 5. Fusion of four cells to a large symplast in the parenchymatous tissue of the perianth leaf of *Anthurium Scherzerianum*. Union of the nuclei of the upper two cells. The nucleus of the third and a piece of that of the fourth cell can be seen at the other pole of the young cyst cell. Oil immersion, ocular 7.

but rather in the protoplasm. Sometimes a single colony occupies the entire width of the cyst (Pl. II, Figs. 6 and 7).

During the development of a large cyst the nuclei of the neighbouring cells exhibit a curious phenomenon. They become distorted and homogeneous in appearance and show a tendency to fuse. But even in those parts of the tissue where a fusion has not yet taken place, the nuclei of the cells apparently unaffected are homogeneous and are located near or alongside the cell wall, which therefore indicates a tendency to fusion with neighbouring cells (Text-figs. 2, 3, and 4).

During or even very shortly before the formation of crystals the

nucleus of the cyst undergoes certain changes. It may assume lobed, spiral, or twisted forms, as shown in Pl. II, Figs. 10, 12, 13, 14, and 15, and Text-figs. 1, 3, and 4.

It is not unlikely that this amoeboid change of form is associated with chemical or physical changes in the constitution of the cell, for it is well known that nuclei can be stimulated by physical or chemical means so as to undergo pathological fusion, and this I believe is the case in *Anthurium*.

In general the form of the nucleus has nothing to do with cell fusions; nuclei of abnormal form may occur independent of the latter, and such abnormal nuclei have been found in many pathological cases. It is most probable that in the cyst nuclei of *Anthurium* such a pathological condition is to be attributed to physico-chemical causes, for, as we have seen, the crystallization of calcium oxalate begins in the immediate neighbourhood of the nucleus, which at the time is located in a dense mass of protoplasm. The nuclei are therefore closely associated with crystal formation. In the raphid-bearing cells, as ordinarily observed in many plants, the nucleus has not a spherical form as in the neighbouring cells, but is more or less elongated (Pl. II, Figs. 14, 15, and 16).

In this case also we have to deal with change of form which is perhaps associated with some physico-chemical cause. The normal nucleus passes over into a pathological state. In the cyst nuclei of *Anthurium* the pathological condition is made evident by the spiral and lobed condition. It is of further interest that the production of calcium oxalate crystals decreases with the final cessation of the activity of the latter, i. e. when the contents of the nucleus become homogeneous and are about to dissolve. From this fact it is clear that there is a relation between the cyst nucleus and crystal formation. The relation between the form of the cells and cell growth and the position and function of the nucleus can be clearly determined and followed in the case of these crystal symplasts. It may be emphasized that the formation of these peculiar symplasts, whose origin is, in all probability, to be attributed to abnormal physico-chemical processes in the tissue, takes place, not in the outer parenchyma cell layers of the perianth leaves, but for the most part in the inner layers. In the outer layers mainly polyhedric crystals are formed, and neither cell association nor nuclear fusion is to be observed.

The formation of crystal cysts may be observed not only in all the vegetative organs of *Anthurium scandens* and *A. Scherzerianum*, but also, in all probability, in other species of the genus *Anthurium*.[1]

[1] The occurrence of similar crystal symplasts in the parenchymatous tissue of the floral organs of *Arisaema triphyllum*, which I had the opportunity to observe in the early part of this season, may also be noted here.

IV. Discussion.

Having described the origin of such a cyst, it may be well to compare it with the process of development of an ordinary raphid-bearing cell, particularly in view of the fact that in both cases abnormal regulatory phenomena are principally dealt with. The two kinds of crystal cells differ principally from each other in that only in the former do cell and nuclear fusions take place. A comparison will at least yield further information as to the behaviour of the cell elements with reference to the crystal structure.

In this comparison of the origin of a simple raphid cell and that of a cyst, three prominently important points are to be noted:

1. The behaviour of the nucleus.
2. The behaviour of the protoplasm.
3. The formation of the crystal colonies.

1. *The Behaviour of the Nucleus.*

From the behaviour of the nuclei with respect to the protoplasm it will undoubtedly be possible to arrive at a conclusion as to whether the protoplasm suffers through the formation of crystals or not, or, in other words, whether or not normal physiological conditions or processes are interrupted.

Rosanoff (42) and later De la Rue (10) in *Hoya carnosa* were the first to observe crystal-bearing cells, and they noted the survival of the nucleus after crystal formation. De Bary (9) also mentioned briefly the survival of the nucleus of such cells which have not yet lost their vitality, i. e. whose cell contents are not yet filled with crystal substance.

In the cell, during the formation of the crystal, there arise entirely different conditions, physico-chemically and physiologically, which certainly affect the form and the position of the nucleus.

Kohl (20), who studied such raphid cells, especially in *Hyacinthus orientalis*, *Vanilla planifolia*, and Orchid bulbs, found that the nucleus, immediately after appearance of vacuoles, assumed a vertical position adjacent to the cell wall, where it remained during its cell existence (p. 274). According to Kohl the raphid bundle is always formed in the centre of the cell, and especially in the cells where a contraction of the protoplasmic wall covering has taken place. In these cases he points out that the nucleus is in direct connexion with the raphid bundle. Zacharias (65) and Johow (19) made the same observation in raphid-bearing tubes of Monocotyledons and Dicotyledons.

It was possible to determine from my observations on *Anthurium*, that the position and form of the nucleus in the crystal cysts are influenced by abnormal physiological processes, i. e. by the growth of the calcium oxalate

substance within the cell. At the appearance of the vacuoles the nucleus is pushed aside. Crystal formation influences the position of the nucleus, if not directly, at least indirectly. It is a fact, however, that the position of the vacuoles depends entirely upon the physico-chemical conditions, while the position of the nucleus is a purely physical or chemical phenomenon. The nucleus of an ordinary crystal or raphid-forming cell has, for this reason, a position adjacent to the wall; in fact, along the longitudinal wall. It is very seldom seen at either of the ends. However, in the cyst cell the nucleus may frequently be observed at one end of the cell. The large hypertrophied nucleus is always in contact with the crystal or with the raphid colonies which lie in various directions, though masses of protoplasm may often be very dense and concentrated.

The nucleus in a cyst cell differs in its position from an ordinary prism or raphid cell, in that in the former there results a general physiological influence radiating towards the nucleus, equal in all directions, whereas in the ordinary prism cell the influence is exerted outward from the point on the cell wall at which the nucleus lies.

The fusion of adjacent neighbouring protoplasts with a particular protoplast creates a certain pressure upon the nuclear mass of the latter, thus causing it to assume a position which depends upon the direction of the pressure exerted by neighbouring protoplasts. The nucleus may be kept upright or forced into some other permanent position by reason of the mass of calcium oxalate crystals which develop at the centre of the cell. The change of position of the nucleus is thus the result of simple mechanical conditions.

Heidenhain (17) and his school observed that in the case of resting cells containing a great deal of granular material, the nuclei come to lie flat on the lower side of the cell. However, when secretion begins the nuclei raise themselves somewhat from the base of the cell, finally becoming bubble-like, and do not stain as heavily as before.

Nussbaum (38) and later Heidenhain (17) declare that these conditions arise from purely mechanical causes, because the nuclei in the resting cells (*Drüsenzellen*) are mechanically influenced by the accumulation of the secreted material in the body of the cell, and make it possible therefore—on account of the accumulation of the secreted material—for the nuclei to move again into original position of equilibrium. It is doubtful whether or not the granular material in the resting cells mentioned by Heidenhain should be compared with the calcium oxalate substance discussed here. Although it is more or less certain that they are substances of different chemical constitution, it is a matter of fact that both are secreted material deposited in the cell; they both have a certain influence on the nucleus.

While it is easier to compare to a certain extent the above-mentioned

case described by Heidenhain with conditions prevailing in the ordinary calcium oxalate crystal cell, it is very difficult to compare the behaviour of the nucleus in the former case with that of the crystal cyst. In the formation of the cyst cell an activity in different directions takes place, i. e. a fusion of a whole cell complex occurs whereby the control of the movement or position of the nucleus in the original young cyst cell is very often lost.

Other abnormal conditions inside and outside the original cyst are added, i. e. the accumulation of the secreted material in the cell body, which is of course pronounced in the fusion with neighbouring cells; the study of the behaviour of the nucleus is thus much more difficult. Under these circumstances an additional and essential factor should be kept in view, which is certainly of some importance in regard to the behaviour of the nucleus in the cases of function of crystal substances in cells, generally speaking.

In the crystal cell there is an accumulation of secretion material which is evidenced by the crystallization of the oxalate substance by which water molecules are stored. The protoplasm is consequently deprived of molecules of water. Finally, the nucleus is also influenced by the withdrawal of water from the protoplasm, whereby the regulatory phenomena are disturbed.

There also can be no doubt that the stimulus, be it an external or an internal one, first affects the protoplasm and then directly or eventually the nucleus. However, the irritation may influence only the plasma or both it and the nucleus. This of course depends entirely upon the quality and the quantity of the stimulus. The changes in form of the nucleus are therefore brought about indirectly and do not result from the direct effects of the irritation.

Fuchs (13) found in the raphid cells of *Galium Mollugo*, *Asperula tinctoria*, and also of *Hydrangea Hortensia* and *Aloe*, that the nucleus in the crystal cell is of an elongated and more or less spindle-like form, whereas the nucleus in *Mesembryanthemum crystallinum* was round and entirely indistinguishable from the nuclei of other cells. It may be that in the latter case the nucleus was only temporarily round, and had not yet been influenced by the irritation or would not be influenced at all. From the work of Fuchs it does not appear whether or not the nucleus keeps its round form or whether it changes in form later.

As far as can be ascertained, no one has undertaken to systematically observe the behaviour of the nucleus during the development of such crystal cells. In any event, the observations of Fuchs are not sufficient to lead to any definite conclusion.

The temporary spherical form of the nuclei of the crystal cysts in *Anthurium* offers an interesting example of changes of form under the influence of irritation stimuli. After the fusion of the nucleus of the

neighbouring cell with the nucleus of the young cyst, the nucleus of the latter displays a more or less round form. Later, it stretches lengthwise and takes a position along the wall, and indeed, most frequently, at one of the ends of the hypertrophied cell. Because of the abnormal regulatory processes in this condition of cyst development, the nucleus is pushed to one side.

The fusion of the protoplasts reminds one of the normal fusion of living protoplasts or of the ontogeny of non-septated (36) and septated sieve tubes, and also of the processes of the sexual cell fusions. This is likewise true of normal fusion processes, found for example in the pith of the hypocotyl and epicotyl of the bean stem which has been treated with violet or diffused light,[1] and also of the cell fusion during the formation of secretorial cysts and tubes. Cell fusion may finally take place after infection, as for example in the case of *Heterodera Schachtii* (25). Küster suggests that it is very difficult to decide whether the material which dissolves the cell wall is produced by the parasite or by the host cells themselves. In the crystal symplasts there occurs a similar solution of perforated or defective cell walls. The cell-wall dissolving material is after all probably produced in this case in the symplasts themselves. It is certainly more difficult to find the reason for the fusion of two parenchymatous cells with a young cyst cell, than to determine the cause of the fusion of two or more endosperm cells. Since it might be suggested that, in the latter case, there is a solution which is of enzymic nature and concerns the solution of reserve products, it is a matter of fact that, by the fusion of nuclei of other neighbouring cells with this hypertrophied nucleus, the latter becomes richer in water, and for that reason assumes its original form again.

However, if the fusion ceases and the formation becomes more active, the combination with water begins again and also becomes more and more active; the nucleus, as a consequence, undergoes an elongation during which it takes various amoeboid and convoluted forms. Probably the increase in surface area of the nucleus enables it to absorb water molecules more freely at the proper time, so that it regains its original form. A circumstance in favour of this view is the fact that as a cell becomes older and approaches the end of its functional ability it becomes more devoid of water than before, particularly in cells of a secreting character such as those here discussed. In addition to the above suppositions relative to the withdrawal of water, other circumstances and factors may affect the change in form of the nucleus. The change in nuclear form in the calcium oxalate cyst reminds one of the change in nuclear form in the

[1] Paper read at the meeting of the American Association for Advancement of Sciences in New York, 1916.

living leucocyte during its movements through the various connective tissues.

In the last case, according to Heidenhain, the change of form is effected by intermittent pressure exerted in rapid succession by the plasma body. Intermittently this causes considerable surface enlargement and surface changes which can only be explained by a high degree of elasticity and pliability in the nuclear membrane. It is entirely probable that in the case of crystal cysts there is a rapid successive exertion of pressure due to the tendency which exists in the young cells to unite with adjacent cells, and due also to the same tendency towards union of the nuclei.

A union or fusion of the neighbouring nuclei with the young cyst nucleus can also be brought about, followed by the change in form of the large nucleus thus formed. Another important case in which the loss of water in the nucleus might play a part is the change of nuclear size in the young parenchyma cells. We know, for example, that the cell nuclei, together with the nucleoli in the growing cells, which have stopped so as to divide, sometimes often grow and then diminish in size later (see Schiller on *Antithamnion*, 48, p. 285). Among other examples of nuclear changes which, according to Schiller's observation, depend in all probability on the physiological processes in the cell, are those of certain regenerated cells which result from injury. In each cell there may be several nuclei lying like a string of pearls, one after the other.

This observation resembles that of Neměc (36) in the plerome of *Ricinus communis* and *R. borboniensis*. Here these nuclei very often fuse together, and from such a mass only one large nucleus results, which may be divided again. These nuclei, which lie in a straight line like strings of pearls, have, according to Neměc, a nuclear mass in direct relation to the size of the cell (pp. 128–9). Neměc also puts forward another view, for he adds: 'Wir haben jedoch gesehen, dass Zellen, die mit gleicher Anzahl von Kernen versehen sind, eine beträchtliche Verschiedenheit in ihrer Grösse zeigen können, welcher Umstand mit der eben entwickelten Annahme nicht übereinzustimmen scheint. Aber erstens hängt die Kernteilung auch mit der in der Zelle enthaltenen Zytoplasmamasse zusammen. Zweitens, ist diese nicht einfach der Zellgrösse gleich, da die Pflanzenzellen zahlreiche Vakuolen enthalten, deren Grösse es bedingt, dass eine recht grosse Zelle weniger Zytoplasma enthalten kann, als eine andere ebenso grosse oder sogar kleinere' (p. 136). In other words, the size of the nucleus depends entirely on the age or the physiological conditions in and immediately around the cell.

Schiller stated that all forms of nuclei occur in *Antithamnion cresc.* var. *tenuissima* and *A. plumula*, although the cell form remains the same.

It also appears very clear from the observations of Kohl (20), Van Bambeke (64), Lidfors (27), and other investigators, that the nucleus possesses

an active tendency to change its form, and I find this to be the case from my own observations. As stated, this does not depend on the cell form but only on the physiological processes there. This is demonstrated by the difference between the nuclear form of the common or raphid cell and those of its neighbouring cells which are without crystals and of the same size.

I agree with Schiller that the supposition of Miehe (35), Haberlandt (15), Rosen (43), and other investigators, that the nuclear forms are due to tension which is brought about by the change in cell form, is entirely wrong.

Smolak (49) writes about his observations in *Prunus domestica*, var. *Victoria*, with regard to the change in nuclear form in the palisade and spongy parenchyma cells, which were affected by the silver-leaf disease. He says on p. 145: 'While the septum-like nuclei appear in the palisade cells rather frequently, yet this is not the case in the spongy parenchyma. On the contrary, in the latter tissue, the nuclei commonly show sharp-pointed lobes as above described. Probably the form which the disorganized nucleus takes may be dependent on the form of the cells.' On p. 150 the author further says: 'The abnormal increase in size of the nucleus is—to judge by the manner of its occurrence—connected with the increase of metabolism, just as it is, for instance, in the large nuclei in the glands of animals or in healthy tissues of the seaweed *Antithamnion* (Schiller, 14), where the surface of the nuclei is much increased in size by enlargement and change of form or development of lobes.'

I agree with Smolak that the increase in size or very often the change in form may be connected with the increase of metabolism. The change in nuclear form, as it is demonstrated by his figures, shows that it is not due to the increase of metabolism, but to pathological phenomena which are no doubt caused by toxaemia.

In his first paragraph he contradicts himself by assuming that in an organized cell the changes in nuclear form are dependent on the cellular form, but I believe that it has no relation to cell form. On the contrary, it is influenced by the metabolic processes either physiological or pathological therein.

The behaviour of the contents of the raphid cell nucleus with respect to the nucleolus leads to the supposition of water loss. The more water the nucleus is deprived of, the larger becomes the nucleolus, which is to be regarded as nothing more than a secretion of the nucleus, that is to say, a metabolic product.

The nucleoli appear especially large in the hypertrophied crystal cells, whereas, as previously stated, they do not assume any unduly large proportions nor become very conspicuous in the nucleus of a normal parenchyma cell. Because of this abnormal nucleolar size in such hypertrophied crystal cells very often there results, in all probability, a special process in the

pathological cell. For example, the nucleolar proteins might change into secretion products.

According to Haecker's (16) theory of the structure of the nucleus, the nucleolus is merely a splitting off or intermediate product due to the change in material. These products are removed from the nuclear content as a sort of secretion during the rest period at the beginning of mitosis, and may be either in combined or uncombined form. Haecker therefore concludes: 'Inasmuch as the need for living substance increases when division becomes more active, as a necessary consequence there follows the production of living substance in the form of large and—because of the small size of the nucleus—compact nucleoli.' The opinion of Haecker that the nucleoli are living substances is, however, contradictory to his assumption that they are cleavage products or secretions.

Other important evidences of the secretory-like character of the nucleolus are that the nucleolar substance increases considerably as soon as the nucleus is affected by the abnormal concentration of acidity in the hypertrophied cyst cell, and also the appearance of the death and dissolution of the nucleus, which is very striking as soon as the nucleolus has filled the entire volume of the nucleus.

Although the abnormal increase in size of the nucleolus gives us some information as to the end of its existence, the opinion is that its contents are nothing but an assimilation or secretion product. This also finds support in the abnormal size of the nucleolus in the primary endosperm nucleus, which I have occasionally observed in the embryo-sac of *Gunnera* shortly before division (see 40, Plate IV, Fig. 28). But inasmuch as we have to deal in the latter with a normal or regular phenomenon and the division of the nucleus directly follows the increase in size of the nucleolus, one should make a distinction between the two.

After this discussion of the rôle of the nucleolus in the hypertrophied cyst nucleus we may accept the theory of Haecker with the proviso that the nucleolus is not regarded in pathological cases as an intermediate but as an end product of the metabolism; his theory deals exclusively with an excretion of the nucleolar substance during the division of the nucleus under normal conditions.

Other cytologists, like Carlier (2) and Heidenhain (17), believe that the nucleolus is nothing more than an organized excretion product of nuclear metabolism, and that this substance should be separated from the chromatin.

Wilson (60) agrees with Haecker that the nucleoli may be regarded as a transformation product of the chromatin or as a chemical cleavage product or secretion. Wilson says: 'It seems not improbable that nucleoli are tributary to the same general process, perhaps serving as storehouses of material formed incidentally to the general nuclear activity, but not

of further direct use.' The expression 'storehouses' certainly gives the impression that the nucleolus contains reserve products, which are stored in them to be used later for a specific purpose.

Carnoy and Le Brun (4, 5, 6) stated, as a result of their investigations on the germinal vesicle of Amphibia, that the nucleoli represent products from which the chromosomes are derived.

Botanists, more especially Zimmermann (67), Reinke,[1] Went,[1] Farmer,[1] also O. Hertwig[1] and others, agree to this view-point. Strasburger and his school, who were at first of the opinion that the nucleolus is used especially as construction material for spindles, accepted later the dominating theory at that time, that the nucleolus is of importance as the source of chromatin. Although the authors mentioned observe the superposition and the approximation of the nucleolus to the chromatin substance, particularly in the spireme stage, other investigators were able to determine its position along the nuclear wall and separated from the chromatin substance.

Schürhoff (57), who adheres to the theory that the chromatin is derived from the nucleolus, says on p. 56 of his paper: 'Das Stadium der Synapsis ist nun dadurch ausgezeichnet, dass sich die Gesamtheit aller fädigen Elemente des Kerns dem Kernkörperchen anlegt. Wenn wir hier von den verschiedenen Theorien, die sich auf die Wechselwirkungen der einzelnen Chromosomen beziehen, absehen wollen, so lässt sich andererseits nicht in Abrede stellen, dass es in diesem Stadium zu lebhaften Beziehungen der künftigen Chromosomen zu dem Kernkörperchen kommt.' On p. 57 of his paper, however, he says regarding the behaviour of nucleoles during synapsis in the embryo-sac mother-cell of *Lilium Martagon*: 'Allerdings beobachten wir im Stadium der Synapsis regelmässig, dass das Kernkörperchen der Kernwand direkt anliegt, so dass während dieses Stadiums ein Auftreten von nucleolar Substanz nicht ausgeschlossen wäre, doch finden sich im umgebenden Zytoplasma keine Anzeichen für eine derartige Abgabe von Substanz.' In this Schürhoff contradicts himself. Between the chromatin substance and the nucleoles of the nuclei of the connective tissue of the pollen sac of *Arum maculatum*, he observed threads which join them and which after staining take the same colour as the chromatin, according to the statement on p. 63 of the same paper. As soon as the chromatin increases its staining power the nucleolus loses its intensive colour. On account of these two observations I believe that the threads are nothing more than chromatin formed from the substance of the nucleoli.

Even though the staining of the nuclear material is the same as the chromatin, it is very difficult to accept Schürhoff's opinion that both are the same chemical substance. It may be possible that the threads are

[1] Mentioned by Zimmermann.

formed to facilitate the transmission of secondary substance resulting from the activity of chromatin substance.

On the other hand, one finds no reason to regard the difference in staining between the nucleoles and the nucleus as evidence for Schürhoff's view of the latter.

Contrary to the statements of botanists thus far mentioned, Van Wisselingh, in his recent paper on *Zygnema cruciatum* (62), says that the chromosomes arise from the nuclear network and not from the nucleoles (p. 10).

Arthur Meyer (33 and 34) regards the nucleoles as a part of the nucleus, consisting of protein substances which resemble the nucleo-proteins and which belong, probably, to a special chemical group.

Regarding the latest view-point among the zoologists, it is worth while to refer to the publications and discussions of Schreiner (52–4) and Hogben (18).

Schreiner mentioned an excretion of particles through the nuclear wall by way of the nucleolar threads in the fat cells of *Myxine*, thus giving rise to stainable plasm rods. This author, referring to his own results, says on p. 138: 'Diese Untersuchungen haben als Resultat ergeben, dass das häufige Auftreten von feinen Plasmakörnchen in der unmittelbaren Nähe innerhalb der Kernmembran von der Nukleolarsubstanz herstammt.' Schreiner believes that these granular bodies or fuchsinophil plasma elements are active in the process of excretion, and that the fat substance is indirectly formed from the nucleolus.

Hogben (p. 286), Loyez, and Hegner (see Hogben) stated that the secondary nuclei originate from the granules which have been ejected from the nucleus of the oocyte nurse cells and oocyte follicles and transferred as *chromidia* into nucleoform bodies by the cytoplasm of the egg. He finally regards the nucleoles as transitory structures.

Schiller (47) agrees with Haecker, and adds: 'The nucleoli are still separated in soluble form from the nucleus during the inactive period of the latter as a kind of secretion at the beginning of the mitosis.' He also believes that the increase of nuclear substance, i. e. division, depends upon favourable external conditions.

Regarding the division I agree entirely with him, but not in regard to the increase under certain circumstances, as in the cyst cells in the parenchyma tissue of *Anthurium*. It is a fact that in pathological cases the nucleolus becomes larger with every following division. In this case, no doubt, the influence of abnormal conditions are of importance.

Zacharias (66) stated that the advanced age of the leaves of *Galanthus* occurs together with the decrease of the nucleolar substance of the nucleoli. This statement may hold true so far as it concerns *Galanthus*, but not always in other cases. Changes in form and size of nucleus and nucleolus are very different, according to the nature of the pathological circumstances.

At the end of this discussion of the behaviour of the nucleus and nucleolus we may come finally to the conclusion that the abnormal change in form of the nucleus depends directly upon water loss. Perhaps also short intervals of pressure, which may occur in the cell as the result of purely mechanical or physico-chemical causes, may play a part, as may also abnormal acidity, which in turn may be caused by infection. The specific normal behaviour of the nucleolus indicates, after all, that the latter is undoubtedly only a final product of metabolism.

2. *The Behaviour of the Protoplasm.*

We come now to the second point of our discussion, or the behaviour of the protoplasm during the development of crystal cells. This behaviour, especially in an ordinary raphid cell, has not as yet been made the subject of a special study.

As a result of observations we know of course that as soon as the calcium oxalate appears in the vacuoles the normal movement or activity of the protoplasm is disturbed, which is no doubt the cause of the lateral position of the nucleus. As already stated, the young cysts are characterized by their extraordinary density of protoplasm, in which numerous vacuoles gradually appear, instead of the large one which is usually observed in normal raphid cells. These vacuoles then fuse into one large vacuole in which crystal colonies are developed. The concentrated mass of protoplasm which appears so conspicuously in the young cysts reminds one of that of a large embryo-sac cell with its much smaller protoplasmic content. It clearly indicates an infected or pathological tissue. This abnormal accumulation of protoplasm may perhaps be explained by assuming that during or before the fusion of two protoplasts a concentration of the protoplasmic mass is effected, the cause of which is as yet unknown.

This concentration of protoplasm may be caused by the calcium oxalate which is present in liquid form and concentrated there as a result of the accumulation of that substance from a number of neighbouring cells by endosmosis, or through some other chemical or physico-chemical process which has previously taken place.

However, it is unquestionable that the extraordinary concentration existing in two adjacent protoplasts constitutes evidence of a high degree of acidity in the protoplasm of these cells, inasmuch as there is no question here of embryonic tissue or of endosperm cells.

It is not a question of wall dissolution, as has been observed in pathological cases, but of their bursting. This may perhaps be caused by a too great osmotic pressure. Irregular physiological processes take place in a certain cell complex which probably lead to the bursting. Whether or not these processes have their origin in abnormal external

conditions remains unknown. In a normal raphid cell we do not find such plasma concentrations as we found in the young cyst cells, and usually only one central vacuole filled with mucilage is developed where the raphid colony is gradually formed.

3. *The Formation of the Crystal Colonies.*

In *Anthurium* the wall of the polyhedric crystals and that of each raphid crystal serves as a plasma wall, and is in all probability of hemicellulose. This can be easily tested on sections treated for some time with a ferric ammonium sulphate solution, by which the crystals are dissolved.

In normal cells the presence of the cellulose wall around the crystal colonies probably means that it serves as an enlargement of the inside surface of the cell, an enlargement which has obvious physiological significance. On account of the separation of the crystal complex from the living part of the protoplast, the surface of the latter becomes larger, thus serving as a protection to that part of the cell which has not been affected. As a result of the irregular processes, we observe in the crystal cells an accumulation of the crystal substance in the form of raphid colonies near, beside, or behind one another.

The first investigator who studied *Anthurium* in relation to the formation of calcium oxalate in the parenchymatous tissue of the perianth leaf was Kohl (21). He made the statement that, owing to the insufficient assimilation in the cells of that organ, and also because the conflicting influence of salts which are formed later is excluded, primary calcium oxalate crystals are only to be found, while the secondary and tertiary are dependent on the influence of light, and are consequently formed in other parts of the plant. According to Kohl the primary crystals usually in raphid form are in tubes, which are for the most part parallel to the main axis of the petals. Furthermore, Kohl studied some plants among the Aroideae, where primary crystals occur in the form of polyhedric crystals. The author therefore studied both the cells containing the polyhedric and those containing monoclinic crystals.

Inasmuch as Kohl did not study the history of the development of the crystal cells in *Anthurium*, and was content only to make a statement of the crystal types formed in this plant, it was important to make a thorough study of it.

As he states, the petals of the flower are the best available material for this purpose, and I decided, therefore, to make a special effort to study those parts of the plant very carefully. However, no difference could be observed between the form of the calcium oxalate crystals in the petals and those of the leaves, stem, and roots.

Furthermore, the so-called crystal tubes (*Kristallschläuche*) of Kohl are not tubes at all, but are hypertrophied cells which are formed in the parenchymatous tissue after the fusion of a number of cells of a certain cell complex. They have not the form of tubes only, as Kohl contends, but they are round, oval, or curved, according to the position of the cells.

Having studied the developmental history of the crystal cysts, I have decided to make a careful study of other so-called *Kristallschläuche* described by Kohl and others, and shall not be surprised to observe the same phenomena.

According to Dalitsch's study on the leaf anatomy in the Aroideae, the raphids are formed in those cells which lie vertically on each other. Such cells are fused together, and the crystals are embedded in a dense mass of protoplasm. Dalitsch (p. 345) observed this in *Philodendron longi-laminatum*.

In Fig. 13, Plate III of his paper (8) the author demonstrates a case of several raphid colonies in a parenchyma cell near the epidermis of the leaf.

Recently a similar case has been described by Porsch. Engler (12), who mentioned Porsch as the author of the crystal cells in *Philodendron Sellowii*, did not give the title or the year of issue for that particular publication (p. 28). Probably neither Dalitsch nor Porsch saw any nuclear fusion, and did not carefully study the development of the crystal cysts, but, with Hansteen, regarded the fused cells as crystal tubes.

The crystal colonies in *A. scandens* and *A. Scherzerianum* are not in a common sac, as with those found in the regular raphid cells just discussed. Besides this distinction, every needle is separated from the plasma by a plasma and not a cellulose wall.

The solution of the cell wall in pathological cells is of course the cause of the formation of a number of crystal colonies in a single crystal cell, and probably the cause of their position, because the pressure of the protoplasm of the neighbouring protoplasts acts doubtless in different directions, whereas the fusions of the protoplasts do not all take place at the same time. The result is that here we are concerned principally with abnormal regulatory phenomena located in certain cell regions of the tissue.

On the piercing of the cell wall between two or three contiguous parenchyma cells, cell fusion takes place, and the calcium oxalate, together with the plasma substance, is forced near to the fusion point of the nuclei, the position at which the first crystal colony is to be formed.

It is highly probable that the union takes place in the immediate vicinity of the break in the cell wall or possibly at the opposite side of the break in the wall. Immediately after the union it may be that, in the first case, the two nuclei are carried along in the direction of the plasma movement, after which they fuse together. The first colony which appears indi-

cates the direction of the plasma pressure and the direction of concentration of the calcium oxalate.

A fact which serves as a logical argument is the striking peculiarity that, while the extreme outside parenchyma cell rows contain less water and consequently show mostly polyhedric crystals, the inside cell tissues contain more water, and therefore show monoclinic crystals.

There are formed, therefore, two rings which resemble those of Liesegang (29–31). This striking relation between these different crystal regions and the colloid theory of Liesegang can be observed in other cases, i. e. in two neighbouring cells with calcium oxalate crystals of different forms. This phenomenon is very common in nearly any plant tissue. Although an indirect application of the theory of Liesegang is possible in these cases of different crystal formations in the parenchymatous tissue, there is not yet sufficient material at hand to make a suggestion for a direct relationship between the colloidal theory and the cell fusion before the crystal formation.

The formation of calcium oxalate crystal cysts, whereby large cell complexes of the parenchymatous tissue are destroyed, must eventually have an effect on the physical appearance of the plant. Although such has not yet been observed, it is certain that the plant will show some effect as soon as the normal function of the vesicular elements are disturbed by the increase in the formation of the cysts.

Summary.

After having studied the crystal cysts of the genus *Anthurium*, the following final statements can be made:

1. Two different crystal regions separated from each other exist in the parenchymatous tissue of the perianth leaves of *Anthurium scandens* and *A. Scherzerianum*, of which the outside region consists of polyhedric crystals, while the inside is characterized by monoclinic raphid crystals with more water than the former.

2. The region of the monoclinic crystals is further distinguished from the other where polyhedric crystals occur, in that in the former fusion of cells and their nuclei occurs and precedes the crystal formation, while in the latter no fusion takes place.

3. Symplasts are formed as a result of cell and nuclear fusion, whereby often the whole parenchymatous tissue of the organ is dissolved.

4. The formation of the symplasts consists mainly in the gradual increase in the density of the protoplasm of two neighbouring cells and the subsequent hypertrophy of their nuclei. The latter assume positions on opposite sides of a common cell wall. After this cell wall has been broken

through by the increased physical pressure in one or both of the cells, the fusion of the nuclei takes place.

5. The large nucleus resulting from the fusion of these nuclei keeps its more or less round form as long as it shows a tendency towards fusion. It may be amoeboid, spiral, and lobed, and takes up a position along the cell wall, and very often at one end of the hypertrophied cell.

6. During the fusion of the nuclei several calcium oxalate crystal colonies are formed in different positions immediately around the hypertrophied nucleus.

7. At the end of the crystal formation in the symplast the nucleus takes a prolonged or oval form, while the nucleolus enlarges considerably, the nucleus becoming thereby homogeneous. Finally, the hypertrophied nucleus dissolves and the crystal colonies remain in the cyst cell or symplast.

8. From the latter it appears clear that the behaviour of the nucleus, i. e. the cell and nuclear fusion, is in relation to the separation of the different crystal regions.

9. It appears also, from the increase in size of the nucleolus at the end of the struggle for existence by the nucleus of the symplast, that the nucleolus is only an end product of metabolism.

10. The formation of these symplasts is an extraordinary pathological phenomenon; it is the first case known in which the formation of calcium oxalate crystal colonies is accompanied by cell and nuclear fusion.

In conclusion, I wish to extend my thanks to Drs. N. Kopeloff, H. C. Sands, and Henry Keller for their friendly help in preparing this paper for publication.

EXPLANATION OF FIGURES IN PLATE II.

Illustrating Dr. Samuels's paper on *Anthurium*.

The drawings were made with an Abbe apparatus from sections of the affected parenchymatous tissue taken from the perianth leaf of *Anthurium Scherzerianum*.

Fig. 1. Beginning of the formation of the hypertrophied cell which may be distinguished from neighbouring cells by the extraordinary content of plasma and the size of the nucleus immediately following the fusion process. Oil immersion, ocular 8.

Fig. 2. The fusion of several nuclei in a young cyst. Oil immersion, ocular 8.

Fig. 3. The fusion of two large cyst nuclei lying in a plasma ring. Oil immersion, ocular 12.

Fig. 4. The union of two large cyst nuclei which are lying in a plasma ring. Oil immersion, ocular 12.

Fig. 5. Young symplast in the central part of the perigone leaf. Nuclei after the fusion lying in an extraordinarily large protoplasmic mass, and moved more or less to the one extremity of the cell. Oil immersion, ocular 8.

Fig. 6. Symplast from the second layer of the parenchymatous tissue. Nucleus at the side wall of the cell. Raphid colony with curved crystals. Oil immersion, ocular 8.

Fig. 7. Symplast with nucleus at one of the poles of the cell, connected by plasma threads with the protoplasm wall, and situated in a dense mass of protoplasm. Oil immersion, ocular 4.

Fig. 8. A cyst in the parenchymatous tissue of a perigone leaf. Fusion of a cyst with a neighbouring cell and the fusion of their nuclei. The cyst occurs as a space in the tissue. Crystal colonies developed in different positions. Oil immersion, ocular 8.

Fig. 9. Cyst from the central part of the perigone, with the form of an embryo-sac and with protoplasm threads in the cell. Two nuclei united, i. e. lobed nuclei, lying along the side wall. Crystal colonies in more or less different positions. Oil immersion, ocular 8.

Fig. 10. Cyst nucleus in lobed form. Oil immersion, ocular 12.

Fig. 11. Fusion and union of a symplast nucleus with other nuclei in the parenchymatous tissue. Crystal substance already formed. Oil immersion, ocular 12.

Fig. 12. Shell-formed symplast nucleus from the parenchymatous tissue. Oil immersion, ocular 12.

Fig. 13. Lobed cyst nucleus lying at the side wall of a parenchymatous cell of the perigone leaf. Oil immersion, ocular 12.

Fig. 14. Cyst nucleus with spiral form and lying along the side wall of the cell of the same tissue. Oil immersion, ocular 12.

Fig. 15. Spherical cyst nucleus stretched out and lying along the side wall in the protoplasm mass of a cell of the anther tissue. Oil immersion, ocular 7.

Fig. 16. Nucleus of a symplast stretched out and lying along the side wall of a parenchymatous cell with newly formed raphides. The nucleolus has grown extraordinarily. Oil immersion, ocular 12.

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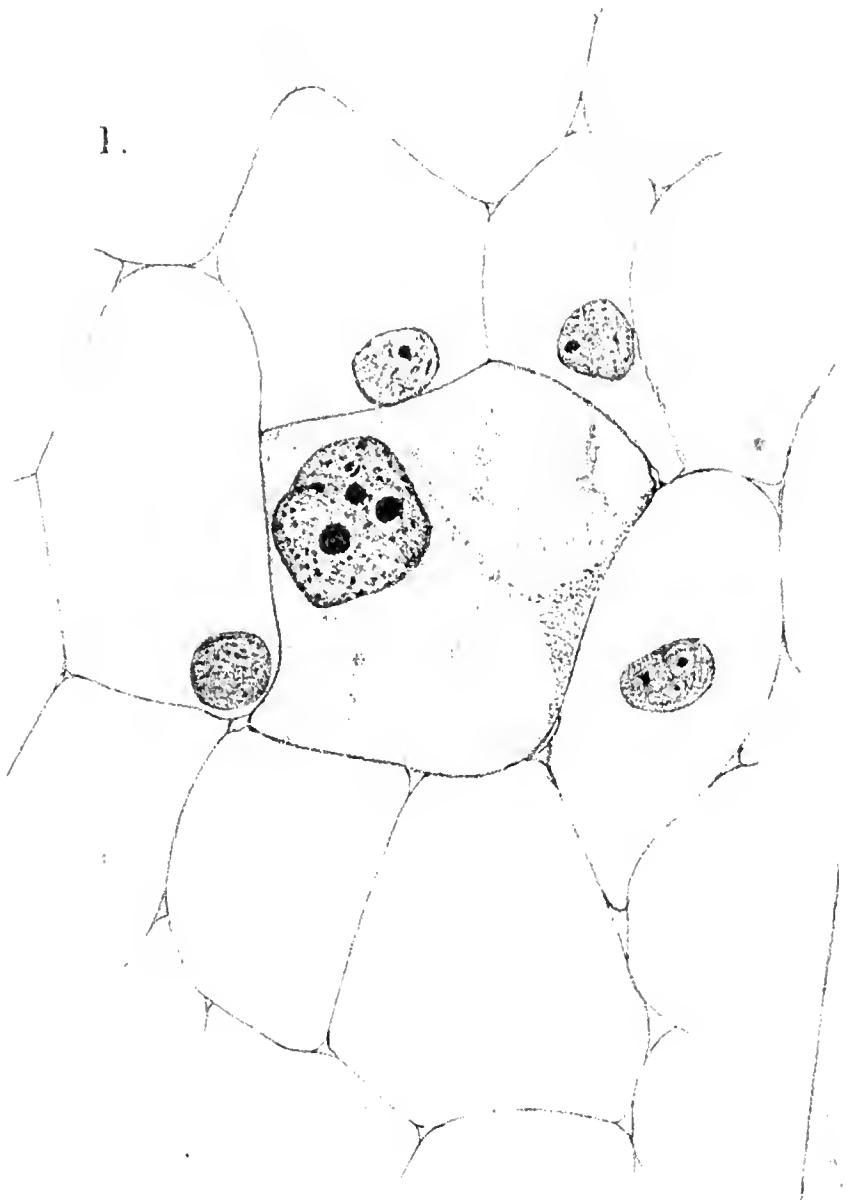
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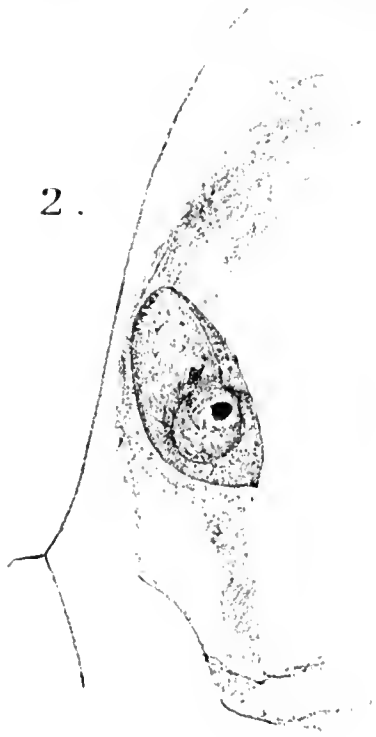
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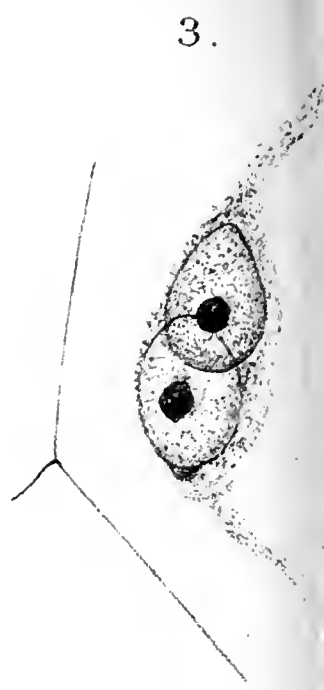
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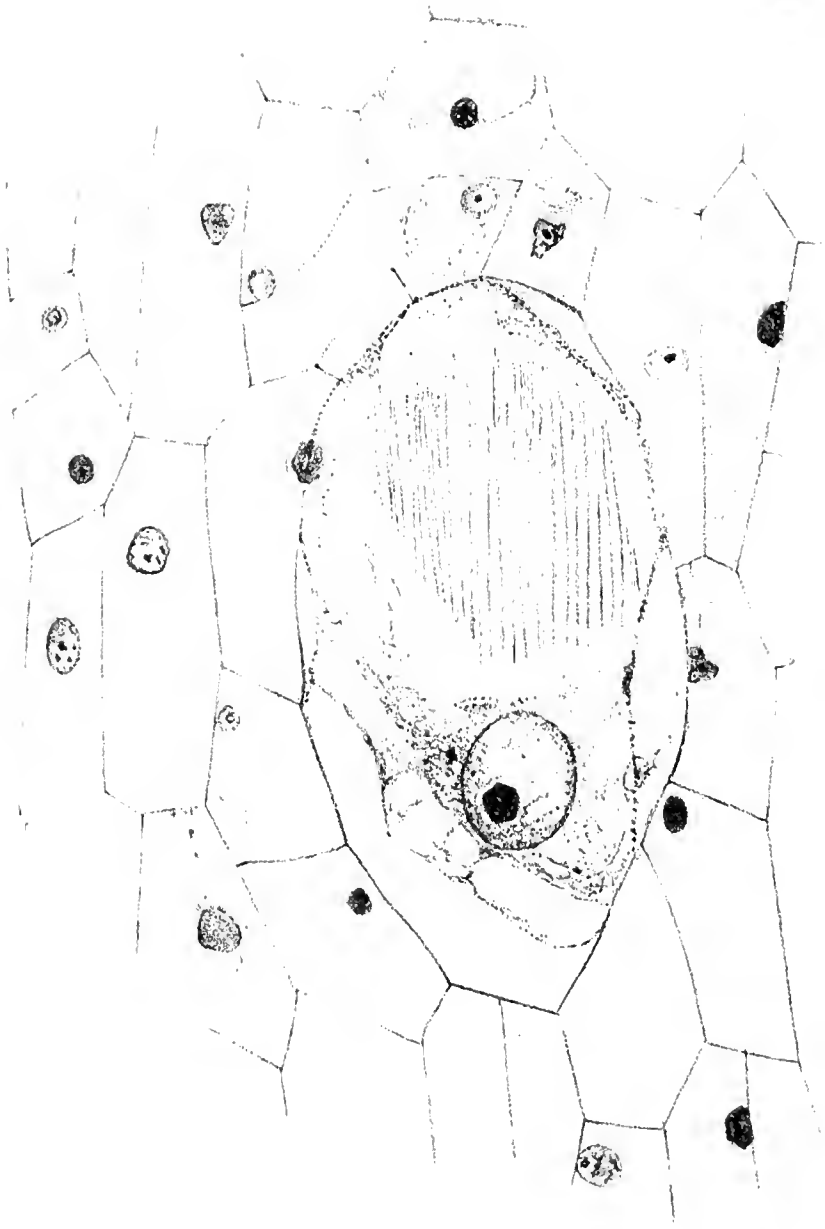
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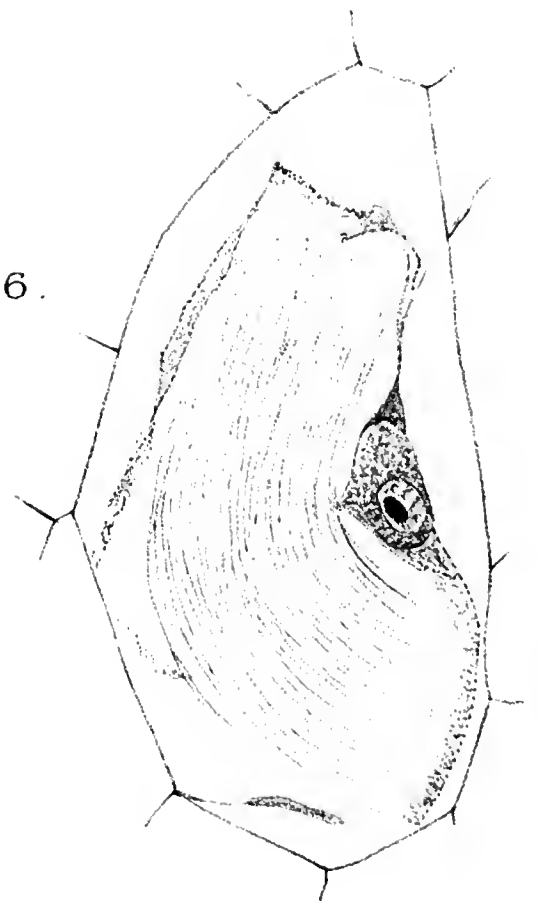
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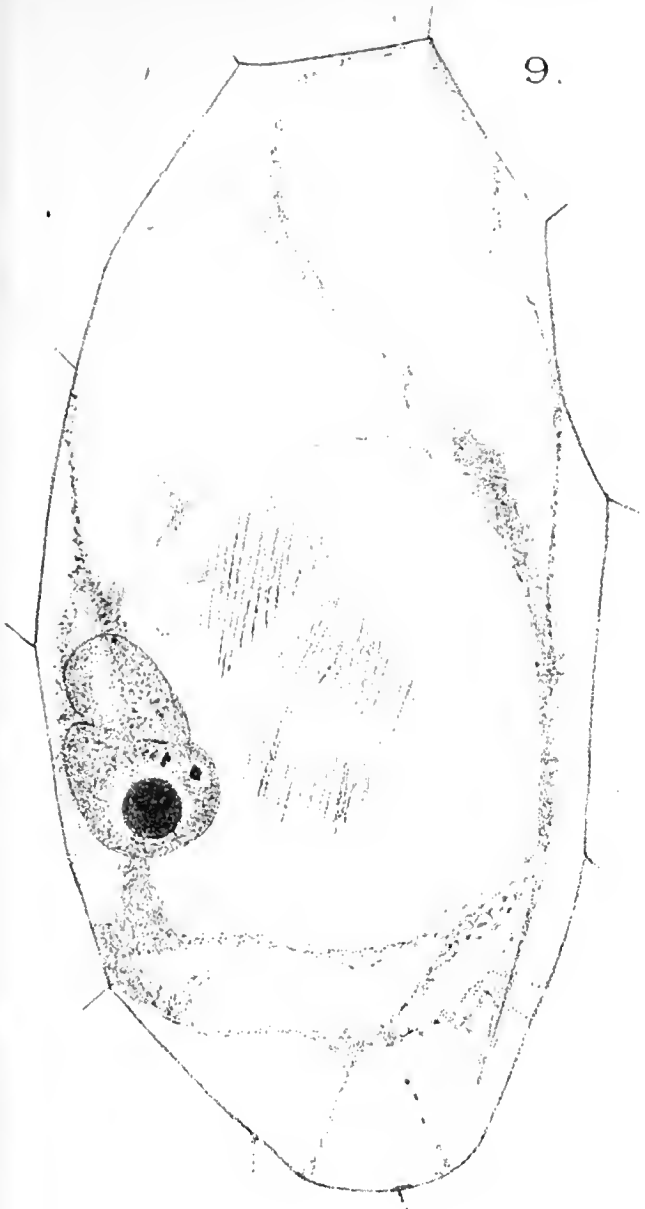

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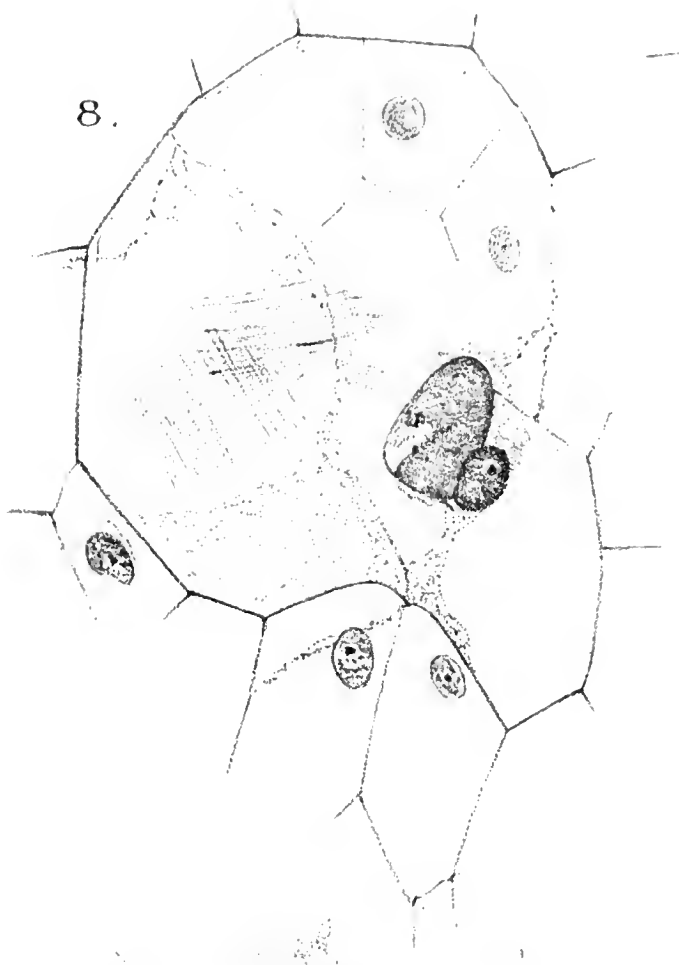

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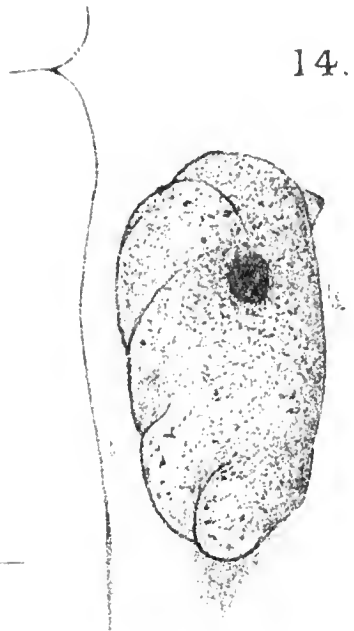

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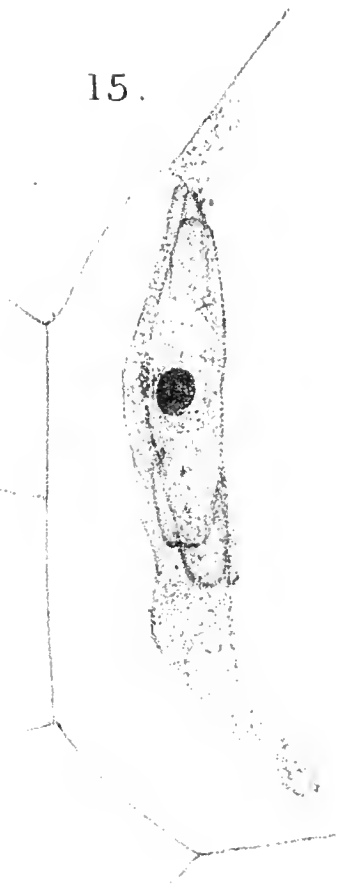

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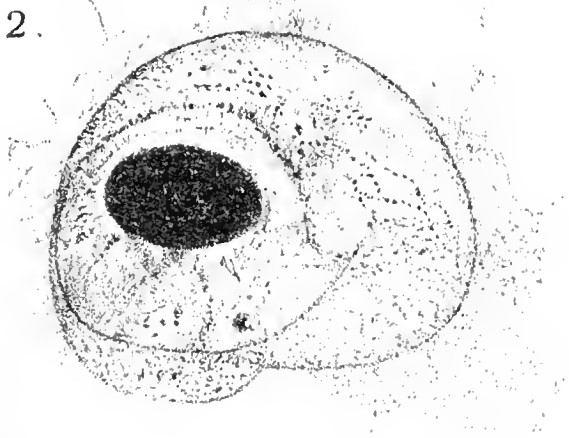

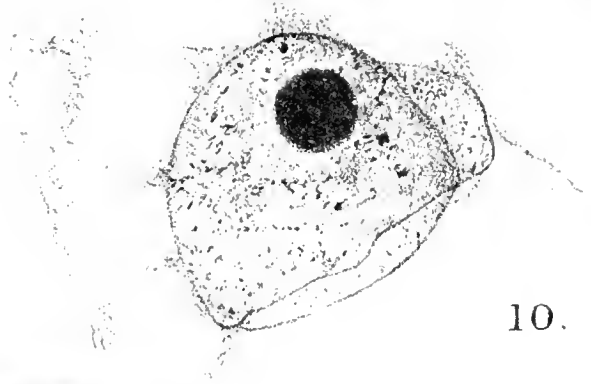

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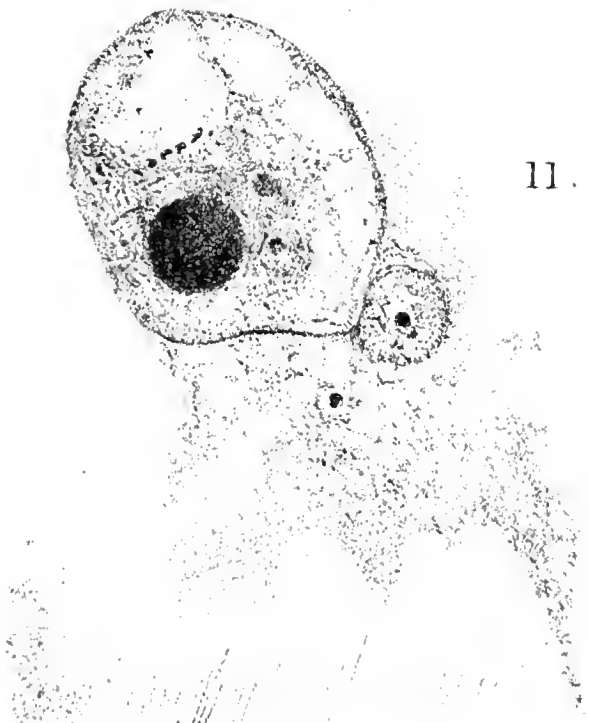

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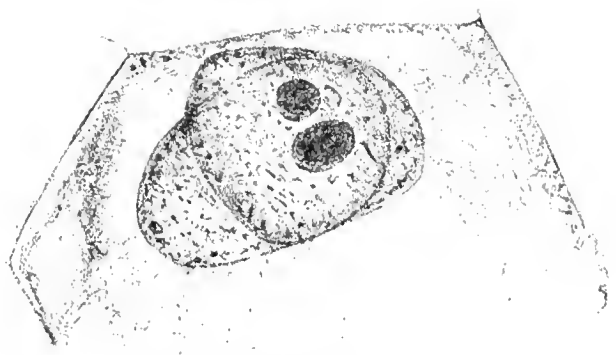

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The Relationships of the Different Types of Angiospermic Vessels.

BY

W. P. THOMPSON, PH.D.

With eleven Figures in the Text.

IT was recently demonstrated that in some angiosperms the simple or 'porous' perforation of the vessel has been evolved by the loss of bars from the scalariform type. At the same time it was pointed out that the scalariform type itself may either represent a retention and slight modification of the primitive scalariform pitting of the older gymnosperms and pteridophytes or may have been secondarily evolved by the fusion of circular bordered pits. Subsequently Miss Bliss (1) has called attention to the fact that in some cases the simple perforation seems to have been formed by the haphazard fusion of circular perforations derived from bordered pits after the manner which I had described as occurring in Gnetales (5). She considers that in some cases circular bordered pits have fused in horizontal rows to form scalariform pits which by a slight modification have become scalariform perforations, and these by a further fusion have become simple, while in other cases the circular bordered pits have fused haphazardly to produce simple perforations. The two methods are simply variations of the same fusion process. Brown (2), on the other hand, maintains that scalariform pits and perforations are not secondarily derived by pit fusions, but are retentions of the primitive scalariform pitting, and that the circular pits of angiosperms, as of lower plants, have been formed by the breaking up of scalariform pits through a reticulate condition.

It was therefore desirable that a comprehensive survey of angiospermic vessels be made to determine how the vessel had been evolved in each group, how commonly haphazard fusions had occurred, their significance in the general evolution of the angiospermic vessel, and their relationship to the process described for Gnetales. In this survey it became clear that the existence of certain other types of perforations required emphasis.

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The Widespread Occurrence of Transitions between Scalariform and Simple Perforations.

Transitions between scalariform and simple perforations (Fig. 1) are present in a great many species belonging to many different families. In a considerable number of species both scalariform and simple perforations may be found in the adult wood, and almost invariably these woods also show transitions between the two types. In many species simple perforations only are found in the adult wood but scalariform ones are present next to the pith, and these species generally show beautiful stages in the loss of scalariform bars in the region just outside the scalariform vessels. In almost every family in which species are found characterized by simple perforations and others characterized by scalariform ones, species may also be found showing the intermediate stages.

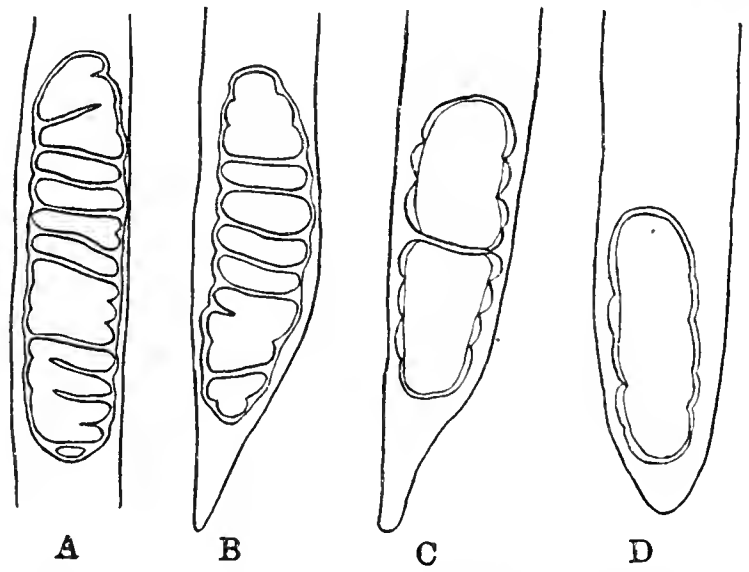

Fig. 1. A–D. Transitions between scalariform and simple perforations in the vessels of *Symphoricarpos occidentalis.*

While on the whole the transitions are commoner in the lower families they can be found even among families placed very high systematically. In the Caprifoliaceae, for example, beautiful cases are quite common. In Fig. 1 a group of transitional perforations are shown as seen in *Symphoricarpos occidentalis* (Hook.). For the details of the process the reader is referred to my earlier paper (5). Altogether I have noted such transitions in many species distributed among twenty-eight families from Casuarinaceae to Compositae. It may be concluded that at least in the great majority of angiosperms the uniperforate vessel has been evolved from the scalariform type by the loss of bars from the latter.

Vessels with Reticulate Perforations.

In some species there are perforations which are neither simple nor purely scalariform but which are in some degree reticulate. The reticulations are of several different sorts. The simplest kind is that which is not uncommonly associated with the scalariform type. Wherever scalariform pits or perforations are found, from ferns to angiosperms, there is a tendency for contiguous bars to be joined occasionally, either by a more or less definite cross-piece or as a result of their being not quite parallel. In some cases these connexions between contiguous bars become fairly frequent, and a more or less intricate net results. But the essential

scalariform nature of such perforations is evident. Particularly intricate examples are found in some Compositae (Fig. 2) and Ericaceae, in *Sauraja*, *Myrothalamus*, *Tetracera*, *Alsodeia*, *Cornus*, and *Tropaeolum* (Fig. 5). They are of the same nature as the reticulate pitting which frequently accompanies the scalariform pits of the side walls. It is well known that in the older vascular plants, at least, the multiseriate circular pits have been derived from scalariform pits through such reticulate conditions.

Another kind of reticulate perforation is found in a few species. We may take as examples of these the vessels of *Cordia* illustrated in Fig. 3. Here the perforation is in the form of a rather regular uniform net. The individual openings in the net are approximately isodiametric and about the same size as, or slightly larger than, the bordered pits on the side walls of the same vessel. The whole net is about the same size as the simple per-

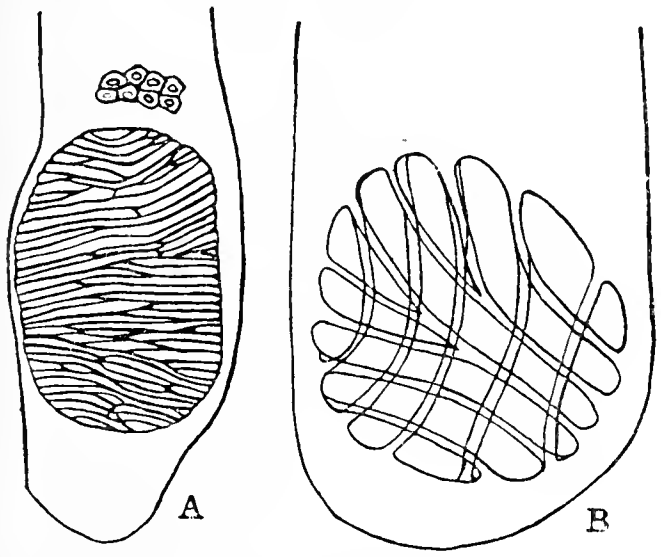

FIG. 2. A, B. Reticulate perforations in *Helianthus* sp.

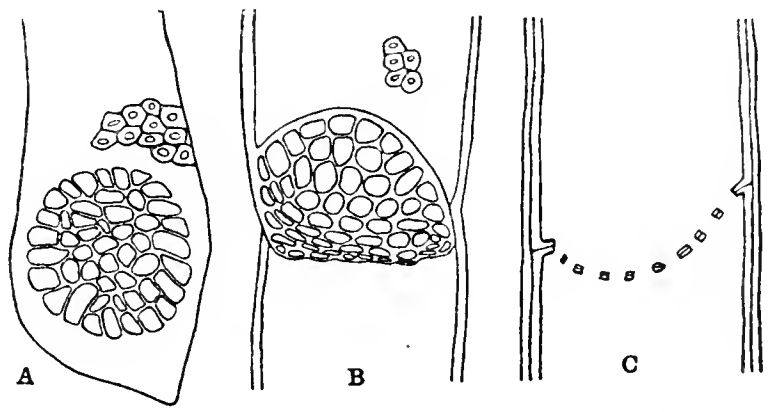

FIG. 3. A, Reticulate perforation from *Cordia myxa*. B and C, from *Cordia suaveolens*.

foration of other types. In many cases the net sags down at the centre into the vessel-segment below it. This is shown in face-view, Fig. 3, B, and in section in Fig. 3, C. In these perforations there is no indication whatever of the scalariform condition or of the fusion of pits to form the individual openings. In fact these openings are little larger than the bordered pits of the side wall, and appear to have been formed by the loss of the membranes of the pits accompanied by a slight amount of enlargement.

These reticulate perforations have been observed in several species of *Cordia* (Borraginaceae), *Tecoma radicans* (Fig. 4, A) (Bignoniaceae), *Vitex alata* (Verbenaceae), *Bougainvillea speciosa* (Fig. 4, B) (Nyctaginaceae), and *Boerhaavia* sp. (Nyctaginaceae). They never occur to the exclusion of other types. In fact in every case observed the great majority of perforations are of the simple type, though examples of the reticulate type are present in most sections. Their occurrence is very sporadic. I have carefully examined many other species in the families to which the above-mentioned forms belong without finding any trace of them. They occur in forms where the great majority of perforations are simple.

Reticulate perforations of this type appear to be quite different from those described above as frequently accompanying the scalariform type. They show no trace of the scalariform condition. It is, of course, conceivable that they are of the same nature as those previously described, and that the reticulation of the latter has gone much farther and the individual pores rounded up. In *Tropaeolum* one sometimes finds perforations approaching the *Cordia* type along with more numerous ones which are nearly scalari-

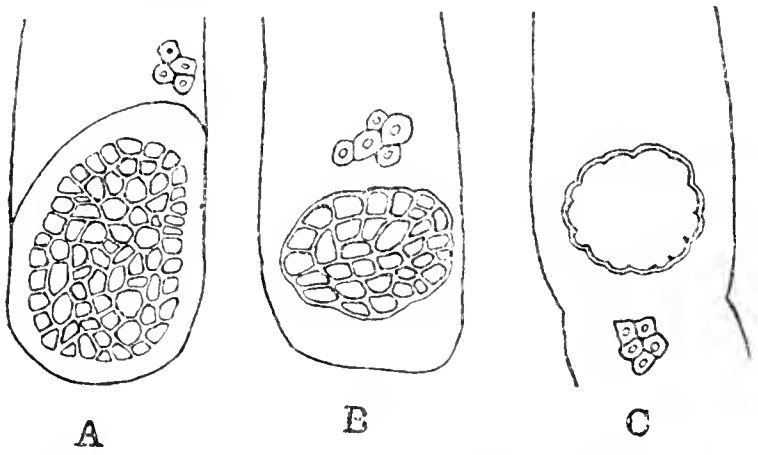

FIG. 4. A, Vessel from *Tecoma radicans*. B and C, from *Bougainvillea speciosa*.

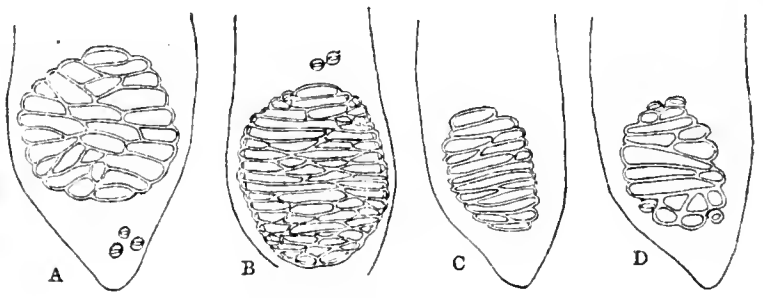

FIG. 5. A–D. Scalariform-reticulate perforations from *Tropaeolum*.

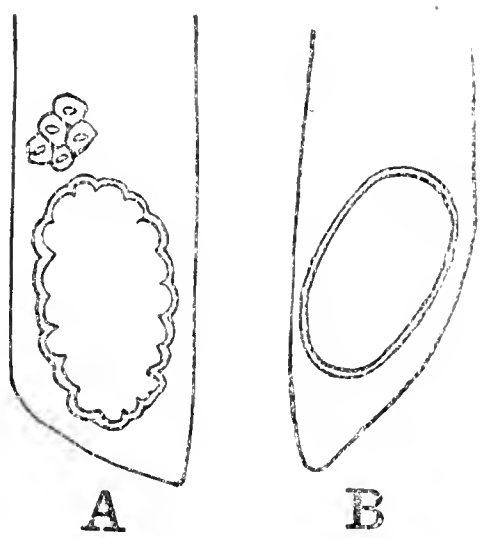

FIG. 6. Vessels from *Cordia callacosa*, A showing evidence of the loss of reticulations.

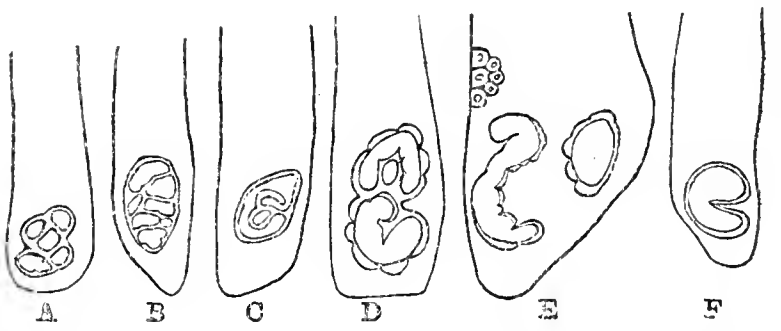

FIG. 7. A–F. Stages in the disappearance of reticulate perforations in *Potentilla monspeliensis*.

form (Fig. 5). But the origin and relationships of the different types will be discussed in later paragraphs.

In some cases there is evidence that the reticulate perforations of the *Cordia* type have disappeared and given the simple type. Appearances such as Fig. 6, A, are common next to the pith in *Cordia callacosa*. The scalloped edge of the perforation appears to be the result of the loss of a net. A similar perforation is shown in Fig. 4, C, drawn from *Bougainvillea speciosa*. In these forms I have never seen any evidence of the individual pores of the net fusing; apparently the whole net is lost at once.

In some species a very irregular net appears to be associated with a fusion of perforations, as pointed out by Miss Bliss. Such conditions may be found in certain members of the Rosaceae, and are illustrated in Fig. 7 from *Potentilla monspeliensis*, and in Fig. 8 from *Cydonia*. Somewhat

similar conditions have been observed in *Tropaeolum* (Fig. 9), and in *Pelargonium paelargium* collected at Cape Town. From these figures it seems clear that small perforations are fusing irregularly and producing large simple perforations. In many vessels in the region where this fusion is proceeding the perforations are scalariform or nearly so (Fig. 8), while in

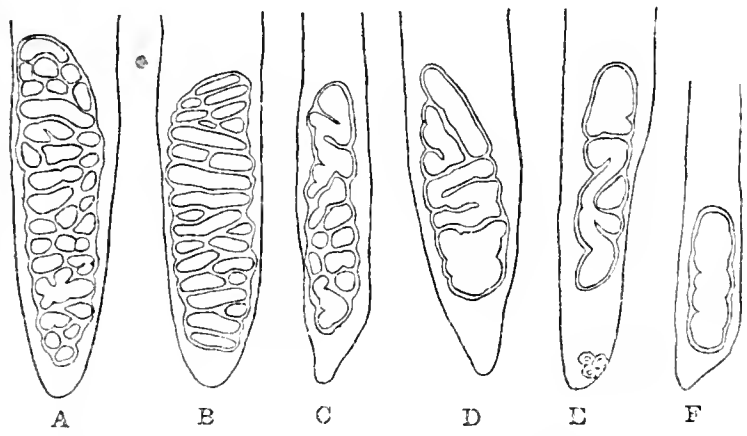

FIG. 8. A–F. Scalariform-reticulate perforations and their fusion in *Cydonia*.

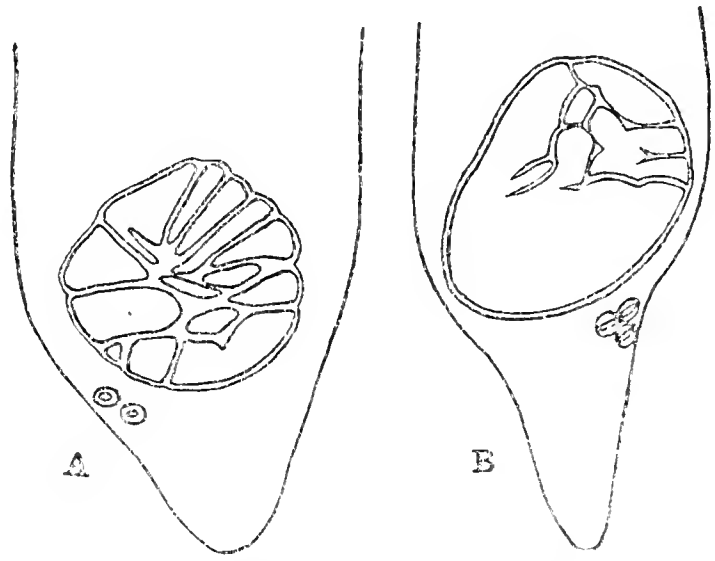

FIG. 9. A and B. Loss of reticulations in vessels of *Tropaeolum*.

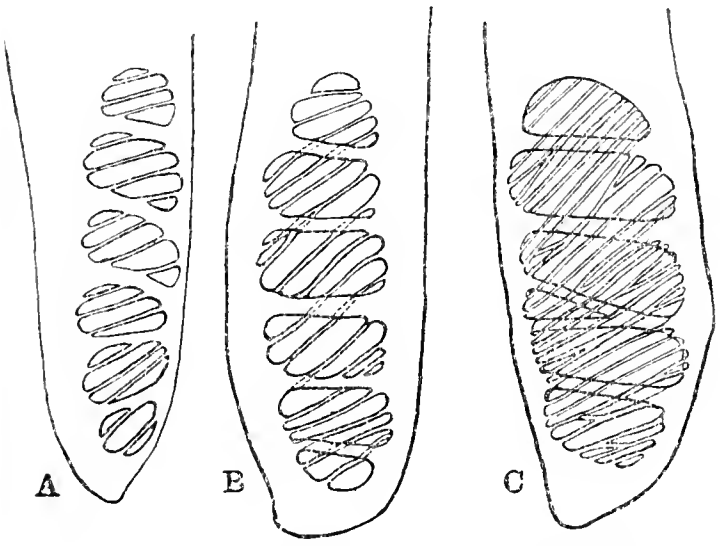

FIG. 10. A–C. Perforations in *Epacris coriacea*.

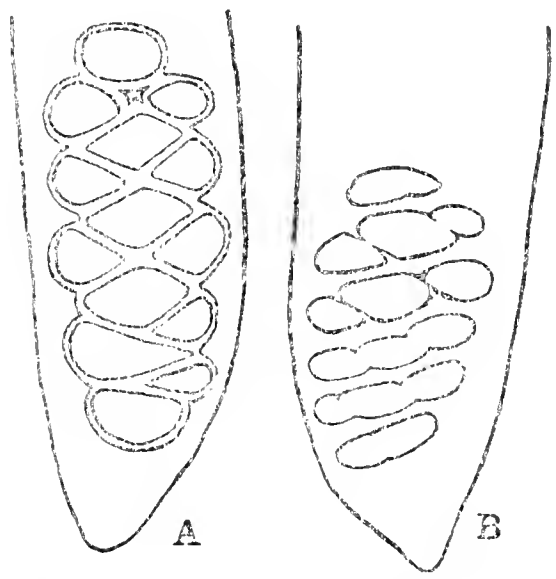

FIG. 11. A and B. Perforations in *Epacris coriacea*.

other vessels the reticulate condition which frequently accompanies the scalariform is evident. Such fusions are relatively rare and are usually found in herbs.

Still another type approaching a net is figured by Solereder (4) for *Epacris*. In this case there are peculiar clusters of perforations as shown in Fig. 10, A. Careful examination of many vessels shows, however, that these are really due to scalariform bars inclined at different angles on the two sides of the wall as seen in Fig. 10, B and C, and Fig. 11, A. Frequently the bars on one side are larger and farther apart than those on the other side. Sometimes the finer bars can be traced right across the larger ones, but in other preparations they cannot be so traced. In some cases (Fig. 11, B) the bars on one side disappear, while those on the other side remain.

Discussion.

The significance of these reticulate perforations in regard to the evolution of the vessel depends on their relation to the scalariform type, and this in turn depends on the origin of that type. I pointed out in an earlier paper that the scalariform perforation may have originated in two ways: it may represent a slight modification of the primitive scalariform pitting, or may have been derived by the fusion in horizontal rows of circular pits.

Miss Bliss has presented evidence that the scalariform pit and perforation in angiosperms have originated in the second way—by the fusion of circular pits. It is necessary to assume, on this view, that the circular pits were originally formed from the scalariform ones by the reticulation of the latter, and that in angiosperms the reverse process has occurred, and the circular pits gone back again to scalariform by a fusion process. She believes also that the irregular nets, as seen in Rosaceae, &c., are simply a variation in this fusion process.

Brown (2), on the other hand, holds that the scalariform condition is primitive in angiosperms, being a survival of the exactly similar pitting of the older gymnosperms and not a most remarkable instance of reversed evolution. The scalariform pit is being discarded for the circular one. It is first discarded on the side walls because, since these walls are in contact with a variety of cells differing in shape, size, &c., the scalariform pit loses its alinement. Under these conditions the circular pit is more adaptable and comes to prevail. On the end walls, however, which are in contact with walls like themselves, the scalariform type remains and becomes the scalariform perforation.

Miss Bliss's chief argument is the occurrence of intermediate stages between multiseriate and scalariform pits. Admittedly there are abundant examples of these transitions in angiosperms. But obviously the transitions may be read in either direction. Bliss reads them from circular to scalariform and Brown from scalariform to circular. The decision must be made on other evidence.

Brown states that in conservative regions the scalariform pit prevails, and that the multiseriate circular type characterizes the more specialized parts, while Bliss contends that the reverse is true. I am bound to state that in my experience with a large number of species Brown is correct. The order from the pith outwards as I find it is the familiar one of lower plants, spiral, scalariform, reticulate, multiseriate. And in a large number of species the scalariform pitting persists through the secondary wood with or without the circular type. It is, of course, possible in some forms to find scalariform pits in the older wood and compare them with carefully selected circular pits near the pith somewhere else. It is necessary to take into

consideration differences in the rate at which the transformation of one type into another takes place. In one radial row it may take place more rapidly than in another. Also the first vascular elements of one row may be farther out than those of another. Therefore sections which are slightly oblique and include more than one row may give very deceptive appearances. When the transitions are studied carefully in a single row of cells it is found that the scalariform pitting appears first, is succeeded by the reticulate type, and this in turn by the multiseriate circular type, just as in ferns and gymnosperms, and that in many forms the scalariform type persists through the secondary wood.

Moreover, adequate comparisons of the secondary wood of specialized and primitive families shows that scalariform pitting when it occurs is generally in the latter (such as Piperaceae, Monimiaceae, Cupuliferae, Magnoliaceae, Bruniaceae). As in other cases, the different tendencies in the evolution of wood structures are expressed in different degrees in different cases. But in general highly specialized woods show multiseriate and not scalariform pitting on the side walls.

Miss Bliss also argues that since in some cases in ferns the end walls are reticulate while the side walls are scalariform, therefore the end wall is more specialized than the side wall. Consequently in those angiosperms which show the scalariform condition on the end wall and multiseriate pits on the side wall, the scalariform must represent the more advanced condition. It seems to be stretching the doctrine of conservative regions rather far to say that one side of a cell must in all cases be more conservative than another side. Moreover, as a matter of fact there are plenty of reticulate side walls in ferns and many dicotyledons with scalariform side walls. The true explanation of the occurrence of multiseriate pits on the side walls and scalariform perforations on the end walls of the same segments appears to be that the scalariform pit is being discarded for the multiseriate, that since the side walls in dicotyledons are in contact with a great variety of smaller cells the scalariform pits loses its alinement and quickly becomes broken up, and that since the end wall is in contact with a similar end wall the scalariform condition tends to remain in this position.

The view that the scalariform pit is primitive in angiosperms and not derived secondarily by the fusion of multiseriate circular pits is then supported by (1) the extreme improbability of such a remarkable example of reversed evolution, (2) the succession of vessels with the different types of pitting and transitions in the first-formed wood, and (3) the occurrence of the scalariform type in primitive families and the nearly universal occurrence of the multiseriate type in highly specialized families.

If the scalariform perforation is primitive in angiosperms, being a retention and slight modification of the scalariform pitting of primitive gymnosperms and pteridophytes, there has been no fusion process in its

production, and consequently the various reticulate conditions cannot represent a variation of such a fusion process. Reticulate perforations of the type found in Compositae, Ericaceae, &c., appear to be modified reticulate pitting which is commonly associated with scalariform, and which in some cases is derived from the latter as a passing phase in the production of multiseriate pitting.

The uniform nets of *Cordia*, *Tecoma*, &c., are relatively very rare and sporadic, and occur in highly specialized families (Borraginaceae, Bignoniaceae, Verbenaceae), so that they appear to have no significance in the evolution of the vessel as a whole. Moreover, there is no evidence of pit fusions in these cases. The individual pores are of the same size or only slightly larger than bordered pits. The whole net appears to drop out at once. They are found in only a few of the vessels. It seems probable that they represent a special condition. Apparently the vessel-segments start to form alternate pitting on all walls, and then the membranes drop out at the ends. In some cases the net seems to be due to the fact that the end of one vessel-segment abuts on the side wall of another overlapping segment. The end becomes perforated, but the side on the other element retains its alternate pitting, in many cases losing the membranes. Sometimes two adjoining vessels have lateral communications, and it is not unnatural that in such conditions the reticulate condition derived from alternating pits should occur.

As for the fusions in *Cydonia*, *Potentilla*, &c., in some cases at least they appear to be due to the loss of reticulations in the same way as the bars disappear from scalariform perforations. It is to be noted that frequently both scalariform and reticulate conditions are found in the same perforated area which is breaking down to form the simple perforation. The scalariform pit has been transformed into the multiseriate through the reticulate condition, and there appears no reason why this transformation could not have begun in occasional forms before the pits became perforate. At any rate the reticulate condition frequently accompanies the scalariform, and may break down to produce the simple perforation in the same way that the scalariform does. But even if we grant that they are real fusions of multiseriate bordered pits, it seems that they could have little significance. They are of such rare and sporadic occurrence, and are found in such specialized forms (chiefly herbs), that their existence can be of little real importance, in view of the evidence that the scalariform type is primitive and the widespread occurrence of transitions between scalariform and simple perforations.

It is undoubtedly true that in the great majority of angiosperms the uniperforate vessel has been derived from the scalariform by the loss of the bars, and the balance of the evidence shows that the scalariform perforations are the result of a slight modification of the primitive scalariform

pits. These latter have also on the side walls become broken up to form multiseriate circular pits through a reticulate condition. The reticulate end-wall perforations are associated with this, or in some cases may represent special anomalies.

In Gnetales, on the other hand, the uniperforate vessel has undoubtedly been produced by the fusion of smaller perforations derived from circular bordered pits (5). There is no scalariform or reticulate condition in the secondary wood. Occasionally, since the pits are haphazardly arranged, two perforations derived from bordered pits may, in specially selected places, stand on the same horizontal level. But to use such specially chosen portions of an end wall as examples of the scalariform condition, as MacDuffie (3) has done, is to use the term scalariform in a sense quite different from that which it has always conveyed.

Summary.

1. Transitions between scalariform and simple perforations are found in the vessels of numerous species of angiosperms belonging to many different families from the lowest to the highest.

2. In a few species reticulate perforations accompany the other types. In some cases they are like the reticulate pitting which frequently accompanies scalariform pits; in other cases a regular net with isodiametric individual openings is found. In the former the individual openings may fuse irregularly and produce simple perforations; in the latter the net appears to be lost as a whole.

3. The evidence, which is reviewed, indicates that the scalariform *pitting* of angiosperms is a retention of the primitive scalariform pitting of the older gymnosperms and pteridophytes, and is not produced secondarily by the fusion of circular multiseriate pits. On the other hand, it has given rise to the circular pits of the side walls through a reticulate condition. It has also given rise to the scalariform *perforations* of the end walls.

4. Since the scalariform perforations are not the result of a fusion, the reticulate perforations are not the result of a variation in this fusion. They represent rather a special or abnormal condition generally found in specialized forms.

5. The perforations of Gnetales are not genetically related to any of the types found in angiosperms.

University of Saskatchewan.

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Age and Area:

A Reply to Criticism, with Further Evidence.

BY

J. C. WILLIS, M.A., Sc.D., F.R.S.,

European Correspondent, Botanic Garden, Rio de Janeiro.

With five Figures in the Text.

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GENERAL.

WITH a paper in October, 1921 (10), I began a second series of communications, of wider scope than the first ten, though also based upon Age and Area. My first intention was to continue the series in logical order until I had published the whole theory of Age and Area and its many implications, but when I fully realized that to do this would take perhaps six or seven years, I determined to bring out a book dealing chiefly with Age and Area, and incidentally with its further implications. To this course I was also strongly urged by various friends. The book (11) has now appeared, and contains original matter that would have sufficed for at least another dozen papers. A gap is thus made between this paper and the last, which I must ask my readers to regard as filled by the publication of the book.

At a joint session of the Zoological and Botanical Sections at the meeting of the British Association at Hull in 1922, there was a discussion upon 'The Present Position of Darwinism', understanding thereby the theory of Natural Selection. At the meeting I continued the attack upon Natural Selection which I have persistently carried on since 1902 (cf. 5.

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pp. 441–4, and especially 7, 12). Papers based upon Age and Area were read by Mr. G. Udny Yule and myself, the title of mine being 'On the Inadequacy of the Theory of Natural Selection as an Explanation of the Facts of Geographical Distribution and Evolution'. We had read papers upon somewhat similar lines at a meeting of the Linnean Society in February (abstract, 13), and the change of view-point of the biologists since that meeting was clearly marked. Many of the speakers were concerned less with defending Natural Selection than with attacking Age and Area, which was variously described, then or in subsequent newspaper articles, as 'worth less than nothing', 'utter balderdash', 'a few well-known facts distorted', and 'a theory whose career has already ended', with more to this effect.

As usual the objections were mostly due to a misunderstanding of the hypothesis. In spite of the fact that throughout I have made clear that it must not be applied to less than ten species at once, and those allied, and that these must only be compared with other groups allied to the first, persistent and somewhat pathetic attempts are made to apply it to individual cases, or to unrelated forms. As an instance of the kind of argument employed, I may quote from the second review of my book in 'The Times' the sentence (which falls into both errors) 'Is the barn-owl, almost world-wide, more ancient than the three-toed ostrich, found only in South America?'. In my book, upon p. 62, I have given an illustration of the way in which, though only dealing with fives instead of tens (as should always be the case), Age and Area is inapplicable to three out of five cases, although all species are following the law with complete exactness, a thing that is somewhat improbable in real life. In chapter ix I have devoted much attention to this difficulty, and I have repeatedly pointed out, there and elsewhere, that inapplicability to single cases does not in the very slightest degree invalidate the theory.

The single-species difficulty seems a stumbling-block to a very great number of people, and it is needful once more to emphasize the fact that one cannot reduce statistics to individual cases—the very name statistics indicates this. To take an illustrative case from ordinary life: no amount of study of individual members of the Goddard family would tell us much about the origin of the family, and it might be very difficult to make out anything, so far as I know, from history (corresponding to palaeobotany). We might ultimately know every detail about their anatomy and physiology, and even about their genetics, but none of these would be likely to give a clue. But supposing that we add up the numbers of Goddards in every county, in the more slowly-moving section of the population, the farmers, we obtain in this way *statistics* which show that they form 0·55 per cent. of the farmers in Berkshire, and no more than 0·31 in the adjacent counties, whilst they almost disappear beyond Dorset, Derby, and Norfolk. Con-

sequently one may infer with fair probability that Berkshire was the first original home of the name (2), for not even the most enthusiastic supporter of Natural Selection and adaptation would contend that a Goddard was especially adapted to Berkshire. The biologist fights shy of statistics, except when he wants to prove something in his own work, and at other times is apt to believe in the old gibe that statistics can be made to prove anything—a gibe which really refers to statistical fallacies, into which the untrained experimenter with statistics is very apt to fall. But in the case of the statistics upon which Age and Area is based, the fact that the work has been taken up *con amore* by a statistician of Mr. Udny Yule's reputation should be sufficient to show that there is no fallacy involved, as one or two speakers at Hull seemed to imply. There are very many problems, and those often problems of wide range, which can only be solved by means of statistics, but from their very nature these statistics cannot be applied to individual cases. Statistics show that the average Scot is about 20 lb. heavier than the average Englishman, and it does not in the very slightest degree affect this result to say, 'Oh, but Smith weighs 13 st. and MacPherson only 10'. And the bulk of the single-species arguments against Age and Area are almost exactly similar to, and equally as valid as this.

It is probable enough that *no two species*, allied or not, travel at exactly the same speed in their diffusion over the surface of the earth. But each will, on the average of long periods, travel at more or less of an average rate, so that in twice the time it will cover twice the distance. A species *A* may travel at the rate of 50 miles per 10,000 years, say, and in 20,000 will cover a distance of 100 miles, while *B* may only travel 5 miles in the 10,000 years, and 10 in 20,000, requiring therefore 200,000 years to cover a distance traversed by *A* in 20,000. The area covered will be the same, yet *B* will be ten times as old as *A*. But both fall in with the Age and Area theory, and with equal exactness.

If one were to take a group of closely allied species, e.g. species of similar habit belonging to the same genus, it would seem probable that as they will all have much the same mechanism for dispersal, and somewhat the same type of reactions to surrounding circumstances, they will spread at rates not very widely different. And taking at least ten will do away with the small differences that will occur between them, so that one group of ten will give much the same result as another group of ten allied to the first. One group, for example, might show rates of spread represented by 1, 3, 2, 1, 4, 3, 2, 1, 2, 3, and another by 3, 2, 1, 4, 2, 4, 1, 3, 2, 1. On adding these up one finds the first to give an average of $22/10 = 2{\cdot}2$, and the second of $23/10 = 2{\cdot}3$, a very small difference. If one deal with species always in *groups* of at least *ten allied forms*, and compare only with similar *groups allied to the first*, one will find that Age and Area is closely followed.

That it *is* closely followed is shown by the extraordinary success of

predictions based upon it and upon it only, of which further examples are given below. It is necessary to make once more clear that the success of prediction upon so large a scale as has now been effected proves the supposition of Age and Area so completely that it can only be displaced by finding some other hypothesis that will also explain the facts and make the predictions—a thing that no one has as yet attempted.

The general principle of Age and Area, when once grasped, seems almost axiomatic, as I have already pointed out on various occasions, and would probably be accepted with little difficulty were it not, among other reasons, for the lingering influence of Natural Selection, which has always insisted that competition was the chief agent in determining the areas occupied. In fact, it has been predicted by two of my supporters, and various reviews, letters, and notices have led me to expect the same, that within a short time Natural Selection will be quietly dropped, and people will say that Age and Area has long been obvious to the meanest intelligence, and that there was no need to make such a fuss over a simple axiom.

Another objection which was strongly urged at Hull was that endemics must be regarded as chiefly relics. I have already devoted much attention to this point, e. g. in my book, p. 88, but it evidently requires further emphasis. People who work in much detail with individual species, and come across many which are obviously of relic nature, are apt, more or less unconsciously, to exaggerate the number and importance of these cases, while they do not properly realize that the number of local species to which no stretch of imagination can apply the term relics is enormous. There are in Brazil alone 240 local species of *Eugenia* and 200 of *Paepalanthus*, to take merely a couple of genera. Five or six such genera would equal the whole number of species that are usually regarded as relics. It cannot be made too clear that the relics are outnumbered by about 60 or 70 to 1, so that when dealing with large numbers, as in statistical work, they are quite lost in the crowd. The objection is really a single-species objection in another form. It has been pointed out in the book, e. g. on pp. 94, 243, that in dealing with such forms from an Age and Area point of view, one must obviously include the 'fossil' area.

As I pointed out at Hull, no one has attempted to answer the many queries that I have propounded upon this subject in (8), p. 349, in the book, p. 89, and elsewhere, though one speaker said that time enough (four years) had not yet been allowed. If in four years no one can answer one of these questions, which could now be multiplied if required to a hundred or more, the chance of any serious reply seems but a small one.

A paper in the July number of the Annals (3) gives a striking illustration of the kind of work that will probably mark the next stage in the development of Age and Area. There Mr. J. R. Matthews takes the flora of Perthshire. At first glance it seems as if it had been distributed quite

independently of Age and Area, but when analysed it appears that 82 per cent. of the flora belongs to the more recent 'lowland' type, and has closely followed Age and Area in its distribution, while the remaining 18 per cent. 'are essentially arctic-alpine or boreal plants. These, as proved by the fossil record, are relics of a northern flora formerly more widespread, and a gradual elimination since glacial times has produced their present discontinuity of distribution.' But if one were able to determine the whole fossil record of these species, one would find, I have not the slightest doubt, that in their time of spreading they also followed Age and Area. Mrs. Reid, our leading palaeobotanist of the Tertiary, wrote to me about this paper, and allows me to quote the following sentence: 'Mr. Matthews . . . has proved that, though Age and Area on the surface appears not to hold particularly well, it is not because it really fails, but because it is masked by a disturbing cause, and when that cause is discriminated and eliminated, there stands the law fully evidenced.'

In actual fact, the results of the Age and Area law may be seen even on this receding highland flora, by working with larger areas, as is done below in dealing with the flora of Dublin, where it is shown that the plants of this highland flora that occur in Dublin and Wexford can be mainly predicted from determining those that occur in Wales at corresponding distances from Duncansby Head.

The general proposition put forward in Age and Area—that the area (sum of individual areas) covered by a group of ten or more allied species depends upon its age (sum of individual ages), whether within the country concerned, or (taking the whole world) from the time of its first evolution—is now being accepted by many biologists, but the further deductions which logically follow from its acceptance are still a bugbear to many, and I have myself come across two or three who say that they would accept Age and Area were it not for these inevitable sequels.

The general standpoint taken up with regard to the relations of Age and Area to the theory of Natural Selection, which was set forth in very limited time at Hull, has been more fully elaborated in a paper in the 'Nineteenth Century' (12), to which reference may be made by those who desire a simple statement of the chief points of difficulty that Age and Area has brought up for the latter theory to surmount if it can. It has long been recognized that Natural Selection has only been repelling attack with greater and greater difficulty, and that it is hopelessly unable to explain the results of work upon Mendelian lines. Age and Area, which has opened a range of facts as widespread as those of Mendelism, and as inexplicable by Natural Selection, has thus brought a formidable attack to bear from a new quarter and with new forces.

The acceptance of Age and Area, with its allied proposition of Size and Space, makes easily comprehensible many phenomena which have long

been regarded as insoluble problems in geographical distribution. No theory based upon adaptation to conditions could hope to explain why the genera and species were distributed as they are—why, for example, they overlapped one another in such a casual way, some occupying large, some small, areas, and no two coinciding. It was almost impossible to conceive that conditions should overlap in this extraordinary way (cf. the map of the distribution of *Ranunculus* in New Zealand in 8, p. 343, and in 11, p. 156), and perhaps even more impossible was it to understand why, as a rule, a genus retained its area unbroken, except by large barriers like expanses of sea. If there were much killing out of species by competition, one would expect a good many genera to show broken areas.

So utterly hopeless did it seem to be ever to find explanation of the simplest facts of distribution upon the theory of Natural Selection, that for many years work upon general distribution had been practically abandoned, or confined to speculation with little fact to back it (cf. Hooker's remark, 11, p. 104). Practically the only important work of the last twenty years has been that of Guppy, which has led him to conclusions diametrically opposed to the Darwinian theory. Even so simple a fact as that *Coleus elongatus* is confined to the summit of Ritigala in Ceylon, while *C. barbatus* occurs there and also ranges tropical Asia and Africa—a fact which can be matched from almost any large genus of animal or plant—is quite incapable of explanation by the theory of Natural Selection.

If one adopts the very simple theory of Age and Area, which has been placed in a position of great strength by the fact that it can be, and has been, so successfully employed in making predictions, all this is made clear. If we start with a hypothesis A, and from it deduce that B must therefore occur, and then find, upon examining the facts, that B *does* occur, we get a very strong argument indeed in favour of A. When a hypothesis A has been successfully used in this way on many occasions, it can only be disproved by producing a new hypothesis X, which will explain what A explains, and allow at least the same predictions, or as many, to be made. Successful prediction goes a long way in proof of the correctness of the hypothesis with which we set out, when carried out many times, without failure, as has been the case with Age and Area. This method of proof, however, is as yet somewhat new and unfamiliar to most biologists, having been only rarely employed, and then mostly in connexion with work upon breeding on Mendelian lines. Age and Area has now been used to make more than a hundred predictions about geographical distribution and endemism. As every one of these has proved correct, within comparatively small limits, very strong evidence—not *a priori*, but in support of a rival hypothesis—is now required to displace it.

Age and Area imagines that species spread, under the pull of the many and various factors acting upon them, at a rate which over long periods of

time is slow and uniform, or fairly so, and which thus allows acclimatization to go on at the same time. Age thus becomes a measure of dispersal, or vice versa, but, as already explained, the measure probably differs for every species, though all will spread, if unimpeded, about twice as far in 100,000 years as in 50,000. The spread is everywhere impeded by ecological and physical barriers, and will ultimately be stopped in one or more, or all directions, by one of these barriers, perhaps most often by a physical barrier, or by a sudden change of climate. There seems no reason to suppose, however, when one looks at such a map as that of the species of *Ranunculus* in New Zealand, that most species have reached their limits of dispersal.

Age and Area, then, implies that, on the whole, area occupied increases with age, and that as a general rule the very localized species are not the failures, as used to be imagined, but the young beginners. Whether the increase with age is in direct proportion or not, we do not yet know. There seems reason to believe that at first, when a species is probably represented by few individuals on a small area, the rate of dispersal will be much slower than when it becomes more common, but when once it has reached reasonable commonness on a considerable area it would seem probable that further dispersal will be at a more or less uniform rate.

The study of the localized species (cf. maps in 8, p. 343, or 11, p. 156) shows that they appear within, or very close to, the boundaries then occupied by the genus, but in what to us at present appears a casual way. A consideration of the map of the New Zealand species of *Ranunculus* upon p. 156 of my book, and of the diagram upon p. 76, will make this clear. Even in the case of the most localized species of all, confined to a mere spot of ground (cf. p. 151), where the conditions now existing must be practically the same as those under which it arose, one can see no special reason for its appearance, nor anything of special local adaptation in the characters that distinguish it (cf. pp. 225–7).

Now, as has been shown in the book, chaps. xvi, xx, genera appear to obey the same rules as species, also appearing in this casual manner; and they spread in the same way with age, increasing their number of species on the whole as they do so. As has already been frequently pointed out, no two genera or species will spread at the same rate, though all will follow the law of Age and Area. The result will therefore be what we actually see in nature—a vast multitude of more or less rounded areas, overlapping one another in every conceivable way, and of every possible size.

One of the most essential differences between this and the Natural Selection position is that under Age and Area one need no longer engage in the hopeless task of finding differences between species in characters that are of importance in the struggle for existence. If they exist, well and

good; but obviously they rarely exist in any marked degree, or the figures for distribution would not come out in such definite agreement with those that would be expected were Age and Area alone operative. Under the latter supposition we regard most species as not yet having necessarily reached their limit possible of dispersal.

Under Age and Area species are supposed to owe their differences in distribution not primarily to differences between *themselves*, but to differences in the time at which they were first evolved, or first appeared in the country under consideration, and secondarily to differences in the average rate at which they are dispersed. Into this latter factor there, of course, enter all those differences due to dissimilarities between the species themselves. Trees, for example, will spread at a slower average rate than herbs, Cruciferae probably more slowly than Compositae, and so on. By taking the species always in groups of ten, and comparing these with other tens allied to the first, chance differences between them will have their effect reduced to a minimum.

It will be seen that this method of looking at species and their distribution substitutes for the essentially 'vital' character of distribution under Natural Selection a much more 'mechanical' conception, that species owe their distribution primarily to their age, a distribution checked only by barriers, mechanical or ecological. On the old theory species were regarded in general as having reached their limit of possible distribution; on the new they are regarded as usually in process of expanding their areas, and generally with extreme slowness. On the old theory species of very limited area were looked upon as owing that smallness to the competition of other more successful types, and therefore as the failures in the struggle for existence; on the new, they usually owe it to the circumstance that they are still comparatively young, and have not had time to occupy larger areas.

Now the relative values of these two theories can be quickly tested by putting them to the proof, and using them to make predictions as to distribution. If only one of them can be thus used, and at the same time proves to be successful in its predictions, then it is clear that the evidence in favour of that theory becomes very strong. This is exactly what happens in the present case. No one has ever suggested that Natural Selection can be used for prediction, whereas Age and Area has already been used over a hundred times with success. So easy is it to make predictions, and so regularly are they successful, that it is almost literally true to say that one may at the present time 'shovel' in results in the study of distribution. The criticism that has been directed against Age and Area is of the arm-chair *a priori* type; but if the critics would take the trouble to try the methods for themselves, I feel certain that they would not long decry them. In the present paper I make some predictions of larger range

than hitherto, and it will be seen that they are successful, and that their results have bearings upon many problems of distribution.

There were many minor criticisms brought up at Hull, and in reviews of my book and elsewhere, and some of these will be dealt with in later papers.

The Criticisms of Mr. C. Tate Regan (with a Note on Distribution of Family Names).

According to the very careful report of the papers and discussions made by my friend Mr. G. Udny Yule, and according also to my own notes, Mr. Tate Regan 'questioned whether areas gave a true hollow curve; he thought that the curve was probably modal, and appeared J-shaped owing to the grouping of the lower portion. He did not consider that hollow curves had any particular significance; you could obtain them from all sorts of data, e.g. from the numbers of occurrences of surnames in the London Telephone Directory. He emphasized the importance of sterility and of isolation in evolution. Dr. Willis's views did not explain adaptation, which could only be explained by selection of slight favourable variations.'

It will be seen that this is much less a defence of Natural Selection and the other features of the Darwinian theory than an attack upon Age and Area. That Mr. Tate Regan himself holds the general principle propounded in Age and Area is clear from the following extract from the chapter on Geographical Distribution in his book (4) upon 'The Freshwater Fishes of the British Islands', 1911, p. 271: 'The fact that species which have a very wide and a very similar distribution on the mainland of Europe and Asia have very dissimilar distributions in the British Islands can only be explained by supposing that our islands were connected with each other and with continental Europe comparatively recently, when our eastern, and probably our southern, streams were tributaries of continental rivers and received from them the fishes which they contained; only nine or ten of these had reached Ireland before it became a separate island, and the distribution of the rest in Britain at varying rates according to circumstances has not yet proceeded long enough to spread them all over the island.'

This paragraph (which I threw upon the screen at the meeting of the British Association at Hull) is so clear a statement of Age and Area that, had I not made a brief preliminary statement four years earlier, I should feel inclined to ascribe priority to Mr. Tate Regan. His phrase 'at varying rates according to circumstances' so clearly expresses what I have tried once again to make clear above—that no two species travel at the same rate, but that all follow as closely as possible Age and Area, spreading twice as far in twice the time (when the time is long)—that I should like to have Mr. Tate Regan's permission to adopt the phrase for future use. The sentence that concludes the quotation gives the rest of the proposition of

Age and Area—'has not yet proceeded long enough to spread them all over the island.' There is no suggestion here of competition, of adaptation, or of any of the other outstanding features of the Darwinian theory.

The author of this paragraph explained that he imagined all the fishes to arrive at the same time, or in other words that they were all at the same time at the line of junction with the Continent. This of course means that though in Britain they might be distributed 'at varying rates according to circumstances', this was not so upon the mainland, where they all simultaneously reached the same line. Or if this interpretation be rejected, then we must suppose that those fishes that first reached the line of division must have waited there until all had arrived, so as to effect a kind of Norman conquest of the English rivers. And this although England was attached to the Continent for a very long period.

The only reasonable explanation of the distribution of the freshwater fishes, as of the plants, is that they came over so soon as they reached sufficiently far down the Seine and the Rhine, &c., each one spreading at its own average rate, when long periods are considered, and that the dispersal in Britain has been mainly determined by the time factor, so that, in the words of Mr. Tate Regan, 'Ireland has only ten of the twenty-two species. . . . In Britain there is a considerable diminution in the number of species towards the north . . . in the Northern Highlands, as in the outlying islands, there seem to be no indigenous true freshwater fishes . . . a similar decrease . . . takes place from east to west. . . . Quite a number . . . are absent from Wales west of the Severn system. . . .'

With regard to the question whether areas give a true hollow curve, the matter seems to me one of comparatively small importance. It is really a matter of the size of the units of area employed. Taking fairly large units, as I have done, there can be no question that the greatest number of species are found, in any genus of reasonable size, upon the smallest areas. But if one try to divide these units into smaller ones, one finds only a very few genera with a sufficient number of species to enable such a thing to be done. It is obviously unfair to divide the smallest area into yet smaller without at the same time doing the same thing to the larger areas, and there are so few in these larger areas that one may often find many of the smaller units without any species in them at all. Suppose, for the sake of example, that we divide the flora of New Zealand by smaller areas than at present (cf. 11, pp. 64, 77, &c.). To take the first large genus, *Ranunculus*, one finds the endemic species to have the following ranges:

830, 670, 580, 570, 540, 460, 420, 340, 340, 320, 310, 280, 260, 220, 180, 170, 90, 60, 60, 20, 20, 20, 10, 10, 10, 10, 10 (miles of the length of New Zealand).

It is clear that the smallest unit, ten miles of longitudinal range in

New Zealand, contains five species, the second (20) three, while the whole 100 miles from 101 to 200, or any of the higher hundreds, never contains more than four. The areas are obviously concentrated towards the bottom. If one made the unit one mile instead of ten, it is clear that there would still be five species in the first ten classes, while in the one hundred classes from 601 to 700 there would only be one species. I think that one may say that the curve is not modal, but hollow, but to get a curve at all one must work with units of appreciable size. If one take all the genera in the New Zealand flora that have over 20 species, and add up their endemics, one gets the result that while the endemic species with a range of 100 miles or less are no less than 105, those with a range from 101 to 200 miles or any of the other hundreds never exceed 38. Splitting up the 105, one finds that 43 have a range of ten miles or less; more, that is, than in any hundred above the first. Of these 43 I feel confident that more will be near to the unit of one than to ten miles of range, but much more detailed study is yet needful before a definite decision can be given on this point. The flora of New Zealand is too small for a proper decision, and one requires that of a continent.

The importance of the question lies in the fact that if the curve be shown to have a mode at, say, five miles, with diminishing numbers below that (when large numbers are taken), it will mean that that is the probable size of area upon which species commenced, and that those with smaller areas are perhaps dying out. Even so, however, the area is too small to have allowed of Natural Selection of infinitesimal variations. Judging from the great numbers of species that are confined to very small areas indeed, I should imagine, however, that a smaller unit than five miles of range will be found to show the maximum.

Mr. Tate Regan did not think that the hollow curves had any particular significance. Such a statement can only be attributed to an insufficient consideration of the matter. Can it for a moment be supposed that all the first fifteen families of flowering plants should give such a series of curves for sizes of genera as those in Fig. 1, without a *very* definite reason? In no single instance does the curve for one family even approach closely the curve for another, though the points of origin are only five squares apart upon the tracing-paper. Why should every family, animals and plants alike, show such a large proportion of monotypes? Why should they all turn the corner between the threes and the fives? These curves would not show such an exact similarity, and, for animals and plants alike, always turning the corner between the figure for three species and the figure for five, unless there were some overmastering reason for it.

Not only are these curves hollow curves, but they are all hollow curves of the *same mathematical type*, and when plotted logarithmically they give straight lines or close approximations thereto, which is a thing that does

not necessarily happen with a hollow curve. The fact that the logarithmic curve is a straight line (Figs. 2, 3) indicates that the genera have been formed by a close approximation to the rule of compound interest, a conclusion which has very important consequences, to which I shall refer in later publications.

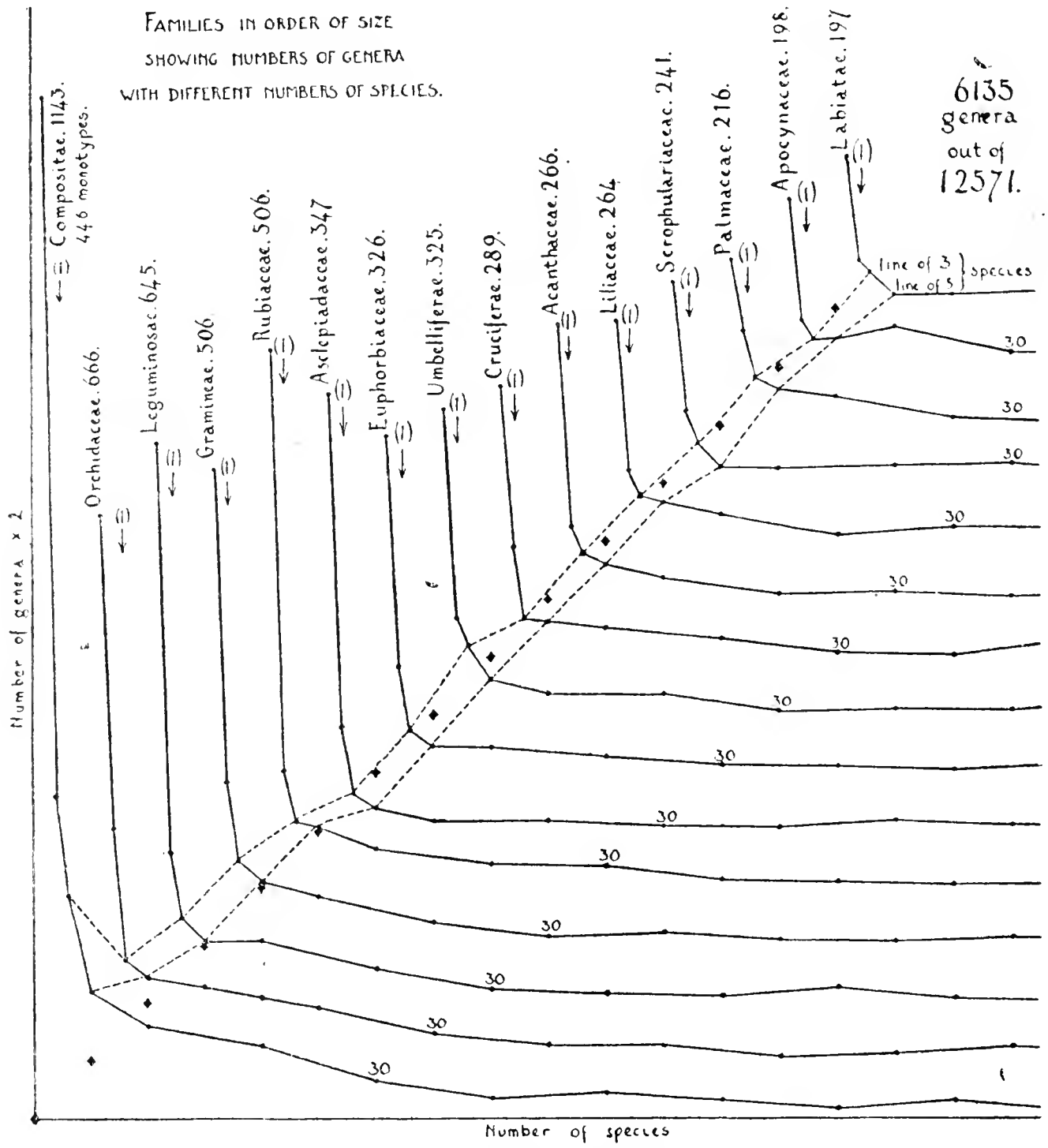

FIG. 1. Hollow curves exhibited by the grouping into sizes of the genera in the first fifteen largest families of flowering plants. Each curve is diagonally above the preceding one, as indicated by the heavy black dots (points of origin). Note that the curve almost always turns the corner between the point marking the number of genera with 3 species, and that marking the number with 5 (indicated by the dotted lines). The number after the name of the family shows the number of genera in it.[1]

Mr. Tate Regan quoted the case of the names in the London Telephone Directory as giving a hollow curve like the plants, evidently thinking that there was no rule which governs the origin of names, and that they showed no distribution like plants. The answer to this criticism has been made very easy by the existence of a book of which Mr. Tate Regan was evidently

[1] Fig. 1 is reproduced by permission from Willis (11); the block kindly lent by the Syndics of the Cambridge University Press.

ignorant, and which was written by our leading distributionist, Dr. H. B.

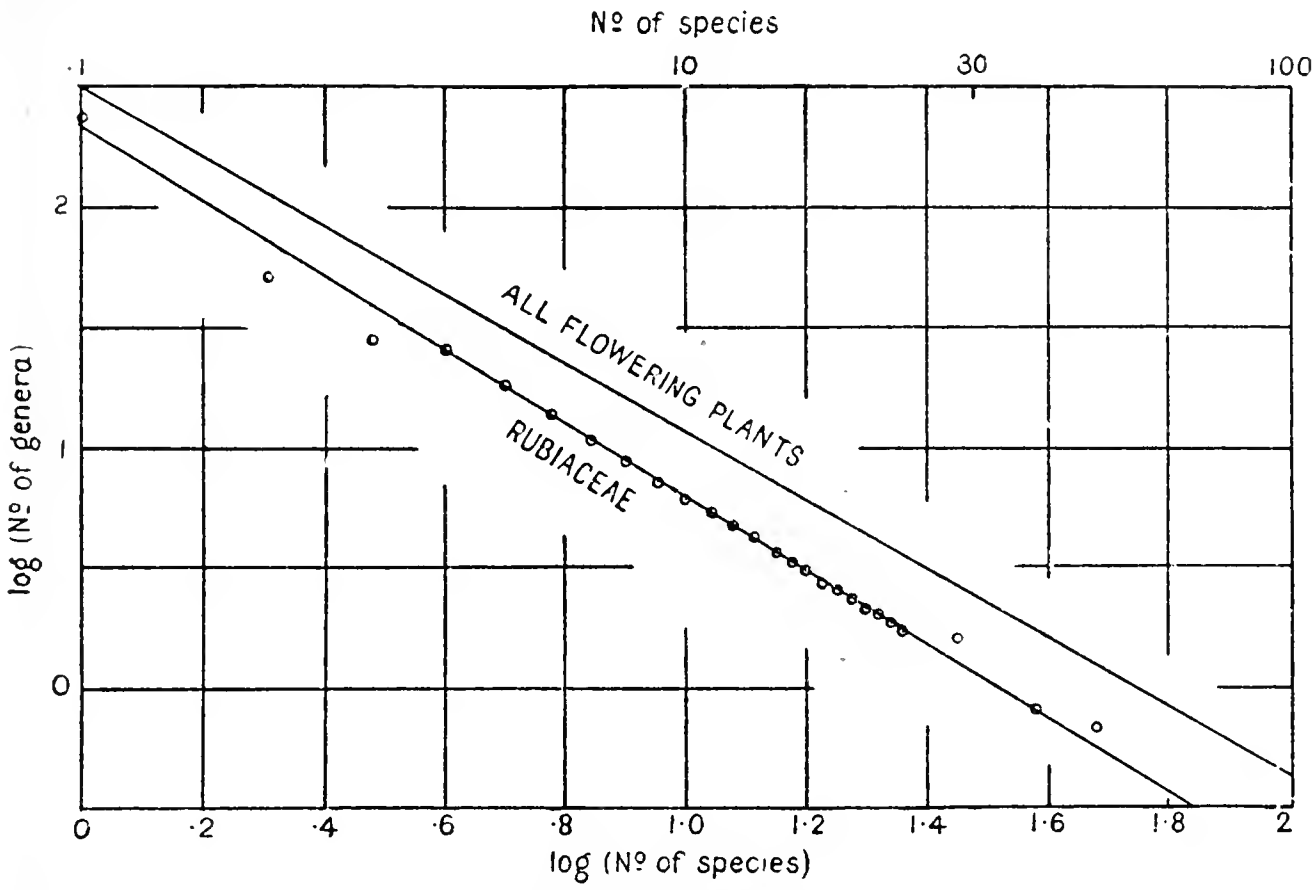

FIG. 2. Logarithm curve for Rubiaceae (from Willis, 'Dictionary') with corresponding curve for all flowering plants beside it (from Willis, 'Age and Area').[1]

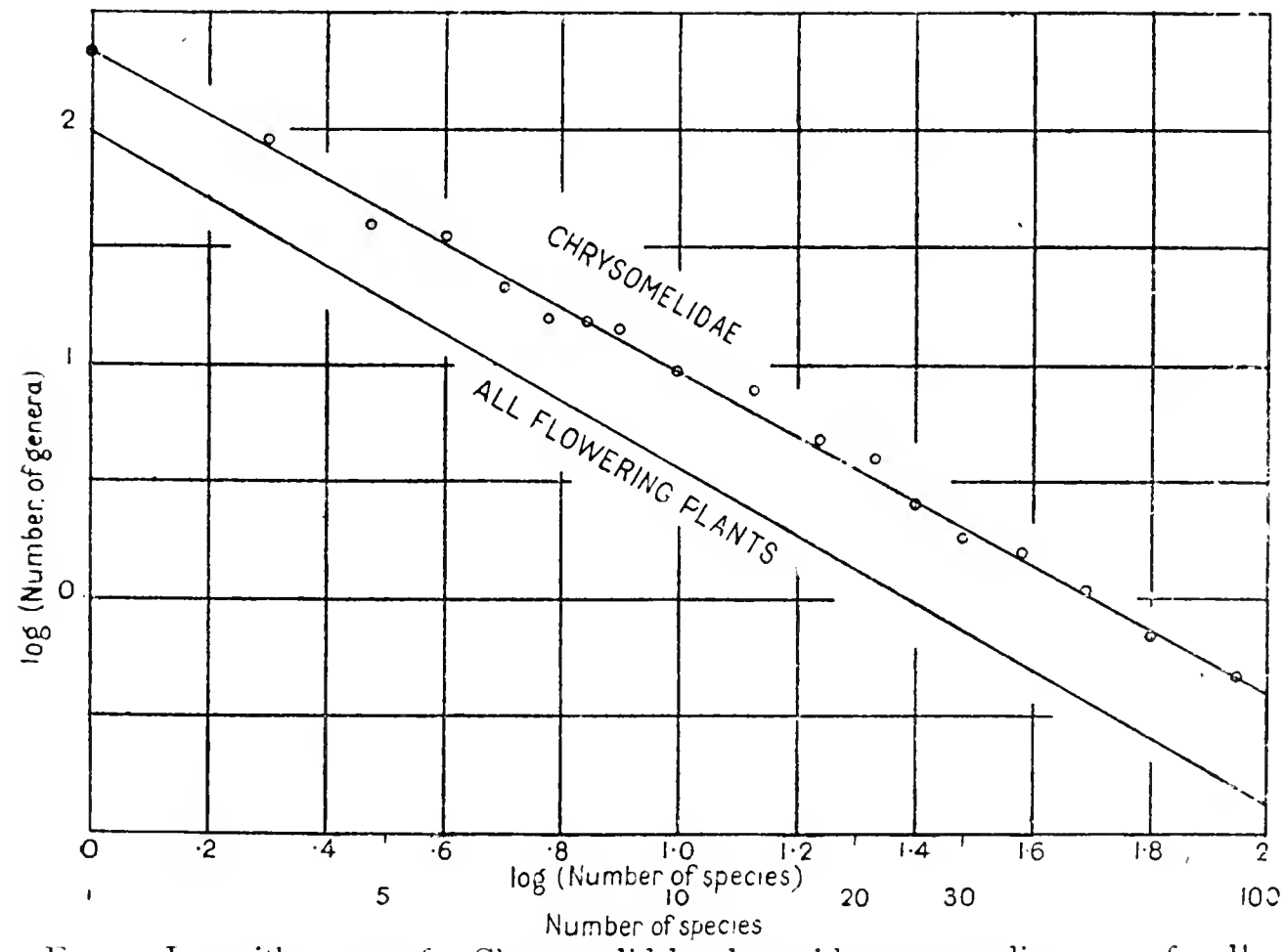

FIG. 3. Logarithm curve for Chrysomelid beetles, with corresponding curve for all flowering plants beside it (from Willis, 'Age and Area').[1]

Guppy—the 'Homes of Family Names.' The names in the Telephone Directory were found to give a hollow curve, the great bulk of the names

[1] Figs. 2 and 3 are reproduced by permission from Willis and Yule (13); the blocks kindly lent by the Syndics of the Cambridge University Press.

being rare, but a few, like Smith, Brown, Jones, and Robinson, being very numerous. But the reason for their distribution is evidently the same as in the case of plants. Some names are older than others, and will thus be more widely distributed and common, whilst, just as in plants, the large ones will tend to gain upon the small at an accelerated rate (cf. book, p. 34). Further, polyphyly, or multiple origin, is more likely to occur with names than with species.

The most slowly moving class of people in Britain is undoubtedly the class of farmers, many of them yeomen of very ancient descent upon the same spot. Guppy has made a special study of the distribution of their names in England and Wales, with statistical figures of frequency. Taking all names that occur in any county to a frequency (in the class of farmers) of 7 per 10,000, or more, he finds 3,925 names represented. When tabulated by frequency, these show:

Reaching a commonness of 7 per 10,000 *in*	*Number of names.*
1 county	2,441
2 counties	650
3 ,,	268
4 ,,	130
5 ,,	83
6 ,,	69
7 ,,	45
8 ,,	36
9 ,,	28
10 ,,	15

The numbers afterwards show a few trifling irregularities, but show some for every number of counties up to 36 (8 for 15 counties, 4 for 20, 5 for 25, 3 for 30, 1 for 35), and there is also one each for 39, 40, and 41 counties.

In other words, the distribution of these names is closely similar to the distribution of the species of a genus (cf. many examples in my book in ch. xvi), beginning with a great many on the smallest unit of area, and diminishing rapidly at first and more slowly later. The same general rule has evidently guided both distributions. Take, for example, opening the book at random, the name Halfacre. It reaches a frequency of 20 per 10,000 in Berkshire, but does not reach 7 anywhere else. It therefore may be presumed with good probability that the name originated in or near Berkshire. In the same way Hadfield (frequency 52) occurs in Derbyshire, Haffenden (18) in Sussex, Hadley (22) in Worcester, Haggett (9) in Somerset, none of these reaching a commonness of 7 anywhere else. But if one take such a name as Smith, one finds it common in nearly all counties, though varying from 300 in Worcester to 22 in Somerset.

Now let us look at the figures in another way. For each name and each county Guppy gives the proportion per 10,000 when it is above six. Let us begin with the names that are given for one county only. These

show an average proportion of 15·8 per 10,000 names (of farmers) in the county. But if we split up the 2,443 into ten groups we find:

Names beginning with		*Average proportion per name per* 10,000 *names of farmers.*
A, B	326 names	15·4
C	208 ,,	15·7
D, E, F	262 ,,	15·9
G, H	329 ,,	16·9 (maximum)
I, J, K, L	241 ,,	15·8
M, N, O	225 ,,	16·6
P, Q, R	255 ,,	14·9 (minimum)
S	237 ,,	15·4
T, U, V	191 ,,	15·5
W, Y	169 ,,	15·0

A most extraordinary resemblance is thus to be seen among these ten groups whose individual size is given in the table. The whole range of average occurrence is only from 14·9 to 16·9 per 10,000. The larger figure for G and H is due to the occurrence there of unusually large proportions of 'stay-at-home' names. These are, however, well distributed over the country, the only regions in which there are none being Wales and some of the Eastern Counties.

If we go on to the names which are above a density of six in two counties, we find, taking the larger number of the two in each case, that the proportions vary in the same ten groups from 18·5 to 25·6, with an average of 21·9. Thus the minimum here is a good deal higher than the maximum for the names of one county only. In the same way the 'threes' show a range yet larger again (corresponding to the diminishing numbers) with an average of 27·1.

It will be noticed that the average proportion in the county with the largest representation shows an increase with the number of counties covered by the name, and when we tabulate the figures we get the following interesting result:

Number of counties in which a commonness of 7 *per* 10,000 *is reached.*		*Average commonness per* 10,000 *in the county with the maximum.*
1	2,443 names	15·8
2	650 ,,	21·9
3	268 ,,	27·1
4, 5	213 ,,	35·8 [1]
6, 7, 8	150 ,,	40·8
9–12	72 ,,	64.6
13–20	78 ,,	90·2
Over 20	53 ,,	144·1

[1] The fours show a maximum proportion of 33·8.
,, fives ,, ,, ,, 38·8.

In other words, the more widely distributed over the country the name has become, the more common is it in the region where it appears to have started (what was said above about polyphyly being borne in mind). This is exactly parallel to what is seen in plants and animals, just as was the

other phenomenon described above, and seems to me completely to answer Mr. Tate Regan.

Mr. Tate Regan then went on to emphasize the importance of sterility and of isolation in evolution—points to which there will be few dissentients. It may be pointed out that the large mutations postulated by my supposi-

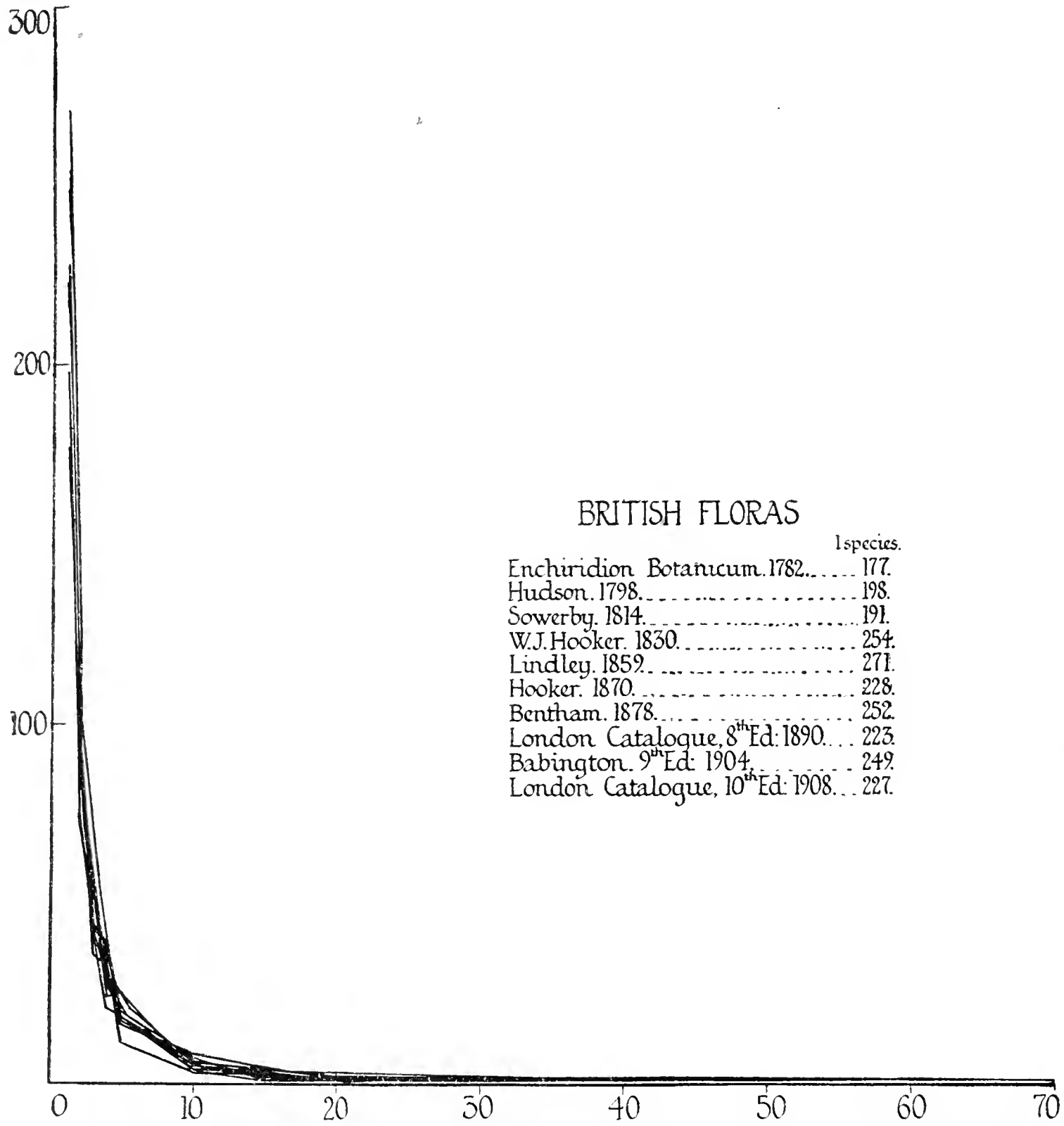

FIG. 4. Hollow curves exhibited by the grouping into sizes of the genera in ten British Floras, 1782–1908.

tions (11, p. 215) will be much more likely to be mutually sterile with their ancestors than would smaller ones.

Finally Mr. Tate Regan objected to Age and Area because it did not explain adaptation. This, however, no theory since the older theories of Darwin and Lamarck has attempted to do. Personally I do not think that adaptation is nearly so well marked as is often supposed (cf. 6), and it has often been pointed out, e.g. by de Vries on p. 224 in my book, and by

Mr. J. T. Cunningham at Hull, that it shows chiefly in generic and family groups, rather than in species, so that any theory of evolution that begins with the species, as does the Darwinian, must fail in its explanation. But a separate paper is required to deal with so large a subject.

Some Statistics of the British Flora, to show the Trifling Differences due to 'Lumping' and 'Splitting', or to Continued Work upon the same Flora.

An objection often brought up (cf. 11, p. 98) is that my figures, if not indeed accidental, depend upon the fact that the floras used are by a particular type of systematist. It is contended, in other words, that the flora of a 'lumper' will give a very different result from that of a 'splitter'. Others contend (cf. 11, p. 84) that the figures will be vitiated by further work upon the floras concerned.

I have already mentioned that in fact neither of these objections can be supported when the actual facts are examined, but in order to show their inapplicability in a striking way, I have taken at random ten British floras which I found in the University Library in Cambridge, ranging from 1782 to 1908, or over a period of 126 years during which much work has been done, and including the work of such 'lumpers' as Bentham, and such 'splitters' as Babington and the editor of the London Catalogue.

Though in some cases hardly a genus or a species has the same limitation in two of these floras, it will be seen at once that the general result is one of the most extraordinary similarity. There cannot be the slightest doubt that the curve is the same curve in all cases, with only unimportant variations. All the floras show the same hollow curve. And from this I think that one may assume that a similar result would follow in any other case, as indeed I have found to be the case in other instances.

The actual figures for the distribution of the genera in these various floras into sizes are given below, commencing with the flora that shows the greatest number of genera represented in Britain by one species only.

	Flora.	*Genera of* 1.	2.	3.	4.	5.	10.	*Larger.*	*Largest.*
A.	Lindley, 1859	271	102	52	25	18	5	3/14	1/65
B.	W. J. Hooker, 1830	254	83	50	27	21	6	3/14	1/66
C.	Bentham, 1878	252	84	49	22	23	4	4/15	1/47
D.	Babington, 9th ed., 1904	249	92	43	37	20	5	2/14	1/97
E.	J. D. Hooker, 1870	228	90	53	27	18	6	3/15	1/69
F.	London Catalogue, 10th ed., 1908	227	83	42	27	20	7	4/14	1/130
G.	London Catalogue, 8th ed., 1890	223	90	35	32	16	3	8/11	1/72
H.	Hudson, 1798	198	76	44	20	18	4	2/16	1/35
J.	Sowerby (Smith), 1814	191	74	44	38	18	6	3/15	1/57
K.	Enchiridion Botanicum, 1782	177	82	54	26	11	3	3/15	1/38

It is clear that though the 'splitter' pours contempt upon the 'lumper',

and vice versa, both really work upon what are essentially the same principles, and that they produce results of the most extraordinary parallelism.

The Distribution of Genera by Sizes in Definite Areas; a Difficulty for the Supporter of Relicdom.

A commencement has been made with this subject in my book, upon p. 189, where it is shown that in South America, to take one instance, there exist, confined to this continent, 18 per cent. of the world's monotypes, but only 16 per cent. of the ditypes, 13 per cent. of the genera with five species, 11 per cent. of those with ten, and so on, the proportions steadily diminishing. To explain this by Natural Selection appears to me an impossibility, for the same phenomenon is shown by other continents and islands, and Natural Selection could not produce such a regular and mechanical result, nor would relics be likely to exist in a steadily diminishing proportion, with the greatest number at the point of death.

But other problems arise out of these facts, which are perhaps even more difficult for the natural selectionist. How explain, for example, the fact that the actual numbers and proportions (from my Dictionary, 4th ed., as usual) of the genera confined to the three most isolated continents are:

	Africa.		*North America.*		*South America.*	
Total of genera	1,740		1,308		1,739	
With 1 species	835	48 %	612	47 %	887	51 %
2 ,,	254	14	224	17	263	15
3 ,,	136	7	111	8	125	7
5 ,,	97	5	58	4	87	5
10 ,,	56	3	42	3	59	3

The percentages are counted downwards; 48 % of the African genera are monotypes, 51 % of the South American.

Not only do the proportions decrease regularly, but they are closely the same for all three. Neither of these facts can be explained by Natural Selection, though they are quite simple to Age and Area, if we imagine genera to be 'casually' formed at different spots.

Or, to take another case, why, on the theory of Natural Selection, should Australia, which is a large island, have only 241 out of its 565 endemic genera monotypic, or 42 per cent.,[1] while Java has 57 out of 59, or 96 per cent.? The smaller islands than Australia, taken as a whole, have 1,037 out of 1,582 endemic genera monotypic, or 65 per cent. The explanation of relicdom is somewhat hardly pressed to explain such cases as this, or the still more difficult case that while Java has 96 per cent. of its endemic genera monotypic, Socotra has 89 per cent., Japan 80 per cent., Ceylon 76 per cent., Madagascar and New Zealand 70 per cent., New Caledonia 57 per cent., and the Hawaiian Islands merely 31 per cent. (cf. figures on

[1] i. e. less than Africa, North America, or South America.

p. 175 of my book). Why should there be so many more relics, both in number and in proportion, upon the islands that are nearest to the mainland and least isolated? The greater proportion of larger endemic genera upon the more outlying islands shows clearly that these genera were formed at an earlier date (on the whole), and thus, having had longer time in which to expand, have grown larger.

Why has tropical America, a comparatively continuous area, 13 per cent. of its endemic genera monotypic, while the much broken Old World tropics have only 6 per cent., and tropical Asia 36 per cent.? The general distribution of genera by sizes in different areas, more fully described in my book, offers very serious problems to the supporter of relicdom for most endemics.

Prediction applied to the Flora of the British Islands.

The predictions hitherto applied with such success have been mostly in reference to the flora of New Zealand and its surrounding islands, and as the criticism has been made that though prediction may succeed in such regions that have been comparatively little interfered with by man, it would be valueless in such regions as the British Islands, an attempt at prediction is here made for the latter. The attempt is to forecast the flora of the county of Dublin (a region of which we possess a good and recent flora (1)) from what is known of the distribution of the plants of the adjacent island of Great Britain. For the latter there is no more recent general authority, so far as I know, than Watson's 'Topographical Botany', 2nd ed., 1883, and I have kept rigidly to this. I have not the time available to consult all the innumerable papers in which more recent work is enshrined, nor is it necessary for such work as the present, for, as will be seen below, the prediction is perhaps more successful than were any of those which I made in regard to the flora of New Zealand.

It is generally admitted that Britain and Ireland were at one time united to one another and to the Continent, and received their floras thence. The nearest point to the Continent, and that a point not uncentral (in all probability) as regards the land union, is the South Foreland in Kent. For the purpose of prediction let us assume that this was the centre of the main continental (northward-moving) invasion, and draw circles round it as a centre. If we take a circle that includes the county of Dublin, it will be seen that it also includes the counties of Wicklow and Wexford, and then crosses Scotland by the south of Ayrshire, through Lanark, Peebles, and Edinburgh, reaching the North Sea near Dunbar in Haddington.

The prediction now is obvious. One will expect to find the flora of the south-east corner of Ireland made up, so far as this invasion is concerned, of those plants which reach Kent, and also reach Ayrshire or Haddington.

But there are many plants in the British Islands that seem to have

entered from the north-east. Let us, for the sake of prediction, suppose that this invasion centred at Duncansby Head, and draw a circle round that

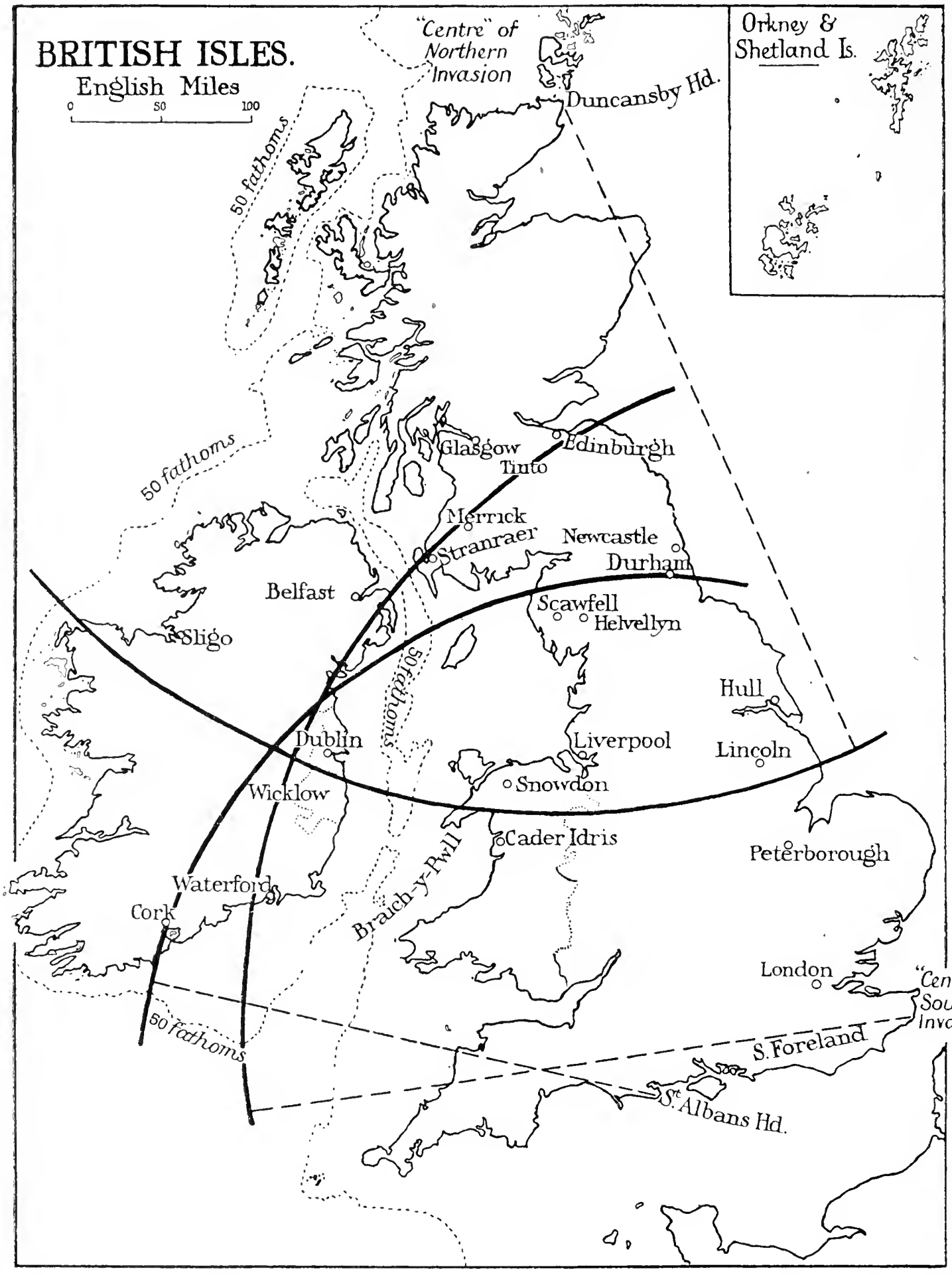

FIG. 5. Map of the British Isles to show ranges of plants reaching co. Dublin.

to include Dublin county. This circle crosses England and Wales from Merioneth to the Wash, and one will expect the Dublin flora to contain those species that reach to this circle in Britain. There may have been other invasions, and the directions may not have been exactly as here

imagined (for example, there might have been invasion from the direction of St. Alban's Head, as shown in the third circle), so that this is really only a rough approximation. But on this basis let us see to what extent the flora of Dublin is predicted. The flora is helped out in a few instances by that of Wicklow and Wexford, especially in regard to mountain species.

Taking from Watson those plants that fulfil one or other of the two conditions laid down above as needful, one obtains a total of 739 species. Many of these are now regarded as aliens or introductions of some kind, and it would lead too far to go into the question in detail. Of these 739 our region includes 564 regarded as fully native, or 72 per cent. A further 47 (6 per cent.) are marked by Colgan as colonists, but all of them except *Anthemis arvensis* are marked in the 'Cybele Hibernica' as native in Ireland. Still another 49 occur in Dublin, marked by Colgan as Casuals, Denizens, or Aliens, and the Cybele marks 27 of these as native in Ireland. We thus find in all, $564+46+27 = 637$ out of 739, or over 86 per cent., predicted. Of the remaining 102, the Cybele gives 45 as native in parts of Ireland, so that only a few plants remain that are not found. Of the species found in Dublin, and *not* predicted, 40 in all, 12 are marked by Colgan as Aliens, Casuals, or Colonists. Considering the effects of man's occupation for so many centuries, this result must be looked upon as very successful prediction.

One may even apply prediction nearer home. A circle with centre at the South Foreland, and including Dorset, also includes much of Derbyshire, and one will therefore expect a great similarity between the floras of these two counties. Taking the floras of Mansell-Pleydell and of Painter, and comparing, one finds the species the same for page after page, the two counties having no less than 1,305 in common, i.e. 88 per cent. of the flora of Dorset, and 95 per cent. of that of Derby. Of the remainder of the Dorset flora, 84 are seacoast species, which one will not expect to find in Derby, and in Derby 23 are Highland types that cannot occur in Dorset. If we omit these from the respective floras, the 1,305 species in common represent 93·2 per cent. of the flora of Dorset and 97·2 of that of Derby. A more detailed study of invasions, and of local distribution in England, would probably make the approximation even closer; this is merely given as a rough illustration, and was chosen simply because I happened to have at hand the flora of Derbyshire.

A Correction of Previous Work upon the Flora of Ceylon.

Mr. J. S. Gamble, C.I.E., F.R.S., informed me in conversation that in the course of his work upon the flora of Madras, a number of species had been discovered there which in my Catalogue of Ceylon Plants are recorded as endemic to Ceylon. It at once struck me that upon the theory of Age

and Area these would probably prove to be mainly among those recorded as possessing an unusually low degree of rarity (for endemics), and I asked for a list, which Mr. Gamble has kindly furnished. The species so far recorded are:

Dillenia retusa	*Microtropis Wallichiana*	*Osbeckia rubicunda*
Uvaria macropoda	*Vitis glyptocarpa*	*Casearia coriacea*
Xylopia parvifolia	*Sapindus erectus*	*Heracleum zeylanicum*
Alphonsea sclerocarpa	*Crotalaria Walkeri*	*Hedyotis Lessertiana*
Garcinia echinocarpa	*Eugenia Fergusoni*	*Urophyllum zeylanicum*
Calophyllum trapezifolium	*Eugenia olivifolia*	*Ixora Thwaitesii*
Erythroxylon obtusifolium	*Osbeckia buxifolia*	*Emilia zeylanica*
Olax zeylanica		

If one note, from Trimen's 'Flora', the recorded rarity of each of these in Ceylon, one finds that of the 22, there are VC 1, C 4, RC 11 (i.e. 16 below average rarity), RR 3, R 2, VR 1 (i.e. 6 above average rarity). The 22 species show an average rarity of (22/70) 3·18, much below the average for Ceylon endemics of 4·34. Their removal alters this to (787/3,448) 4·38. As only one place of decimals was employed in my calculations, it will be seen that the figures remain unaltered—a fact which may help to impress upon some objectors how little difference in the statistics is made by the alteration of a few individual figures. Incidentally, by removing 11 from the old list of 139 RC, and only 3 from the 136 RR, the change removes the only break in the regularity of my figures which has so far shown itself when dealing with considerable numbers.

Summary.

This paper is chiefly concerned with a reply to the criticisms upon Age and Area that were made at the Hull meeting of the British Association, and special attention is once more directed to the most fruitful sources of difficulty, for little new criticism was brought up. People insist upon applying Age and Area to individual cases, or to unrelated forms, and in both instances, as has frequently been pointed out, this will only lead to error and difficulty. The law is only applicable to groups of at least ten allied forms. The other great difficulty is the view that endemic forms are chiefly relics, yet, though the latter are common enough, it is not properly realized that they are quite lost in the crowd when large numbers are considered.

Several original pieces of work are then inserted to illustrate what has been said. A definite reply is made to the criticism of Mr. Tate Regan, who was the leader of the opposition at Hull, and who made several animadversions upon Age and Area that do not seem to me to be justified. Incidentally a brief analysis of Guppy's work upon distribution of family

names is made, and it is shown that they have followed rules similar to Age and Area and Size and Space.

Some statistics are given of various British floras published from 1782 to 1908, and it is shown that the curves for sizes of genera for ten floras of this period agree very closely indeed with one another.

The next section is concerned with the distribution of genera by sizes in definite areas. Among other things it is shown that the nearer to the mainland an island is, the larger is the proportion of monotypes (usually supposed relics) among its endemic genera—a very difficult phenomenon for the advocate of relicdom to explain.

Further illustration of the ease and success of prediction is then given by predicting more than 86 per cent. of the flora of Dublin from what is known of the distribution of plants in Great Britain; and a further prediction that the floras of Dorset and Derby (apart from coast plants in the one, and highland in the other) will be much the same is borne out to the extent of 93 per cent. in Dorset and 97 per cent. in Derby.

Finally, a correction is added to previous work upon the flora of Ceylon. Twenty-two species, formerly supposed endemics, have been found in Madras, and prove to be, as would be expected by Age and Area, on the whole decidedly on the 'Common' rather than the 'Rare' side in their Ceylon distribution.

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Observations on the Action of X-rays on Plant Cells.

BY

MAUD WILLIAMS.

Introduction.

THE present work arose out of experiments upon the changes produced when certain plant cells are immersed for long periods in solutions of electrolytes (1). An empirical formula had been obtained connecting the concentration of a particular salt solution employed and the time of immersion needed to produce a specific change in the cells studied. This change, which is also employed in the present work, was judged to have taken place when a 0·1 per cent. $K_2Cr_2O_7$ solution was able to enter the cells and combine with the tannin contents to yield a precipitate within a time limit of three minutes. The results obtained for series of salts made it seem desirable to study the influence upon living protoplasm of electric charges of one kind only, unhampered by the presence of undissociated molecules or of salt ions.

Treatment with radium seemed a possible way of attaining this object. The ordinary radium bromide preparations give off α particles (positively charged), β particles (negatively charged), and X-rays of a very hard type. By using the preparation encased in mica or platinum the α particles are absorbed and the cells can be submitted to the bombardment by the negatively-charged particles, which travel with great velocities, and to the action of the X-rays. It is obvious that the influence of the β particles alone can be estimated only when the effects of the X-rays themselves are known.

Although it is not suggested that treatment of plant tissues by either X-rays or by radium is likely to be of practical importance, the investigation of cell behaviour when under these radiations is of great interest from the following points of view:

(*a*) Possible stimulation.

(*b*) The coagulation of colloidal matter.

(*c*) The action of etheric radiations of very short wave-length upon the colouring matters of cells.

The second of these matters is that of chief interest in the work undertaken, but various interesting changes have been observed, and it is proposed to deal with the experiments in a series of short papers.

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Section I. Changes produced in Cells of *Saxifrage umbrosa* by X-rays.

1. Historical survey.
2. Nature of the X-rays, factors to be considered.
3. Methods used.
4. The series of changes produced.
5. Summary.

1. The general interest aroused by the discovery of X-rays by Röntgen in 1895 led to the action of these rays on various plant and animal structures being studied at an early date. There are many conflicting statements in the work of the earliest workers, which is only to be expected when one considers the fact that the actual nature of the rays was then unknown and experiments could not be carried out under any standard conditions.

In 1897 observations upon *Vallisneria spiralis* under the radiation from a 'gas bulb' were published by Lopriore (2). This observer found that treatment for thirty minutes produced an acceleration of circulatory rate, but there was a return to the normal rate when the radiation ceased. Treatment which lasted one hour caused the protoplasm to become yellow, granular, and coarsely vacuolated, while after two hours of radiation, although the cells were still living and the protoplasm circulating, the chlorophyll bodies were becoming colourless. The suggestion of stimulation implied by the acceleration of circulation is supported by the fact that other workers increased the rate of opening of buds by exposing them to X-rays, while it is well known from the medical side that small doses of radiation may accelerate cell division of tumours in animals.

Although Lopriore did not reach the stage in which cells were actually killed while under the radiations, lethal actions were soon discovered during work on animal cells, and Schaudinn (3) found that treatment for fourteen hours caused amoebae to round themselves off. It was found that many bacteria were peculiarly resistant to these rays, although so powerfully affected by ultra-violet light.

Meanwhile analogous experiments were being made upon cells exposed to radium, and although these will be dealt with in a later paper, attention must here be called to the work of Packard (4), because he suggested that the presence of chlorophyll increased the resistance of the cells, and that the presence of light was a factor of importance in his tests on chlorophyll-containing cells.

2. It has now been established that X-rays are waves of the same type as light waves but of much shorter wave lengths than those of either visible or ultra-violet light.

As produced in the original forms of 'gas bulb' these waves are heterogeneous; homogeneity can be obtained by 'filtering' through a suitable thickness of aluminium. On consideration of the more recent work upon light and stimulation it becomes apparent that when one deals with any radiation of the same type as visible light one must be prepared to consider both intensity and 'quality' (i.e. wave length) as important factors in the action upon plant cells.

In any attempt to obtain quantitative results it is necessary to maintain the 'past history' of the material as uniform as possible, to expose cells in the same state of development, to carry on the experiments in darkness, and to use X-rays of homogeneous nature.

3. The material already used in the work on electrolytes referred to earlier proved very suitable for examination under X-rays. When strips were torn from the upper surface of the petiole of *Saxifraga umbrosa* the cells were in mature condition and very constant in shape. They were particularly clear and easy to examine, and remained in active circulation if kept for as long as forty-eight hours in distilled water (free from copper), and for longer still when kept in tap water. The strips were sufficiently large for division longitudinally for certain tests where accurate control was needed.

For exposure a number of strips was mounted under a cover-slip 1·5 in. by 0·75 in.; if quantitative results were needed this cover-slip was tested for uniform thickness by spherometer measurements. The glass slide was held in a wooden rack with sides to darken it, at a known distance from the focus spot of the X-ray bulb. The bulb itself was contained in the usual way in a lead-lined box with an opening only at the focus region so that scattered rays could not influence the material.

For preliminary work the rays were obtained from the ordinary gas bulb, in which constant output is impossible. This work served to show the changes produced and the order in which they occurred. More accurate work was then undertaken with the Coolidge tube. This modern type of bulb gives heterogeneous rays, and the penetrating power depends upon the difference of potential maintained between the electrodes. The X-rays are produced by the action of electrons given out by a heated spiral of tungsten wire, and the intensity is constant if the temperature of the wire be constant. The heating is carried out by means of an electric current, and the strength of this must be maintained steady in order to give a constant temperature.

Since the penetrating power of the rays depends upon the difference of potential between the electrodes, it can be tested by a 'spark-gap' in circuit with the bulb, and the generator must be adjusted to keep its value constant.

If the radiations are then passed through a centimetre thickness of aluminium one can obtain an output constant in intensity and quality.

A range of intensities is possible if one places the slide at different distances from the bulb, since the intensities then fall off inversely as the squares of the distances.

4. In following the series of changes strips were removed from the slide after various times of exposure and examined both with the high power and with the paraboloid form of ultra-microscope.

The first change seen was an increase in the rate of circulation, of a type which must be defined clearly. In this material there is no distinct movement of rows of chloroplasts as in some plants, but circulation of the protoplasm is shown by the movement of bright particles, which focusing shows to be near the wall, along lines parallel to the long axes of the cells, and a similar movement along protoplasmic threads leading to the centre of the cell. In all discussion of circulation only bright particles of similar size and appearance, moving along lines parallel to the long walls, are considered.

As exposure proceeded there was an increase in the amount of Brownian movement executed by these particles. Since the temperature was steady and the sizes and optical properties of the particles remained constant, this increase in movement would appear to denote a fall in the viscosity of the medium. As there was a forward motion imposed upon the particles by the movement of the protoplasm it appeared as though relative values of the viscosity at different stages could be obtained by observations on the curves executed by the particles, according to the method used by Svedberg (5). Unfortunately the amplitudes of the vibrations were too small to make such estimations possible.

Longer exposure to the rays caused a diminution of circulatory rate, and finally cessation.

It was hoped to obtain figures of quantitative value by plotting graphs of circulatory rates after various times of exposure, but the movement in the plant cells proved to be influenced by too many factors to make success likely, as the nature of the tests did not allow time to compare the specimen with its own control at each stage.

Measurements were made to find whether the stimulation indicated by increase in circulatory rate was followed by a depression when treatment ceased before there was any outward sign of injury in the cells. For each of these tests strips were divided longitudinally; half was exposed and half kept as a 'control'. Circulatory rate was measured in each at intervals—readings being taken after ten minutes' exposure to the light of the microscope mirror, and measurements being made as quickly as possible on central cells in the strip.

The table below shows some representative experiments. In no case was the exposure long enough to produce visible injury either after exposure or at the end of twenty-four hours.

It will be seen that considerable changes took place in the circulatory rates of the controls during the time of the experiments, but in every case the irradiated specimen ultimately showed a depression in rate compared with its control. Precisely analogous results will be shown later in a section dealing with the action of radium on the same material. The effects do not agree with the observation of Lopriore that circulation became normal after cessation of treatment.

CIRCULATORY RATE AND INFLUENCE OF X-RAYS.

Coolidge tube. Heating current 3·5 amps.
Cover-slips 0·035 cm. thickness.
Specimens mounted in tap water.
Slide placed at aperture of box enclosing the tube, darkened except for glow of the bulb.

Date of exposure.	*Time of exposure.*	*Milliammeter reading.*	*Control.*		*Specimen.*		*Time between observations.*	*Control.*		*Specimen.*	
			No. of readings.	*Average time 20 div.*	*No. of readings.*	*Average time 20 div.*		*No. of readings.*	*Average time 20 div.*	*No. of readings.*	*Average time 20 div.*
1922.	min.	milliamp.		secs.		secs.	hrs.		secs.		secs.
Feb. 1	10	1	16	19·1	16	20·1	24	16	18·1	16	27·3
Feb. 15	3	0·6	16	22·1	16	18·1	24	16	25·1	16	26·9
Mar. 1	3	0·6	16	18·1	16	13·9	24	16	16·4	16	21·7
Mar. 23	5	0·2	16	19·9	16	17	24	16	16·3	16	16·9

As treatment was extended beyond the time needed to accelerate the circulation other changes occurred. The protoplasm began to leave the cell wall, very gradually at first. In the early stages of this change the protoplasmic surfaces were parts of smooth curves and the clear appearance was retained.

To test whether this shrinkage was of reversible nature treatment was stopped in some cases and camera lucida drawings made of representative cells. The material was then transferred to a fresh supply of tap water and drawings made at intervals with the same focusing arrangements. The area of the protoplasm in these cells was estimated with a planimeter. Not only was there no return to the normal condition, but shrinkage was found to be progressive more than an hour after treatment.

In specimens in which the shrinkage stage had been reached it was noticed that the pink colour possessed by some of the cells was beginning to fade. A possible explanation of both shrinkage and loss of colour was to assume the permeability had been modified and that solutions were escaping from the cell, so lessening the sap pressure.

To test the truth of this assumption experiments were made upon pieces of epidermis torn from the under surface of the leaves of the plants. One strip from each leaf was exposed and the other kept in tap water as a control.

Immediately after exposure to the Coolidge tube the specimens were

mounted in fresh tap water, and ten stomata, chosen at random, were measured for the stomatal ratio, i.e. the ratio of the width to be the length of the slit.

The control was then dealt with similarly, and both strips kept in fresh supplies of water, in darkness for twenty-four hours. Fresh measurements were then made, care being taken of course to leave each strip over the mirror of the microscope for the same interval before beginning the tests.

Exposure for ten minutes to the Coolidge tube with a reading of two milliamps was found to reduce this ratio very perceptibly, while twenty-four hours after treatment the difference between experimental strip and control was greatly enhanced. On one occasion with more penetrating rays (reading one milliamp) the average for the stomatal ratio was reduced to 0·39 against a value for the control of 0·55. These results therefore confirmed the view that shrinkage was due to decreased sap pressure and consequently to changed permeability.

There was still the possibility that the loss of red colouring might be partly due to the destruction of the colouring matter. This was tested by preparing a water extract of beetroot to give the same pigment. From this, by dilution, a series of concentrations was prepared.

A tube of extract was then darkened by thick black paper and exposed to a strong dose of rays of duration longer than that found to cause loss of colour in the plant cell. When the liquid was matched against the graded tubes it was found to be unchanged in depth of colour and its absorption spectrum was observed to be identical with that of the original solution. The fact that the chloroplasts were not affected in this material, while those in *Vallisneria* were found by Lopriore to become yellow, led to tests on chlorophyll extracts similar to those on the red colouring anthocyanin being made.

Exposures up to fifty minutes were made with the Coolidge tube; again no change in intensity was discovered, and the absorption spectrum was identical with that of the control both immediately after exposure and again a week later. Where discoloration has been found to occur in other plants it is possible the cause has been the action of acids released by the changes in permeability and not a direct action of the radiations.

Attention was next paid to the changes in the appearance of the protoplasm on still longer exposures as circulation began to slow down. The shrunken surfaces became more and more irregular, and an increased amount of light was scattered when the cells were viewed with the ultra-microscope. In some cells great vacuolation was found.

Finally a stage was reached when, within three minutes, a solution of $K_2Cr_2O_7$ of 0·1 per cent. could produce a precipitate with the tannin of the cells. The ultra-microscope showed that this precipitate appeared both within the shrunken mass and in the corners of the cells from which the

protoplasm had retreated. The tannin must therefore have been capable of diffusing out as well as the $K_2Cr_2O_7$ diffusing into the protoplasm.

Work which has already been done points to a relationship between intensities used and time of exposure needed to bring the cells to this last stage. It is hoped to deal with this quantitative aspect in a later paper.

Summary.

Strips of tissue from the upper surface of the petiole of *Saxifraga umbrosa* were subjected to X-rays.

1. Small doses of radiation are said to accelerate circulation, but in this material a depression follows, and there is no return to the normal in twenty-four hours.

2. There is evidence of a lowering of the viscosity of the protoplasm in the early stages of radiation.

3. From the time any change is seen in the protoplasm that change is irreversible.

4. There is no direct influence of the rays upon the anthocyanin or the chlorophyll.

5. The protoplasm allows the diffusion of solutes from the vacuole, and appears to become coagulated.

6. By the time a precipitate can be obtained with the potassium dichromate used as a test for increased permeability all movement of the protoplasm has stopped.

The Sir John Cass Institute, London.

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Fertilization in Sphaerocarpos.

BY

H. W. RICKETT.

With Plates III and IV and three Figures in the Text.

Introduction.

THE details of the process of fertilization have been very thoroughly studied in examples of all the great groups of plants save the Bryophyta. In this group no complete cytological account of fertilization has been published for any form. A few scattered observations, ranging from the beginnings of cytology until recent times, make up the literature on the subject. This condition is partly due, no doubt, to technical obstacles in the study of the mature archegonium, owing to the formation of a large quantity of mucilaginous material through which the antherozoid passes to the egg and which renders fixation difficult.

The earliest account of fertilization in a Liverwort was given by Strasburger (1870) for *Marchantia polymorpha*. He described the egg as having an apical colourless receptive spot. He figured the swarming of antherozoids around the end of the neck of the open archegonium, in the extruded mucilage, and was able to distinguish single antherozoids in the neck. They were too small for him to follow farther. As to their fate when they reach the venter, he says:

'Doubtless they are taken in by the receptive spot. Whether here, as in ferns, one spermatozoid is enough to complete fertilization could not be made out; from the size of the egg and the receptive spot in comparison with that of the spermatozoid, I might conclude that there are here a larger number of the latter taken into the egg.'

The first to concern himself with the cellular details of the sex organs of a Liverwort was Kruch (1891), who gave a careful and fairly complete account of the development of the sex organs and of fertilization in *Riella Clausonia*. His paper is of especial interest in that his account of fertilization can be very easily harmonized with that which is here presented for *Sphaerocarpos*, a form thought by many to be closely related to *Riella*.

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He observed no receptive spot in the egg. He found that, of many antherozoids that enter the archegonium, only one penetrates the egg. This at once enlarges greatly, and becomes surrounded by a homogeneous hyaline area whose outer limit is not well defined. The body of the antherozoid then segments, first into four, then into eight threads; he thought that the eight threads result from the longitudinal division of the four seen in the earlier stage. The clear area now possesses definite boundaries, which thus outline the male nucleus. About the same time, the female nucleus also forms eight chromatin threads. The two nuclei become almost equal in size. He observed them in contact, but did not see their actual fusion. The entrance of an antherozoid causes the appearance of tangential divisions in the cells making up the wall of the venter.

Cavers (1904), studying *Fegatella conica* (*Conocephalum conicum*), by introducing a drop of water full of swarming antherozoids into a preparation containing mature archegonia was able to follow the opening of the archegonium, the extrusion of the mucilage, and the passage of the antherozoid down the neck. He could not distinguish the antherozoid after it had penetrated the egg. Large numbers of antherozoids reached the egg and caused the latter to exhibit a rocking movement.

Several stages in the fertilization of *Riccia natans* (*Ricciocarpus natans*) were observed and figured by Garber (1904). Numerous antherozoids were seen caught in the mucilaginous matter extruded by the archegonium. The egg is concave at the end nearest the neck, and, after the archegonium has opened, is somewhat shrunken away from the walls of the venter, though not nearly so much so as is usually described for *Riccia*. The fertilized egg swells and its end extends up into the neck canal. Garber saw the two sexual nuclei lying in the egg cytoplasm. The male is about half the size of the female nucleus, and stains more darkly because its chromatic contents are more closely crowded together. These contents include one or several large bodies and some smaller granules. The female nucleus contains a large body in the centre, and apparently a fine reticulum surrounding this. There are conspicuous plastids with starch grains all through the egg cytoplasm. According to his account, the male nucleus approaches the female nucleus and becomes embedded in it, but it is a question whether his figures really prove this point. In the absence of statements as to the thickness of his sections, or as to whether the two nuclei were at the same focal level, the nuclei may equally well be interpreted as being one above the other, no fusion occurring at this stage. As in *Riella*, the entrance of the antherozoid is followed by divisions in the cells of the venter, so that the latter consists of two layers of cells before the first division of the zygote.

Humphrey (1906) saw one stage in the fertilization of *Fossombronia longiseta*, which is noteworthy as being markedly different from anything

reported for any other Liverwort. He figures a cross-section of the archegonium and egg, with the antherozoid, a long, thin, curved body, lying in the egg cytoplasm, with one end in contact with the egg nucleus. This antherozoid resembles the free-swimming cell, and has no resemblance to a resting nucleus, or even to the antherozoid immediately after its entrance into the egg as described by Kruch and by the present writer. Humphrey reported that the egg of *Fossombronia* has a well-defined receptive spot, but did not describe the latter. He described the large egg nucleus as possessing but one nucleolus and a small amount of chromatin.

Meyer (1911) obtained several fertilization stages in *Corsinia marchantioides*, which showed the male and female nuclei lying side by side in the egg cytoplasm. In the earliest stages observed, the male nucleus has already rounded up and is surrounded by a clearly defined membrane. In its centre is a small mass of chromatin granules, from which linin threads extend towards the periphery. Later the chromatin granules become spread throughout the nucleus, which is now filled with a complicated fine network. The large egg nucleus is similar in structure. The cytoplasm of the egg is quite dense, and filled with vacuoles which are arranged somewhat radially about the nucleus as a centre. The egg was somewhat shrunken under the influence of the fixing fluid, and was surrounded by a clearly defined fertilization membrane after the entrance of the antherozoid.

Miss Black (1913) saw one stage in *Riccia Frostii* similar to that just described for *Corsinia*. The male nucleus lay in the egg cytoplasm beside the egg nucleus. Its structure was that of a resting nucleus. She also figures one egg which she interprets as having been fertilized and in which the nuclei have already fused. Its nucleus is large, and the chromatin is collected in a cord or in short segments, with a fine network occupying the remaining space. This, she says, is typical of a great number of eggs observed. She also reports the division, after the entrance of the antherozoid, of the cells that make up the wall of the venter—the basal cells usually dividing first.

Miss Graham (1918) figured male and female nuclei in the cytoplasm of the egg of *Preissia quadrata*. The male nucleus is smaller than the female. The chromatin in both is massed in the centre of the nucleus, and seems to be in the form of small masses or rods. She was chiefly interested in the discovery of centrosomes in the egg during fertilization, to which reference will be made in another connexion.

Woodburn (1920) shows one stage in fertilization in *Reboulia hemisphaerica*, which is general is similar to those just described. The nuclei are stated to be in the resting condition, though the figure is not very clear.

Sharpe (1921) figures one stage in the fertilization of *Anthoceros*. The male nucleus lies within the egg cytoplasm, in contact with the egg nucleus.

Both nuclei are in the resting condition. In the cytoplasm of the egg lies an elongated plastid.

In the Mosses the state of our knowledge of fertilization is even more unsatisfactory.

The entrance of the antherozoids into the archegonium was seen by Roze (1872) in *Sphagnum cymbifolium*, and by Arnell (1875) in *Discelium nudum*. The former figures many antherozoids clustered about the opening of the neck, several passing down the neck, and one in the venter just touching the egg. They enter the neck with the ciliated end ahead, and retain the cytoplasmic globule at the posterior end, carrying it with them into the venter. Arnell described the entrance into the venter of many antherozoids which impart to the egg a rocking movement. After this ceases, the surface of the egg is papillose, owing to the incompletely absorbed antherozoids. He was unable to distinguish the antherozoids after they had penetrated the egg.

Gayet (1897) also saw the entrance of many antherozoids into the archegonium of *Fissidens incurvus*, but discerned no rocking movement of the egg. Only one antherozoid penetrates the egg. This becomes first crescent-shaped, then spherical, and finally fuses with the egg nucleus, while the thin envelope of cytoplasm with which it has been surrounded fuses with the cytoplasm of the egg. The female nucleus possesses at this time four very distinct chromosomes, which seem drawn towards the male nucleus. Gayet followed the same history in *Bryum capillare* up to the crescent-shaped stage of the male nucleus. He also described abnormalities in the archegonia of various Liverworts, such as supernumerary ventral canal cells, and reported the fertilization of the ventral canal cell instead of the egg in *Marchantia*.

The Leeuwen-Reijnvaans (1908 *a*) described a remarkable series of events in the fertilization of *Polytrichum*. According to their account, reduction divisions occur in both antheridium and archegonium. The egg and the ventral canal cell then fuse, and the cell resulting from this fusion is fertilized by two antherozoids, this double fusion restoring the sporophytic number of chromosomes. The antherozoids maintain a slender form in the cytoplasm of the cell formed by the fusion of the egg and the ventral canal cell, but take on the appearance of resting nuclei when they come in contact with the female nucleus. The membranes of the three nuclei then disappear. In a subsequent paper the same authors (1908 *b*) described a similar 'double reduction' in *Mnium*. The spermatogenesis of *Polytrichum* has been investigated by Walker (1913), and that of *Mnium* by Wilson (1911). Both of these authors reported nuclear divisions of the usual type, without reduction. Walker also found that the egg and ventral canal cell of *Polytrichum* do not regularly fuse, and attributes the results of the Leeuwen-Reijnvaans to their technical methods.

In *Sphagnum subsecundum*, according to Bryan (1920), fertilization is replaced, at least in some cases, by a fusion of the ventral canal cell and egg. The possibility that a process of this type may be widespread among the Bryophyta makes it all the more desirable that the complete history of fertilization be followed in a number of forms.

Material and Methods.

The account that follows is based on a study of *Sphaerocarpos Donnellii* Aust. The plants used were grown on soil in three-inch pots, which were kept in Wardian cases in the greenhouses. Most of the cultures originated in single sporelings or in single plants isolated from larger cultures. Cultures of each sex were thus kept separate until it was desirable to bring about fertilization. Male and female plants were then transplanted to the same pot. After about a week they had become firmly attached and were flourishing in the new soil. By flooding these mixed cultures with sterilized distilled water, the plants having been kept submerged about fifteen minutes, numerous sporophytes were obtained. The sporophytes first became visible under a hand-lens as small, spherical, whitish bodies in the bases of the older involucres. The time necessary for them to attain this development varies from two to eight weeks from the time of flooding.

The fixing fluid that gave the best results was made according to Flemming's medium formula, modified by the addition of 3 per cent. urea crystals. Satisfactory preparations were obtained by using only the most healthy cultures, by fixing during the cooler seasons of the year, and by taking special precautions in dehydration and in paraffin infiltration.

To obtain a series of stages of fertilization in progress, plants from cultures that had been flooded were removed and fixed every few hours after flooding. Several trial sets of fixations sufficed to work out the best technique, and to disclose the approximate time involved in the process of fertilization. Four complete series were then made, covering periods from a few minutes to five days after flooding, at intervals of from two to four hours. These series were designated as *A*, *B*, *C*, and *D*. The account that follows is based on all four of these series, which after close examination proved to correspond very closely in all essentials.

The fixing plants were embedded in paraffin in the usual way, and sections were cut 7 μ in thickness. The stain most used was the Flemming triple combination of safranin, gentian violet, and orange G. The contents of the venter of the archegonium showed a marked affinity for the stains, especially for the safranin; so that it was possible only to dip the slide in the safranin and then necessary to destain in acid alcohol. An immersion of about a minute in gentian violet gave good results. Substitution of light green for orange G did not give as clear preparations. Heidenhain's iron-alum-

haematoxylin was also used. This stained the chromatin and nucleolus very heavily—almost too heavily—but left the cytoplasm practically untouched. When light green was used as a counterstain with the haematoxylin, the cytoplasm became visible, but could not be seen so clearly as with the triple stain.

Sections were made in a longitudinal vertical direction through the plants and seriated so as to include all of every plant. Only a few of these sections contained archegonia of the right age, and the others were discarded.

Free-swimming antherozoids were prepared for study by introducing them into a drop of water on a slide, inverting this slide over a phial containing 1 per cent. osmic acid, and allowing it to dry, afterwards staining in dilute gentian violet.

Observations and Discussion.

Since in *Sphaerocarpos* fertilization proved to be a slow process, and since the history was worked out from a large number of instances, it was found convenient to divide the process into a number of *phases*, each distinguished by some well-marked change in the male nucleus, in the female nucleus, or in both. The nature of these changes will be taken up as the phases are severally discussed. It must be borne in mind, of course, that these changes are in reality continuous, and that the limits of the phases are therefore purely arbitrary, constituting merely a method of handling the large amount of data. The number of cases in which fertilization was observed, with the distribution of these cases over the time covered by the process, and their classification into phases, is shown in Table I. Reference will be made later to the information deducible from such a statistical arrangement of the data.

Structure of the Mature Archegonium.

Mature archegonia appear, as has been pointed out elsewhere (Rickett, 1920), just behind the growing point of the thallus. Between them and the group of initial cells are other archegonia that have not yet reached maturity, and farther back on the thallus are others which, in the absence of fertilization, have disintegrated.

The structure of the archegonium is that typical for the Liverworts. The neck is regularly curved, often forward, owing to the more rapid division of the cells on one side. The venter is large and rounded, and consists, before fertilization, of a single layer of cells. The neck consists of six rows of cells, as described by Campbell (1896) and Cavers (1911). Hy (1884) and Gayet (1897), on the contrary, state that there are but five rows of cells in the archegonial neck.

TABLE I.

Cases of Fertilization.

Phase.	*Series.*	*Time after flooding. Hrs. Min.*	*No. of cases.*
1.	C	0 45	1
	A	0 55	8
	C	1 30	9
	A	1 40	8
	C	2	4
			30
2	C	4	6
	C	6	5
	A	7	4
	C	8	1
	C	20	2
	C	25	1
			19
3.	C	14	2
	C	18	4
	A	18 30	2
	C	22	2
	C	28	1
	C	32	1
			12
4.	C	28	2
	A	28	5
	B	30	5
	C	32	6
	B	36	1
	B	38	22
	B	40	9
	C	40	4
	C	42	3
	C	44	4
	B	44	15
	C	46	3
	C	48	5
	A	48	15
	C	50	1
	C	52	2
	C	53 30	2
	B	64	1
	D	65 30	1
	D	67 30	2
			108

Phase.	*Series.*	*Time after flooding. Hrs. Min.*	*No. of cases.*
5.	C	28	1
	C	42	5
	C	44	1
	B	49	2
	B	52	10
	C	53 30	2
	B	56	8
	C	59 30	3
	B	60	7
	D	62 30	5
	C	64	1
	D	65 30	1
	C	66	29
	D	75 45	1
	D	84	1
			77
6.	C	59 30	1
	B	60	4
	B	64	1
	D	71 30	1
	D	73 45	1
			8
7.	B	60	3
	B	64	2
	D	65 30	8
	D	67 30	11
	A	68	9
	D	75 45	7
	D	84	1
	B	105 30	1
			42
8.	B	64	2
	A	68	1
	D	65 30	1
			4
9	B	64	1
	A	100 30	4
			5
10.	D	71 30	1
	D	84	2
	B	105 30	2
			5
		Total	310

The egg is large and ovoid, measuring about 40 by 20 μ. It does not quite fill the cavity of the venter. This is partly because of the disintegration of the ventral canal cell. There seems to be no considerable

shrinkage due to fixation, for in living archegonia freshly dissected out the egg may be observed through the wall of the venter as a darker body lying free in the cavity. The action of the fixing fluid is shown, however, by the slight irregularities in shape of several of the eggs figured. The cytoplasm of the egg is dense, of a fine structure, and stains very deeply. The nucleus lies in the centre of the egg, and is also of an ovoid form, measuring about 13 by 10 μ. In the telophases of the last division of the central cell, which gives rise to the egg and the ventral canal cell, the nucleus contains a small mass of very deeply staining chromosomes, so tightly packed together that nothing can be made out of their structure or number. In the resting nucleus of the egg the chromatin is still in a close mass in the centre, about the nucleolus, surrounded by a clear space which in turn is bounded by the nuclear membrane. Certain strands and granules are to be found in this clear area. Occasionally a nucleus is found having a more open structure, and in such cases the chromatin is seen to be still largely in the form of rods and threads. One nucleus of the latter type is shown in Pl. III, Fig. 2. In the centre is a large nucleolus.

There is no indication of any receptive spot in the cytoplasm of the egg. This corresponds with Kruch's (1891) account for *Riella*. A receptive spot has been described by Strasburger (1870) for the egg of *Marchantia*, by Humphrey (1906) for that of *Fossombronia*, and by Campbell (1888, 1918) for the eggs of *Targionia*, *Funaria*, *Pilularia*, and several ferns. In all these forms it is described as a more hyaline area of the cytoplasm near the apex of the egg. In *Sphaerocarpos* there are many cases (see especially Figs. 4 and 9) in which the distal end of the egg (the upper end in the figures) is flattened or even slightly concave. Garber (1904) described a similar concavity at the distal end of the egg of *Riccia natans*. It is possible that what is known as the 'receptive spot' appears only under the influence of certain technical methods. The flattened or concave portion of the egg in my preparations may be indicative of a local structural difference, which under a different fixation might appear as a hyaline area. There are conflicting statements as to the presence of a receptive spot in the eggs even of the same form, for Durand (1908) denies the existence of such a spot in *Marchantia*.

At maturity the four canal cells that fill the neck, together with the ventral canal cell, break down, and the mucilaginous material derived therefrom streams out from the mouth of the archegonium. This material may be seen in preparations as a densely stained, coagulated, somewhat stringy mass, filling the neck canal and part of the venter, and extending out from the neck. The egg is now ready for fertilization, and apparently remains so for only a short time. If not fertilized, the egg disintegrates, this change being evidenced first by a great increase in its (already marked) staining capacity, by a massing together of the nuclear materials, and

finally by a contraction of the whole egg into a small body which lies free at the base of the venter. During this process of disintegration the distal concavity is usually more pronounced than previously, while at the other end the egg is quite sharply pointed. The remainder of the archegonium persists unchanged in appearance for a long time after the egg has disintegrated.

The age of the archegonium may be judged approximately by the stage of development of the involucre. At the time of the maturity of the egg, the involucre is merely a cup around the base of the archegonium, extending sometimes to the base of the neck. Archegonia showing fertilization stages thirty-six hours after flooding are usually completely enclosed by the involucre, and after three days from the time of flooding the involucre extends a short distance beyond the end of the neck. The continued growth of the involucre is not dependent upon fertilization, although its form is considerably affected by the development of the sporophyte. A short distance behind the growing point archegonia containing eggs in the first stages of degeneration may be found, surrounded by involucres about thirty-six hours old (judging from the above account based upon cases of fertilization). From this time on the involucre enlarges rapidly, while the archegonium finally withers; so that the posterior part of the thallus is covered with the large cylindrical involucres, enclosing at their bases the remnants of unfertilized archegonia. The rate of growth of the involucre is so variable (compare Text-figs. 2 and 3) that estimates of its age based on its size, and consequently of the age of the archegonium based on that of the involucre, must be taken very cautiously. But it seems probable that the time during which an archegonium may be fertilized is limited to a period of from twenty-four to forty-eight hours after it reaches maturity, at which time the entrance of antherozoids is made more likely by the fact that the archegonium is not yet completely enclosed by the involucre.

An archegonium with its involucre is represented in Text-fig. 2. In this case the egg has been fertilized. Not all of the neck is shown, since its distal end curves, and portions of it appear in succeeding sections.

Each archegonium is usually surrounded by its own involucre; but on one plant I found two involucres each of which contained two archegonia. Two archegonia were found also which contained apparently two eggs each, side by side in the venter, and of about equal size. Several cases of similar abnormalities are to be found in the literature. Gayet (1897) reports that two superposed ventral canal cells are sometimes found in *Sphaerocarpos*. He found two ventral canal cells also in *Marchantia*, in this case side by side. Campbell (1896) reports the occurrence of two superposed ventral canal cells in *Geothallus tuberosus*.

Penetration of the Antherozoid into the Egg.

If one observes a male culture under a binocular microscope, many involucres may be seen to extrude from their tips small globules of whitish sticky material. These globules may be picked off with a needle or fine brush and deposited in a drop of water on a slide, or the whole plant may be transferred to the drop for a short time. The drop of water, upon examination with a high power of the microscope, proves to contain numerous antherozoids, which after a few minutes disentangle themselves from the surrounding viscous material and begin to swim around in the water. If killed and stained in the manner already described, their form is that shown in Text-fig. 1.

TEXT-FIG. 1. Antherozoids shortly after having emerged from the antheridium. × 1300.

An antherozoid consists of a slender rod, variously curved or coiled, bearing two cilia attached near the anterior end, and a cytoplasmic vesicle at the posterior end. This vesicle is, apparently, soon lost. The cilia are not attached at the same point. At the anterior end of the body of the antherozoid is a less deeply staining portion. One cilium is attached at the anterior end of this body, the other just posterior to it. Measurements of the cilia of the antherozoids figured show a difference in length between the two cilia of each individual; the anterior cilium measuring quite constantly 44 μ, the posterior one from 46·5 to 50·5 μ; an average difference of 3·6 μ. The average length of the body of the antherozoids figured is 18 μ, the individuals varying from 16 to 20 μ. The body is 0·5 μ thick.

Of all stages in fertilization the actual entrance of the antherozoid into the egg is the most difficult to observe. The neck at this time is filled with the mucilaginous material described above, and the space in the venter above the egg with the remains of the ventral canal cell; all of which forms

a dense, stringy, deeply staining material filled with various small bodies and granules. I have not succeeded in detecting the free-swimming antherozoid in its passage through these regions, though I have examined many slides made from material fixed at various short intervals after flooding. Neither have I seen a single case in which I can say with certainty that the antherozoid was penetrating the egg. The membrane of the latter is at this time very delicate, and, either naturally or as a result of fixation, the surface layer of the cytoplasm is somewhat 'frayed out' into the surrounding space; so that instead of a clear boundary of the egg there is a confusion of little strands and granules which seem to belong to the cytoplasmic reticulum. Among these bodies the entering antherozoid may in some cases lie concealed. The thinness of the egg membrane at this time has been noted also in *Riccia sorocarpa* by Kny (1866), who thought that the membrane is resorbed, at least at the distal end of the egg.

The first sign of the presence of the antherozoid in the egg is the occurrence, in fixations made fifteen, twenty, and forty-five minutes after flooding, of a slender curved body lying in the peripheral regions of the egg cytoplasm and hardly to be distinguished from the latter. Such a case is shown in Pl. III, Fig. 1. In several cases these bodies were parallel to the surface of the egg. Some were in the distal end of the egg (Fig. 1), some in the basal end, and still others in one side. This makes it doubtful whether the concavity sometimes seen in the distal end of the egg, described above, has any real relation to the entrance of the antherozoid. If it really represents the receptive spot described for other forms, then the receptive spot, in *Sphaerocarpos* at least, is not the only place at which antherozoids can penetrate the egg. It is noteworthy that these bodies which I have interpreted as antherozoids correspond in their variable position in the egg cytoplasm to the male nuclei clearly seen in later stages, which similarly may approach the female nucleus from above, from below, and from the side. In one case, a slender strand of the mucilaginous material that fills the venter extended across the ventral cavity from the archegonial wall to a point on the surface of the egg near where the antherozoid lay inside the egg.

First Phase.

The antherozoid first appears clearly and unmistakably discernible as a small, rod-shaped, often curved body lying in the egg cytoplasm, surrounded by a clear space of varying dimensions, whose boundaries are not clearly defined. This body is dense, staining most readily with safranin, and apparently homogeneous; I have found no evidence of a differentiation of its contents at this stage. It may be pointed at one or both ends, but usually both ends are rounded. It measures about 6 by 1·5 μ. This stage is illustrated in Fig. 2.

Whether this is the whole antherozoid or only its nucleus could not be

determined. Neither at this nor at any later stage could I discern anything in the egg cytoplasm that might be interpreted as the remains of the cytoplasm of the antherozoid; yet it is certain that the rod-shaped body just described becomes transformed into a typical nucleus. Probably the cytoplasmic portions of the antherozoid are absorbed by the egg cytoplasm immediately after its penetration. Possibly the clear space mentioned above is occasioned by the occurrence of this change, though it seems more probable, in view of later changes, that it is due to an extrusion of material from the dense body itself. Taking what seems to be the most probable view of the origin of the latter body, I shall refer to it in future as the antherozoid nucleus, or as the male nucleus. A comparison of its dimensions with those of the free-swimming antherozoid shows that it has contracted greatly in length, while increasing in thickness.

In one case (one and a half hours after flooding), the male nucleus was not surrounded by a clear space, and was slightly more slender than usual. This is the only indication found of what must be an early stage in an extremely rapid process—the transformation of the antherozoid, or of its nucleus, into a large, dense, rod-shaped body. Indeed, the rapidity of all these first processes and changes is impressive. One case in which the male nucleus was already clearly distinguished in the usual shortened form was found forty-five minutes after flooding. The absence of clear stages showing the penetration of the antherozoid is no doubt partly due to the rapidity with which it takes place.

In another case (one and a half hours after flooding) the egg cytoplasm shows a slight depression in its surface, near one end of the male nucleus, which now lies within. This probably marks the place where the antherozoid entered the egg.

The egg at this time shows little change from the condition already described. The cytoplasm varies in appearance. In some preparations it has the appearance of a fine, complex reticulum embedded in a homogeneous mass (Fig. 2). This apparently reticular structure may of course be in reality alveolar. An alveolar structure does indeed appear in later stages; but in these cases the open spaces are large, regular in shape, and clear, whereas at the early stage now under discussion they are small, irregular, and take a deep stain. It seems probable, therefore, that the structure here is reticular in nature, with a dense homogeneous ground substance. Even at this early stage, the cytoplasm of different parts of the egg may vary in structure. In one case, the cytoplasm is noticeably denser about the nucleus, and shows a more open structure in the peripheral region. This is suggestive of what is to come later. A similar structure has been described by several authors. Gayet (1897) says that the egg of *Sphagnum* is formed of cytoplasm having a reticular structure, the meshes of which are denser about the nucleus. Campbell (1918) figures and describes the ripe egg of

Targionia as having a denser zone of cytoplasm surrounding the nucleus. Miss Graham (1918) reports that the outer zone of the egg of *Preissia* is more coarsely alveolar than the portion around the pronuclei. In *Nephrodium*, according to Yamanouchi (1908), the cytoplasm of the egg has a finely fibrillar structure, very dense about the nucleus and vacuolate towards the periphery.

It is of course impossible to draw from fixed material any final conclusion as to the actual structure of the protoplasm in the living state. But since the stages herein described were all seen in preparations made in exactly the same way, with the same reagents, and since, as will be seen, there is a progressive series of changes in the cytoplasm, it is evident that, if not the structure, at least the substance, of the cytoplasm must undergo changes during the process of fertilization. The whole series of changes lends support to the idea that there are two kinds of substance present in the cytoplasm, corresponding perhaps to Strasburger's (1892, 1893) 'trophoplasm' and 'kinoplasm', which at first are uniformly distributed throughout the cytoplasm, but of which one later becomes more or less localized and differentiated for its special functions.

Eggs observed at this stage measured about 50 by 20 μ. The average difference in length (10 μ) between these eggs and mature unfertilized eggs is probably significant, and indicates that the egg begins to enlarge at this time. This is in accordance with the account of Garber (1904) for *Riccia natans*, and of Campbell (1918) for *Riccia glauca* (?), both of whom described the egg as enlarging immediately after fertilization.

The female nucleus is also larger than that of the unfertilized egg, measuring about 17 by 12 μ. Its structure is essentially that already described. Fig. 2 shows an egg whose nucleus, although the antherozoid has already entered, contains traces of chromosomes which have persisted from the last preceding division. Usually, however, the egg nucleus has rather the appearance shown in Figs. 4 and 5, containing numerous small, irregular masses, granules, and rods, some scattered through the nuclear cavity, but most of them massed in the centre about the large nucleolus and almost hiding it. The nucleoli, of which there may be one or two, often appear irregular in shape, but this is probably due to the fact that masses of chromatin are grouped about and in contact with them.

There is no definite relation between the respective positions of the male and female nuclei. Just as, apparently, the antherozoid may penetrate the egg at any point, the male nucleus may lie in any part of the cytoplasm, and at any relative distance from the plasma membrane and from the nucleus of the egg. Most frequently the male nucleus is somewhat towards the distal end of the egg, and about midway between the egg nucleus and the outer boundary of the cytoplasm.

This phase extends through about two hours from the time of flooding.

Second Phase.

The next phase is marked by a change in shape of the male nucleus, which now becomes ovoid. This is shown in Fig. 3. Its structure and position do not change. It is of this form for about eight hours. It becomes somewhat shorter, being now about 5 μ in length ; but its thickness almost equals its length, so that it is evident that a considerable enlargement has taken place.

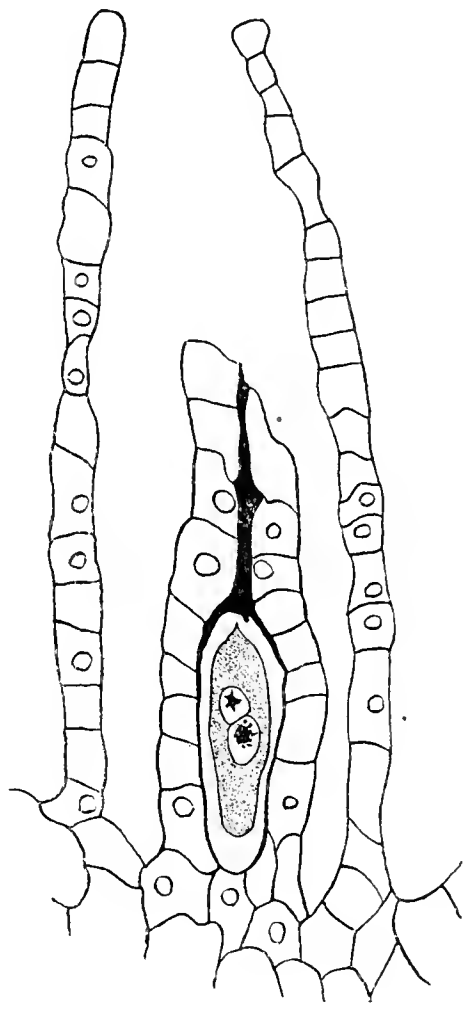
The female nucleus is the same in appearance as in preceding and succeeding phases (Figs. 4 and 5). The egg cytoplasm is also unchanged in many cases, but in others it becomes coarser, showing a deeply staining, irregular reticulum. This is formed of threads of varying thickness, sometimes straight, sometimes very much twisted and curved, with granules of very various sizes at the points of junction of the threads and often at other points along them. This condition is more usual in succeeding phases, and is illustrated in Fig. 8, which represents the fourth phase. Such a structure may, of course, be considered as an alveolar one, greatly distorted by the fixing agent. My chief reasons for considering it a true reticulum are that a very clear alveolar structure is to be seen in later stages, and that eggs at this stage, though fixed in the same way, present quite a different appearance.

Third Phase.

During the next twelve hours, the male nucleus is approximately spherical (Fig. 4), and sometimes has the appearance of a dense mass of granules rather than that of an absolutely homogeneous body as heretofore. This structure may be especially evident at its surface, which is often rough, with tiny protrusions, instead of having a clear smooth outline. In most cases, however, the structure of the male nucleus is apparently the same as in the previous stages, except that the clear space surrounding it is now for the first time bounded by a definite membrane. This membrane is continuous with the cytoplasmic reticulum and is probably cytoplasmic in origin. The male gamete is now, therefore, represented in the egg by a typical nucleus, consisting of a nuclear membrane, a dense central mass containing chromatin, and a clear material filling the rest of the nuclear cavity; this clear material is analogous to the nuclear sap of the ordinary vegetative nucleus, and is probably formed by extrusion from the denser central mass of the nucleus.

The egg retains the same appearance as in the previous phases, as to both its nucleus and its cytoplasm (Figs. 4 and 5). Its surface has lost that 'frayed' appearance characteristic of the earliest stages, and a clearly defined membrane is present. Without microchemical tests, it is impossible to say whether or not a cell wall is present. In all later stages, and even in the young embryo consisting of several cells, there is no thicker boundary of the

egg or of the free surface of the cells of the embryo than appears at this time. Meyer (1911) describes and figures the egg of *Corsinia marchantioides* as forming about itself a distinct membrane after receiving the antherozoid. Comparing his figure with the fertilized egg of *Sphaerocarpos*, the egg of the latter takes up far less room in the venter, and what seems to correspond to the 'fertilization membrane' in *Corsinia* is the heavily staining mucilaginous material that filled the cavity of the venter and the neck during the first phase of fertilization and that remains as a dense layer appressed to the inner wall of the venter and filling the neck (Text-fig. 2). In a subsequent paper Meyer (1913) again refers to, and figures, the fertilization membrane, this time in *Plagiochasma rupestre*. Here the resemblance is even stronger to what I have seen in *Sphaerocarpos*, and the suspicion seems justified that the so-called 'membrane' may really be the mucilaginous material of the venter, which looks like a membrane belonging to the egg only when the latter completely fills the venter.



TEXT-FIG. 2. Archegonium surrounded by involucre and containing an egg in the fourth phase of fertilization. Thirty-six hours after flooding. × 320.

A fertilization membrane has been described for several other Liverworts. Kny (1866) figures and describes a fertilized egg of *Riccia sorocarpa* as surrounded by a fairly thick layer or membrane. Garber (1904) says that the egg of *Riccia natans* surrounds itself with a membrane immediately after fertilization. The same is reported for *Fegatella conica* by Cavers (1904), and for *Targonia hypophylla* by Campbell (1918). Cavers says that the membrane is of cellulose. These authors do not, however, go into details on the subject.

The position of the male nucleus during this phase is in general the same as in previous phases, but in one instance the two nuclei were seen in contact.

Fourth Phase.

There now begins a marked change in the male nucleus. The condition mentioned above, in which the mass of this nucleus seems to be granular in structure, is more often seen, and often the structure is looser, so that distinct granules may sometimes be made out, with lighter areas between them (Figs. 10 and 11). At the same time, threads project from the edge of the dense part of the nucleus and extend across the clear space to the nuclear membrane (Fig. 8). Sometimes there are but one or two of these threads,

sometimes they are present throughout the nucleus, so that the latter has the appearance of a rough wheel with a large hub and irregular spokes (Fig. 7). Finally, the whole male nucleus may resolve itself into a compact reticulum which fills the whole of the space within the membrane, no part being noticeably denser than any other. This structure is illustrated in Fig. 12, a very early stage of the next phase. The reticulum is composed of granules linked together by threads which are almost as thick as the granules, so that the whole structure is still fairly dense and compact. The chromatic material of the nucleus now takes a violet colour in the triple stain, whereas in former stages it stained more readily with the safranin.

This series of changes is not found in succession throughout the period occupied by this phase. It seems probable that each nucleus must pass through these steps in order to attain the reticular structure; but some of the later stages are found in some nuclei at the beginning of the period, while others, perhaps in the same plant, still resemble those in the preceding phase. On the other hand, the earlier stages may be found throughout the period.

It is in this phase of the process, in fact, that the times at which the various changes take place begin to be variable. Series *A* and *B* furnish most of the cases in which the chromatic mass of the male nucleus is contracted and dense, while in Series *C* the more open reticular type of structure is more common. This variation between series suggests not only that the time necessary for the different phases may vary, but that the extent of the changes undergone by the nucleus may differ in different cases. It may be that in some cases the male nucleus never forms an open reticulum. The cause of this variation may lie in temperature or other external conditions, for each of the series was fixed at a different time of year from any of the others. And again, it is possible that the procedure of fixing varied sufficiently to account for these differences.

Another complication enters in the fact that in Phase 5, next to be described, the male nucleus seems to contract again, and presents an appearance similar to what is seen during Phase 3 (Pl. IV, Fig. 14). This makes it difficult to say, in the case of such a contracted nucleus occurring during the present phase, whether it is a delayed or an advanced stage. In one case, unfortunately torn in the preparation, the male nucleus seemed to contain a single much-coiled thread, along which were scattered granules in a single row.

Some variation begins to appear also in the female nucleus. In general, the chromatic substance is still massed in the centre, and little can be made out of its structure; but occasional nuclei are found in which more of the chromatin is distributed through the nuclear cavity in the form of threads and granules, with larger masses here and there. Typical nuclei of this phase are shown in Figs. 6 and 9. Sometimes also the changes characteristic

of Phase 5 are seen to be beginning here—a grouping of the chromatic material into the general shape of a continuous thread. In Series *A* most of the cases of fertilization 48 hours after flooding, a time which falls within Phase 5 in the other series, belong here as regards the structure of their nuclei.

In the cytoplasm the tendency towards an alveolar structure becomes more marked. There are also many cases showing the coarsely reticular structure described under the second phase (Fig. 8). Few eggs, however, show the fine, more homogeneous structure characteristic of the first phase. Some of the preparations belonging to this period show the beginning of a phenomenon which is characteristic of the next phase, and which will be more fully discussed later. This is a massing of a more dense and finely divided material in the cytoplasm near the poles of the female nucleus, leaving the rest of the cytoplasm often distinctly alveolar. The beginning of this process is shown in Figs. 9 and 12. Very rarely indications of this change are found in earlier stages. In Series *C* several eggs showing the characteristics of the second phase, and fixed only a few hours after flooding, show areas in the cytoplasm, near the poles of the nucleus, the substance in which seems more homogeneous than the rest of the cytoplasm and stains more lightly. Mention has already been made, under Phase 1, of a case in which the cytoplasm was denser and of a finer structure immediately around the nucleus, more open in the peripheral region. The most remarkable instances of the localization of a differentiated portion of the cytoplasm were found in Series *A*, 48 hours after flooding. In these cases there is an aggregation in the cytoplasm, in the regions normally occupied by the polar caps just described, of distinct granules of considerable size. They stain blue, and are conspicuous in the preparations. They are usually arranged in more or less regular rows radiating out from the poles of the nucleus. They are connected by a delicate system of slender threads. The whole structure seen under the low power of a microscope suggests at once two asters formed of radiating fibres; however, there is no distinctly recognizable body present at the centre of the radiations, either in this or in earlier or later stages. This condition is shown in Fig. 11. It is not universally found during this phase even in Series *A*, and is met with only rarely in the other series. As it sometimes occurs in later stages, further discussion of it will be postponed.

The condition of many of the female nuclei in eggs which show these cytoplasmic granules is peculiar. The contents are stained red instead of violet as usual, and are in the form of rounded bodies scattered throughout the nuclear cavity, with traces of a very fine reticulum. In Fig. 11 nine of these bodies are visible. The remainder of the same nucleus, seen in the adjacent section, shows several more. They have an appearance suggestive of small nucleoli, and recall the structures present in the nucleus in some of

the first stages of degeneration. This, together with the sporadic occurrence of the granules in the cytoplasm, suggests that the whole phenomenon may be artificial or pathological in nature.

It is the fourth phase which seems to correspond with most of the stages of fertilization in Liverworts observed by other workers.

Fifth Phase.

It was seen in the consideration of Phase 4 that there is a great variation in the time of occurrence of particular stages relative to the time after flooding. This variation now becomes even more marked, so that, although the next set of changes occupies roughly the next 20 hours (to 66 hours after flooding), this time may vary very greatly.

The male nucleus, as mentioned above, apparently undergoes a contraction, and appears once more as a small mass of granules surrounded by a clear area (Pl. IV, Fig. 14). It is probable that this condition is usually preceded by the open reticulate condition, since the majority of the cases of this phase was found in the same series (*C*) as afforded most of the instances of the reticulate condition.

The characteristic change in the female nucleus during this phase is the organization of chromosomes. The first indication of a change is that the chromatic substance, heretofore (in most cases) densely massed in one part of the nucleus, begins to spread out or unravel slightly, and becomes collected in several large masses and a larger number of smaller ones, which in their shape and position with respect to one another suggest that a continuous thread or spireme is being formed—or at least a series of chromosomes or short threads arranged roughly end to end. The substance of these groups of material is still a mass of fine granules and delicate coiled and twisted threads. The rest of the nuclear cavity is not entirely clear, for much of what seems to be the same stainable material is scattered through it, often in the form of a very delicate and rather regular reticulum bearing granules at the points of junction of the threads. Some slight indications of the beginnings of these changes can be seen in Fig. 13; the chromatic substance is still densely massed in the centre of the nucleus, but within it can be distinguished some more massive bodies arranged roughly in chains. In the nucleus represented in Fig. 14 an apparently continuous spireme has been formed. The outline of the latter is rough, and it is not homogeneous in structure. In many cases it seems to be continuous with the delicate reticulum which fills the rest of the nucleus. A nucleolus is not recognizable at this stage; if present, it does not differ from other structures of the nucleus either in form or in reaction to the stain. In Figs. 16 and 17 the spireme has apparently segmented into chromosomes; the outlines of the latter are much smoother than that of the spireme shown in Fig. 14. In Fig. 17 the X-chromo-

some described by Allen (1919) is suggested by a segment which is much longer and thicker than any of the others.

The two nuclei are usually in contact during this phase.

In many cases the staining method which best showed the nuclear details imparted to the cytoplasm only a very slight coloration, so that no details concerning its structure could be made out. In all cases, however, in which the cytoplasm was deeply enough stained to be carefully studied, certain well-marked changes were taking place in it. These changes are therefore assumed to be typical of this phase. The changes in question, as has already been indicated, involve the massing of a finer and more homogeneous portion of the cytoplasm at opposite ends of the egg nucleus. This differentiated material now takes the stain quite readily, but nothing can be made out of its structure save that it is practically uniform—apparently a mass of fine granules, possibly, in the living state, a colloidal liquid uniform in composition throughout its extent. In some cases there are faint indications of a reticular structure within these polar caps (Fig. 9). At their boundaries they merge into the cytoplasmic reticulum. The peripheral region of the cytoplasm is very vacuolate, and its substance is mostly concentrated into large and conspicuous ray-like structures, of a fibrillar nature, which radiate out from the boundaries of the polar caps. The whole appearance suggests very strongly a flowing of certain materials in the cytoplasm towards the female nucleus, and their aggregation there in a dense mass. A typical case of this process is shown in Fig. 9; another, in not quite so advanced a stage, in Fig. 12.

In the later stages the polar caps become more dense and apparently even more homogeneous, so that it is no longer possible to discern a reticular structure in them; they enlarge so as to surround completely the two nuclei and to occupy the larger part of the egg. The boundary of the material of the polar caps is now much sharper; outside it the cytoplasm is alveolar, the alveoli being fairly regular in shape and uniform in size. The condition is illustrated in Fig. 16. An indication of the ray-like structures formerly present is still seen in the presence of blunt, more or less conical projections from the dense into the alveolar cytoplasm, extending sometimes quite to the outer boundary of the cell.

In three cases the central mass of cytoplasm was replaced by a mass of granules, more or less radially arranged, as in those cases in Phase 4 described above.

It is while these changes are going on in the egg that the cells of the venter begin to divide, so that the wall becomes composed of two layers of cells. This division does not occur at the same time in all the cells, and the time at which it begins varies greatly. It might perhaps be expected that the beginning of these cell divisions would be correlated with some definite stage in the fertilization process. As a matter of fact, in the majority

of cases division of the wall cells does begin at the time that the polar caps begin to appear. Fifty such cases were counted; while only six showed a division in the wall of the venter at a time when the formation of polar caps had not begun. Twenty-seven eggs had formed their polar caps without any sign of division in the wall of the venter, but in many of these cases the polar caps were formed in advance of the usual time. It seems probable, therefore, that the division of the cells of the venter, if not stimulated by the changes just described in the egg cytoplasm, is at least usually coincident with them. The division is complete, and the venter consists of two layers or cells, in all cases observed in which the chromosomes are organized in the sexual nuclei.

Sixth Phase.

This phase includes the final steps in the organization of the chromosomes of both nuclei, the disappearance of the nuclear membranes, the splitting of the chromosomes, and the division of the latter to form the two daughter nuclei of the first embryonic division. Next to the penetration of the egg by the antherozoid, this is the most difficult of all the phases to observe. This fact is evident from a glance at Table I. Only eight cases are listed under this phase. Of these, four show the nuclear membranes intact, but enclosing fully organized chromosomes (Fig. 16).

The organization of chromosomes in the male nucleus was not seen. No transition stages were obtained between the granular condition last described and that of those nuclei which contained a clearly defined spireme or chromosomes lying within the nuclear membrane. The change is probably, therefore, very rapid; and the suggestion is inevitable that the spireme and chromosomes are organized during the contraction period of the nucleus—much as the *gemini* of the heterotypic prophases are organized in synizesis during the reduction division.

One case was observed in which the chromatic substance of the male nucleus was in the form of an apparently continuous spireme, coiled within the nuclear membrane. This is shown in Fig. 15. In another case, shown in Fig. 16, instead of a spireme there were eight distinct chromosomes, the regular gametophytic number for *Sphaerocarpos* (Allen, 1919). One of these is very small, and only by good fortune in the making of the section did it escape being masked by one of the larger ones. This is undoubtedly the Y-chromosome described by Allen. It is not a cross-sectional view of a long chromosome, since it disappears completely from view with a slight change in focus.

The female nuclei in these cases had not quite completed the organization of their chromosomes; the outlines of the latter were not quite so sharp, nor was their consistency apparently so uniform, as was the case with those of the male nuclei. Hence it would seem that, although the female

nucleus begins to organize its chromosomes earlier than the male, the completion of this process requires a longer time in the female nucleus, and that, before it is finished, chromosomes have been organized in the male nucleus with great rapidity. This difference may be connected with the large amount of material which is thrown off by the female nucleus and which takes no part in the formation of chromosomes. This material has been described under the preceding phase as visible in the form of small granules and in traces of a very fine reticulum scattered through the nuclear cavity; in the metaphase of the first embryonic division (Fig. 18) the cytoplasm contains many small granules which take the same stain, and which are in all probability the same bodies as were previously observed in the female nucleus.

In one of the cases above noted the central dense portion of the cytoplasm surrounding the female nucleus was replaced by a collection of the peculiar granules described under Phase 4, much as is shown in Fig. 11.

One preparation shows two groups of chromosomes lying in the cytoplasm, but is not sufficiently clear to enable me to determine whether or not the nuclear membranes are still present. Another preparation shows a dense mass of chromosomes surrounded by a slight clear area, but is also not sufficiently clear to be of great value. These two cases illustrate this phase without making very clear what is actually taking place.

Two preparations showed the chromosomes lying free in the centre of the dense central mass of cytoplasm. One of these belonged to Series *B*, and was fixed 60 hours after flooding. The chromosomes are in a small group, and only eleven could be counted with certainty (Fig. 18; one chromosome is in the adjacent section); the others (sixteen is the full sporophytic number) must either have been dragged out of place by the sectioning knife and lost, or were entirely concealed by some of the larger ones. The large X-chromosome is clearly visible. Two of the chromosomes seem to be split. Close examination of the cytoplasm showed it to be streaked in appearance, with alternate light and dark strips running in a fairly regular course. Definite fibres could not be seen. It is possible that this structure is an artifact resulting from the action of the fixing fluid on what was when living a typical spindle; but this suggestion is rendered less probable by the fact that the same preparations show spindles with clearly defined fibres in the vegetative cells of the thallus (Figs. 23 and 24), and in succeeding embryonic divisions (Fig. 22). It appears, therefore, as though the spindle of the first embryonic division were masked by a dense homogeneous material possibly nutritive in nature, or, more probably (to judge from the manner of its first appearance), a formative material in which the fibres originate. The streaked appearance of the whole mass of material may indicate the position of fibres which are themselves invisible as such.

The other preparation of a similar stage was found in Series *D*, fixed $73\frac{3}{4}$ hours after flooding. It is shown in Fig. 19. Fourteen chromosomes

are visible, of which two are thicker and less regular in outline than the others, and may each represent two chromosomes close together. Many of the chromosomes are split, the split being visible through only a part of the length of each chromosome. The large X-chromosome is plainly visible. One chromosome is very small, and, owing to the fact that it has divided, almost ring-shaped; this may be the Y-chromosome contributed by the male parent.

The most important question suggested by a consideration of these stages and of those immediately preceding them is: Do the sexual nuclei actually fuse, after the chromosomes have formed, but while their membranes are still intact; or do the membranes break down simultaneously, leaving the chromosomes free in the cytoplasm? On this point I have no positive evidence. No stages were found suggesting that fusion was taking place. When any nuclear membranes were present at all, the boundary separating the two nuclei (now always in contact) was always sharp and clear. The next stage found showed the chromosomes in one group, with nothing to indicate which were of paternal and which of maternal origin (except in the cases of the X- and Y-chromosomes), and surrounded by no membrane at all. If an actual fusion of the nuclei occurs, it must be very rapid, and the fusion nucleus must lose its membrane almost immediately. It seems more probable that there is no actual fusion, but that each nuclear membrane disappears independently of the other, though perhaps at the same time. In this connexion it is interesting to note that Miss Black (1913) reports that the nucleus of the fertilized egg of *Riccia* probably undergoes a period of rest, during which the chromatin is collected into a densely staining cord or series of segments, while there may be less deeply staining and more finely divided material scattered throughout the rest of the nucleus. This description at once suggests my cases, during Phase 5, in which the female nucleus is undergoing the first steps of spireme formation. Evidently *Riccia* differs from *Sphaerocarpos* in that its male nucleus has at this stage already fused with the female nucleus. The similarity is rather striking, and there is a possibility that, in *Riccia* as in *Sphaerocarpos*, the male nucleus is still separate from the female at this time, but was overlooked.

Seventh Phase.

This phase includes the anaphases and telophases of the first nuclear division of the zygote and the division of the latter into two cells. The wall formed in this division is always transverse, as in all other Liverworts (except *Anthoceros*) in which this division has been described.

The daughter nuclei during the telophases are much smaller than either of the sexual nuclei just before the division. Because of the degree to which their chromatin is massed together, I could not follow the details of reconstruction. The daughter nuclei shortly pass into a resting state, and then

except for their smaller size, resemble the egg nucleus in the first or second phase. The chromatin is arranged in the form of small granules and delicate twisted threads, without any definite reticular structure (Fig. 20).

As the daughter groups of chromosomes separate, and the daughter nuclei are formed, the central dense mass of the cytoplasm seems to pull out between them, leaving only some coarse strands extending from one daughter nucleus to the other. In some cases these strands are fine enough to resemble the central fibres of an ordinary spindle, though they are never very numerous. Each daughter nucleus is still surrounded by a mass of homogeneous cytoplasm, which radiates in all directions so as to form a sort of star-shaped figure. It is interesting to note that the figures of Campbell (1888) for *Pilularia globulifera* and of Meyer (1911) for *Corsinia marchantioides* illustrating two-celled embryos show a condition of the cytoplasm in the daughter cells very similar to that just described for *Sphaerocarpos*. The condition is illustrated in Fig. 20.

In seven cases, all belonging to Series *D*, the dense substance was replaced by masses of granules as previously described. These are not arranged regularly in rows, and seem larger and closer together than those, for instance, shown in Fig. 11; but they maintain in the cell the star-shaped arrangement just described. Often they are very closely massed upon the new wall separating the daughter cells.

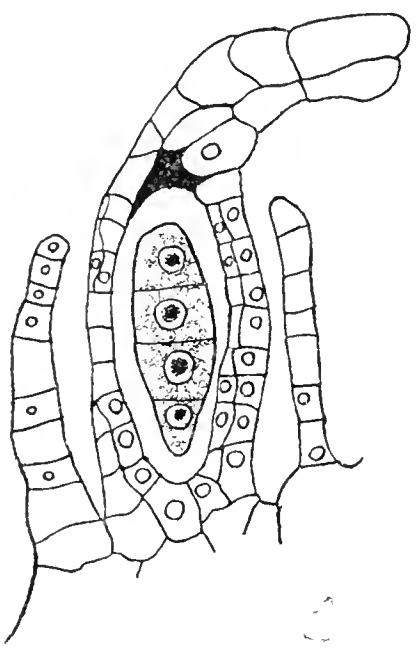
No cases were observed in which the cell plate or dividing wall extended only partly across the cell. In all cases in which a division membrane was present at all, it cut the cell completely into two, and was fairly distinct and heavy.

The two-celled embryo measures about 75 by 25 μ, and is therefore considerably larger than the egg in the first phase of fertilization. The enlargement is gradual, the egg in each successive phase being a little larger than in the preceding one (compare Figs. 2, 8, 18, and 20. Many sections, of course, do not pass through the greatest length of the egg).

Eighth Phase.

In each of the four cases found showing a division in the two-celled embryo, one of the nuclei had begun to divide before the other. In three of these cases the upper nucleus was dividing, in the fourth the lower. Very distinct fibres were visible in three of these cases, arising in the cytoplasm of the dividing cell and spreading out from two fairly definite poles over the nuclear membrane. The chromatin was observed both in the form of a spireme, still enclosed by the nuclear membrane, and in the form of chromosomes arranged in an equatorial plate upon a typical spindle. The latter stage is illustrated in Figs. 21 and 22. Fig. 21 shows a polar view of the equatorial plate. The chromosomes are more slender than in the first embryonic division. Fifteen chromosomes can be counted; of these, either

of two apparent chromosomes may really be two whose ends are close together. The X-chromosome is plainly visible, also, close by it, the Y-chromosome. Fig. 22 shows the spindle very plainly, but the chromosomes are too close together to be counted. Even here, however, the X- and Y-chromosomes are evident, close together as in the case just described. There seems no doubt that dividing nuclei of the embryo contain the full sporophytic number of chromosomes (16). For comparison with these sporophytic groups I have included figures of division stages from cells of the wall of the venter. Fig. 23 shows an equatorial plate; the chromosomes (except the X-chromosome) seem to be cut in cross-section; eight of them are visible, seven being small rounded bodies, and the much longer and thicker X-chromosome extending up and down the spindle. Fig. 24 shows an anaphase, in which eight daughter chromosomes are moving towards each pole of the spindle. In several cases the two daughter chromosomes of a pair are still attached to each other at one end. Here also the X-chromosome is easily distinguished.



TEXT-FIG. 3. Archegonium containing a four-celled embryo. Eighty-four hours after flooding. × 320.

Ninth Phase.

This phase includes embryos which have undergone two cell divisions. The four cells are in all cases that I have observed arranged in a row, as illustrated in Text-fig. 3. This account agrees with that of Campbell (1918), but is contrary to the description of Cavers (1911), who says that the 'embryogeny is of the Marchantiales type'—that is, the young embryo passes through an octant stage. Some unpublished work of Miss E. M. Ritchie indicates that there is some variation in the plane of the second division in the embryo of *Sphaerocarpos*. All the cells are of approximately the same size, and do not differ essentially in appearance from those formed by the first division, except that the cytoplasm is apparently alveolar in structure, and shows only slight traces of the dense material that surrounded the egg nucleus during Phases 5 and 6.

Tenth Phase.

This phase is merely a convenient grouping of all stages that were observed subsequent to those already described. The embryo has become a mass of cells, a longitudinal division having occurred in some cells. Since the present paper is concerned only with fertilization, no further discussion of embryological development will be included.

Time Relations.

Mention has already been made of the fact that sporophytes become large enough to be readily visible from two to eight weeks after flooding. This indicates that the speed of the process of fertilization and the rate of development of the embryo may vary under different conditions or in different cultures. The cultures that took the longest time (eight weeks) to produce visible sporophytes were those from which Series *C* was made. This fact coincides exactly with the cytological data, for, although the fixations in this series were carried on until 66 hours after flooding, no stages were found farther advanced than Phase 5, while in the other series fixations made 60, 64, and $65\frac{1}{2}$ hours after flooding disclosed many examples of Phases 6 and 7 (Table I).

The earlier stages seem to proceed at a quite uniform rate, so that it is possible to assign definite time limits to the first three phases. Phase 4 shows considerable variation; and Phase 5 still more. Finally, Phase 6, which includes the actual division of the fertilized egg, seems to occur over a wide range of time. It is also noteworthy that the preceding phase (5) is quite prolonged, as though the changes that go on therein require a long time for their completion, while the division itself is rapid. It is evident that, after a long period of slow and gradual change, during which time the egg nucleus organizes its chromosomes, the egg cytoplasm takes on a definite structure, and the male nucleus is in a condensed state, the further processes leading to the first division—the formation of chromosomes by the male nucleus and the disappearance of the nuclear membranes—take place with almost explosive suddenness. Furthermore, the time necessary to complete the slow preliminary changes and to initiate the actual division varies very greatly with the individual. These time relations are illustrated (Table I) by the small number of cases of Phase 6 as compared with the number of cases referable to preceding and succeeding phases. This is not because fewer slides were made covering this time, since the instances of Phase 6 that were found occurred often at the same time, sometimes on the same plants as afforded numerous instances of Phases 5 and 7.

A few antherozoids are often seen in the cavity of the venter outside the egg in later stages, when fertilization has already taken place. These retain their normal form, and to all appearances were alive at the time of fixation. This fact suggests the possibility that some of the variation in time of the different stages in fertilization may be due to a variation in the starting-point—that is, that some antherozoids may not enter the egg until several hours after flooding. This, however, is rendered improbable by the uniformity in time of the first two or three phases. It is also possible, as is suggested by an exceptionally premature case of Phase 5 (Table I), that fertilization has sometimes taken place in the material studied before flood-

ing. This, however, must have been rare, if it occurs at all, again for the reason that only cases of Phase 1 are found within the two hours following flooding. We may therefore, I think, conclude that the variation in the times at which stages are found is due to variations in the rate of the process rather than in the time of its starting.

Doubtful Cases.

In a number of the cases listed in Table I, the male nucleus was not to be seen. The female nucleus and cytoplasm, however, showed all the changes characteristic of the fertilization process, at one phase or another. Since no eggs are found in this condition in material which has not been flooded or afforded other opportunity for fertilization, these are thought to be true cases of fertilization in which the male nucleus was accidentally lost in making the preparation. In one case the male nucleus was found apparently inside the female nucleus. Closer examination revealed the fact that it was at a higher focal level than anything else in the egg; and that in the next section there was a clear space surrounded by a distinct membrane, the latter in contact with the membrane of the female nucleus. Evidently the knife had caught the chromatin of the male nucleus without cutting it, dragged it out of its proper place, and left it in a section in which it did not belong. Something of this sort may have happened in some of the doubtful cases referred to.

Here arises the question whether parthenogenesis occurs. The number of doubtful cases referred to above amounts to about 6 per cent. of the total (19 out of 307 cases). If this proportion of all the sporophytes formed resulted from parthenogenesis, we should expect to find a fair number of sporophytes in purely female cultures. As a matter of fact, the reverse is true. In the great majority of female cultures no sporophytes are found; and in cases where they do occur, their origin is always traceable to a male plant that has somehow found its way into the pot, or to an accidental flooding of several cultures (including males) at the same time, or to some similar accident. It might be supposed that the act of flooding the plants may in some way furnish a stimulus by which a few eggs are enabled to develop parthenogenetically. This, however, seems rather far-fetched; especially as female cultures have often been flooded by accident, without the resultant appearance of any sporophytes.

There seems, therefore, little doubt that the development of an egg into an embryo is in all instances the result of a sexual process—that is, that parthenogenesis never occurs, and that cases that might seem to suggest it are due only to accidents of technique.

Polyspermy.

In something over 8 per cent. of the cases of fertilization observed, more than one male nucleus was present in the egg cytoplasm. These cases are

assembled in Table II. They were most numerous in Series *C* and *D*, and in Series *C* were found mostly in the earlier stages.

Most of these cases show two male nuclei instead of one. In the earlier stages both male nuclei frequently appear normal, and the egg is in the condition that is usual for the phase in question. In several cases there are differences between the two male nuclei. In one instance one was less homogeneous than the other, although the time elapsed since flooding was short (4 hours). In one very early case (1½ hours after flooding) no less than six male nuclei were observed in one egg, mostly of irregular shapes. In two other cases there were four; in one of these instances the shapes of the male nuclei were irregular; in the other, two of them were close together within one clear space.

Table II.

Cases of Polyspermy.

Series.	*Time after flooding.* Hrs.	Min.	*No. of cases.*	*No. of male nuclei.*
A	0	35	1	3
C	1	30	1	6
C	1	30	1	2
C	2		1	2
C	4		2	2
C	4		1	4
C	6		1	4
C	6		1	2
C	18		2	2
A	28		1	2
C	32		1	2
B	40		1	2
A	48		1	2
D	62	30	1	several
C	64		1	3
D	65	30	3	several
D	67	30	5	,,
D	84		1	,,
			26	

In slightly later stages there is more frequently a difference between the male nuclei. The difference suggests sometimes that one is developing in the normal way, while the development of the other is arrested in the earliest phases. In one such case, found 18 hours after flooding, one male nucleus has the appearance normal for this time; the other is long, narrow, and pointed at the ends, the form characteristic of Phase 1. In another case one male nucleus is distinctly smaller than the other. Sometimes, however, both of them, three in one case, appear quite normal. The presence of the additional male nuclei seems not thus far to have disturbed in any way the normal development of the female cell.

In the later cases of polyspermy, most of which come from Series *D*, the female nucleus shows signs of degeneration. This is evident in a lump-

ing together of its contents and a loss of finer structure. Similar changes have taken place also in the cytoplasm. The male nuclei in these instances have not reached an advanced stage of development, but appear as in the three earliest phases of the normal process (sometimes as in Phase 5), save that certain irregularities in shape are sometimes evident. The condition is illustrated in Fig. 25.

It seems clear that more than one antherozoid penetrates the egg in a fairly large proportion of cases. The occasional occurrence of polyspermy is perhaps connected with the rapidity with which the antherozoids reach the egg, and with the delicacy of the membrane of the latter. It seems clear also that the entrance of more than one antherozoid disturbs the normal process. The male nuclei themselves are arrested in development at an early stage, and the egg later undergoes degeneration, in much the same way as when it is not fertilized. There is a possibility, however, suggested by some of the above-described cases, that one antherozoid may continue its development in a normal way while the other remains in a condition characteristic of an earlier phase and finally breaks down and disappears. In any case, it is safe to conclude that never does more than one male nucleus take part in the formation of the zygote nucleus.

In many animals polyspermy is a normal occurrence. In other animals the entrance of more than one spermatozoon may be induced by various means; such artificial polyspermy usually causing a degeneration of the egg nucleus. In plants, however, polyspermy has been reported, so far as I know, only in *Fucus vesiculosus*, by Farmer and Williams (1898), and in *Onoclea struthiopteris*, by Mottier (1904). Farmer and Williams noted polyspermy in only three cases of a thousand studied. Their figure shows two antherozoid nuclei apparently fused with the egg nucleus. In *Onoclea* Mottier figures two antherozoids, one penetrating the egg nucleus in the normal way, the other lying coiled in the cytoplasm, surrounded by a clear area.

Cytoplasmic Radiations and Granules.

The cases that have been mentioned from time to time in the description, which show more or less regularly radiating rows of granules in the cytoplasm of the egg, are brought together in Table III. They appear only infrequently—in about 6 per cent. of the total number of cases observed—and in several different phases.

These granules are of spherical or ovoid shape, of considerable size, and seem to be, at least in many cases, united by fibres. The latter fact, as well as their general appearance, makes it improbable that they are of the nature of starch grains. They stain violet in the triple stain. In preparations stained with iron-alum-haematoxylin and light green, they appear as hyaline spheres, quite clear when in focus, black when out of focus.

TABLE III.

Cases showing Cytoplasmic Granules.

Phase.	*Series.*	*Time after flooding.* Hrs. Min.	*No. of cases.*
5	C	40	2
4	A	48	7
7	D	65 30	2
8	D	65 30	1
5	C	66	1
6	D	71 30	1
7	D	75 45	4
			18

It may be that these bodies represent an excess of food material in the cytoplasm. On the other hand, their appearance may be due to slight differences in the action of the fixing fluid, which in turn are perhaps due to slight differences in the condition of the cytoplasm. The substance that is usually present instead of the granules seems to be, as already mentioned, a dense, homogeneous, presumably colloidal material; and the appearance of a system of granules may be caused by a coalescence of one of the phases of the colloid, leading to the production of large droplets.

Both the rows of granules, and, in some cases, the radiating processes extending out from the polar caps, present under the low power of the microscope an appearance similar to that of two asters. It is interesting in this connexion to recall that centrosomes have been described in several Liverworts, notably in *Pellia* and *Marchantia*, in various tissues of both sporophyte and gametophyte. Centrosomes, with distinct radiating fibres, have been described in the fertilized egg of *Preissia quadrata* by Miss Graham (1918), and in that of *Riccardia pinguis* by Florin (1918). In the former case the radiations which form the aster are definite, easily visible fibres, and in the centre is a small definite body. In the latter they are apparently close rows of fine granules, which merge together in small dense masses ('centrosome-like bodies') near the poles of the nucleus.

In *Sphaerocarpos* such rays as are present, though fibrillar in nature in some stages, are much less regular than those described by these workers, and converge upon no definite bodies, but pass into the large dense masses of material to which I have referred as the polar caps. In later stages these rays are not at all fibre-like, and are continuous with the material which now surrounds the female nucleus. The whole appearance suggests, as I have said before, a flowing of material towards the female nucleus. But it is possible that an actual centrosome with its aster may lie concealed in the cytoplasm. At all events, it is evident that marked chemical and physical changes take place in the cytoplasm of the egg at both ends of the nucleus, which are later concerned with its division, and that these changes

may express themselves, in different plants, by influencing in different ways the structure of the cytoplasm; the difference may also be exaggerated by differences in technique.

The concentration of certain materials in the cytoplasm about the egg nucleus may be a phenomenon of general occurrence. Mention has already been made, under Phase 1, of the variety of forms in which this has been partially described or figured. It is interesting also to note that Gayet (1897) describes the egg of *Sphagnum* as containing in its cytoplasm numerous rounded bodies, which he calls chromatophores. The cytoplasm of the fertilized egg of *Preissia quadrata*, as described by Miss Graham (1918), is in a similar condition, and the rounded bodies are more numerous around the nucleus. It is possible that the rounded bodies described by these authors bear the same relation to the denser portions of the cytoplasm noted by others as do the granules of the egg of *Sphaerocarpos* to the more usual homogeneous mass that surrounds the nucleus.

Fertilization in the Bryophyta.

The various cases, mentioned in the introduction to this paper, of fertilization in Liverworts observed and figured by previous writers harmonize very easily with the complete history as here presented for *Sphaerocarpos*. Although in most of the cases previously described only one stage, or several stages almost alike, were seen, and although various interpretations were placed upon these few stages, it seems possible that fertilization may follow the same course in many Liverworts as it does in *Sphaerocarpos*. At all events, in all cases (except two) so far described, the male nucleus enters into a resting condition before fusion with the female nucleus; and whether an actual fusion occurs, and in what condition the nuclei fuse, is not known with certainty in any Liverwort, not excluding *Sphaerocarpos*. Of the two exceptions mentioned, one is the case of *Riella* described by Kruch (1891), in which the male nucleus seems to proceed to the organization of chromosomes immediately after its entrance into the egg and while it is taking on a spherical form. More modern methods might perhaps throw more light on what actually takes place here. The other divergent case is found in *Fossombronia*, as reported by Humphrey (1906). Here the antherozoid, or at least its nucleus, retains its vermiform shape after entering the egg cytoplasm. Its subsequent behaviour was not observed. Some unpublished and as yet incomplete results of Mr. A. M. Showalter (to whom I am indebted for allowing me to see his figures) show what is apparently the same condition in *Riccardia pinguis*, and also seem to indicate that the still vermiform or coiled antherozoid penetrates the female nucleus, much as is the case in some ferns (Shaw, 1898; Yamanouchi, 1908). These two last-cited cases among the Jungermanniales suggest that the behaviour of the nuclei in fertilization may be quite different for the Jungermanniales and

the Marchantiales, which latter group includes all the other Liverworts in which fertilization has been thus far described except *Riella*. Should this suggestion be verified, it is important from a phylogenetic standpoint to note that the history of fertilization in *Sphaerocarpos* and *Riella* resembles that found in the Marchantiales.

If, as seems likely, there is no nuclear fusion, *Sphaerocarpos* constitutes in this respect a unique case among plants. Such a behaviour of the nuclei as seems to hold in *Sphaerocarpos* is characteristic of the gamete nuclei of many animals, reaching its extreme expression in such cases as that of *Cyclops*, wherein the male and female nuclei not only furnish independent groups of chromosomes, but these persist as two distinct groups, and can be followed through the division of the zygote, and in some cases through several succeeding divisions. Among plants, the closest approach yet noted to such a condition is found in *Pinus Strobus*, as described by Miss Ferguson (1904). Here the male nucleus is received in a concave depression in the surface of the (much larger) female nucleus. The chromatin is then organized into a spireme in each nucleus, while the boundary between the two is still very plain. The nuclear membranes then 'fade out', leaving the two spiremes on the spindle. The distinction between the two parental groups of chromosomes becomes lost in the equatorial plate of the first division.

Fusion of nuclei while the latter are in the spireme state has been described by several writers—as by Miss Weniger (1918) for *Lilium philadelphicum* and *L. longiflorum*, by Sax (1916) for *Fritillaria pudica* (in rare cases). The separate formation of chromosomes in two groups by the male and female nuclei after they have completely fused has been reported by Blackman (1898) for *Pinus silvestris*, by Murrill (1900) for *Tsuga canadensis*, by Hutchinson (1915) for *Abies balsamea*, and by Sax (1918) for *Triticum*. Chamberlain (1899) figures an egg of *Pinus Laricio* which contains two separate spiremes. In many of the higher plants that have been studied, however (Chamberlain, 1916; Sax, 1916, 1918), the two sexual nuclei fuse completely while in the resting state. This is apparently characteristic for the Thallophyta (a review of cases of fertilization in a number of the lower plants is given by Mottier, 1904). In *Polysiphonia violacea*, however, Yamanouchi (1906) reports that the male nucleus contains distinct chromosomes when it fuses with the female nucleus; the latter is in a resting condition. In the ferns, so far as known (Yamanouchi, 1908), the two nuclei fuse completely, and the fusion nucleus passes into a resting condition before the first division.

Sphaerocarpos furnishes yet another instance in support of the doctrine of the individuality of the chromosomes, since the eight chromosomes that are formed separately in each nucleus are undoubtedly the same as those that pass on to the spindle and finally form the daughter nuclei of the first

division. It is also worthy of note that, in spite of the immense size of the female nucleus and the much smaller size of the male, the chromatic material which actually enters into the sporophytic nuclei is contributed approximately equally by each parent (except for the difference between the X- and Y-chromosomes), since a large part of the substance within the female nucleus passes into the cytoplasm after the nuclear membrane disappears.

Summary.

1. The antherozoid nucleus, after entering the egg, assumes the shape of a thick, curved rod surrounded by a clear space (Phase 1). After about two hours it takes first an oval shape (Phase 2), then, about eight hours later, a spherical shape (Phase 3). Its body is in these stages dense and homogeneous. These changes occupy in all about twenty-two hours.

2. The clear space surrounding the antherozoid nucleus becomes bounded by a membrane. The male nucleus now expands somewhat and its chromatic material assumes a more or less reticular structure, resembling that of the female nucleus at this time (Phase 4). In this condition the nuclei remain about twenty-four hours.

3. The female nucleus then proceeds to the slow organization of chromosomes, while the male nucleus again contracts and loses its visible structure (Phase 5). In the egg cytoplasm, meanwhile, dense caps of homogeneous material appear opposite each pole of the female nucleus. These processes occupy on the average about twenty hours, the time being very variable.

4. The polar caps in the egg cytoplasm enlarge until they completely surround the two nuclei, which now lie in contact. The male now organizes its chromosomes very rapidly, and the membranes of the two nuclei break down probably at the same time. Eight chromosomes can be counted in the male nucleus. A spindle cannot be distinguished in the cytoplasm on account of the dense mass of material there present. The first division proceeds very rapidly (Phase 6). About sixteen chromosomes can be counted. The first partition wall of the young embryo is transverse, as are the two succeeding ones in the majority of cases.

5. Polyspermy occurs in about eight per cent. of the cases of fertilization observed. This, at least usually, results in the degeneration of the egg.

In conclusion I wish to express my great indebtedness to Professor C. E. Allen, at whose suggestion and under whose supervision this work was done.

Department of Botany,
University of Wisconsin.

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EXPLANATION OF FIGURES IN PLATES III AND IV.

Illustrating Dr. Rickett's paper on Fertilization of *Sphaerocarpos*.

PLATE III.

All figures were outlined with the aid of a camera lucida, at a magnification of 2,000 diameters. Lenses used were Zeiss comp. oc. 12 and Zeiss apochr. obj. 2 mm., N.A. 1·40. The sections were 7 μ in thickness. The upper end of the figure in every case corresponds to the distal end of the egg.

Fig. 1. Antherozoid shortly after its entrance into the cytoplasm of the egg. Ten minutes after flooding.

Fig. 2. Egg showing the first phase of fertilization. Two hours after flooding.

Fig. 3. Male nucleus in the cytoplasm of the egg during the second phase. Seven hours after flooding.

Fig. 4. Third phase. Eighteen hours after flooding.

Fig. 5. Remainder of female nucleus of preceding figure.

Fig. 6. Female nucleus of egg during the fourth phase. Thirty-eight hours after flooding.

Fig. 7. Male nucleus during the fourth phase, showing open type of structure. From the egg containing the nucleus shown in Fig. 6.

Fig. 8. Fourth phase. Thirty hours after flooding.

Fig. 9. Fourth phase, showing the formation of polar caps in the cytoplasm. Forty-four hours after flooding.

Fig. 10. Male nucleus from the egg of Fig. 9 (in adjacent section).

Fig. 11. Egg during the fourth phase, showing radiating masses of granules replacing the polar caps in the cytoplasm. Forty-eight hours after flooding.

Fig. 12. Very early example of the fifth phase. Fifty-six hours after flooding.

Fig. 13. Remainder of female nucleus of preceding figure, showing the first indications of spireme formation.

PLATE IV.

Fig. 14. Male and female nuclei in the fifth phase. Sixty-two and a half hours after flooding.

Fig. 15. Male nucleus which has its chromatin in the form of a spireme. Seventy-one and a half hours after flooding.

Fig. 16. Early example of the sixth phase. Each nucleus a group of chromosomes surrounded by a membrane. Cytoplasm definitely organized for the first division. Sixty hours after flooding.

Fig. 17. Remainder of female nucleus of preceding figure.

Fig. 18. Sixth phase. Nuclear membranes have disappeared. Sixty hours after flooding.

Fig. 19. Sixth phase. Equatorial plate of first division. Seventy-three and three-quarter hours after flooding.

Fig. 20. Seventh phase. Two-celled embryo. Sixty-eight hours after flooding.

Fig. 21. Chromosomes in the second division of the zygote. Polar view of equatorial plate. Sixty-eight hours after flooding.

Fig. 22. Same as Fig. 21. Side view showing spindle. Sixty-four hours after flooding.

Fig. 23. Gametophytic chromosomes in a dividing cell of the venter. Side view of equatorial plate.

Fig. 24. Same as Fig. 23. Anaphase.

Fig. 25. Polyspermy. Male nuclei as in fourth phase, egg beginning to degenerate. Several more male nuclei appear in adjacent sections. Sixty-seven and a half hours after flooding.

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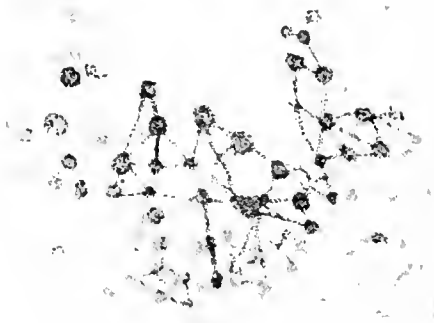

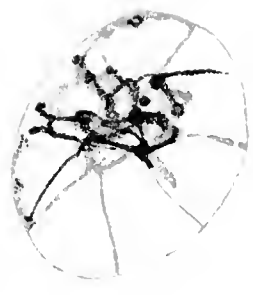

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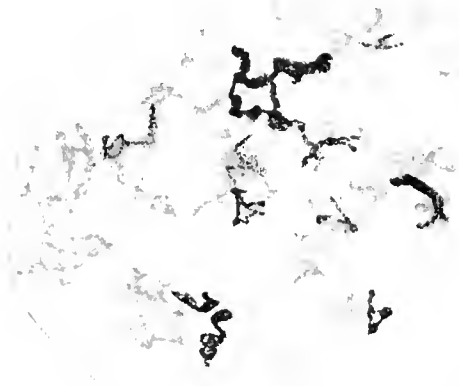

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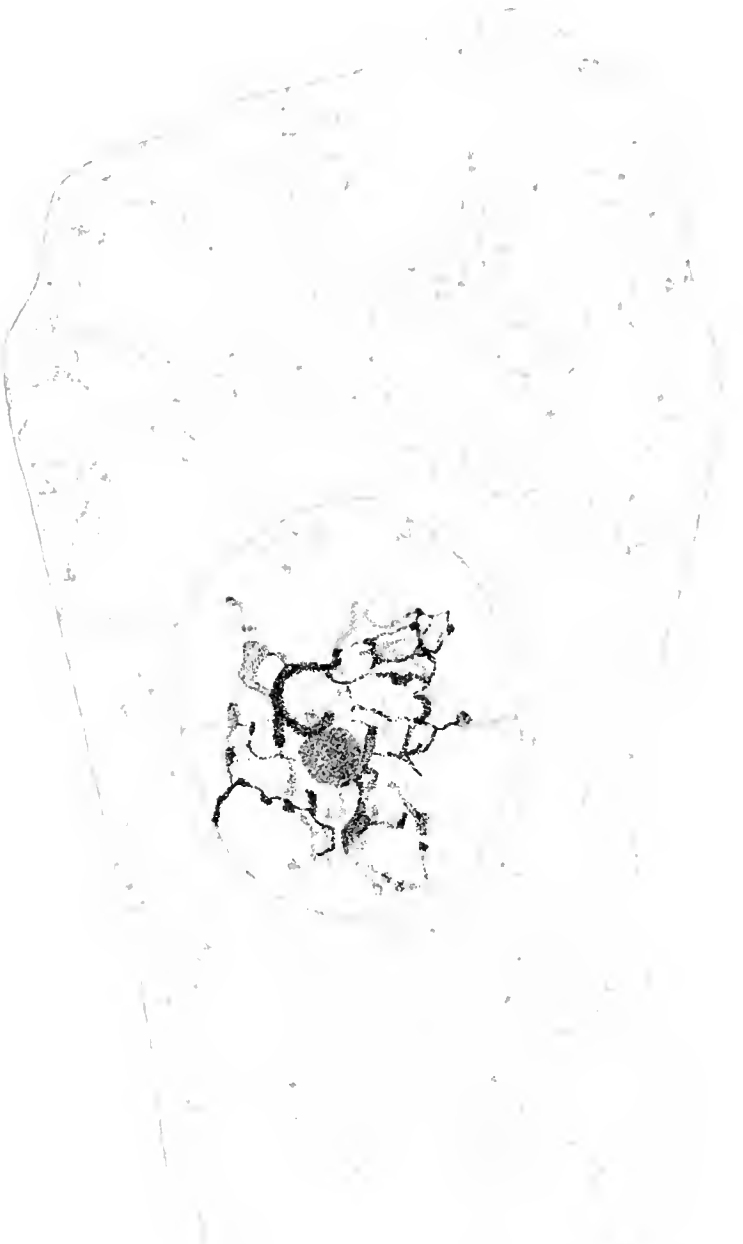

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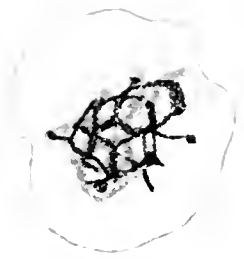

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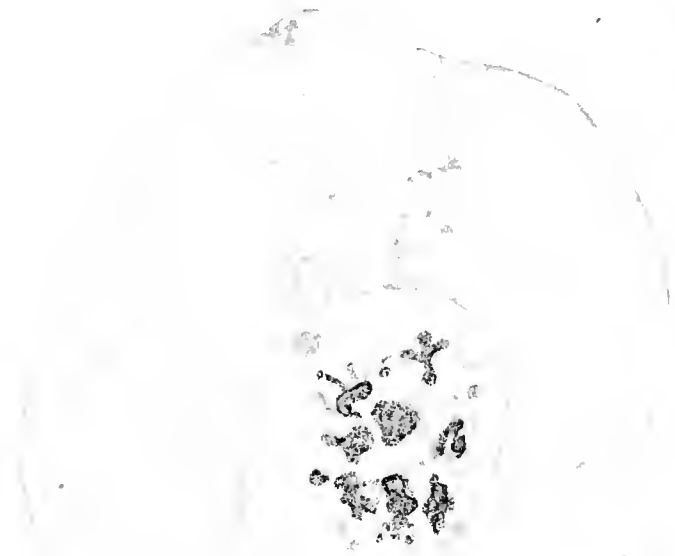

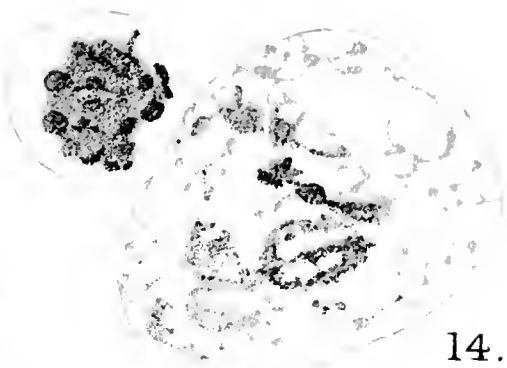

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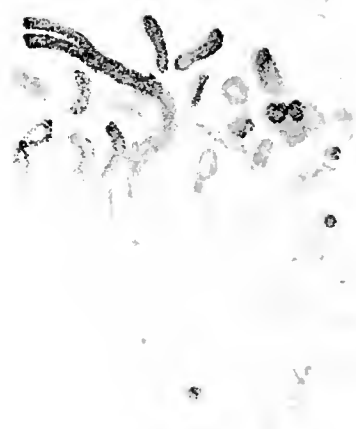

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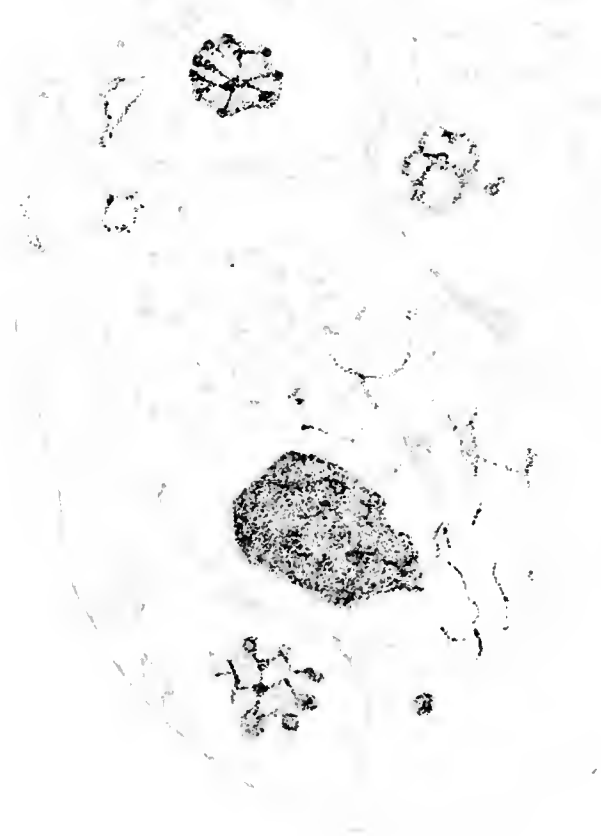

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Studies in Growth. IV. Correlations in Development.

BY

W. H. PEARSALL.

With six Figures in the Text.

THE earlier papers of this series (1, 2) dealt with certain aspects of the growth of yeast and of roots upon cuttings. As a result of the conclusions drawn from those special cases, it appeared to be possible to

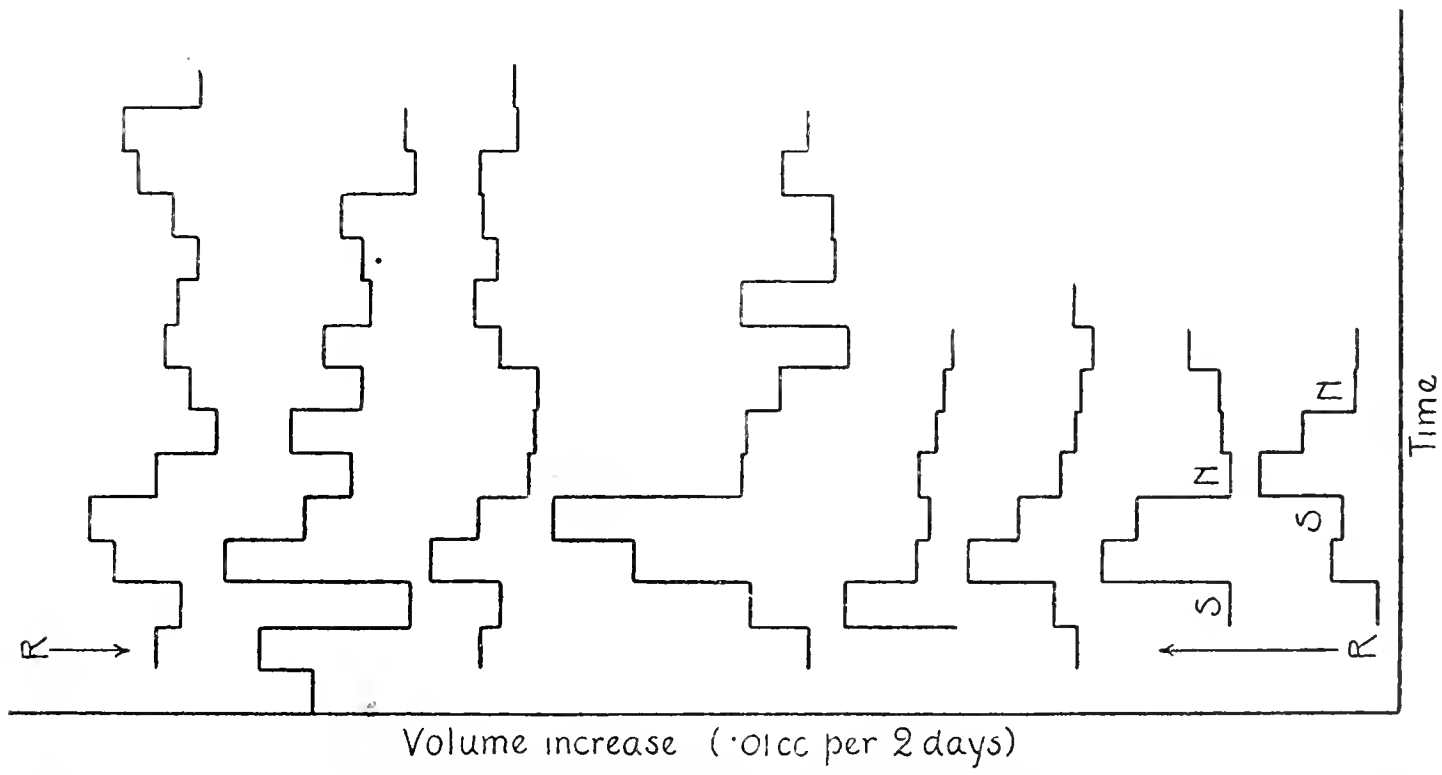

FIG. 1. Rate of growth of bean roots in volume. R indicates appearance of secondary roots; stem removed at S; new shoots appear at N.

explain some of the earlier data obtained in these studies, which had until recently seemed to be of little value.

The first measurements undertaken in this connexion were those of the volumes of roots grown from Broad Beans (*Vicia Faba*, L.), the root volumes being measured in a volumometer as previously described (8). In the first series of observations, begun on July 31, 1920, the beans were grown in Shive's optimum three-salt culture solution (9), this being renewed every two days. The volumometers were kept in a water-bath, at an approximately constant temperature of 15° C. (± 1°). The water-bath was covered so that the roots were in the dark. The whole apparatus was set up in an artificially warmed greenhouse.

[Annals of Botany, Vol. XXXVII. No. CXLVI. April, 1923.]

The results of the measurements upon these beans are given in Table I (Fig. 1) as rates of growth (i.e. increase in volume per two days). There are insufficient data for the analysis of these curves, but they show the characteristic depression of the rate of growth following (in the case of volume measurements) the appearance of secondary roots. The rate of root growth then increases once again, when a second factor causes a second drop in the growth rate, which subsequently becomes roughly constant. The appearance of tertiary roots was not responsible for this second depression of the growth rate and consequently some other factor is involved.

TABLE I.

Volume Increase of Bean Roots (in $\frac{1}{100}$ c.c. per two days).

	Series I (August).				*Series II (September).*			
Days	1	2	3	4	5	6	7	8
2	29 R	51	30 R	18 R	R	27 R	18 S	10
4	16	74 R	18	47	70	37	77	32
6	48	5	50	101	34	78	62	26 S
8	59	91	31	138	30	55	17	64
10	29	54	6	59	35	36	23	45
12	0	33	3	57	24	29	23	21
14	12	61	0	41	21	28	38	20
16	24	27	19	8	19	21	20	
18	18	46	30	58		30		
20	9	23	20	14				
22	20	27	36	16				
24	37	38	38	40				
26	44	2	11	27				
28	7	7	13	0				

R = lateral shoots appear. S = shoot removed.

(These continuous volumometer readings were obtained in collaboration with Professor J. H. Priestley and Miss D. Armstead, to whom the author is very much indebted.)

A second series of results by the same methods was obtained in September, 1920. More detailed notes on the whole plants are available, and it is possible to recognize the distinctive features of the rate curve more clearly and to correlate them with other growth factors. The results are also embodied in Fig. 1.

The first measurements were taken at the time of the appearance of the secondary roots, except in the case of No. 5, where the roots developed rather earlier. The growth rate, at first therefore low, rose rapidly, and then in all cases again fell to a low level as in the previous series. The second drop in root growth was very definitely associated with the rapid development of the stem, which grew rapidly after the appearance of secondary roots, so that the depression of the root growth rate corresponded with the maximum production of stem and leaves. The demands of such a developing shoot on food supply from the cotyledons must be equal to or greater than those of the roots, and the rapid development of the shoot seems to imply, therefore, a diversion of the food supply from the

root apices and a consequent limitation of their rate of growth. If the stem for any reason stopped growing, we should expect the roots to grow at an increased rate.

In Nos. 7 and 8 of this series the shoot was removed, and a marked increase in the rate of growth for the next four days was the result of this operation. The rate of root growth then fell again to a low rate, and about the fifth or sixth day new shoots were visible, the falling off in root growth apparently being due to their development.

These assumptions were examined further by growing peas (*Pisum sativum*) and determining the average weight of stems and of roots at fixed intervals. In order to avoid complications due to photosynthetic effects on dry weight the peas were grown in the dark. This does not appear to alter materially either the rate or the total amount of root growth. Attempts were also made to eliminate some of the extreme variability observed in some earlier experiments. Two main types of variation were usually observed: (1) variability in germination, presumably due to variations in the thickness and permeability of the seed-coat; (2) variability in subsequent development. To get a moderately uniform and fairly representative set of growing peas, about three thousand peas were spread out and soaked in sufficient Shive's optimum three-salt solution to just cover them. On germination about three hundred of these were selected, having roots of average and similar length (1·5–2·5 cm.), the extreme variants being thus eliminated. The selected plants were then grown with their roots dipping through a perforated plate into the Shive's solution. The cotyledons rested upon filter-paper and the seeds were kept in incubators at fairly constant temperature and humidity. The nutrient solution was changed every three days.

In Series III no further elimination of variations was made, but in Series IV to VI no determinations were made until the appearance of secondary roots—and then the material was graded according to the length of the shoots, equal numbers of plants with very long or very short shoots being discarded. In this way only about 100 to 150 plants were left, the extreme variants having been eliminated at two stages in the growth-cycle on two criteria, (1) root length, (2) stem length.

The samples for determination of the dry weight were taken in lots of ten. The attachment to the cotyledon was treated as part of the root—since it increases in weight in proportion to the root—and after cutting the plant away from the cotyledons at the junction of the attachments and cotyledons, the stem was also cut off just above the attachment. The roots, stems, and cotyledons were then dried for six hours at 100° C. and parts of each plant weighed separately. The average dry weight and probable error of the set of ten plants were then determined.

A modification in the method of sampling was introduced in the case of Series III, IV, and VI. In these series, equal numbers of the extreme

variants were selected for each determination, the criterion being stem length. In this way, the plants remaining for the later determinations approximated more and more closely to the average of the material; the later determinations are probably more reliable, and their probable error is smaller. The probable error was not determined for the earlier readings (stem weight) in Series IV, V, and VI, since the individual weights are too small to measure accurately.

Results.

Series III was carried out at a temperature of 25° C. (± 0·5°). In this an effort was made to obtain the complete growth curve and the results are recorded in Table II (Fig. 2). The figures for root growth show the characteristic stoppage of root growth previously recorded for roots on *Tradescantia* cuttings and for Broad Bean roots (volume measurements). The roots finally ceased growing about the ninth day, and no tertiary roots appeared either at this time or later (such as were found in *Tradescantia*). The stem at the ninth day, however, was growing at the maximum rate, and it was still growing at the conclusion of the observations. No marked increase in stem weight took place until the appearance of secondary roots, after which the elongation of the stem was very noticeable.

Table II.

Average Dry Weight of Peas (green wrinkled variety).

Series III (25° + 1° C.).			*Series IV* (15° + 1° C.).		
Day.	*Shoot.*	*Root.*	*Day.*	*Shoot.*	*Root.*
1	1·0 mg.	2·1 mg.	4	1·8 mg.	9·8 ± 0·39 mg.
1·5	1·3	2·8	$4\frac{1}{2}$	2·2	10·1 ± 0·37
2	1·4	4·8 ± 0·11	$5\frac{1}{2}$	3·5	10·7 ± 0·40
2·5	1·9	6·0 ± 0·20	$6\frac{1}{2}$	5·2	13·0 ± 0·42
3	3·0	7·4 ± 0·29	$7\frac{1}{2}$	7·6 ± 0·40	16·4 ± 0·55
3·5	3·5 ± 0·04	8·7 ± 0·40	9	14·2 ± 0·39	21·3 ± 0·57
4	3·5 ± 0·07	11·2 ± 0·32	11	25·0 ± 0·52	26·2 ± 0·72
4·5	5·7 ± 0·30	12·2 ± 0·71	12	31·0 ± 0·77	27·6 ± 0·71
5	6·2 ± 0·30	12·4 ± 0·47	13	35·7 ± 0·79	26·1 ± 0·90
5·5	7·6 ± 0·35	13·1 ± 1·0	14	40·6 ± 0·95	26·6 ± 0·83
6·5	12·7 ± 1·0	16·1 ± 1·1	16	46·5 ± 1·10	27·8 ± 0·71
7·5	18·4 ± 1·2	20·4 ± 1·8			
8·5	30·0 ± 2·4	28·3 ± 1·8			
9·5	41·8 ± 2·9	32·2 ± 1·9			
10·5	47·6 ± 2·9	31·1 ± 2·0			
11·5	52·0 ± 3·2	32·0 ± 2·4			

Consideration of the rate curves for this series showed that prior to the appearance of secondary roots there seemed to be a negative correlation between the stem and root growth rates. When the stem rate increased that for the root decreased. After the appearance of secondary roots, stem and root growth both increased rapidly and roughly proportionately until the stem reached its maximum rate, when the root stopped growing,

although the stem continued to grow at a decreasing rate. Since the stem still continued to grow rapidly it is clear that food supply from the cotyledons was not the factor preventing the further growth of the roots, and we must then suppose either that the stem entirely absorbed the cotyledonary food supply, preventing any material from reaching the roots, or else that some other factor preventing root growth was in operation. No evidence has been found for the latter assumption.

Possible objections to this series of observations were three in number.

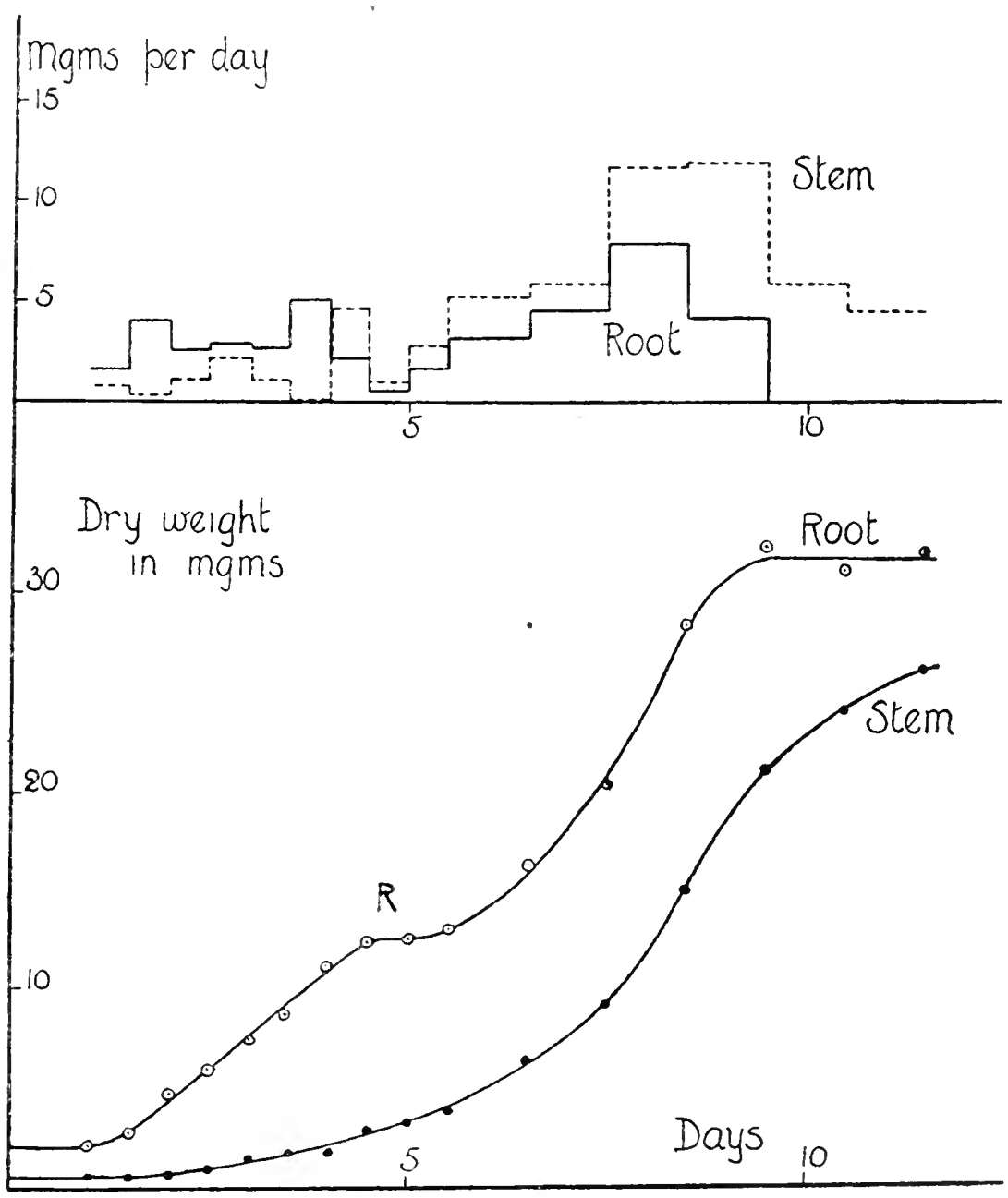

FIG. 2. Growth of stem and root (Series III) in peas at 25° C. Secondary roots appeared at R. Stem curve reduced to half the vertical scale. (Rate curves above.)

(1) The variation of the plants and probable error of the determinations were so high that arguments based on the growth rate were barely justifiable. (2) The determinations were few in number after the appearance of secondary roots. (3) At the high temperature employed, the period of rapid growth was passed through so quickly that it was difficult to see whether or no any correlation existed between stem and root growth. Another series (IV) of determinations was therefore undertaken, the first objection above being met by the reduction of the variations as described in a previous paragraph (p. 263). The observations were carried out at lower temperatures

(15° C. ± 1°), and the estimations were only begun after the appearance of secondary roots. The results obtained were similar to those of Series III (see Table II and Fig. 3), but in this case the stem had only completed about

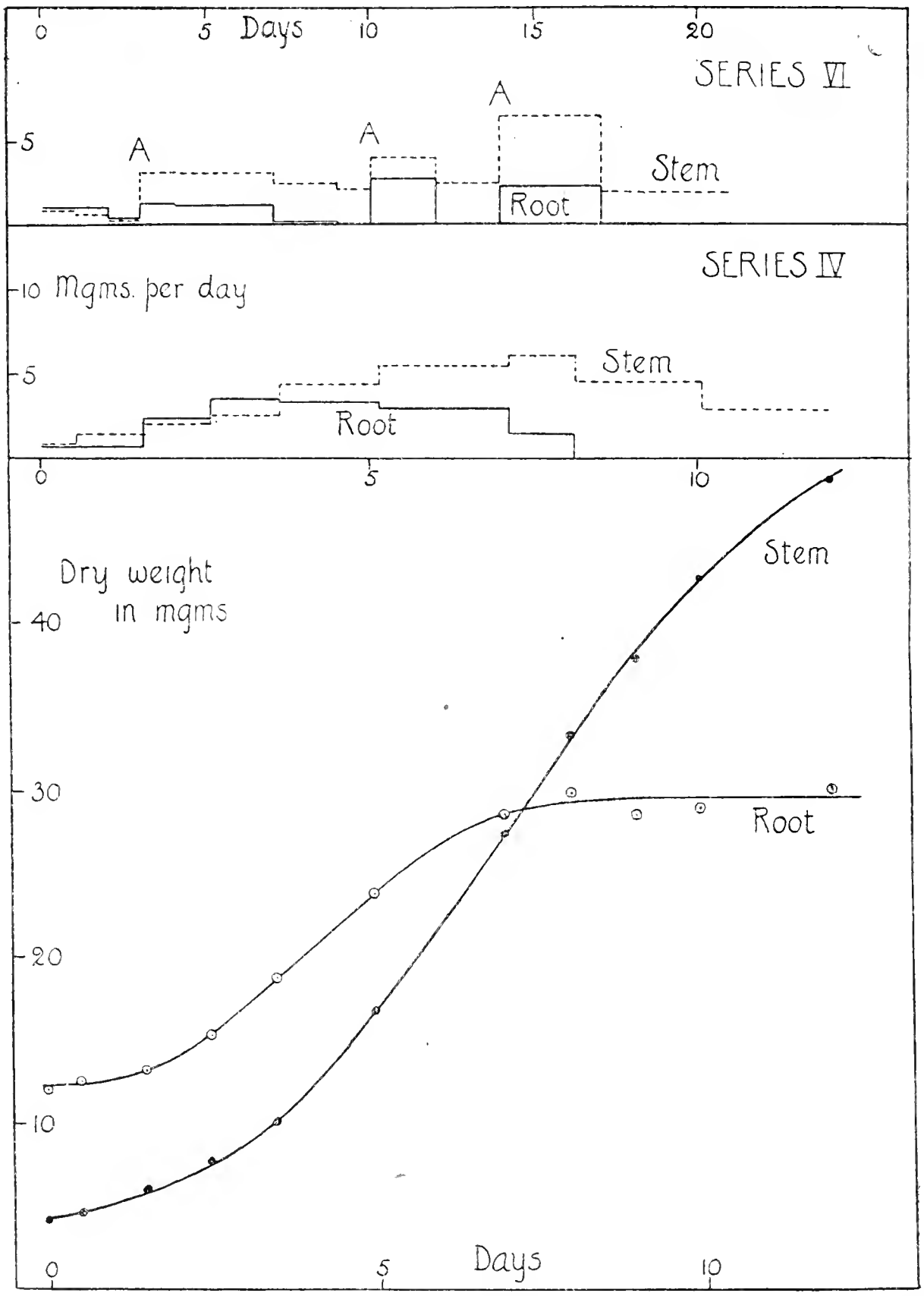

FIG. 3. Growth of stem and root in peas (Series IV) at 15° C. after appearance of secondary roots. Rate curves of Series IV and VI are given above. In VI the nutrient solution was aerated at A.

half its growth by the time the roots stopped growing. The rate curves seem to show a limiting of the rate of root growth when the stem growth rate becomes high. As the stem rate gradually increases, that for the root proportionately decreases, and this is quite definitely in harmony with the view that the food supply is diverted to the stem apex—ultimately causing a stoppage of root growth.

An additional series (V), at the same temperature but using another variety of pea (round yellow) is given in Table III and Fig. 4. The results of this series are of the same type. Root growth after the appearance of secondary roots maintains a constant and apparently limited rate, while the stem rate is rising to a maximum. When the rate of stem growth shows a marked decrease root growth practically ceases.

TABLE III.

Average Dry Weight of Peas (round yellow variety).

Series V (15.0° ± 1.0° C.).			*Series VI* (14.8° ± 1.2° C.).		
Day.	*Shoot.*	*Root.*	*Day.*	*Shoot.*	*Root.*
0	2·7 mg.	8·6 ± 0·2 mg.	0	2·5 mg.	12·2 ± 0·2 mg.
1	3·1	9·1 ± 0·3	1	3·4	13·2 ± 0·4
2	4·1	9·7 ± 0·4	2	4·0	14·3 ± 0·3
4	8·3 ± 0·4	14·3 ± 0·4	3	4·2	14·7 ± 0·4
7	25·2 ± 1·0	20·5 ± 0·7	4	7·2 ± 0·6	16·1 ± 0·6
8	32·5 ± 0·9	21·7 ± 0·6	7	16·3 ± 1·0	20·0 ± 0·6
10	50·0 ± 1·4	26·2 ± 0·8	9	21·3 ± 1·8	20·1 ± 0·5
12	64·3 ± 1·7	29·8 ± 0·9	10	23·5 ± 1·2	20·0 ± 0·4
15	78·1 ± 2·1	32·0 ± 0·8	12	31·5 ± 1·2	26·0 ± 0·8
17	81·0 ± 2·7	31·9 ± 0·8	14	36·6 ± 1·3	25·8 ± 0·8
			17	56·0 ± 1·45	32·4 ± 0·9
			21	63·2 ± 1·6	31·9 ± 1·0

The possibility existed that the limiting of the oxygen supply to the roots might be the reason for the limited growth rate. This seemed to be disproved by the high growth rate obtained in the roots of Series III at a much higher temperature (25° C.), though otherwise under precisely similar conditions. Secondly, no increase in the rate of root growth was obtained by replacing the nutrient solution more frequently or by aerating it. In the third place, when oxygen deficiency did appear it produced a distinct stoppage of growth. This may be illustrated by Series VI (Fig. 3), in which the solution was left unchanged, but aerated on the third, tenth, and fourteenth days. The dish holding the solution was in this case much smaller and deeper, the plants being crowded together. The lateral roots grew vigorously on the surface of the liquid, but, except after aeration, the main root and submerged laterals showed little or no growth. The effects of deficient oxygen supply were very marked in this case, and it is of interest to notice that the stoppage of root growth produced was associated with a marked reduction in the rate of stem growth—a feature not observable in the preceding series of observations. The facts clearly suggest, therefore, that the limitation of root growth rate in the earlier series was not due to deficient oxygen supply.

Further, since changing the nutrient solution more frequently produced no effect, and since there was no change in the results when the nutrient solution was reduced to one-fifth of the strength used in the series cited, it

does not appear that the supply of nutrient salts was the limiting factor. The assumption that the stem was almost monopolizing the organic food supply, to the partial exclusion of the roots, seems best to fit the facts, and one would then expect the stem to go on growing after root growth had ceased, a significant feature of all the cases investigated.

The question can be considered from an entirely different standpoint.

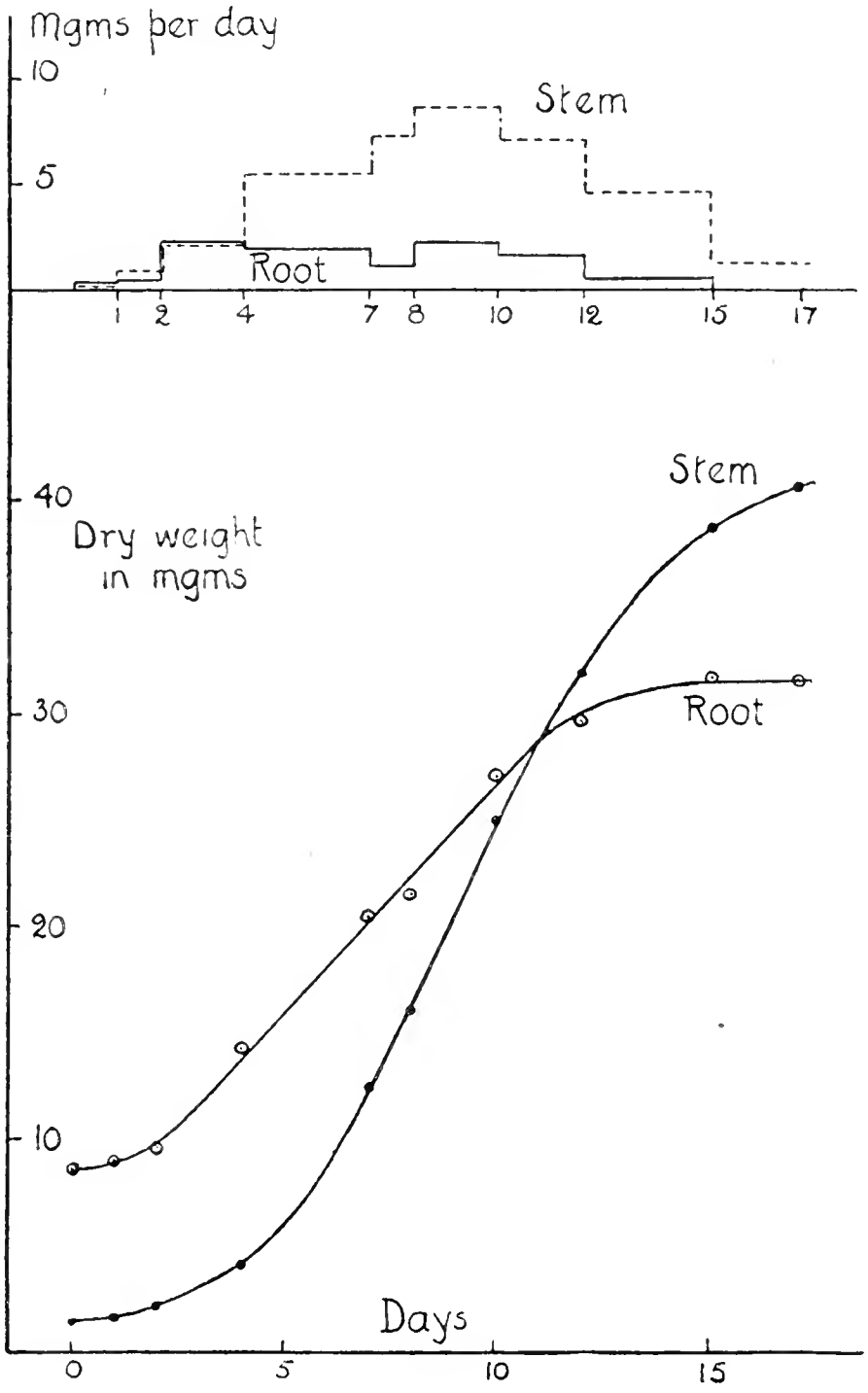

FIG. 4. Growth of stem and root (Series V) in peas after appearance of secondary roots. (Rate curves above.) Stem weight reduced by one-half.

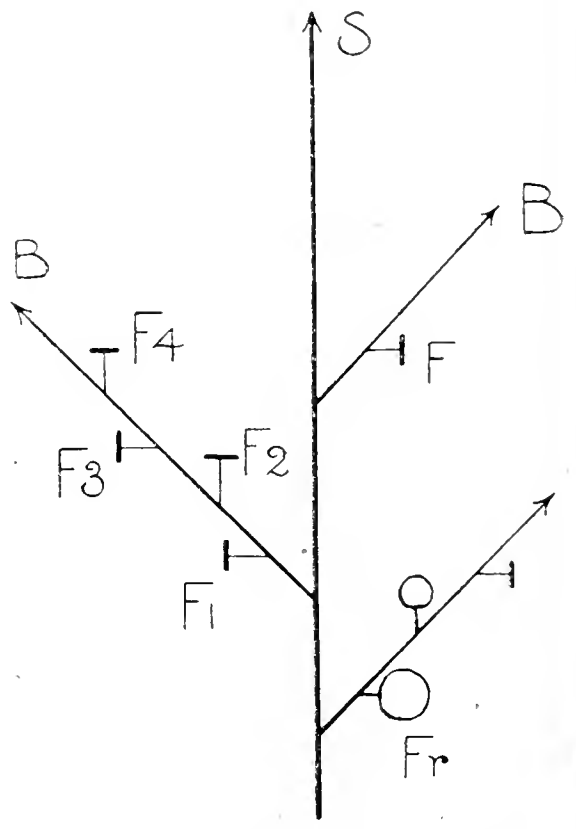

FIG. 5. For explanation see text.

After secondary root development, the total area of root meristem possessed by a plant is increased enormously and the rate of growth of the roots might therefore be expected to increase proportionately.

An examination of the rates of growth (by volume) before and after secondary root development in Series I (Nos. 2 and 3) shows that this is not the case, and a similar result is apparent when the following table is examined. This table shows the average growth rate of pea roots before and after secondary roots appear.

TABLE IV.

Average Dry Weight Increase per Day of Pea Roots.

	Before secondary roots.	*After secondary roots.*
Stem in light 15° C.	1·6 mg.	2·1 mg.
	1·5	1·5
	1·6	2·2
Stem in dark 15° C.	2·4	2·9
	1·7	1·9
	1·6	1·5
Average 15° C.	1·7	2·0
Stem in dark 25° C.	3·3	6·1

These results do not include periods when root growth was seriously decreased. They are obtained by finding the average growth rate from curves such as are given in this paper. For example, the figures at 25° C. are taken from Series III, the periods being $1\frac{1}{2}$ to 4 days and $6\frac{1}{2}$ to $8\frac{1}{2}$ days. The growth curves are practically straight lines between the points taken.

The only case in which a markedly increased average growth rate results after secondary root formation is at the higher temperature, and in this case we are probably justified in assuming that the rate of hydrolysis of food materials would be at least twice, probably about three times, as rapid, compared with that at a temperature of 15° C. Preliminary estimations of the temperature coefficient of protein hydrolysis in bean cotyledons seem to support this assumption. Thus it appears probable that at a temperature of 15° C. food supply is the factor limiting root growth after the development of lateral roots. Since the forms of the curves for stem growth approximate fairly closely to those for the normal autocatalytic reaction—which is now recognized as being a form of curve common in growth processes—it does not appear to be justifiable to assume any limitation of growth rate in the case of the stem, apart from that implied by a comparison between growth and an autocatalytic reaction. Hence the food supply to the root must be regarded as the residue remaining after the stem has withdrawn its supply.

The point of view developed above is in agreement with the earlier results of Kny (4) and Townsend (10). The papers of the former are somewhat unsatisfactory, but this author considered among other things the effect of removing the shoots from seedlings on the amount of root developed. This treatment, so far as any conclusion is possible from Kny's results, generally causes increased root growth in both *Zea Mays* and *Vicia Faba*, though in the latter case this effect is delayed. Townsend (10) also gives data for the effect of shoot removal on the rate of root growth in the same two plants. The roots of seedlings thus treated were 26 to 33 per cent. longer than those of normal plants five days after treatment. His results seem to be beyond suspicion, and justify the view that removal of the shoot allows an increased rate of food delivery to the root meristem. It

may be pointed out in passing that the subsequent development of new shoots quickly reduces the rate of root growth to the original lower level.

The establishment of this negative correlation between shoot and root growth may apparently be extended much farther as a result of Townsend's work, although this author attributes his results to some stimulus caused by injury. He found that in seedlings the removal of the root tip accelerated the rate of stem growth, and that the removal of a leaf in a young *Phaseolus* resulted in an increased growth rate of the remaining leaf. The hypothesis that food supply is the factor limiting growth in these cases appears to be too suggestive to be lightly dismissed.

Correlation in the Cotton-plant.

Further, this hypothesis appears to have equal value in explaining the form of the flowering and fruiting curves in the cotton-plants. These curves have been exhaustively investigated by Balls and Holton (1, 2) and by Harland (3), and their papers form a valuable contribution to the study of growth. They conclude that edaphic factors limit the growth rate of the cotton-plants studied (in Egypt and St. Vincent), and according to Balls (2) the limiting factor is probably the supply of nitrates. With a limited rate of food supply the rapid growth of one set of organs should therefore mean a correspondingly decreased growth rate in other parts of the plant.

Thus in Fig. 5, where S = stem, B = branch, F = flower, and Fr = fruit, the sum of the growth of S, B, F, and Fr, will be a constant when the rate of food supply is constant. If, then, the growth of S ceases, the growth rate of B, F, and Fr will increase proportionally ; or conversely, if the growth rate of any organ, say Fr, increases, the rate of growth of the others (S, B, and F) should decrease.

If it is assumed that the supply of substance from the roots is a limiting factor in the total growth of the stem, &c., then in the case figured the growth of the fruit (Fr) would proceed at its maximum speed, since it draws first on the supply stream, while some or all of the organs B, F, and S would grow at a reduced rate, the rate being least in those farthest from the source of supply X. Thus the rate of growth in the stem apex (S) would, other things being equal, be the lowest in the case cited.

In Egyptian and Sea Island varieties of cotton, the development of stem apex, branches, flowers, and fruits occurs essentially as in the figure given. The exhaustive data of Balls and Holton (1, 2) and of Harland (3) are therefore available for a further analysis of these assumptions.

Their results are in all cases expressed as the average plant of a large crop, the number of plants being sufficient to eliminate most sources of error. They give the average number of flowers opening per plant per day, and the average number of bolls (fruits) maturing per plant per day. For

one series of experiments Balls also gives the average length of the stem. This set of results has, therefore, been used, since it possesses the additional advantages of having been obtained in a long and favourable season (1913).

In the cotton-plant the flowers are borne successively on lateral branches. The oldest and longest branches at the base are the first to produce fruits, which appear while the upper branches are still producing flowers. Since the general arrangement of the plant is as in Fig. 5, the appearance of flowers ought to curtail the growth of the stem apex, and the development of fruits should reduce the rate of flowering, if the supply of

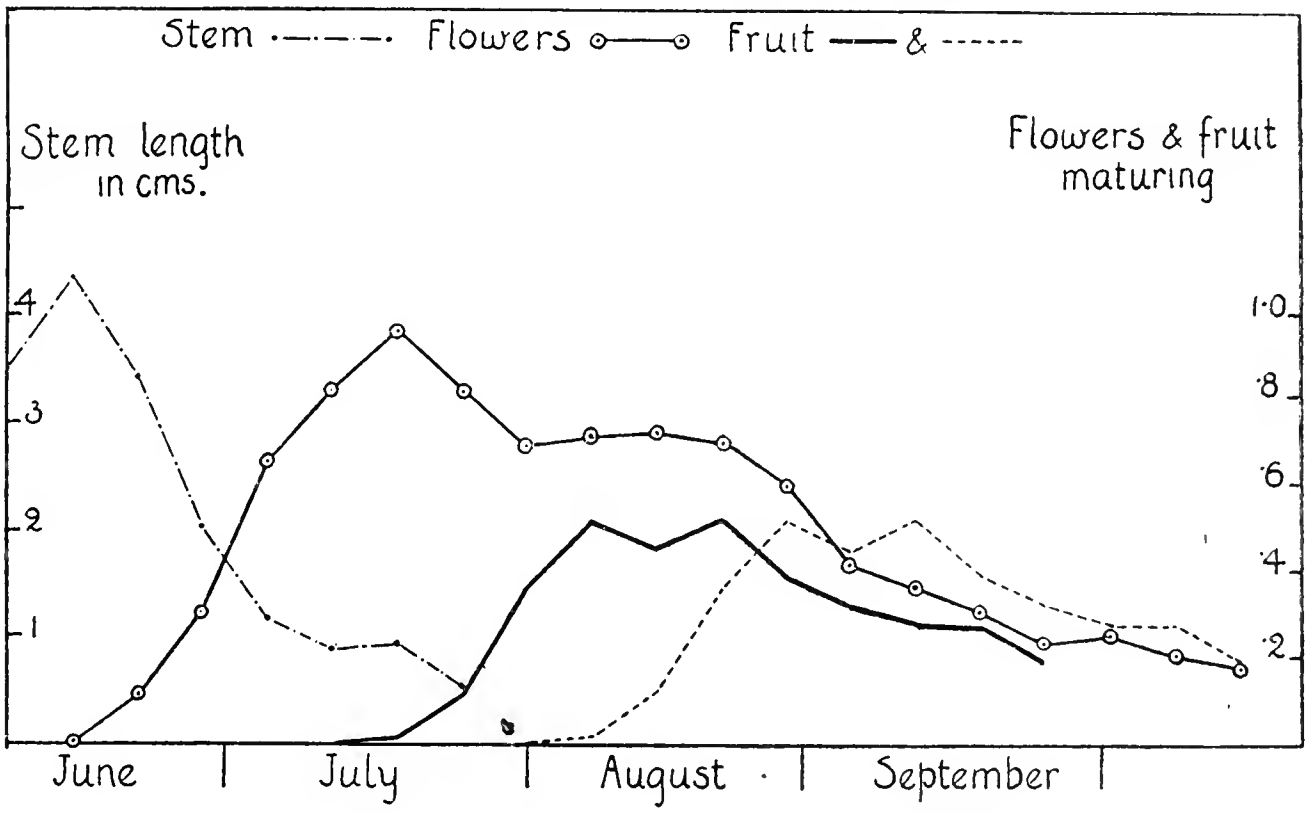

FIG. 6. Stem length, flowering and fruiting curves in Egyptian cotton (from the data of Balls and Holton), giving average increase per plant per day. The continuous fruiting curve is put forward three weeks (see text).

food material from the roots is the limiting factor in the total growth rate of the plant.

The results of Balls and Holton (1, 2) for the Middle Period sowings in the sowing date experiments of 1913 appear to justify this assumption. They are given as weekly averages (Fig. 6), for increase in stem length, flowers opening, and fruits maturing per plant per day. It is apparent that the decreased rate of growth of the stem after June 13 coincides with the development of flowers nearer the base of the plants. After July 18 the flowering rate also decreases, and this is followed by the development of the fruits. The flowering and fruiting curves do not actually represent the effects of the developing fruits on the flowering curves. The flowers give rise to *mature* fruits about seven weeks later (Balls, loc. cit.), but the fruits grow most rapidly about two (or two and a half) weeks after flowering. Since the flowers take four weeks to develop, we can assume they are growing most rapidly about a fortnight before they open. Hence the most

rapid growth of the fruits takes place four (or four and a half) weeks after the most rapid growth of the flowers. Thus, roughly, the *food demands* of the fruits will follow those of the flowers by about four (or four and a half) weeks, instead of by seven weeks, and to represent the effect of fruit development upon the flowering curve, from this point of view, the fruiting curve needs to be transposed three (or two and a half) weeks earlier (i. e. seven to four). The drop in the flowering rate then appears to be directly related to the increase in the numbers of fruits, and the horizontal portions of the curves also coincide, representing a condition of equilibrium when constant proportions of the food supply are used respectively by flowers and fruits. This balance seems to be maintained throughout the final decline in growth rates, which according to Balls is due to the rising water table, root asphyxiation becoming marked at the beginning of September.

The smaller variations can be eliminated and the main features of the curves retained if the figures are presented as total stem length, number of flowers and of fruits produced by the average plant at different times. The dependence of stem length upon the time of flowering, and of the total number of flowers produced upon the appearance of fruits, is equally clearly indicated by this alternative method of presentation.

The same results are obtained from the other sets of figures given by Balls and Holton (1, 2), and also from those of Harland (3), for cotton grown in St. Vincent, though in the latter case rather abnormal rainfall occurred in the middle of the flowering period. Harland's data are of interest, since they show a higher rate of flower production when decreased fruit production took place, showing that fruit production had depressed the rate of flowering.

We may pass on to consider other aspects of the development of cotton-plants which seem to fall in line with the views put forward here. The valuable data of Harland (3) are of particular interest in this respect, since he analyses statistically the growth of various parts of the plants, though attempting no explanation of his results.

Referring to Fig. 5, on any branch B the order of flowering is F_1, F_2, F_3, &c. If the growth rate of the branch is uniform then time intervals, F_1–F_2, F_2–F_3, F_3–F_4, &c., should be uniform. Harland shows statistically that actually in cotton the time interval between the flowering of adjacent nodes (F_1–F_2, F_2-F_3, &c.) increases as the distances from the central branch increase. Hence a point is reached when the rate of growth is practically zero. When it is remembered that each flower may produce a fruit, i. e. a growing-point nearer the source of supply (the stem), it seems possible to explain the falling off in growth rate of later nodes as being due to the growth of fruits lower down the branch, which serve to reduce the rate of food supply to the branch apex.

Harland also shows that, in a precisely similar way, the time interval

between the first and second flowers on a branch increases as the distance of the branch from the lowest fruiting branch increases.

e.g. fruiting branches	1–20	20–30	30–40
Average time interval between first and second flowers (days)	5·1	6·2	7·9

It seems clear that growth on the lowest branches proceeds at a maximum rate, because these branches draw first on the food supplies from the roots, the higher branches only receiving the residue.

In addition, the first node on each branch flowers later than the first node on the branch below it, and these time intervals increase as one ascends the stem. Harland's figures are:

Fruiting branches	1–20	20–25	25–30	30–40
Time interval between first nodes on successive branches (days)	2·2	3·5	3·7	4·6

The individual variation is much greater, the greater the distance from the lowest fruiting branch.

Finally the number of nodes per fruiting branch decreases from below upwards, and the average percentage of fruits maturing to flowers produced decreases from 33–37·8 for nodes 1 to 3 on fruiting branches to 14·9–10 for nodes 4 to 6, and is zero on nodes 7 and 8.

The abortion of terminal buds on branches occurs along with flower-bud and fruit shedding, and may, according to Harland, be considered as being due to the same factors. There seems to be no reason why this and the other facts described should not be referred to decreasing growth rates, consequent upon the limiting of the food supply. In all the cases considered for cotton there are developing growing-points nearer the roots than are the later fruits and flowers or the branch apices. In the case of roots growing from seeds, the growth of the stem limits the food supply to the roots and hence reduces their growth rate. The diminution of growth rate observable in cotton as the growing organs get farther from the base of the stem seems to show an exactly comparable state of affairs.

The effect of manurial treatment has been shown by both Balls (1, 2) and Harland (3) to leave the *form* of the flowering and fruiting curves unaltered, but to increase the *rate* of flowering and fruiting throughout. If more of the limiting nutrient substances are available and are passing up the stem, then more will pass the lower growing-points, and the rate of growth of the later flowers and fruits will increase, so that the rate of flowering and fruiting as a whole will be increased. Thus the manurial results agree with the hypothesis developed here.

Additional Note.

Since writing these paragraphs a valuable statistical analysis of correlation in the cotton-plant has appeared (Mason, 5), which fully confirms the

views put forward above. The author shows, in brief, that removal of the flowers or developing fruit permits a further development of stem elongation. He suggests, however, that this is due to the downward movement of carbohydrates to the fruits. This involves the assumption of a correlating factor which causes the deflexion of assimilates from the apical region to the developing fruit. Two sets of facts demonstrated by Mason seem to be opposed to these assumptions. In the first place, the removal of vegetative branches causes only a slightly increased rate of axis elongation, while flower formation is increased by 50 per cent. Secondly, the correlation coefficients between neighbouring fruiting branches are greatest in the apical region. Both of these facts can, however, be readily explained on the assumption used in the preceding pages, that food supply from the roots is the limiting factor. In the latter case, the flowers would draw first on this supply and the removal of the vegetative branches would thus increase food supply to the flowers, allowing only the excess materials to reach the stem apex. Moreover, the farther the fruiting branches are away from the source of supply the more rigidly would their activity be limited by the food supply and the closer would be the correlation between successive branches.

Summary.

1. Data are presented for the growth of roots from seeds, measured as changes of volume and of weight.

2. The evidence shows that the development of subordinate roots upon roots of seedlings causes a temporary decrease in growth rate. The increase in the size of the root meristems after secondary root production appears to produce no corresponding increase in the growth rate of the whole root. The stem continues to grow after the root has stopped.

3. The observed facts are consistent with the hypothesis previously developed that food supply is a limiting factor in the early periods of root growth from seeds and cuttings. The stem is assumed to compete successfully with the root for the cotyledonary food supply, especially after the development of secondary roots, thus causing a reduction of the root growth rate.

4. The data of Balls and of Harland for growth of stem, flowers, and fruits in cotton are examined, as representing a case in which food supply from the roots is assumed to be a limiting factor.

5. It is shown from these results that in cotton the decreased rate of growth of the stem can be attributed to flowering, and that subsequently the decreased flowering rate can be attributed to the development of fruits nearer the source of supply of the presumed limiting food factors.

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The Distribution of certain Portions of the British Flora.

I. Plants restricted to England and Wales.

BY

J. R. MATTHEWS, M.A., F.L.S.,

Royal Botanic Garden, Edinburgh.

With six Diagrams in the Text.

INTRODUCTORY.

THE flora of the British Isles, although a comparatively limited one, presents many interesting problems in plant-geography. These problems, as is well known, have been discussed from time to time, especially since Edward Forbes (1846) dealt with them in a classic memoir, 'On the Connexion between the Distribution of the existing Fauna and Flora of the British Isles and the Geological Changes which have affected their Area'. Forbes, an advocate of overland migration, recognized five distinct sub-floras and explained their distribution in Britain as a result of successive invasions from the extensive land mass lying eastward, prior to the detachment of Britain from the European mainland. The composition of our flora was also the subject of prolonged study by H. C. Watson (1835, 1847), and largely to his labours, too, we owe a careful analysis of the distribution of our native plants as presented in 'Topographical Botany' (1883). Some of the 'types of distribution' formulated by Watson have been discussed and defined with greater precision in an interesting paper by Stapf (1914), while Moss (1914), in a general account of plant distribution in Britain, brings the work of Forbes and Watson into line with that of recent authors.

All writers on the subject are generally agreed that the British flora is essentially a reduced continental flora, derived from the Continent in relation to past climatic changes, yet there is no unanimous opinion regarding the time and method of arrival of its several elements. A decisive answer cannot easily be given to these questions, for the historical succession is incomplete. A glance at the position, however, may not be without interest.

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Historical Phytogeography.

The problem of the origin of the British flora was attacked by Clement Reid (1899), employing the historical method, but the imperfection of the fossil record made a final conclusion exceedingly difficult. Since that date our knowledge of Pliocene and Pleistocene floras has materially increased. The researches of C. and E. M. Reid (1908) and those of E. M. Reid (1920 *a*) on pre-glacial floras of Western Europe provide reliable evidence of the nature of the vegetation of this country before the onset of the Pleistocene, when the character of the flora underwent a decided change. A comparative review of West European Pliocene floras is given by E. M. Reid (1920 *b*), who shows that the flora during this period changed from one having a high percentage of exotic species, many of which are Chinese-North American, to a flora (represented by the Cromerian at the top of the Pliocene) about 95 per cent. of which is composed of species which inhabit East Anglia at the present day. The author refers to a suggestion made in an earlier paper (1915) in which it is stated that the high land of Central Asia may have acted as a second centre for the origin and dispersal of temperate species in Miocene and Pliocene times, and is led to make the further suggestion that 'much of the living flora of the lowlands of Western Europe has been derived from this source, by dispersal through the Near East, the Caucasus, and the mountains of Southern and Central Europe, or by way of the Mediterranean'. The identity of most of the Cromerian flora with species now occurring in England has been mentioned, and, were it not for the onset of a period marked by increasing cold, there would have been probably no special difficulty in tracing the progressive emergence of our flora to its present state. But the change from heat to cold which culminated in the Ice Age produced a migration southwards, and the central problem of distribution in Britain turns entirely on the course of events throughout this period of climatic change. Information as to what exactly happened during the time of maximum glaciation would provide a definite starting-point in any attempt to solve the problem, but unfortunately the geological record is inconclusive.

There is, however, a steadily increasing body of evidence to show that a redistribution of vegetation must have occurred during Pleistocene times, since in place of the temperate flora which inhabited the south of Britain at the close of the Pliocene we find post-Pliocene fossil floras containing a very pronounced arctic element. The early records of glacial plant-bearing deposits are enumerated by C. Reid (1899), who gives full details, and we shall here refer only to two recently described arctic floras from low latitudes in England. Reid (1916) gives a list of the plants of the late glacial deposits of the Lea Valley, 22 per cent. of which are arctic species. The arctic flora revealed by Marr and Gardner (1916) at Barnwell in the Cam Valley and

referred to a late, if not the latest, stage of the Pleistocene deposits of the district has been fully studied by Chandler (1921), who finds that 42 per cent. of the species are arctic-alpines. Of these the following are worth noting:

Ranunculus aconitifolius, L., *Papaver alpinum*, L., *Draba incana*, L., *Arenaria sedoides*, L., *Arenaria biflora*, L., *Potentilla alpestris*, Hall., *P. fruticosa*, L., *Dryas octopetala*, L., *Saxifraga oppositifolia*, L., *Vaccinium uliginosum*, L., *Primula scotica*, Hook., *Armeria arctica*, Wallr., *Salix Arbuscula*, Fries., *S. Lapponum*, L., *S. herbacea*, L., *S. polaris*, Wahl., *S. reticulata*, L., *Betula nana*, L., *Carex lagopina*, Wahl., *C. ustalata*, Wahl., and *C. capillaris*, L. To these may be added *Oxyria digyna*, Hill, *Scheuchzeria palustris*, L., and *Carex incurva*, Lightf., reported from the Lea Valley, but not on record from the Cam Valley.

The occurrence in England during the Pleistocene of arctic species now extinct in Britain and known only from arctic Europe and Greenland is particularly interesting, as is also the fact that other species of the Cam Valley are unknown from Arctic Europe, occurring only in alpine situations in Central and South Europe.

Whether this arctic-alpine flora existed at the climax of a cold period the evidence from the plants alone, as Miss Chandler points out, is insufficient to say, but the association points to climatic conditions different from those which now prevail in the south of Britain, and clearly indicates that an invasion of northern forms had taken place since the time of the Cromerian flora.

Numerous arctic plant-beds occurring at low levels have been described from Scotland also, the most extensive investigations being those of Lewis (1905–11). Considerable fluctuations in the distribution of vegetation over North Britain during post-glacial times are indicated, for the changes described by Lewis occurred later than the last ice-sheet and give no information about the chain of events during the period of maximum glaciation. In Europe, Scandinavian workers have succeeded in tracing with some degree of precision successive floras which existed during the Pleistocene. A review of the historical sequence is given by Wille (1915) and short accounts of numerous continental investigations are included by Clements (1916) in his discussion of the ceneosere. The general conclusion may be reached (E. M. Reid, 1922) that plant migrations have been brought about in the past under stress of climatic change, and, while Britain undoubtedly shared in the southward movement developed during the glacial period, there is, as already mentioned, no incontrovertible evidence of the exact extent of this movement within our own country. Historical phytogeography does not provide a conclusive answer.

Nevertheless, certain students of the subject have adopted a definite standpoint. Engler (1879) assumes the complete or almost complete

destruction of the pre-glacial flora of Britain and its re-immigration in post-glacial times. Clement Reid also maintains that, with the possible exception of a few arctic plants, our flora is post-glacial in origin. 'We have merely to account', he says (1911), 'for the incoming of our existing flora after an earlier assemblage had been swept away almost as completely and effectually as the celebrated volcanic eruption wiped out the plants of Krakatao.' Stapf (1914) sees 'no way out of the conclusions at which Mr. Reid and many years before him Professor Engler have arrived', although he doubts whether certain elements in the British flora are explained 'by chance and occasional introduction of seeds', as is propounded by Clement Reid.

On the other hand, the hypothesis that much of the flora survived the glaciation of the country is not without its advocates. We have seen that Forbes, as long ago as 1846, regarded our flora as derived from different parts of the Continent by successive overland migrations, and he came to look upon the Iberian element, which is found in the west of Ireland, as the oldest, dating back to a time when a mysterious 'Atlantis' connected Ireland and Spain. Students of the Irish flora and fauna have done much to substantiate Forbes's hypothesis. Zoological evidence bearing on the 'Atlantis' problem is discussed by Scharff (1902), and the same author (1912) disputes the idea of a wholesale destruction of the flora which occupied Britain in pre-glacial times. Kennard and Woodward (1917), dealing with the post-Pliocene non-marine Mollusca of Ireland, also support the survival hypothesis, and Praeger (1910), writing of the Pyrenean plants in the west, describes them as 'relics of a vegetation which once spread along a bygone European coast-line which stretched unbroken from Ireland to Spain'. Further evidence in support of survival from early Tertiary times is provided by three American species—*Spiranthes Romanzoffiana*, Cham., *Sisyrinchium angustifolium*, Mill., and *Eriocaulon septangulare*, With. These occur in Ireland, the last in the west of Scotland also, and it is held that they must be members and relics of a pre-glacial flora which occupied a northern continent linking Europe and America across the North Atlantic.

The problem of the origin and distribution of any flora is so intimately related to the question of slow overland migration versus other possible methods of dispersal, such as water-currents, wind, or migrating birds, that mention must be made of it. A particular case, not incomparable with that of Britain after glaciation, is that of the Faeröes, discussed by both Ostenfeld (1901) and Warming (1903). Both agree that the present flora is post-glacial and that re-colonization from sea-borne or bird-carried seeds has been insignificant. Warming believes the wind is responsible for the introduction of most of the plants now found on the islands, while Ostenfeld holds that invasion took place over a post-glacial land bridge. Holttum

(1922), dealing with the vegetation of Greenland, regards the present flora as post-glacial, and states that of 416 vascular plants recorded, about 60 per cent. are southern types, most of which must have been carried over the sea by natural means.

The extent of former land connexions between Britain and Europe is a little doubtful, but even if it were but slight in recent Pleistocene times, the distance to be travelled by most of those forms which inhabit only the south of England would not be very great. We cannot here enter into the question as to how dispersal has been effected in the case of those plants held to be indigenous. As Guppy (1893) has pointed out, time has long since discounted the various methods, and I would add that the part which man has played from earliest times in the introduction of seeds can never be truly estimated. We cannot really discriminate between 'native' and 'introduced' species if we go far enough back.

More need not be said to illustrate the divergent views which are held regarding the origin and dispersal of our native flora. Only further geological evidence one way or the other will clear the ground for the botanist. The problems remain, and to the fundamental question as to the effect of glaciation on the plant population at the time it seems impossible in the present state of our knowledge to give a definite answer. I should not have reverted to these old-standing problems had not plant-distribution in all parts of the world recently assumed a new interest and a new importance.

The Present Investigation.

The promulgation of the hypothesis of 'Age and Area' by Dr. Willis (1915, 1919), which has now been successfully tested in a variety of ways, opens up a new standpoint in the study of plant geography. In particular, it provides a new angle from which invasions and migrations may be viewed, and it is especially from this aspect that the following analysis has been made and not from any hope to solve the whole problem of distribution in Britain. In order to trace probable lines of invasion and inward spread, a cartographic presentation of the facts of distribution seemed desirable, if not necessary, and most of the points I wish to bring forward will be offered in the form of maps. *The first step to tracing the progress of the creation of vegetation is to know the proportion in which groups appear in different localities, a relation which must be expressed in numbers to be at all tangible.*[1] We are not here concerned with the origin of new forms, but by ascertaining the number of species in different localities we may at least attempt to trace the progress of that invasion by which our flora has gradually been built up.

[1] A statement of Sir J. D. Hooker. Extracted from a chapter by Dr. Guppy in Willis's Age and Area. Camb. Univ. Press, 1922.

As a working basis, a list of species generally admitted as indigenous was compiled from Babington's 'Manual' (1904), omitting micro-species of critical genera, since our knowledge of their distribution is far from complete. But for all the major species the data of distribution are available in Watson's 'Topographical Botany' (1883) and Bennett's Supplement to that work published in 1905. Since then some new records have accrued, but the differences between my own list and that of Druce (1908) or the London Catalogue (1908) are not sufficient to affect the general results when mass distribution is under consideration. Further, the new additions are not likely to affect one area in particular; they will be more or less uniformly distributed.

General Analysis of the British Flora.

The number of British flowering plants, which alone are dealt with in these distribution studies, is 1,377, of which 1,295 occur in England, 1,024 in Scotland, and 944 in Ireland. These figures themselves indicate a diminution in the flora as distance from the Continent increases, and while this is true it is well to remember that numerous species occur in Scotland or in Ireland which are absent from England. Again, England and Scotland share over 100 species which do not extend to Ireland, and 70 inhabit England and Ireland which are not recorded from Scotland. But of plants exhibiting a restricted range by far the largest number appears in England and Wales. This limited portion amounts to 266 species, nearly 20 per cent. of the total. On account of its restricted distribution, a study of this 'English' flora may throw some light on invasions and inward spread of plants in Britain as a whole, since we can learn little in this connexion from those species which now inhabit the majority, if not all, of the 112 vice-counties into which the country is divided for topographical statistics. It is an attempt to work backwards, as it were, picking up clues where we can from those plants which, whatever the cause, still show a limited range within our islands.

Distribution of 266 'English' Plants.

For convenience, species restricted to England and Wales will be referred to as 'English' plants. Their mass distribution is expressed cartographically in Diagram 1, the inset map indicating the European distribution of the same group. The bulk of this English portion of our flora may have come through France, where over 90 per cent. of it is centred. The dispersal *within* England is then very much what would be expected, but when we bear in mind the long period of time during which much disturbance of our native flora has occurred, the interesting feature exhibited

by the map is the remarkably regular diminution of the flora from the south-east, where it is concentrated, towards the north-west, where it is sparse. The *mass* distribution suggests a general invasion and migration north-westwards.

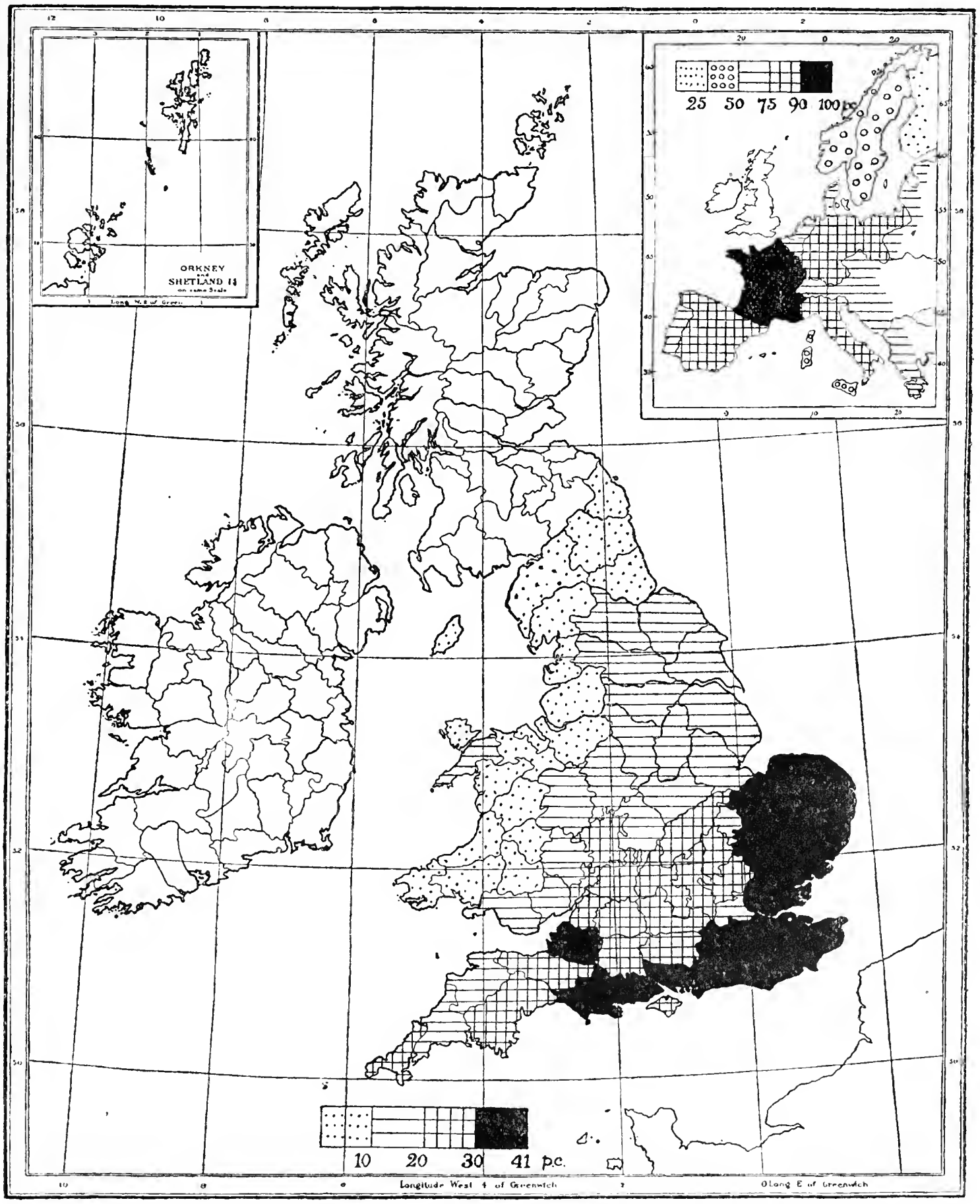

DIAGRAM 1. Distribution of 266 plants of the British Flora confined to England and Wales.

Whether the immigration of the British flora as a whole followed this direction only further analysis will show, but a clue which seems worth following is provided by the map before us.

There is at present little evidence to show whether any of these

'English' species survived the Glacial Period in Britain. Some certainly existed in the country during the Pliocene, being recorded either from the Cromerian or from the Castle Eden floras. These are *Acer campestre*, L., *Inula Conyza*, DC., *Picris hieracioides*, L., *Carpinus Betulus*, L., *Fagus sylvatica*, L., *Stratiotes aloides*, L., *Naias marina*, L., and *Potamogeton trichoides*, C. and S. Others may be regarded as allied in their distribution to that Atlantic element in our flora which is considered by some as of pre-glacial age in Britain. These are *Arabis stricta*, Huds., *Hypericum undulatum*, Schousb., *H. linariifolium*, Vahl., *Physospermum cornubiense*, DC., *Cnicus tuberosus*, L., *Lobelia urens*, L., *Erica ciliaris*, L., *E. vagans*, L., and *Scrophularia Scorodonia*, L. There is no evidence of the occurrence of any of these western plants in glacial deposits. Their survival would mean long occupation of the country, yet they have not spread far during the long interval since glaciation, nor, as has been pointed out by Seward (1911) for the Lusitanian species occurring in Ireland, have they evolved new forms as might be expected of species of antiquity and long isolation.

If we turn to the 'Age and Area' hypothesis we should interpret this restricted English flora as recent. The species which comprise it should, on the average, be among the last to have arrived. If most of them have advanced by way of France and spread no farther than the southern counties of England, it is because they are still comparatively young in the country.

This idea embodied in the 'Age and Area' theory is by no means new, as Willis himself points out in his recent volume on the subject, and it is interesting to find that in 1913, two years before Willis published his theory, Clement Reid had written, 'A hardy fauna and flora seem to characterize the period of the submerged forests [of Britain]; but the absence or great scarcity of characteristic survivors from a former period suggests that even the lowest of these deposits is far removed from the Glacial Period. The arctic species had already had time to die out or had been crowded out; *but the time had not been sufficiently long for the incoming of the southern forms which now characterize our southern counties*' (italics mine). The idea that the southern species occupying the south of England are recent arrivals is here clearly expressed.

It is on much statistical evidence, however, that Willis bases his theory, and an examination of Diagram 1 clearly suggests that numerous species occupy a comparatively small number of counties to produce the concentration in the south-east, and, in contrast to this, few species are so widely dispersed as to occupy all the vice-counties of the country, seventy-one in number. Working out the details and arranging the species in ten classes representing varying degrees of rarity, we obtain the results shown in Table I.

TABLE I.

	Occupying not more than		*Number of species.*	
1.	Seven	vice-counties	129	184
2.	Fourteen	,,	55	
3.	Twenty-one	,,	21	39
4.	Twenty-eight	,,	18	
5.	Thirty-five	,,	11	21
6.	Forty-two	,,	10	
7.	Forty-nine	,,	11	14
8.	Fifty-six	,,	3	
9.	Sixty-three	,,	7	8
10.	Seventy-one	,,	1	

The figures form a decreasing series like those obtained by Willis for endemic species, but since they relate to 'wides' the principle of 'Age and Area' suggests recent arrival, not recent origin, for the great majority of the plants concerned. This is only a broad conclusion, and results which pertain to the mass may not apply to the individual. This is one of the chief objections brought against Willis's hypothesis, and it is often forgotten that the theory is not intended to be applied to single cases and then to draw comparisons. Yet, if it is applicable at all, it is necessary to offer some explanation of those cases which do not conform to the general law. In the present series, the figures which refer to ten degrees of rarity do not produce a very smooth curve, although roughly they follow the hollow pattern type. But to the perfect working out of the 'Age and Area' principle there seems to be some disturbing factor. To discover these disturbing causes is one of the problems confronting the plant-geographer. Relics from past climatic successions, or commingling of more recent assemblages arriving from different directions, or some outstanding ecological differentiation of the flora may be suggested. Competition is perhaps the most potent factor influencing plant distribution. I have already shown (1922), in connexion with a flora having a high percentage of relics, that the 'Age and Area' principle is not particularly obvious until the relic species are eliminated.

But if this English portion of our flora, considered broadly, is at an early stage of invasion, greater precision regarding migrations may be obtained by submitting the rarest class (nearly 50 per cent. of the total as here arranged) to further analysis, since the rarest species will be, in general, the latest arrivals, and the area or areas showing concentration may provide a clue to the direction from which they came. Accordingly, the 129 members of the rarest class have been mapped separately. The result is shown in Diagram 2, where the actual number of species is given for each vice-county.[1] There is a considerable number of outlying species in the north

[1] From my list of data these 129 species occupy, on the average, 3·3 vice-counties. Taking the London Catalogue numbers or those of Druce's List they occupy 3·5 vice-counties.

and in Wales, among which may be mentioned *Actaea spicata*, L., *Viola rupestris*, Schmidt, *Arenaria uliginosa*, Schlecht, *Arenaria gothica*, Fr., *Potentilla rupestris*, L., *Polemonium coeruleum*, L., *Cypripedium Calceolus*,

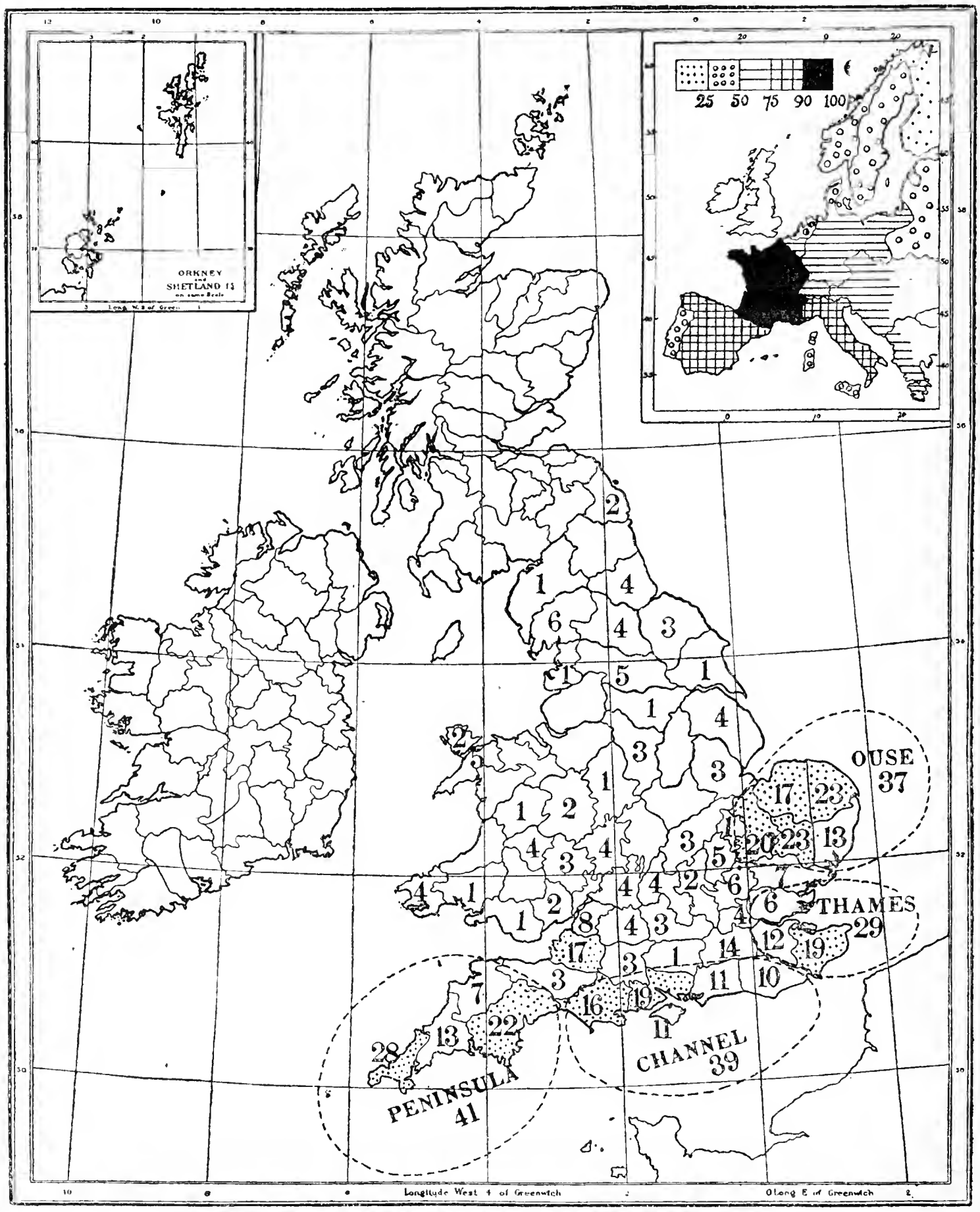

DIAGRAM 2. Distribution of 129 rare 'English' species.

L., *Maianthemum bifolium*, Sch., *Lloydia alpina*, Salisb., *Potamogeton rutilis*, Wolf., *Carex ornithopoda*, Willd., and *Ammophila baltica*, Link. Some of these enter into the montane or boreal flora of England. None of them is known from pre-glacial deposits, and one, *Arenaria gothica*, is recorded from the glacial beds of the Cam Valley. This species, occurring on the Continent

only in Sweden and Switzerland, may be considered a glacial relic, a member of a residual flora, the bulk of which is found farther north, although in Pleistocene times it reached the south of England. Perhaps the other montane or arctic-alpine species are also old, although the fossil evidence is not yet forthcoming. On the other hand, a plant like *Ammophila baltica* of the Durham and Norfolk coasts may have arrived recently, if it has not actually originated in these localities, since it is often regarded as a hybrid. Again, *Potamogeton rutilis* in Anglesea may be a recent immigrant.[1] Such outliers and others that will be mentioned later show the necessity for taking into consideration all the facts which bear on plant-distribution problems. They seem to point in some cases to survival, in other cases to sporadic introduction, rather than to any particular, directed movement. They will interfere, therefore, with the 'Age and Area' scheme in detail. But it is impossible to ignore the fact that most of the very rare flora in England is concentrated in the south-west, south, and south-east. West Cornwall stands highest with 28 species. East Kent has 19, yet this vice-county possesses the largest number when the total of 266 species is analysed. Thus the important point emerges that all the more widely-distributed English species, once having reached Kent, have remained established there. There is no suggestion of a wholesale destruction once colonization has been effected. The dotted areas in Diagram 2 showing density need not be considered the last strongholds of assemblages of plants once more widespread. It seems more reasonable to suppose that they are points at which plants have arrived and have secured a foothold.

If this view is correct, it may be instructive to inquire whether any floral or geographical alliance exists amongst those areas which are especially rich in species. Guided by the results of Diagram 2, we may select a few districts for the purpose of illustrating this point. Four areas are shown enclosed by dotted lines. Each includes several vice-counties, so that the districts compared may not be too small nor the analysis too elaborate. The four regions do not correspond exactly in size with the provinces employed by Watson, being smaller, but it is convenient for reference to use his names, viz. Ouse, Thames, Channel, and Peninsula.

Of the 129 rare species under consideration, 37 occur in the Ouse province, 29 in the Thames, 39 in the Channel, and 41 in the Peninsula. We may now trace the distribution of these assemblages, beginning with the Ouse group. Diagram 3 gives the vice-county details, but, taking the four larger areas proposed, we find that of the 37 species centred in the Ouse province, 11 occur in the Thames, 9 in the Channel, while 7 reach the Peninsula. In the same way Diagram 4 illustrates the distribution of the Thames group of 29 species, of which 11 pass northwards to the Ouse, 13 occur in the Channel, and 7 in the Peninsula. The range of the 39 species

[1] Lately recorded for Shetland also.

concentrated in the Channel area is shown in Diagram 5. Seventeen of these are found in the Peninsula, 13 in the Thames, and 9 reach the Ouse district. Finally, in Diagram 6 there is shown the distribution of the 41

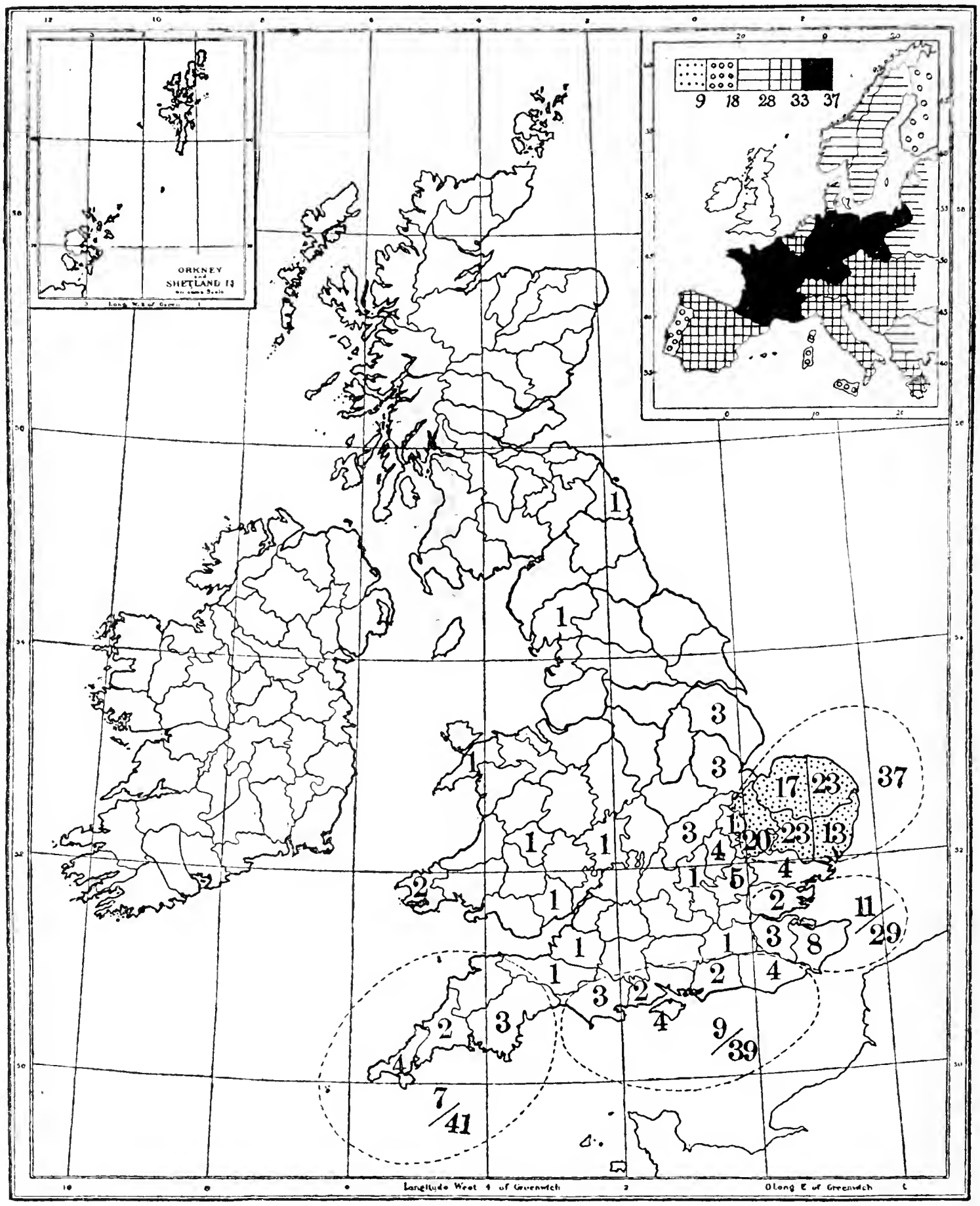

DIAGRAM 3. Distribution of 37 'Ouse' species.

species which have their head-quarters in the Peninsula. Of these 17 are common to the Channel, only 7 reach the Thames, and 7 also appear in the Ouse province.

These facts are summarized in Table II. Since 30 of the 129 rare species do not occur in the districts defined, the total flora of the four areas combined numbers 99 species.

TABLE II.

Ouse.	*Thames.*	*Channel.*	*Peninsula.*
37	11	9	7
11	**29**	13	7
9	13	**39**	17
7	7	17	**41**

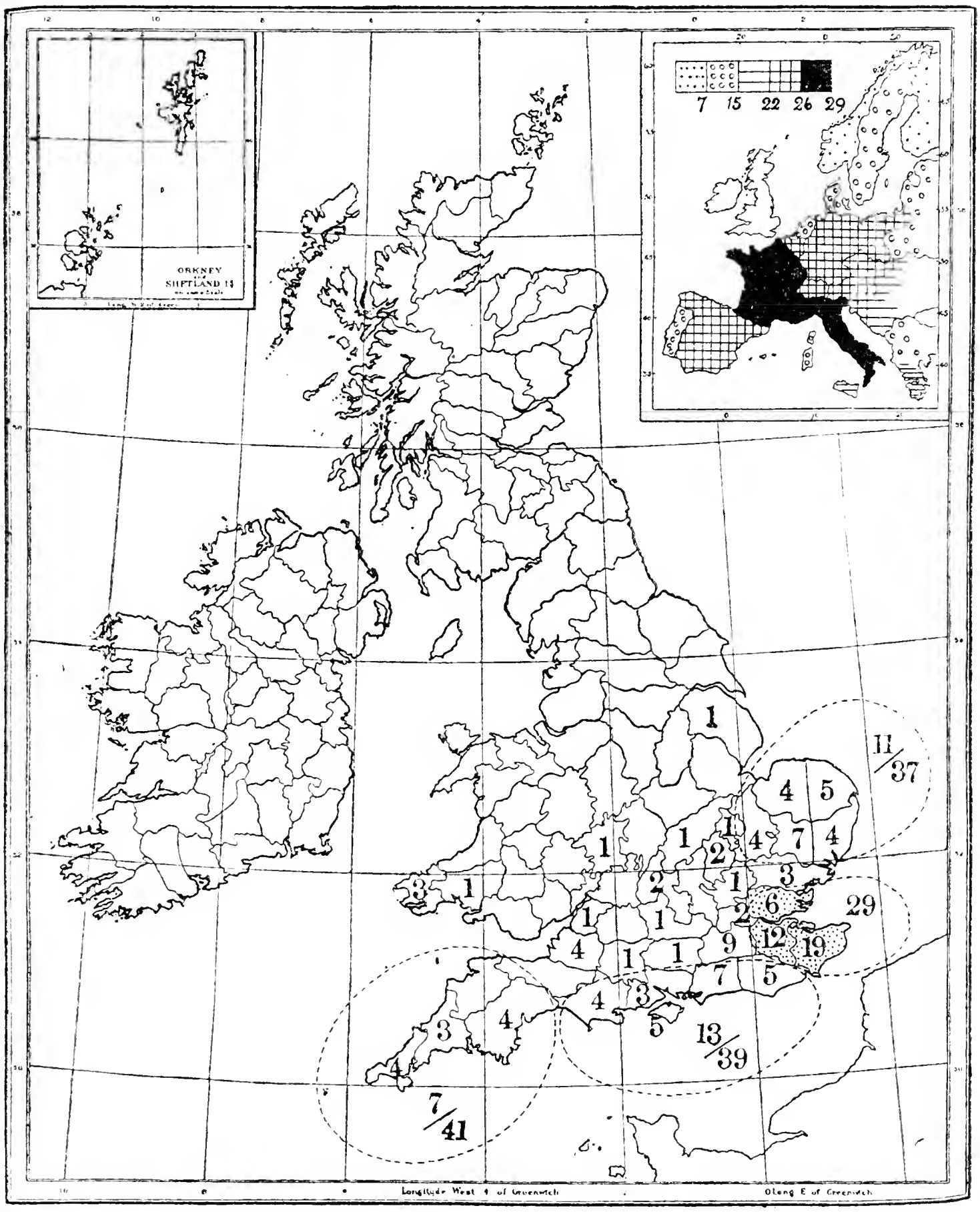

DIAGRAM 4. Distribution of 29 'Thames' species.

It is clear that these small assemblages are fairly diverse in floristic composition. In fact, only two species are common to all the districts, and to the approximately equal floras occupying the most widely-separated regions, Ouse and Peninsula, only 7 species are common. The index of

floral diversity employed by Colgan (1901) is the 'ratio which the total of species not common to both areas bears to the total flora of the two areas combined'. This index for Ouse and Thames is 0·800, for Thames and Channel 0·764, for Channel and Peninsula 0·730, for Peninsula and Ouse 0·901. The greatest difference is between the groups of the Peninsula and the Ouse provinces.

Another striking feature of the results presented in Table II is the pronounced and fairly regular decrease in numbers from the several maxima, whether we take the figures as they stand or in proportion to the group to which they belong, and it is suggested that we may here be dealing with more than one invasion. The presentation of the facts in this form provides, indeed, some definite criterion by which to examine possible lines of invasion, as Willis (1920) has done for New Zealand.

Diagram 3. The 37 plants of the Ouse province are widely distributed in Central and South Europe. Nearly all occur in Germany and France, so the European distribution does not suggest that they should be more prevalent in the east of England than in the south. But a migration from the east is suggested from the range of these Ouse species *within* England itself, and here ecological factors probably play an important part in determining distribution. The introduction of seed and the subsequent germination and establishment of the plant are different things. The former must be happening frequently, the latter rarely, for competition in an area already clothed with vegetation is great. Selection will follow, and so plants are most likely to obtain a foothold in that area where local conditions are most suitable. Once established in the country, the chances for inward spread will be much greater from the English stations than by means of recurrent introductions from the Continent. Diagram 3 illustrates this spread, a pronounced coastal tendency being obvious, for the group possesses a number of littoral forms and a large proportion of species which favour the dry, sandy, or chalky soils found in some of the south-eastern counties. To this may be added the fact that birds as possible carriers of seeds commonly migrate along the coasts of a country. Thus, if Norfolk represents the centre of distribution in England, dispersal has not been uniform in all directions. But, if time is a condition of wide distribution, as it must be, then the small number of Ouse species which have reached the Peninsula may be explained by the fact that they have lacked the time to spread so far. Mathematical precision cannot be expected, and a few outliers are not surprising. Only two Ouse species, *Lythrum Hyssopifolia*, L., and *Hypochaeris maculata*, L., are absent from the Thames and Channel and occur in the Peninsula area. This may be due, though not necessarily, to separate introductions from continental sources, yet, if such occurrences were at all frequent, it is unlikely that the regular decrease from the maximum would be obtained.

Diagram 4. All the Thames species, 29 in number, occur in France, and their concentration in Kent is not unexpected. *Genista pilosa*, L., *Orobanche Picridis*, Sch., and *Cyperus longus*, L., extend to Pembroke, while

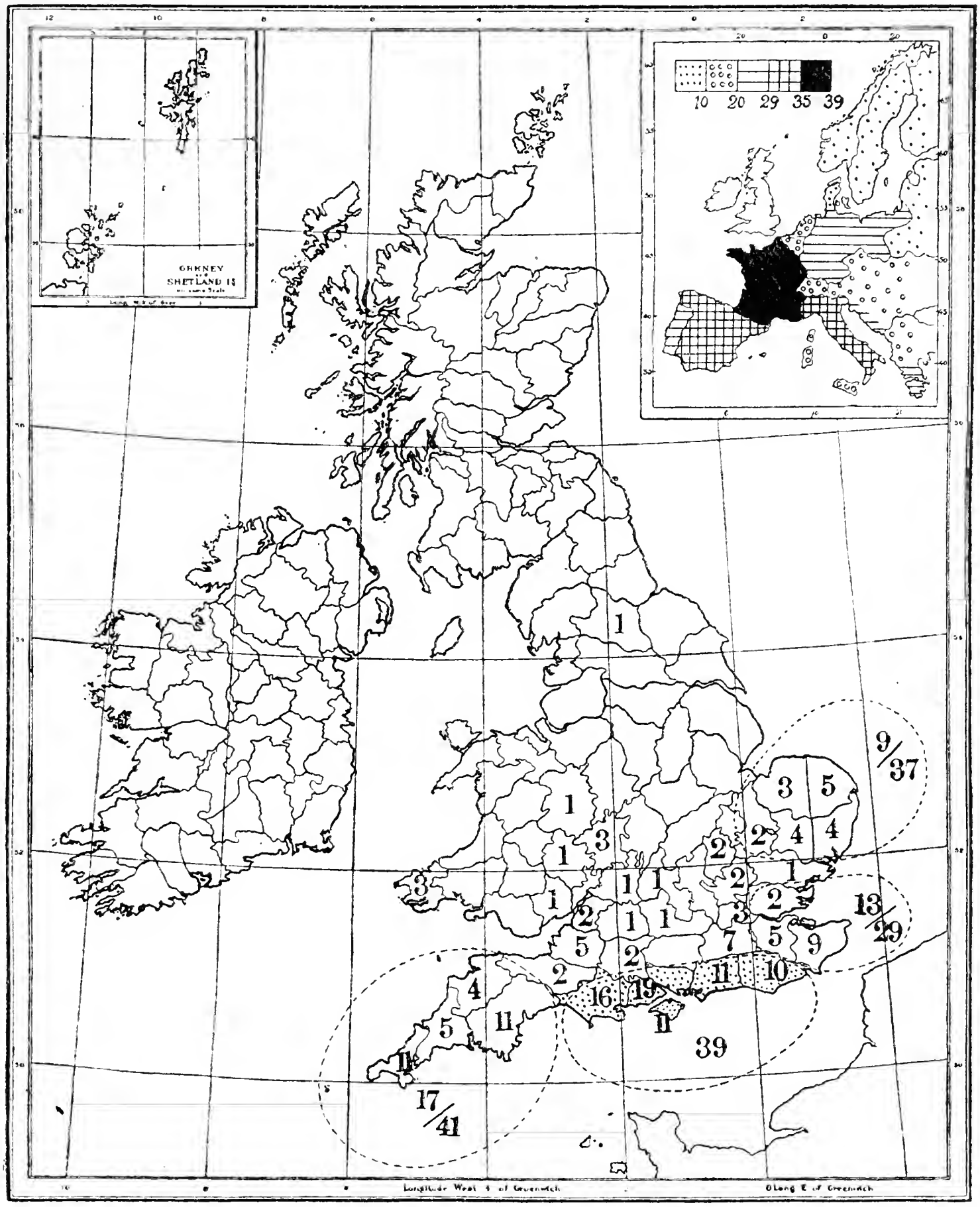

DIAGRAM 5. Distribution of 39 'Channel' species.

Ceratophyllum submersum, L., appears in Carmarthen. Except for these outliers, the thinning out of the Thames species from their head-quarters in Kent is fairly uniform and quite pronounced when the larger provinces rather than small vice-counties are considered.

Diagram 5. All but one of the 39 Channel species are found in France, and 14 are members of the southern element as defined by Stapf

(1914). The group exhibits a sudden diminution in numbers in the interior of England, and a marked coastal tendency east and west.

Diagram 6. The Peninsula species, numbering 41, have a European

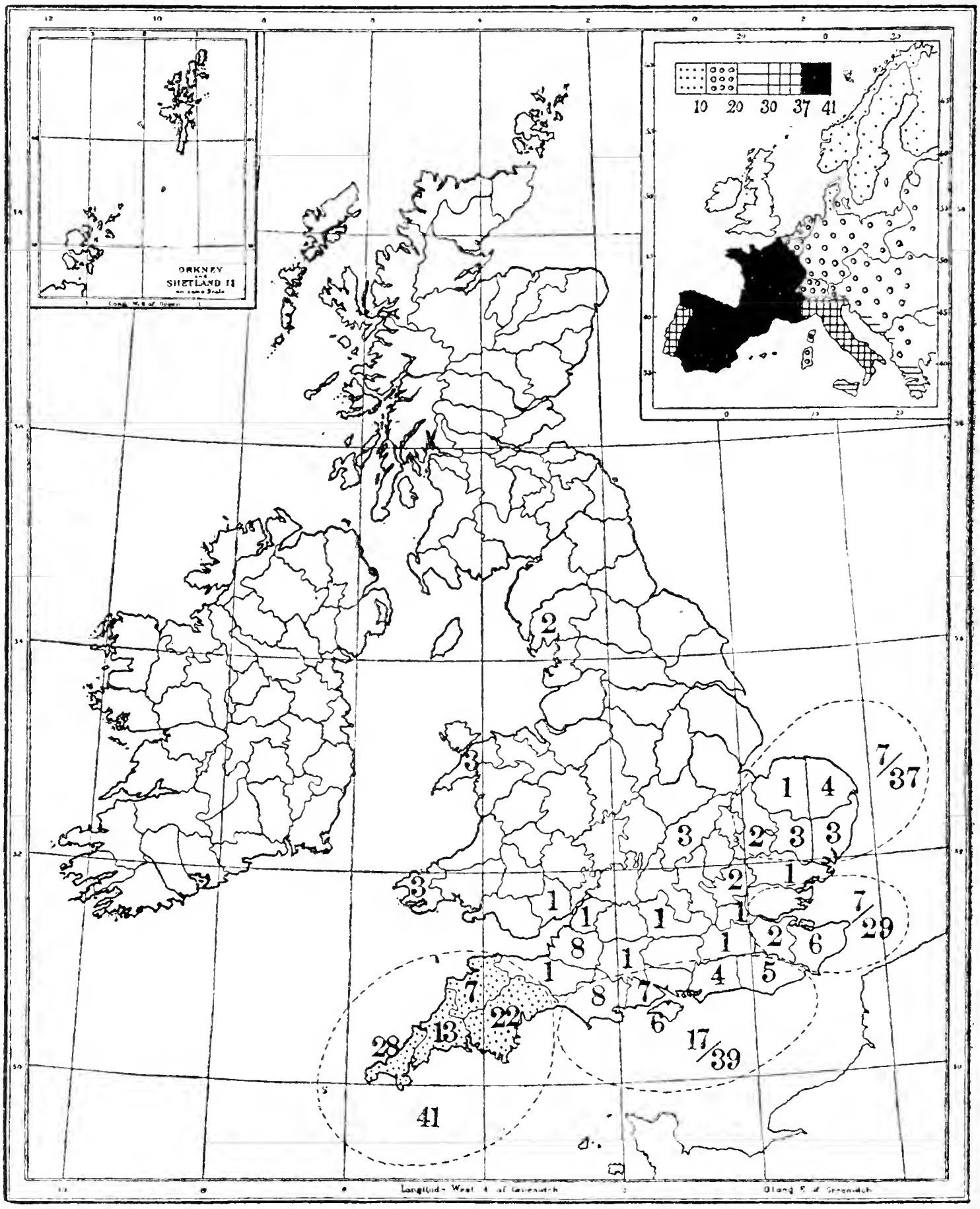

DIAGRAM 6. Distribution of 41 'Peninsula' species.

distribution which is essentially south-western, 22 of them belonging to Stapf's southern element, while 7 are definitely Atlantic. Nearly all occur in France and Spain, and more than half are absent from Central Europe. Their concentration in Cornwall may be attributed, then, to an almost direct northern extension from their chief European centre. Within England they thin out in the *opposite* direction to the Ouse group of plants, as a glance at

Table II or Diagrams 4 and 6 will show. These two series of figures may well suggest the questions whether the two assemblages of plants to which they refer are concentrated in areas where they arrived and became established, or whether they constitute residual groups squeezed into these corners by a process of elimination and disintegration which has been more than remarkably regular. From the former point of view distinct invasions are suggested, although it need not be supposed that every species occurring in the Channel province, for instance, arrived there by direct migration. Common species may occasionally have followed a more circuitous route, arriving, perhaps, by way of the Peninsula or by way of the Thames area.

Attention may next be drawn to an interesting and important feature exhibited by Diagrams 3–6 taken consecutively. The concentration of species in the several districts in England is very fairly matched by the European distribution. The centre of dispersal for the Ouse group is Germany and France, but for the Peninsula assemblage it swings round to France and Spain. In their most general features the maps correspond with those of Stapf (1916), who worked with the southern element of the British flora as determined by its range on the Continent, whereas I have worked with a group whose boundaries are determined by its range in Britain.

The results so far obtained, indicative of the distribution of the rarest species in England, seem consistent with the general principle of 'Age and Area'. It is of importance, therefore, to test the question further by analysing the data for all the English species. Of the total number (266), only 33 are absent from the four provinces we have been considering, i. e. three in addition to the thirty of the rarest class. The dispersal of the remaining 233 through these four areas may be seen from the figures given in Table III.

TABLE III.

Ouse.	*Thames.*	*Channel.*	*Peninsula.*
142	108	101	63
108	**144**	116	73
101	116	**157**	89
63	73	89	**117**

These figures confirm the results obtained from the smaller numbers dealt with in Table II. They also serve to illustrate the general features already expressed cartographically in Diagram 1, where it is shown that the 'English' flora, taken as a whole, is most abundant from Norfolk to Dorset. The Channel province is now seen to possess the largest number of species. It is through this area that the bulk of our English flora may be regarded as having advanced from the Continent, although we have seen that other movements west and north have played a part in the building up of the flora.

It has already been suggested that plants may reach the Channel or Thames region by dispersal along a circuitous route, say through the Peninsula or the Ouse, as well as by direct migration. In the course of time, as a result of the meeting of different migrations, the intermediate districts, Thames and Channel, would attain a larger flora than the areas at the extremities of the region, assuming that plants are arriving in approximately equal numbers and spreading at roughly equal rates—conditions not likely to be fulfilled in nature. But even an approximation to such conditions would produce an average result in which the working out of the Age and Area principle would be obscured if applied to the total flora. In the present case, in fact, we have a group of 233 species distributed through the four districts of the area under consideration as follows:

75 occupy one district;
43 occupy two districts;
62 occupy three districts;
53 occupy four districts.

The figures are not particularly suggestive of 'Age and Area', yet when they are disentangled the applicability of the law becomes more apparent. The rarest species (99) are distributed as follows:

67 occupy one district;
19 occupy two districts;
11 occupy three districts;
2 occupy four districts.

Thus the rare species are seen to follow the general law much more closely. Whatever the cause of the rarity, if these species are the result of different invasions, the admixture in any one district due to migration from different directions will be relatively small. We should expect, then, that the general principle should apply to any particular invasion, although it has to be noted that the numbers involved become much smaller. That this is so has already been indicated in the results shown in Table II, but the facts may now be expressed in the usual 'Age and Area' way. They are given in Table IV.

TABLE IV.

Occupying	41 *Peninsula species.*	39 *Channel species.*	29 *Thames species.*	37 *Ouse species.*
One district	21	14	11	21
Two districts	11	13	7	7
Three districts	7	10	9	7
Four districts	2	2	2	2

The results agree fairly well with Willis's hypothesis. When a species occurs throughout the whole region it is, of course, not easy to determine to

which invasion it belongs. In the rarest class there are only two such species, but in the whole group, as shown above, there are as many as 53. The meeting and commingling of plants following different migratory paths then becomes so pronounced that it obscures any particular movement which may be applicable to certain assemblages considered separately. The distribution of the flora taken as a whole does not apparently follow the 'Age and Area' law, not because the law fails, but because it is obscured.

Discussion.

The data brought together in the preceding pages raise numerous questions about the distribution of plants in England, and I have presented the facts, I hope, without unduly pressing any particular conclusion, for the problem of distribution, even in a small area of the earth's surface, is eminently complex. No attempt can be made to deal with all the questions that arise. The study developed with the aid of the ideas underlying Willis's theory of distribution, and in broad outline that hypothesis seems applicable to the limited portion of the British flora which is under review. To discover invasions has been my chief aim, and while it would be rash to conclude that a definite number of migrations distinct in time and place has been fully demonstrated, there is nevertheless evidence to show that more than one invasion from continental sources has shared in the building up of our native flora. As to the time of these invasions there is little to guide us, although a consideration of all the evidence would suggest that they are comparatively recent. As to place, there seems reason to believe that plants have reached our shores generally along the coastal area from the Bristol Channel to the Wash. Certain areas of establishment are observable, and these show a geographical alliance with continental centres of distribution. Once established in England further spread seems to be effected from the English stations, although the possibility of recurrent introduction from the Continent cannot be entirely ruled out. The coastal tendency is a feature of the distribution of the smaller groups which have been studied in detail, and may be related to the ecological class of the species, and possibly to dispersal by birds which possess coasting habits. Hence species need not radiate uniformly from their centre of origin in the country.

But, allowing for many modifying factors, the results which the present analysis has produced seem capable of explanation along lines suggested by the principle of distribution which Willis has styled 'Age and Area'. An important modification is the exclusion on geological grounds of the boreal element in the flora, a procedure which is justified by the terms of the law itself.

Again, there is a small assemblage of species in North Somerset and Gloucester, few of which come within the boundaries of the areas I have

especially dealt with. *Arabis stricta*, Huds., *Draba aizoides*, L., *Dianthus caesius*, Sm., *Euphorbia stricta*, L., *E. pilosa*, L., *Cephalanthera rubra*, Rich., *Allium sphaerocephalum*, L., *Carex tomentosa*, L., and *Koeleria vallesiana* All. belong to this western group. The first only is western in Europe, the others being generally distributed throughout the Continent. Their localization in Britain is difficult to understand unless it is the result of chance introduction, although it may possibly be due to an extension of that movement which has helped to build up the peculiar element in the Peninsula flora.

Yet, of the total number of 'English' species, only 12 per cent. lie outside the coastal counties from Cornwall to Norfolk. That 88 per cent. should occupy this belt and gradually thin out inland seems strong evidence that immigration has been, on the whole, a fairly definite movement. The process has doubtless gone on for thousands of years, and while occasional introduction may have been the general event, the chances would seem to be in favour of plants reaching points near or having some relation to their chief continental centres. The process may thus approximate to a definite tendency, and we might predict, as is often done, where certain species are most likely to occur. But not infrequently plants will be carried out of and beyond the general trend. They are the particular cases which do not follow the general rule nor conform to the general law. We have seen that in the 'English' portion of the British flora they are relatively few in number.

Summary.

A brief survey of the divergent views held regarding the origin of the British flora is given, and the source and distribution of 266 species which are restricted to England and Wales are then considered. A map illustrates the range of these species, and the general conclusion is reached that this limited portion of our flora has been derived mainly by advance through France in post-glacial times. A detailed analysis shows that nearly 50 per cent. of the group is of great rarity. Excluding some 30 species which are boreal or western outliers (some as relics of a former arctic-alpine flora, others possibly as recent introductions), this rare element is centred along a coastal belt from Cornwall to Norfolk. Certain areas of concentration are found to exist indicating, it is believed, points of arrival and establishment rather than areas of retirement. These features provide a clue to invasions, and details are given for four small assemblages which may be regarded as having followed different migratory paths. An invasion from the east and another from the south are distinguished, and between these two lines the main portion of the English flora has probably advanced. In addition to the cartographic studies presented, the results are expressed in

terms of Willis's 'Age and Area' theory of distribution. It is shown that by trying to discover disturbing factors and allowing for these, the results obtained are consistent with this hypothesis in its broad outlines, although on a first analysis the theory may not appear particularly applicable.

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A Critical Study of Crown Gall.

BY

WILFRID ROBINSON AND H. WALKDEN.

With Plates V and VI and four Figures in the Text.

CROWN GALL is a widespread disease occurring on a great variety of herbaceous and woody plants in relation to wounds, particularly those produced in pruning, grafting, and in the making of cuttings. While of considerable economic importance the disease has acquired even greater interest on account of the far-reaching comparisons which have been made between it and malignant tumours in man by Dr. Erwin F. Smith,[1] to whom we owe most of our knowledge of crown gall.

Toumey (34) gave the first full description of the disease, while Cavara (2), Toumey, and later Hedgecock (7, 8) proved its infectious character by means of extensive inoculations of portions of galls into healthy plants. Erwin F. Smith and Townsend (19) first isolated the causal bacterium from the galls on the Paris Daisy (*Chrysanthemum frutescens*, L.) and a large number of other plants, and proved its pathogenicity by re-inoculation, naming the organism *Bacterium tumefaciens*, Smith and Townsend. They studied its cultural characters fully, and since that time E. F. Smith (19 to 32) has carried out much experimental work on crown gall, and we are indebted to his activity for the accumulation of a vast amount of extraordinarily interesting and suggestive detail regarding the disease. He also early instituted comparisons between the crown gall disease of plants and malignant tumours in man; and a great deal of his experimental work has

[1] Since completing our paper we have seen a recent paper by Smith on Appositional Growth in Crown-gall Tumours and in Cancers, Journ. Cancer Research, vii, 1922. In this Smith describes and figures the development of tumour-tissue by the subdivision of normal parenchyma cells. He states that a narrow strand of tumour tissue is converted into an extension of the tumour as a result of the stimulating effect of the bacteria either within the cells or acting at a distance. He now expresses the opinion that most, if not all, tumour-strands originate in this way, thus receding from his earlier position that tumour-strands and secondary galls originate by the intrusive growth or infiltration of tumour-tissue. He still maintains, however, that the tumour is due to an 'intracellular schizomycete'.

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been carried out from this point of view. He regards most of his results as supporting the comparisons which he has drawn. It will be necessary to refer to these in some detail.

Bacterium tumefaciens is regarded by Smith as a feeble wound-parasite present in small numbers within the cells of the plants attacked, although not demonstrable by direct methods.[1] The organism produces substances which stimulate the tissues to give rise to larger or smaller overgrowths according to the nature and age of the tissues affected. Along with this there is a stunting of growth and ultimately a slow killing of the plant attacked. The main comparisons with cancer and other malignant tumours have been drawn from the experimental production of secondary tumours and of 'teratomas', or crown galls bearing leafy shoots having no relation to preformed buds. The secondary tumours are described as 'growths from tumour-strands bedded deep in normal tissues derived by growth (cell-division), in the form of a continuous chain of cells, from the primary tumour'.[2] Smith's photographs usually show secondary tumours at some considerable distance from the point of inoculation, and he infers that the proliferating tumour-strands have grown intrusively from the point of inoculation to the positions where secondary tumours appear.

In further support of the supposed similarity with malignant tumours he holds that 'secondary tumours reproduce the structure of the tissues in which the primary tumour has developed even when they appear in other organs; thus if the primary growth is in the stem and the secondary growth is in the leaf, the attacked part of the leaf will be converted into a pseudo-stem'.[3] This, and the origin and nature of the secondary galls and tumour-strands, obviously require further investigation before E. F. Smith's interpretations of these aspects of crown gall can be accepted.

The need for re-investigation of these and other comparative aspects of the tumour formation in crown gall was discussed in a paper by Professor W. H. Lang (12) at the meeting of the British Medical Association in Glasgow in July, 1922. We are in complete agreement with Professor Lang's general formulation of these problems, which, as he states, was made in the knowledge and light of this work then in progress. We gratefully acknowledge our indebtedness to Professor Lang for his continual interest and helpful criticism during the course of the work.

The work of some other investigators on crown gall may be briefly referred to before the scope of the present paper is indicated. Jensen (9, 10) has made observations on crown galls produced on beet by inoculation with *B. tumefaciens* and has compared these galls with malignant tumours.

[1] In earlier papers Smith figured the bacteria in small numbers in the cytoplasm of the tumour cells. More recently he has stated that the bodies he previously demonstrated by staining with gold chloride are not bacteria.

[2] Smith (31), p. 417.

[3] loc. cit., p. 417.

According to his view the presence of the bacteria, in the initial stages of the disease, provides the stimulus, which is then continued by the stimulated but uninfected cells behaving as parasitic cells similar to cancer cells. He has also studied another tumour growth on beet, from which it is not clear *Bacterium tumefaciens* can be isolated, and has successfully transferred this tumour between red and yellow forms of *Beta*, but there is little indication of any true infiltration of tumour-tissues in this case.

Kuster (11) states that in secondary galls caused by *B. tumefaciens* conclusive proof of the infiltration of tumour-tissues described by Smith has not been obtained, and suggests that it is more likely the effects are produced by the movement of the gall-producing organisms through the tissues of the host plant. He also denies that the stem-like structure of leaf-galls is derived by the growth of tissues from the stem into the leaf and mentions that such structure is frequently seen in other galls.

Peklo (17) produced tumour-strands and secondary galls similar to those described by Smith by the inoculation of the developing capitula of *Helianthus*.

Friedemann and Magnus (3) have emphasized the fact that crown galls are special irregular developments of wound tissue resulting from the infection by *B. tumefaciens*, and they hold that Smith's figures are not decisive regarding the intrusive growth of tumour-tissue. These two authors, on the grounds of cultural and serological characters, at first thought that certain strains of bacteria isolated from intestinal and some other diseases of man were identical with *B. tumefaciens*. Of the many strains so isolated only one proved capable of producing crown galls on plants. Friedemann (5), however, showed later that this organism was invariably in symbiosis with *B. proteus*, and, since it was only isolated from faeces and not from diseased tissues, that there is no real ground for ascribing to *B. tumefaciens* any pathogenicity to man.

Magnus (16), in a later paper, showed that there is no evidence in favour of the opinion expressed by Blumenthal and Hirschfield (1) that the pathogenic properties of *B. tumefaciens* can be transferred to saprophytic bacteria commonly associated with it.

Riker (18), in a paper which, so far as we know, has not been published in full, states that *B. tumefaciens* can live in soil for at least a year, and that he has obtained microscopic evidence, derived from primary and secondary galls on the raspberry, indicating that the bacteria live in small quantities in the intercellular spaces of the host. He also states that under certain conditions he found the organism able to travel through the vascular bundles.

Levine (13, 14, and 15) has studied the origin of leafy growths on *Bryophyllum* inoculated with *B. tumefaciens*, the behaviour of crown gall on *Ficus elastica*, and the effect of the previous health of beetroot upon the

reaction of this plant to crown gall. These aspects of the disease lie outside the scope of this paper.

The present work has been concerned with crown gall on *Chrysanthemum frutescens* and on *Nicotiana affinis*. *Bacterium tumefaciens* was independently isolated in this country in 1920 by Walkden (35) from naturally-occurring galls on *Chrysanthemum frutescens*. The cultural characters of the organism were fully studied by him, and it was proved by re-inoculation to be capable of causing the disease. Pure cultures so obtained by repeated re-isolations have been used for our experimental study of the disease (Pl. V, Fig. 6).

In the case of *Chrysanthemum frutescens* the development of the galls on the cut surfaces of stems has been studied from the time of inoculation until the galls attain a large size, a strict comparison having been made at every stage with corresponding cut surfaces of uninoculated control stems. This study provided abundant material for an examination of the location of the bacteria causing the diseased growths, and we have obtained new and conclusive evidence regarding this very important matter.

We have critically repeated Smith's work on secondary tumours and tumour-strands both on *Chrysanthemum frutescens* and on *Nicotiana affinis*, and we have obtained galls similar in all respects and similarly distributed to the primary and secondary galls figured by Smith. Experimental evidence, supporting entirely different explanations from those given by Smith of the origin of these secondary galls and tumour-strands, is given below.

Although we have produced, by artificial inoculation both of Chrysanthemum and tobacco, structures precisely similar to the so-called teratomas figured and described by Smith, we do not propose to deal with these in this paper. The interpretation which we place on these so-called teratomas will, however, be briefly referred to in the discussion following the description of our other results.

It will be convenient first to describe the development of artificially produced primary galls on the Chrysanthemum from the earliest stages, then to deal with the distribution of the bacteria in the gall, and finally to describe experiments relating to the origin of secondary tumours and tumour-strands both in *Chrysanthemum frutescens* and in *Nicotiana affinis*.

Development of Galls on Cut Surfaces of Shoots of *Chrysanthemum frutescens*.

The material for the study of the development of galls after the inoculation of cut surfaces of stems was derived from healthy, well-grown plants of *Chrysanthemum frutescens* raised from cuttings and grown in

a temperate greenhouse. For inoculation and as controls we used shoots as nearly as possible similar and equivalent. Where the lower end of a shoot was employed, this was severed from the plant by a clean transverse cut across the middle of an internode, and then, either with or without inoculation, the shoot was planted as a cutting in moist soil or sand. In other cases the apices of vigorous shoots were removed by transverse cuts through a definite internode as near as possible to the apex, and the surfaces so produced were either inoculated or left as controls, the plants being subsequently grown under ordinary greenhouse conditions.

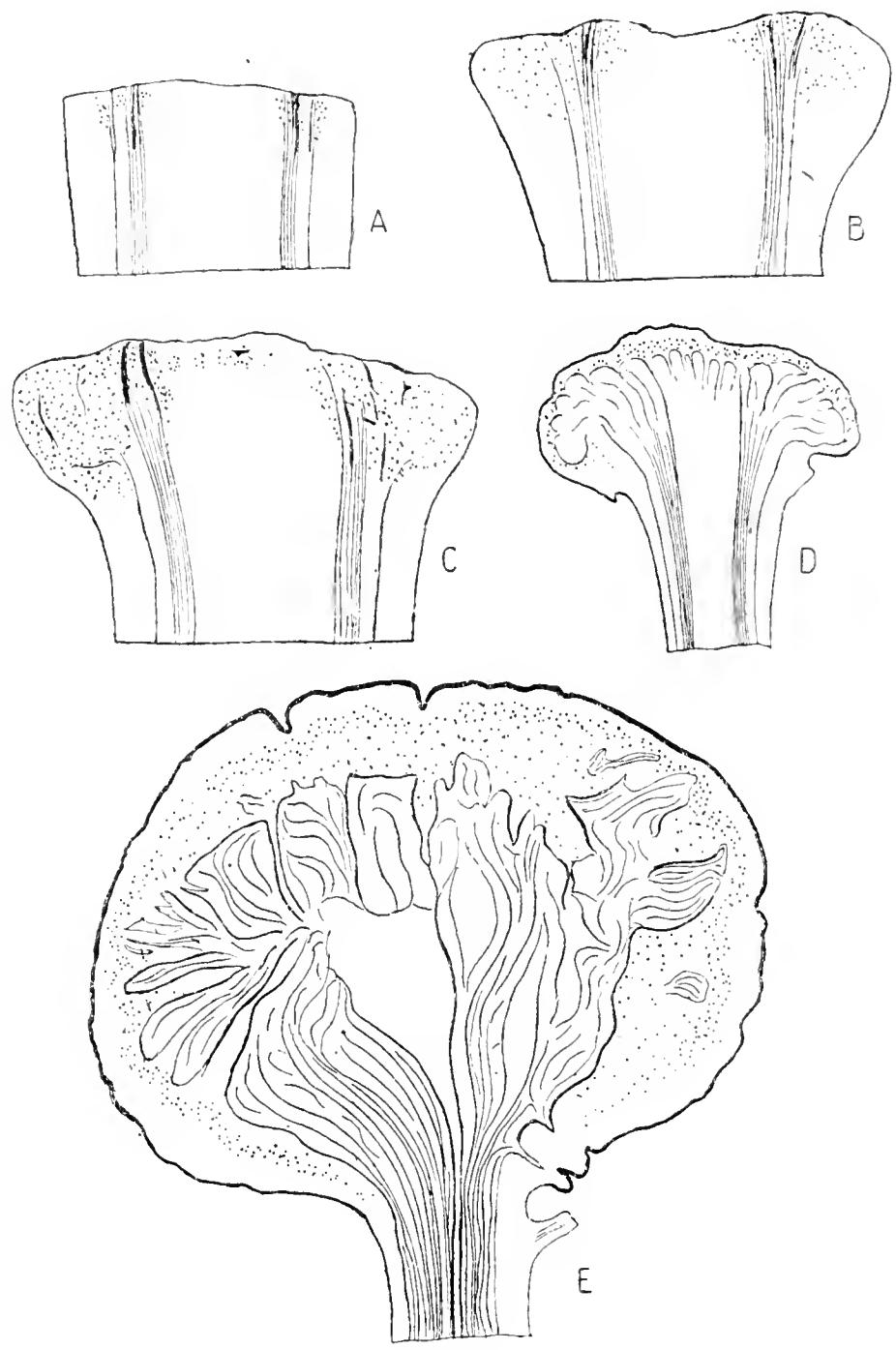
It was found to be immaterial whether the equivalent shoots used as controls were on the same plants as those inoculated or on different plants of the same age, since precisely similar results were obtained in either case. In most of the work, therefore, the control uninoculated surfaces were made on the plant bearing the inoculated shoots. At suitable intervals of time, usually three days in the early stages, small pieces of stem, including either the inoculated or the control surface, were fixed in weak chrom-acetic acid and embedded and microtomed. Sections were usually stained with iron-alum-haematoxylin, followed by orange G in clove oil, or by carbol fuchsin and orange G. In addition, for special purposes hand-sections were examined in water in the fresh condition.

Normal Anatomy of the Shoot.

Pl. V, Fig. 5, is a transverse section of the stem in the region just below the apex, where the inoculations were usually made for the production of aerial galls. The stem is of the ordinary dicotyledonous type. There is a distinct hypodermis and a small amount of collenchyma is disposed in patches. The cortical parenchyma extends about six layers of cells deep and is limited by a definite starch sheath. Mucilage ducts develop at regular intervals in the cortex near to the starch sheath, being usually situated opposite the primary medullary rays between the larger vascular bundles of the ring. The vascular ring consists of larger bundles with smaller ones between, and even at this early stage of development the pericycle is well defined opposite the vascular bundles as fibrous cells with distinctly thickened walls of pure cellulose. At this stage these pericycle cells retain their nuclei and living contents. The phloem of the bundles is a narrower zone within the pericycle and is separated from the xylem by the thin-walled desmogen cells which later function as cambium. The xylem consists of five or six radial rows of metaxylem and protoxylem vessels with conjunctive parenchyma and xylem parenchyma in the protoxylem region. The perimedullary zone is parenchymatous, but is not very clearly differentiated from the parenchymatous pith. The medullary rays are relatively narrow, being at most only eight cells wide.

Changes in Inoculated and Control Shoots.

According to whether the cut surface of the shoot is in contact with the soil when the shoot is treated as a cutting, or the cut surface is left exposed to the air, minor differences in behaviour are observed. The differences



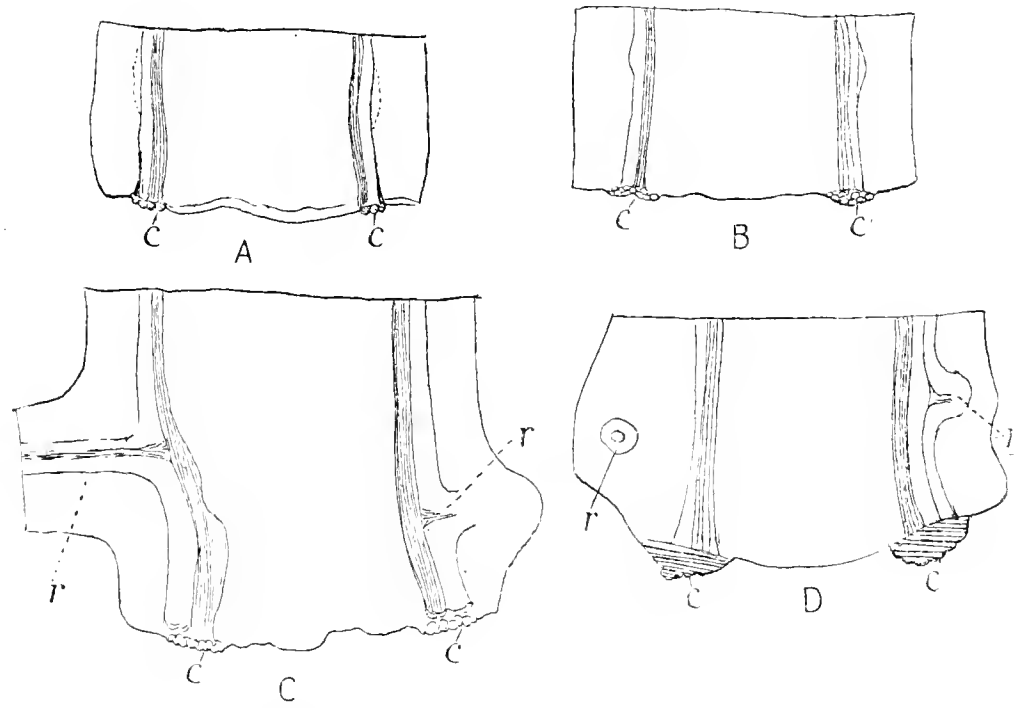
TEXT-FIG. 1. Series of radial longitudinal sections through upper ends of stems at various intervals after inoculation, illustrating stages in gall formation: A after 6 days, B after 9 days, C after 15 days, D after 4 months, E after $5\frac{1}{2}$ months. A, B, and C × 9; D and E × 3.

The dotted areas represent the position of actively dividing tissues. The blackest lines in the vascular bundles and cortex in A, B, and C represent positions where bacteria were observed.

are found in both the inoculated and uninoculated shoots and are partly due to differences in polarity and partly to the different conditions in the soil and in air. Text-figs. 1 and 2 illustrate various stages in the development of galls, which have arisen on the upper ends of stems in air and on the lower ends of cuttings in soil, respectively. The general development is similar in the two cases, but the tumour growth is more superficial in the cuttings, the severed vascular bundles being more quickly covered over by proliferating tissue than in the aerial shoots. It is noteworthy also that in

the former case we have frequently observed, at stages from twelve to fifteen days after inoculation, the ends of gall tracheides extending to the outer surface of the gall. The uninoculated control shoots treated as cuttings develop a small amount of parenchymatous callus (Text-fig. 3),

TEXT-FIG. 2. Series of radial longitudinal sections through lower ends of inoculated cuttings at intervals: A of 6 days, B of 12 days, C of 15 days, and D of 18 days after inoculation. Areas of active-tissue proliferation indicated by dotting. Regions of vascular bundles originally entered by bacteria indicated by darker lines near the ends of the former. × 9.



TEXT-FIG. 3. Series of radial longitudinal sections through lower ends of control, uninoculated cuttings at intervals: A of 6 days, B of 12 days, C of 18 days, and D of 21 days after planting. The vascular bundles are indicated, and the amount of callus proliferation is shown at the ends of these bundles. *c* = callus; *r* = roots. × 9.

whilst the cut surfaces at the upper ends of shoots exposed to air become dry and at most show a very few divisions of the cells adjoining the surface. a bulge of callus never being formed. Even in the control cuttings the amount of callus produced is never great and, so far as we have observed, is always purely parenchymatous in character, the proliferation slowing off after roots are developed behind the callus (Text-fig. 3, C). Text-fig. 3, D,

shows the maximum amount of callus we have observed. It may be mentioned that a greater callus development occurs in cuttings of *Chrysanthemum frutescens* prepared through the nodal region than through the internodal region. In all our experiments the inoculations were made at an internode. Roots do not develop behind the upper cut surfaces of aerial shoots except as rudiments in the late stages of gall development on the inoculated shoots. In such cases the root rudiments arise on the swollen region of the otherwise normal stem immediately below the gall. The region referred to is seen in Pl. V, Fig. 2, although roots have not yet arisen.

When the cut surfaces, either of the upper ends of shoots in air or of the lower ends of cuttings in the soil, are inoculated galls arise and grow rapidly. The growth is usually somewhat more rapid in the case of galls in the air than of those in the soil. The general appearance of such artificially produced galls on aerial shoots is shown in Pl. V, Fig. 1.

The development of such galls from the time of infection will now be dealt with and the changes observed contrasted with those seen in the controls.

Three days after wounding, the shoot (whether control or inoculated) shows marked alterations in the vicinity of the cut surface. The cells actually cut through are dead, and the walls of these, as well as the walls of all the tissues to a depth of two or three cells below the surface, are altered in properties and substance. The walls are much more readily stained by Sudan III, Scharlach R., methylene blue, and haematoxylin than are those of the normal corresponding tissues or than the walls of similar cells immediately after cutting across the shoot. These altered cell-walls, unlike those of the normal parenchyma, are not swollen and dissolved by strong sulphuric acid, but are merely stained brown with this reagent. The changes are doubtless necrosis changes similar to those previously described by investigators of wound reactions. The changes referred to are also shown by the walls of the sieve-tubes, pericycle fibres, and vessels, in these cases extending to a greater depth than in the parenchyma tissues. These cell-wall changes occur whether the wounded surface is in contact with soil or with air, irrespective of polarity, and of whether the surface is inoculated or not. In all cases, however, the maximum effect of the alterations described is attained in three days.

For the purposes of detailed description of the development of the gall, attention will now be confined to the galls arising on the upper ends of inoculated shoots in air. In the inoculated shoots it is evident, even after three days, that the bacteria have entered the open ends of the vessels, of the sieve-tubes, of the young pericycle fibres, and to some extent also the intercellular spaces of the cortex. The walls of the cells, with which the bacteria come into contact, show similar changes to those described above.

Sometimes after three days, but always at six days after inoculation, the abnormal cell-divisions, leading to gall production, have commenced. The cells, particularly of the parenchyma, adjoining the regions of the vascular bundle and the cortex entered by the bacteria, show enlarged nuclei, and themselves either merely enlarge or more frequently become subdivided into smaller cells which maintain a meristematic character.

After nine days the influence of the presence of the organisms has extended laterally from the vicinity of the vascular bundles, both into the cortex and into the pith, and by this time the consequent increase in diameter of the end of the shoot is becoming evident (Text-fig. 1, B). Pl. V, Fig. 7, shows a portion of the upper end of such a shoot after nine days; the dark patches seen indicate the position of bacteria on the inoculated surface, in the intercellular spaces adjoining this, and also in the vessels and in the tissues of the phloem and pericycle.

Pl. V, Fig. 9, which is a portion of a similar shoot fifteen days after inoculation, shows how the passage of the bacteria into the intercellular spaces of the cortex, some distance from the surface, may result in centres of disturbance around which gall-tissue develops by the subdivision of cells. In the Chrysanthemum, however, so far as we have observed in these inoculations, the distance the bacteria extend either along the vessels or intercellular spaces is never more than about 2 mm., whilst in *Nicotiana*, as will be shown below, the bacteria may extend for a distance of some inches.

At the age of the galls shown in Pl. V, Figs. 8 and 9, while a proportion of the proliferating cells continue to be meristematic, others lose their contents and become transformed into tracheide-like cells, with characteristic reticulate or pitted thickening which later becomes lignified. In the pith these gall-tracheides (Pl. V, Fig. 8) extend at right angles to the bundle, and appear progressively from the bundle towards the centre of the pith, being formed from the products of division of pith-cells.

We must regard the effects so produced in the pith as resulting from influences diffusing out laterally from the vascular bundles, for when vertical needle-prick inoculations into the pith of cut shoots similar in age to those used for the other inoculations were made, beyond a slight subdivision of cells surrounding the needle track, no marked production of gall-tissue, including tracheides, was observed.

Later stages in the development of the gall are seen in Pl. V, Figs. 2 and 3, and Text-fig. 1, C, D, and E. The whole structure of galls in these stages is remarkably similar to the callus masses which develop on cut shoots of woody twigs (such as *Populus*) when kept in moist air. The gall-tissue extends completely across the pith, and the vascular bundles and cortex on either side are considerably widened out by the gall, much of which forms irregular woody tissues with intervening parenchyma. The galls are now more or less hemispherical in shape; there is a distinct zone of meristematic

gall-cells arching over the greatly modified end of the stem; and parenchymatous cells are developed externally to this active tumour-tissue. As the gall increases in size, the outermost layers of cells of this cortical region are continually torn apart by the expansion, the layer of dead cells on the exterior becoming very marked in the older galls (Pl. V, Fig. 3, c). There is, however, no production of cork on the exterior of the galls, and, as will be shown below, vast numbers of *Bacterium tumefaciens* are found upon the layers of dead cells on the exterior. The increase in size of the gall, in the later stages at least, seems to result from the presence of the causal bacteria in preponderant numbers in this position.

Apart from the direct effect of the presence of the organisms in leading to the production of tumour-tissue there is also, as Smith's figures show, an extension backwards of the influence some distance from the actual gall. This effect is often manifested in a widening of the vascular ring by more than the ordinary cambial activity in the portion of the stem below the gall as in Pl. V, Fig. 2. A similar effect frequently may be seen in the petiole or midrib beneath a gall borne on a leaf. While dealing with our inoculations of shoots of Chrysanthemum, it may be mentioned that on several occasions we have inoculated axillary shoots growing from positions very near to galls produced by earlier inoculations. We have invariably obtained galls on such shoots, showing that there is no immunity acquired by plants against *B. tumefaciens* following an earlier infection by the crown-gall organism, as has been suggested (Pl. V, Fig. 1, c).

Galls of the general type described above were repeatedly produced by inoculating the cut surfaces of the shoots of healthy plants, and these galls resembled, in every particular, the naturally occurring galls on plants grown in the nurseries. A general distinction must be drawn between the galls described above, whether naturally occurring or experimentally produced, and the secondary galls and tumour-strands to be described below. The former type of gall has a rough exterior, and develops quite differently from the latter type, which has a smooth exterior. The secondary galls and tumour-strands, described by Smith and Peklo and produced by us, are always the result of artificial inoculations and have only been met with in experiment.

The evidence which we have obtained regarding the position of the bacteria in the large galls with rough exterior on the shoots of *Chrysanthemum frutescens* will now be dealt with.

Distribution of *B. tumefaciens* in the Rough Galls on *Chrysanthemum frutescens*.

The difficulties experienced by Walkden (35) and others in isolating *B. tumefaciens* from the interior of naturally occurring galls, after sterilizing

the exterior with mercuric chloride, have been referred to by Walkden in his note on the isolation of the organism. He has pointed out that the isolation is much more readily accomplished if the external surface of the gall is not first sterilized. In isolating *B. tumefaciens* he also found that more numerous colonies of this organism are obtained when portions of the exterior of the gall are used than if the inoculum be taken from the interior of the gall after removing the surface layer. Further, when sections of living galls on aerial shoots of any age from ten days to two months or older are mounted fresh in water, we have found that the external surface of the gall invariably shows a mucilaginous film from which very large numbers of bacteria of the form, size, and general appearance of *B. tumefaciens* can be observed diffusing into the mounting water.

Like Smith and other investigators, we have entirely failed to demonstrate the bacteria, by staining, within the cells of the gall, nor have we obtained any evidence that the bacteria enter the living cells of the host plant. The individuals of *B. tumefaciens* present in the film of mucilaginous material on the exterior of the gall are, on the other hand, readily demonstrated by the ordinary staining methods. In the early stages of the development of galls, after the inoculation of the cut surfaces of Chrysanthemum shoots, it is possible also to demonstrate the bacteria by staining some little distance (for about 2 mm.) along the vessels, and also occasionally in the intercellular spaces of the cortex (e. g. in Pl. V, Fig. 9, at *i.s.*).

The presence of the bacteria in these situations in the early stages of gall-formation is, in part at least, responsible for the shape of the gall which results from the localization of the disturbing influence in definite regions of the stem, i. e. in the vascular bundles and occasionally in *loci* in the cortex. It must be pointed out, however, that in the Chrysanthemum the organisms are not found at any considerable distance from the surface, and soon the form of the growing gall becomes such that the majority of the organisms producing it are localized on its surface. The further evidence for this conclusion which is indicated by the facts outlined above may now be given.

It was repeatedly found that merely dipping the unbroken gall into mercuric chloride solution (1 in 1,000) for ten seconds, prior to breaking it up for making platings, was sufficient to reduce the numbers of *B. tumefaciens* found from two hundred per plate to one. This result was obtained no matter how thoroughly the gall was washed after the treatment with mercuric chloride. The suspicion therefore arose that the active organisms were situated mainly on the outer surface of the gall. To test this the following experiment was carried out :

A gall was taken direct from the plant growing under the usual greenhouse conditions, without any attempt to prevent contamination, and dropped into a tube containing 10 c.c. of sterile water for ten minutes.

The gall was then washed in running water for about four hours (thus attempting to wash off as many organisms as possible from the surface), then placed in a second tube of sterile water for ten minutes; finally the gall was cut into small pieces with a sterile scalpel, and dropped into a further 10 c.c. of water for ten minutes. Platings were then made, using for each plate a loopful of the water from one of the three tubes. The experiment was carried out with a number of actively growing galls from five to eight weeks old, and the results of some such tests are summarized in Table I.

TABLE I.

Platings made to test the number of Bacterium tumefaciens on the exterior and interior of galls, and also the possibility of washing galls free from B. tumefaciens.

		A. *Gall placed for 10 min. in 10 c.c. sterile water.*			B. *Same gall, after washing in running water for about 4 hours, placed in 10 c.c. sterile water.*		C. *Same gall cut into small pieces and placed in 10 c.c. sterile water.*	
Age of gall.	*No. of Pl.*	*Total bacteria.*	*Bact. tume-faciens.*	%	*Total bacteria.*	*Bact. tume-faciens.*	*Total bacteria.*	*Bact. tume-faciens*
Six weeks	1.	178	54	30	12	1	9	5
	2.	333	122	37	13	3	12	3
	3.	192	62	32	15	6	3	0
	4.	212	89	42			15	8
	5.	164	51	32				
	6.	208	82	39				
Five weeks	1.	167	164	98	0	0	3	2
	2.	76	73	97	1	0	4	1
	3.	145	142	98	1	1	6	2
	4.	152	150	98	2	0	6	2
	5.	68	64	94	1	1	3	2
	6.	218	212	98	0	0	0	0
Eight weeks	1.	180	155	86	3	0	19	13
	2.	216	189	88	7	2	3	2
	3.	105	90	85	5	1	7	4
Six weeks	1.	305	159	52	5	1	14	2
	2.	125	47	37	2	0	26	6
	3.	207	154	54				
	4.	279	145	52	6	2	10	0
	5.	147	74	50				

It will be seen from Section A of Table I that there are enormous numbers of bacteria present on the external surfaces of the galls, and of these bacteria a large percentage is always *B. tumefaciens*. Section B of Table I shows that it is possible to remove most of the organisms by washing, though *B. tumefaciens* as well as other bacteria remain in small numbers. Section C of the table shows the small increase in the total bacteria found when the galls are cut into pieces, but there appears no justification from the figures to assume that *B. tumefaciens* is present in greater proportions than on the exterior of the unwashed gall. The num-

bers and also the diversity of the organisms obtained from the broken galls are sufficiently accounted for by the former having lodged in crevices of the rough surface of the gall, or as the residue of the bacteria that originally penetrated some little distance below the surface of the cut shoot at the time of inoculation.

The aerobic character of *B. tumefaciens* may account for the undoubted fact that the bacteria which are introduced to the interior either of vessels or of intercellular spaces of *Chrysanthemum frutescens*, which has relatively small intercellular spaces, do not multiply to any extent nor grow progressively in the interior.[1]

Experiments, using plating methods similar to those described above, have demonstrated with certainty that, for several weeks after inoculation, there is a progressive increase in the numbers of *B. tumefaciens* present on galls left growing on the plants.

The plating experiments thus show conclusively that, in the actively growing galls with rough surface on *Chrysanthemum frutescens*, there are enormous numbers of bacteria situated on the exterior of the gall, that a high percentage of these is *B. tumefaciens*, and that it is reasonable to assume that the presence of these progressively increasing numbers of *B. tumefaciens* on the exterior provides the progressive stimulus which leads to the continued growth of the gall.

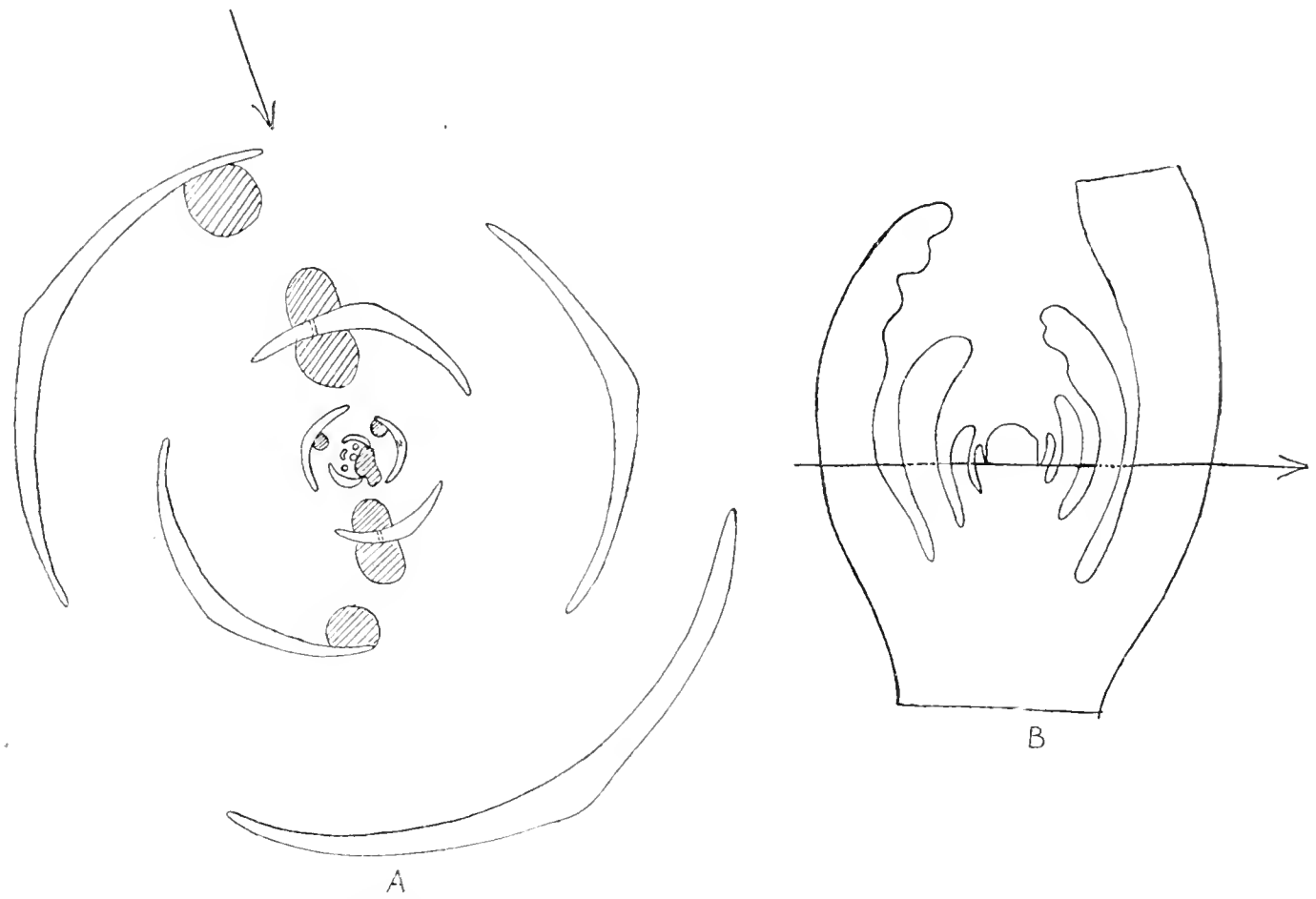
The facts regarding the distribution of the bacteria had obvious bearings upon the origin of the so-called secondary tumours and tumour-strands which have been so fully studied by Smith. These aspects of crown gall were therefore reinvestigated, and may now be considered.

Secondary Tumours and Tumour-strands.

As has been stated in the introduction to this paper, we have succeeded in producing on *Chrysanthemum frutescens* and on *Nicotiana affinis* galls similar in all respects and similarly distributed to the secondary galls figured by Smith. It has not, however, been found possible, as might have been assumed from some of Smith's figures, to obtain secondary galls in *Chrysanthemum frutescens* at a distance from the primary inoculation by inoculating the shoot at some distance from the growing-point. With this plant we have obtained successful results only by the inoculation of the very young tissues in the vicinity of the meristematic apex. This led us to test a view suggested to us some years ago by Professor W. H. Lang, viz. that the appearance of secondary galls and of tumour-strands was due to the subsequent development, growth, and extension of the meristematic tissues under the influence of the bacteria after inoculation.

[1] Below it will be seen that in the shoot of *Nicotiana* the bacteria behave differently in undoubtedly multiplying and progressing in the interior.

In order to obtain as much precision as possible, the inoculations were made into a large number of shoots of similar size, age, and general vigour by means of single horizontal or vertical needle-pricks. Pl. V, Fig. 10, shows the result of such a single horizontal needle-prick inoculation at the apex of a shoot of *Chrysanthemum frutescens* five weeks after the inoculation. Six galls of various sizes at the bases of leaves are seen in the photograph, and in addition the apex was so injured at one side that the terminal bud developed more feebly than usual, and the two nearest lateral buds pro-



TEXT-FIG. 4. A. Phyllotaxy diagram of the shoot of *C. frutescens* shown in Pl. V, Fig. 10. The galls on the various leaves are indicated by the shade areas, and the arrow shows the direction taken by the needle in making the inoculation at the apex.

B. Diagrammatic representation of the presumed path of the needle shown in relation to the longitudinal view of the apical region of the same shoot.

duced shoots. The six galls, which are now widely separated from one another by one or more internodes, have arisen as a result of the inoculation of wounded rudimentary leaves at the apex of the shoot, and have been separated by the subsequent growth of the shoot. *Bacterium tumefaciens* is present in large numbers on the rough exterior of such galls, and can be readily isolated from them. A very slight inoculation wound is sufficient to lead to the production of such galls, and the degree of roughness of the surface visible may in such cases be very slight. Text-fig. 4, A, is a phyllotaxy diagram of the shoot shown in Pl. V, Fig. 10, and it will be seen that the position of the galls on the various leaves is consistent with each leaf bearing a gall, having been wounded and inoculated by the single horizontal needle-prick. The arrows in Text-fig. 4, A and B, show the presumed path of the needle. A very large number of such inoculations was made, and

in most cases, but not in all, where separated galls appeared the phyllotaxy diagram indicated their connexion with the original needle-track.

In a number of other cases, however, both in longitudinal and horizontal inoculations of apices, smooth galls of a different appearance were obtained at a distance from the larger rough galls that were always directly associated with actual wounds. Such galls, which are similar to many of Smith's secondary tumours, are seen in Pl. V, Fig. 11 (*s.g.*), and Pl. VI, Figs. 12 (*s.g.* 1, 2, and 3) and 13 (*s.g.*). Galls of this type only will be regarded as true secondaries. We have never found *B. tumefaciens* on the exterior of such smooth galls either by direct observation or by cultural methods, and, like Smith, we have had considerable difficulty in isolating the organism from the interior, but in this we were successful in two cases. We have, however, in such secondary galls directly demonstrated, by staining, the position of the bacteria in the protoxylem vessels and in the intercellular spaces adjoining these, and further we have found continuity of the organisms from such galls to the primary gall, which arises where the inoculation wound is made. The internal structure of such secondary galls may now be dealt with, and the relations of the infecting organisms to them will be made clearer after secondary galls on *Nicotiana* have been described.

Pl. VI, Fig. 12, shows a portion of a shoot which was inoculated by a single longitudinal needle-prick at the apex ; the rough elongated gall on the side of the shoot is a primary gall (using Smith's terminology), and extending along the midrib of the leaf are three 'secondary' galls in linear series. Pl. VI, Fig. 14, is a longitudinal section of the middle secondary gall on the leaf shown in Pl. VI, Fig. 12, *s.g.* 2. The swelling is originating from within by the subdivision of cells of the parenchyma in a manner exactly similar to that seen in the galls on the ends of shoots dealt with in the first section of this paper. There is, in addition, a more striking subdivision and proliferation of tissues immediately adjoining the protoxylem (*px.*) of the vascular bundle, resulting in the slight general displacement of the tissues of the parenchyma, but there is no evidence of any considerable intrusive growth of tumour-tissue outwards. The elements of the protoxylem (*px.*) stain deeply in the manner characteristic when organisms have been found in them, and the appearances generally are consistent with an influence diffusing out from the protoxylem. Pl. VI, Fig. 15, shows a transverse section of the much older elongated secondary gall (*s.g.*) seen in the deformed leaf in Pl. VI, Fig. 13. The structure is very similar to that frequently figured by Smith for secondary galls on the leaf of Chrysanthemum. It shows the modification of one of the vascular bundles into a radial structure, which was regarded by Smith as evidence of the stem origin of this by the intrusive growth of tumour-tissue from the infected stem out to the leaf in which the secondary gall has arisen. As will be seen below, particularly clearly for *Nicotiana*, we have shown that such galls arise around definite centres of bacteria in the interior

of the structures in which they appear, and we have found no evidence of any invasive growth of tumour-tissue from a distance. In the case shown in Pl. VI, Figs. 13 and 15, we have direct as well as cultural evidence of the presence of the bacteria in the protoxylem region in the centre of the modified midrib, and we have traced the organisms back along the protoxylem to the primary gall by serial microtome sections. The facts thus briefly referred to for secondary galls on *Chrysanthemum frutescens* were made out only after a considerable body of our work had been completed since, as has been mentioned, like Smith, we experienced great difficulty in isolating the organisms from the secondary galls on the Chrysanthemum.

Parallel studies, however, which we carried out on *Nicotiana affinis* enabled us successfully to stain and thus directly demonstrate the bacteria in the interior of secondary galls on this plant, to isolate the organism in large quantities from the interior of such smooth galls, and to demonstrate conclusively the infective migration of the bacteria from the original point of infection for very considerable distances through the plant. These secondary galls on *Nicotiana* will, therefore, now be described.

Cut surfaces of the internodes of the young flowering shoots of *Nicotiana* were inoculated in a manner similar to that described for Chrysanthemum. As in the latter plant, in some cases, a large rough gall arose on the end of the inoculated shoot and, as before, the bacteria were found to be present in very great numbers on the rough outer surface of the gall. More frequently in *Nicotiana*, however, the primary gall arising on the end of the shoot is much smaller; in such cases it is invariably found that a number of smooth galls arise as a series of swellings which extend to a considerable distance below the inoculated surface. Pl. VI, Fig. 16, shows such a series of smooth secondary galls on *Nicotiana*, and it will be seen that these are identical in appearance with similar galls on *Nicotiana* figured by Smith (29, Fig. 24, *x*). Pl. VI, Fig. 16, is quite typical of a large number of inoculated shoots which we have obtained showing secondary galls. We successfully isolated *Bacterium tumefaciens* from the secondary gall (*s.g.* 1) most remote from the inoculated surface and we have also stained these bacteria *in situ* within the galls. Pl. VI, Fig. 17, shows a transverse section of the shoot seen in Pl. VI, Fig. 16, through the swelling (*s.g.* 2) immediately above (*s.g.* 1). In this case there are four centres of disturbance in the cortex giving rise to three secondary galls and a tumour-strand. Serial sections at different levels of the shoot seen in Pl. VI, Fig. 16, have demonstrated the longitudinal continuity, throughout the length of the stem shown, of the tumour-strand and secondary galls seen in the cross-section in Pl. VI, Fig. 17. Around the protoxylem of some of the bundles there are also small disturbances obviously resulting from the presence of the bacteria in these vessels. Pl. VI, Fig. 18, which is taken from the inner face of the vascular ring (at *px.*) of the section seen in Pl. VI, Fig. 17, shows one of these small tumour-strands. Pl. VI,

Fig. 19, shows tumour-strands in the pith of another similarly inoculated shoot of *Nicotiana*. Here the bacteria in intercellular cavities marked *i.s.* 1, *i.s.* 2, *i.s.* 3 are forming a centre from which the stimulating influence is radiating outwards. In these cases it is again clear that there is no true intrusive growth of tumour-tissue: the stimulus merely results in the subdivision of the pre-existing cells of the pith. This is seen even more clearly in Pl. VI, Fig. 20, which also shows the deeply-staining mass of *B. tumefaciens* in the intercellular space which forms the centre for the production of the gall. This figure also indicates that the invaded cavity may be slightly enlarged by the occasional necrosis of cells bounding it, but there are never, in our experience of crown gall, the marked necrosis cavities described for the Olive-knot disease due to *Bacterium savastoni*, Smith. This difference between the two diseases, however, would seem to be one of degree rather than of kind.

We have traced, in serial longitudinal sections, the passage of the bacteria along the very large intercellular spaces which are present in the stem of the *Nicotiana affinis*. Pl. VI, Fig. 21, shows a longitudinal section of a part of a shoot at some distance below the surface which was inoculated eighteen days previously. The dark centres (*i.s.*) in the cortex are clearly seen, with tumour-tissue arising by the subdivision of cells on either side of the bacterial strand. Pl. VI, Fig. 22, shows, more highly magnified, the zoogloeal strand of *B. tumefaciens* seen advancing through a large, longitudinal, intercellular space in the pith of *Nicotiana*. Such zoogloeal strands of the bacteria, which are readily demonstrated by staining, are invariably found in sections of young secondary tumours on *Nicotiana affinis*, and, since detecting them in this plant, we have frequently observed them also in the Chrysanthemum. In the latter plant, however, the intercellular spaces are much smaller and less abundant, and we have not observed the zoogloea to penetrate to a depth greater than two millimetres from the cut surface of the shoot.

In addition to the definite zoogloea of *B. tumefaciens* which have thus been repeatedly observed in the intercellular spaces of both plants studied, it has been mentioned that the bacteria also enter the protoxylem elements of both infected shoots and leaves, and, travelling in these vessels, and also passively carried in them by growth extension, serve as centres for the development of secondary tumours. The secondary galls which we have described and figured (Pls. V and VI, Figs. 11, 12, 13) for *Chrysanthemum frutescens* are of this type. They owe their origin and characteristics to the fact that they were derived by the inoculation of the basal region of leaf-rudiments at such an age that the first differentiation of protoxylem of the leaf-trace had taken place, but the basal growth of the leaf-rudiment had not ceased. Pl. V, Fig. 4, shows a longitudinal section of an apex of *Chrysanthemum frutescens*, and the leaf (*l.*) shows the stage in development at the time of inoculation for results such as are seen in Pl. VI, Fig. 12, to be produced.

At this stage there is a single protoxylem element extending down from the base of the leaf into the shoot, towards its rudimentary vascular cylinder. Secondary tumours arise when the needle-prick introduces the bacteria into this protoxylem element either in the leaf-base or in the stem. If the rudimentary leaves infected are somewhat farther advanced, the needle penetrates the region at the extreme base of the leaf where growth has almost ceased, the organisms are not carried forward in the stretching protoxylem, and, instead of an elongated series of galls, a single large gall arises at the base of each leaf wounded and inoculated. Pl. V, Fig. 10, illustrates this effect. If, on the other hand, the leaf-rudiment is very young, the gall which arises involves the whole rudiment and thus obliterates the leaf. In other cases, where the protoxylem is entered by the bacteria, the young leaf may be so severely wounded that the development of secondary galls is accompanied by a partial arrest of the leaf as in Pl. VI, Fig. 13. The facts thus briefly summarized for the inoculations of leaf-rudiments of *Chrysanthemum frutescens* were obtained by the examination of serial sections of a large number of apices at differing periods varying from three to fifteen days after inoculation by transverse or longitudinal needle-pricks. The observations were controlled in the case of each apex examined by serial sections of a corresponding wounded but uninoculated apex of the same age. The results have shown that the secondary galls on the leaves of *Chrysanthemum frutescens* are largely accounted for by the bacteria inoculated into the young protoxylem, being carried by the stretching growth of this to some distance from the point of inoculation ; our results on tobacco have shown that an actual migration of the bacteria is also possible in the protoxylem. The fact that the bacteria are restricted to the protoxylem in the secondary galls on the Chrysanthemum and that, though staining clearly shows them to be present, they are never so abundant as in the galls on the tobacco, fits with the undoubted difficulty which both Smith and ourselves have had in isolating the organism from these secondary galls on the Chrysanthemum. It would seem that there is, in these cases, little actual multiplication of the organisms after inoculation, while the undoubted very active migration and growth of zoogloeal strands in the shoot of tobacco clearly indicates the reverse. This may be correlated with the much greater ventilation of the tobacco shoot, its larger intercellular spaces affording more favourable conditions for the active multiplication of the highly aerobic *Bacterium tumefaciens*.

It may be mentioned here that measurements of the stem of the tobacco after inoculating the cut surface show that there is very little elongation of the stem such as occurs subsequent to the wounding of an apex either of Chrysanthemum or of the tobacco. In the secondary galls which arise below the inoculated stem-surfaces in the tobacco there is, therefore, no question of growth elongation playing any considerable part in the form and arrangement of the galls.

Secondary galls and tumour-strands essentially similar in all respects to those we have described and figured for *Chrysanthemum frutescens* have, however, also been obtained on the leaves of *Nicotiana affinis*. Numerous needle-prick inoculations of the apices of young flowering shoots of this species of tobacco were made and results similar to those described by Smith have been obtained; but wherever the galls have a rough exterior the organisms are present in abundance on this surface, and when completely smooth galls occur they, like the secondary galls we have described above, have the organisms present in the intercellular spaces and vessels within. For such inoculations, both the enormous stretching by growth of the inoculated meristem and the actual migration and multiplication of *B. tumefaciens* within the vessels and cavities of the stem must be taken into account in explaining the resulting secondary galls and tumour-strands. In the tobacco, as Smith has shown, these frequently burst out to the exterior either from the pith or cortex, and the rough surface, which they then acquire, is richly populated with *B. tumefaciens*.

We have obtained no evidence of the migration of strands of tumour-tissue to any distance at all comparable with that postulated by Smith, but we have shown earlier in this paper that the effect of the bacterial stimulus proceeding from definite centres invariably results in the subdivision and enlargement of cells. There is no intrusive growth of these dividing cells in Smith's sense, although there is often a slight displacement of the dividing cells due to the inequality of pressures developed by the abnormal cell-divisions and enlargements. Pl, VI, Fig. 23, *d*, from the edge of one of the secondary tumours seen in Pl. VI, Figs. 16 and 17, shows one of the most extreme cases of cell-displacement which we observed. Even in this case, however, at *e* it is obvious that the tumour-cells are arising by subdivision of ordinary cortical cells. On the other hand, as we have already pointed out, Pl. VI, Figs. 19, 20, 21, make it clear that the tumour-strands are in no real sense intrusive growths of tissue, but the result of intruding masses of bacteria forming centres of disturbance.

Discussion of Results.

The results of our work do not require extended discussion, but the more general bearings of the new facts we have established must be briefly considered. The study of the mode of occurrence and of the distribution of the bacteria in the galls is a necessary preliminary to the understanding of the manner of growth and development of the latter. Much of the work described in this paper is therefore concerned with obtaining exact knowledge regarding the position of the bacteria.

The demonstration of the bacteria, in large quantities, on the exterior of naturally occurring galls, and also upon those produced in the immediate neighbourhood of inoculation wounds, has explained the earlier difficulties of

ourselves and others in isolating *Bacterium tumefaciens* from the interior of sterilized galls. This distribution of the bacteria also throws light on the form of these galls and their general resemblance to ordinary growths of callus such as occur on the twigs of trees. The organisms present over the hemispherical outer surface of the gall provide the continued stimulus which accounts for the fairly uniform meristematic activity in a region of the gall comparatively close to the surface. This continuity of stimulus explains the differences in the degree of reaction obtained in the galls and in ordinary growths of callus.

The presence of *B. tumefaciens* on the exterior of the galls also explains the extreme ease with which the soil in which diseased plants are growing becomes highly infectious, since the organisms must be washed into the soil whenever water falls on the plants.

The bacteria often associated as zoogloea-like strands have, on the other hand, been traced for shorter or longer distances from the point of inoculation. The course of this bacterial extension is by way of intercellular spaces and protoxylem. The recognition that the formation of tumour-strands and secondary galls follows along the track of invading bacteria leads to a reconsideration of some of the more specific comparisons that have been made between crown gall and malignant tumours.

Smith has held that the intrusive growth of tumour-tissue, which he has described for tumour-strands, is directly comparable to the migratory growth of the diseased tissues in certain forms of malignant disease. We have been unable to find any real intrusive growth in crown gall, but the demonstration of the bacteria advancing in the intercellular spaces and protoxylem fully explains the development of tumour-tissue in the neighbourhood of their path, and also the existence of strands of such tissue connecting secondary with primary galls and the threading of successive secondary galls in a linear series. In our experience the careful examination, by staining methods, of serial microtome sections usually reveals the relatively close proximity of the causal bacteria to the proliferating tissues. There is, therefore, no necessity to assume the stimulation of cells at any considerable distance from the active bacteria. It is unnecessary also to adopt Jensen's hypothesis that stimulated cells removed from the bacterial influence behave in a parasitic manner, as, according to him, do the cancer cells in the animal disease.

It was mentioned in the introduction to this paper that Smith has utilized the appearance of a radial stem-like structure in secondary galls on the leaves of Chrysanthemum for pressing even more closely a further comparison with cancer. He suggests that the stem-like structure occurs in the leaf because the tissues originally inoculated were those of the stem, and the comparison is directly made with those secondary malignant tumours which reproduce in distant organs the tissues of the organ originally diseased.

This idea was originally suggested in relation to the conception that the secondary tumour in the leaf originated from an 'invading destructive growth' (Smith, 23, p. 16) derived from the stem. We have shown that the radial stem-like growth referred to arises by the active division of leaf-tissues on the adaxial side of the vascular bundles in relation to bacteria situated in the protoxylem. There can be no question of simply explaining the transformation of leaf-tissues into those of a 'pseudo-stem' by the fact that the original inoculation was made into the stem.

The appearances in question are in some respects similar to those described by Winkler (36) for the petiolar bundles on *Torenia* when adventitious buds arise on the lamina of the leaf. Here the remains of the desmogen of the leaf-traces give rise to an almost radial bundle by secondary thickening. In crown gall an exactly similar stimulation to this latter almost invariably occurs in the petiole or midrib of the leaf of *Chrysanthemum frutescens* below the position of the primary gall on the leaf. This, like the anatomical change in the petiolar bundle of *Torenia*, seems to be a correlation effect. The facts regarding such structural changes, while of great interest in themselves, do not, in our opinion, support any detailed comparison of the radial structure of secondary galls with the histological results of the active transference of tissues by infiltration or metastasis in malignant tumours.

The origin of leafy or bud-like growths on crown galls apart from pre-formed buds does not, in our view, afford any support to comparisons with malignant tumours. The new growths in plants can only in a very general way be regarded as equivalent to animal teratomas. Where such structures appear in crown gall they are comparable with the adventitious buds or roots that occur in ordinary cases of woody callus, or with the buds that arise very commonly on the cut surfaces of internodes of shoots or on mutilated leaves of *Solanum lycopersicum*, apart altogether from any infection by organisms. Smith's later work has afforded new and excellent examples of this phenomenon. This aspect of the question, however, lies outside the scope of this paper.

The most striking growths of crown gall are always obtained when regions of the plant which are capable of considerable further growth are inoculated. When, as in most of our experiments, the inoculations are made into immature organs the subsequent development of these in the control plant has to be taken into account in dealing with the causation of the effects obtained in the inoculated plant. The changes followed in the normal development of organs behind a growing-point are usually in themselves difficult to understand, but this only makes it the more necessary to consider the effects of the bacterial stimulation in the light of the potentiality for development of these organs. We have found, for example, in the present work that the very different effects produced by the inoculation of

internodes of the stem of *Nicotiana affinis* and of the apex of *Chrysanthemum frutescens* are in greater or less part due to the different potentialities of development and growth in the two cases. In the internodes of tobacco practically no growth in length took place after preparing the surfaces for inoculation, while in the Chrysanthemum the immature leaves and stem of the inoculated bud continued their growth and development, although this took place under the influence of modifications introduced by the presence of the bacteria. We have thus throughout our work found it necessary and instructive to consider the resulting growths in the light of the subsequent development of the host plant after inoculation, and it has been seen that this development plays an important part in determining the form and distribution of the structures which arise. This continued development of plants, as contrasted with animals, must be borne in mind when comparisons such as we have been considering are instituted, and our results show that for this reason alone superficially similar structures, in the two cases, may have quite different methods of origin.

Whilst thus compelled by the facts we have described for crown gall to dissent from the close comparisons with cancer referred to above, we would, nevertheless, agree that there are features in the plant disease which suggest more general comparisons with malignant disease. In both cases, for example, cells and tissues are stimulated to active atypical proliferation, and it is possible that a real insight into the nature of the changes taking place under the influence of *Bacterium tumefaciens* might throw light on the changes taking place as a result of unknown causes in the cells of the animal tumours. We have as yet few facts relating to such features of crown gall, but we regard the cell-wall changes referred to in the body of our paper, and certain precipitation effects we have observed in the gall-tissues, as indicating the direction of further study in this connexion. Similarly we have made some preliminary steps in the investigation of the metabolic changes and the respiratory activity of the plant cells under the influence of *B. tumefaciens*, and we are not without hope that future investigation of the sequence of changes resulting from the activity of this organism upon the tissues of higher plants will throw light upon little-understood problems of tissue differentiation and of development in the healthy plant.

Summary.

1. The development of the galls produced by the inoculation of cut surfaces of *Chrysanthemum frutescens* with *B. tumefaciens* has been traced from the earliest stages.

The effect of the bacterial stimulus is to produce a growth very similar in form, structure, and general appearance to callus growths on woody shoots arising as a result of wounding. At first the bacteria are located on

the wounded surface, and to some extent also in the vessels, and in intercellular spaces of the cortex near to this. The later development of the gall is, for the most part, due to the active presence and multiplication of *B. tumefaciens* on the rough external surface of the gall.

2. Most of the work of Erwin F. Smith regarding the production of secondary tumours has been repeated, but additional results have been obtained which show that the facts bear very different interpretations from those adopted by Smith. Both in *Chrysanthemum frutescens* and in *Nicotiana affinis* we have definitely demonstrated, by staining, zoogloeal strands of *B. tumefaciens* intruding along intercellular spaces and protoxylem vessels forming centres for pathological disturbance and gall-production along the tract. This migration of the causal bacteria in experimentally inoculated shoots of *Nicotiana affinis*, in which little growth extension was possible, fully accounted for the secondary galls obtained. On *Chrysanthemum frutescens* secondary galls have only been produced by inoculating the meristematic tissues near the apices of shoots. In these cases, and also in the galls resulting from the inoculation of the growing-point of *Nicotiana affinis*, the part played by the growth and extension of the wounded and inoculated tissues has been shown to be a very important additional factor in determining the form and distribution of the galls which arise.

3. The primary and secondary galls and tumour-strands arise by a subdivision and subsequent proliferation of pre-existing cells of the host plant in the presence of the bacterial stimulus. There is no invasive growth of tumour-tissue over any considerable distance. The intrusive growth of the bacteria in the intercellular spaces and in protoxylem vessels, together with growth extension of inoculated tissues, fully account for the strands of tumour-tissue connecting the secondary galls with those arising at the points of inoculation.

4. It is held that the results obtained regarding the distribution of the bacteria in the galls invalidate most of the close comparisons which have previously been made between crown gall and malignant tumours.

Barker Cryptogamic Research Laboratory,
University of Manchester.

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EXPLANATION OF FIGURES IN PLATES V AND VI.

Illustrating Messrs. Robinson and Walkden's paper on a Critical Study of Crown Gall.

PLATE V.

Fig. 1. Typical crown galls produced on *Chrysanthemum frutescens* by inoculating cut surfaces of the stems with *B. tumefaciens*. *a* and *b* are galls four months old; *c* is a younger gall produced by inoculating an axillary shoot which arose near to the first gall (*a*). Natural size.

Fig. 2. Radial longitudinal section of a gall (four months old) on the cut surface of a stem. The callus-like growth is shown, and also the extension of the influence leading to an abnormal increase in diameter of the stem below the gall. × 5.

Fig. 3. Radial longitudinal section of a similar gall to that in Fig. 2, five and a half months old. *a*, woody gall-tissue; *b*, actively proliferating gall-tissue; *c*, layers of necrosed cells. × 1½.

Fig. 4. Median longitudinal section through the apex of a shoot of *Chrysanthemum frutescens*, showing leaf-rudiments and parts of young leaves of various ages. The leaf (*l.*) has just differentiated the first protoxylem element of the leaf-trace and is at the stage of development usual when inoculations result in secondary galls on the leaf similar to those seen in Fig. 12, *s.g.* 1, *s.g.* 2, *s.g.* 3. × 50.

Fig. 5. Portion of transverse section of young stem of *Chrysanthemum frutescens* at the level of the first distinctly visible internode below the growing-point of the shoot. × 75.

Fig. 6. Film of *Bacterium tumefaciens* stained with carbol fuchsin. × 1250.

Fig. 7. Radial longitudinal section of stem of *C. frutescens*, showing the cut surface nine days after inoculation. The dark staining of some areas and of cell-walls near the surface is due to bacteria in the protoxylem vessels (*px.*), pericycle fibres (*pf.*), and in some intercellular spaces (*i.s.*). × 75.

Fig. 8. Radial longitudinal section of stem similar to that seen in Fig. 7, fifteen days after inoculation. Shows the region of the pith adjoining the vascular bundle and the progression of the disturbance outwards from the bundles across the pith. Elongated cells (*tr.*) with tracheide-like thickenings are appearing. × 65.

Fig. 9. Radial longitudinal section of stem similar to Figs. 7 and 8, fifteen days after inoculation. Bacteria present in protoxylem (*px.*) and in a line of intercellular spaces (*i.s.*) in the cortex. This line of infection, which is the dark region immediately to the left of the large-celled cortical tissue in the photograph, forms a centre for pathological disturbance. × 50.

Fig. 10. Portion of a shoot of *Chrysanthemum frutescens* five weeks after the inoculation of the apex by a single transverse needle-prick. Six galls at the bases of leaves separated by one or more internodes are seen. The whole of the extension of the shoot above the lowest gall (*g.*) has taken place subsequent to inoculation. Natural size.

Fig. 11. Portion of shoot of *Chrysanthemum frutescens*, showing primary gall (*p.g.*) with rough exterior produced on the stem by needle-prick inoculation. At *s.g.* is a smooth secondary gall on the petiole of the leaf. Natural size.

PLATE VI.

Fig. 12. Portion of shoot of *Chrysanthemum frutescens* inoculated by a single longitudinal needle-prick at apex. Large primary gall (*p.g.*) with rough exterior on stem ; smooth secondary galls (*s.g.* 1, *s.g.* 2, and *s.g.* 3) extending along midrib of leaf. Natural size.

Fig. 13. Portion of shoot of *Chrysanthemum frutescens* inoculated by single transverse needle-prick at apex. Large primary gall (*p.g.*) on stem, and on deformed leaf above this an elongated secondary gall (*s.g.*). Natural size.

Fig. 14. Radial longitudinal section of the smooth secondary gall (*s.g.* 2) in Fig. 12. The tissues on the adaxial side of the protoxylem (*px.*) are subdividing, especially in the immediate vicinity of this. No marked intrusive growth. × 15.

Fig. 15. Transverse section of secondary gall (*s.g.*) on deformed leaf seen in Fig. 13. Tissues of midrib modified to give a radial structure. *Bacterium tumefaciens* was present in the protoxylem region at the centre of this modified midrib. × 15.

Fig. 16. Portion of stem of *Nicotiana affinis*, showing small primary gall (*p.g.*) at the upper end, produced by inoculation of the cut surface ; a linear series of smooth secondary galls is seen extending for some distance below the inoculated surface. *B. tumefaciens* isolated from *s.g.* 1. About five weeks old. Natural size.

Fig. 17. Transverse section of the stem in the region of the secondary gall (*s.g.* 2) seen in Fig. 16. Three incipient secondary galls (*s.g.* 1, *s.g.* 2, *s.g.* 3) and also a tumour strand (*t.s.*) are seen in the cortex. At *r.* a root-rudiment is visible. × 12.

Fig. 18. Portion of the xylem and pith of the section seen in Fig. 17. At *px.* the protoxylem elements are filled with bacteria, and the surrounding cells have begun to subdivide to give rise to a small tumour-strand. × 65.

Fig. 19. Transverse section of similarly inoculated shoot of *Nicotiana affinis* to that seen in Figs. 16 and 17. Three tumour-strands are present in the pith, each having arisen around a centre of bacteria situated in intercellular spaces (*i.s.* 1, *i.s.* 2, *i.s.* 3). × 65.

Fig. 20. Transverse section of similar tumour-strand to those shown in Fig. 19. *B. tumefaciens* present in the large intercellular space (*i.s.*) which forms the centre for the outward radiation of the disturbing influence leading to subdivision of cells. × 270.

Fig. 21. Radial longitudinal section through part of inoculated stem of *Nicotiana affinis* similar to that shown in Fig. 16. The deeply-stained intercellular spaces (*i.s.*) containing bacteria are shown in the cortex with tumour-tissue arising by subdivision of cells on either side of the bacterial strand. The cut surface was situated a considerable distance above the portion figured. × 50.

Fig. 22. Portion of the pith of *Nicotiana affinis* in longitudinal section. Zoogloea-like thread (*z.*) of *B. tumefaciens* advancing in the large intercellular space. × 550.

Fig. 23. Portion of the marginal region of one of the incipient secondary galls seen in Fig. 17. At *d* tumour-cells appear to be intruding between the normal cortical cells, but at *e* the tumour-cells are seen to be arising by subdivision of cortical cells. × 150.

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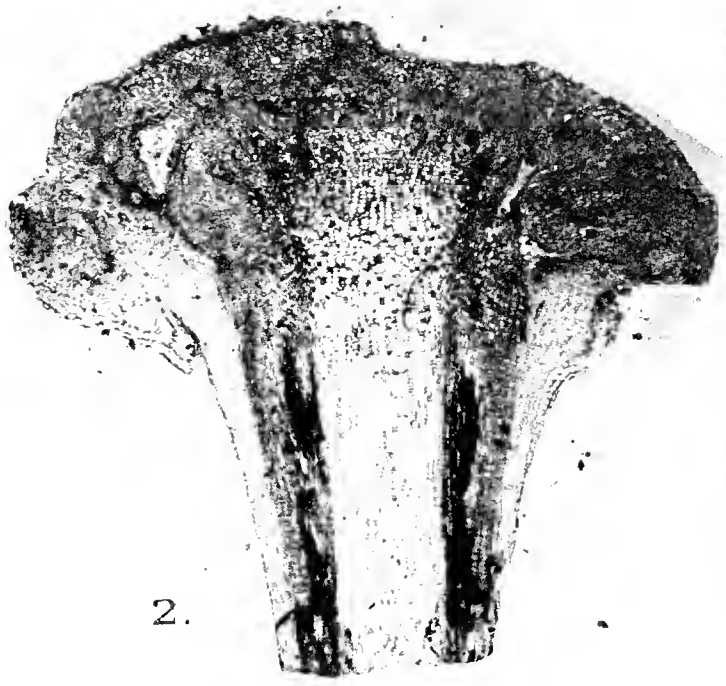

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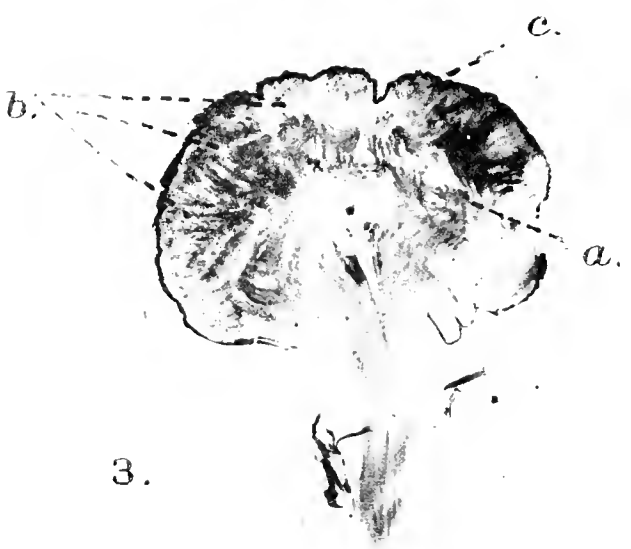

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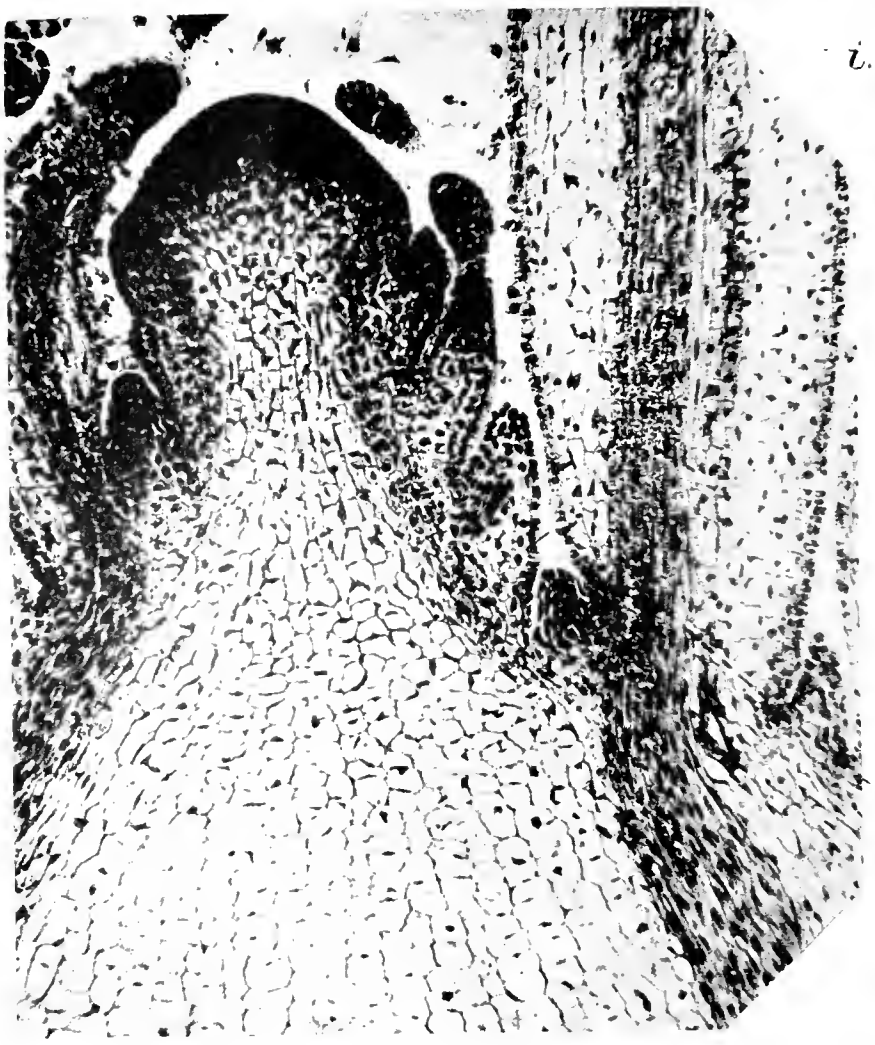

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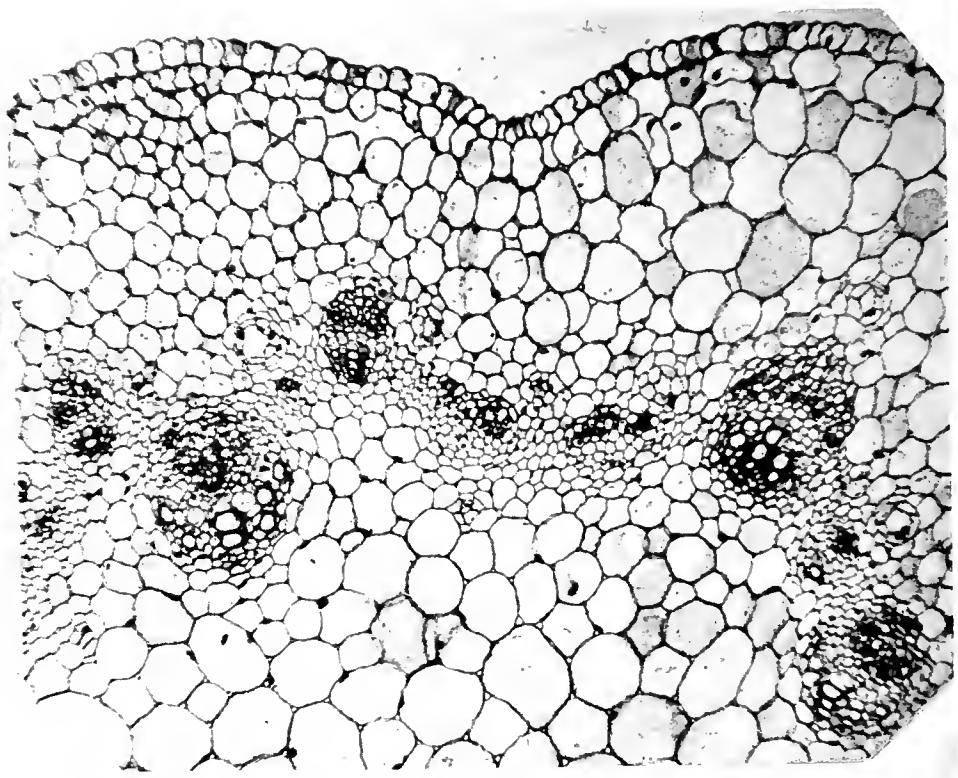

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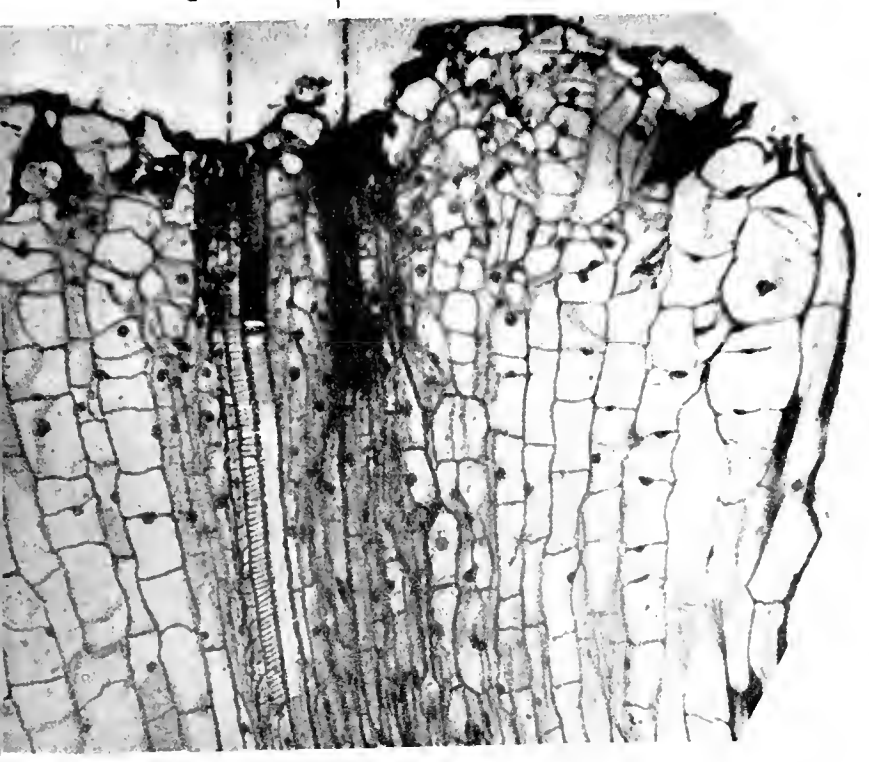

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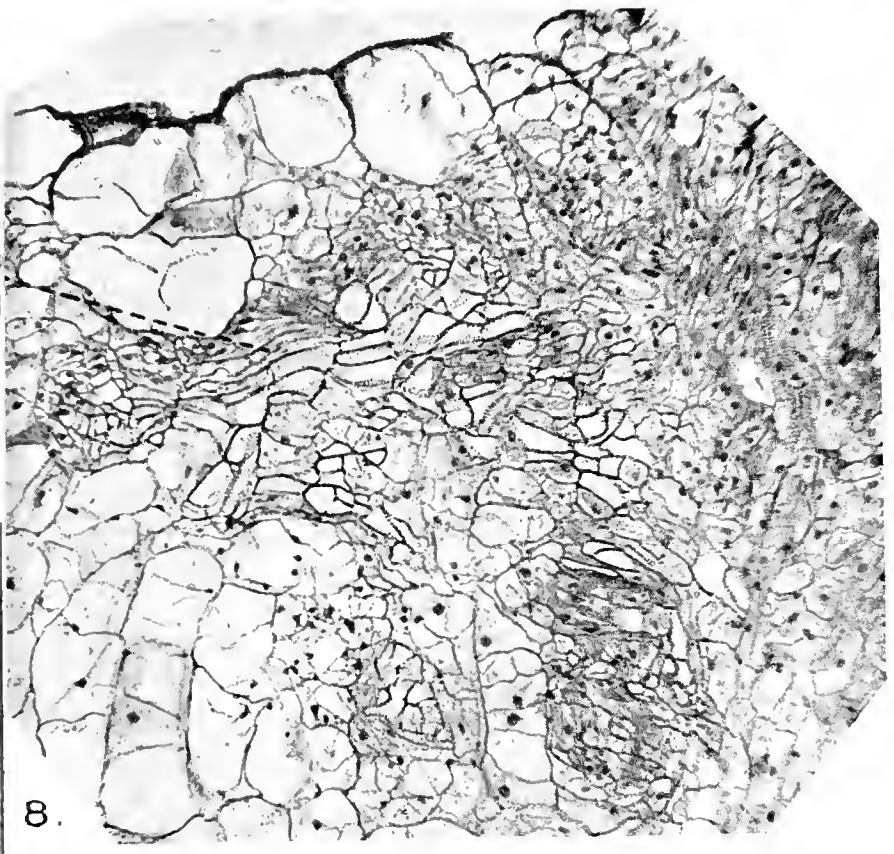

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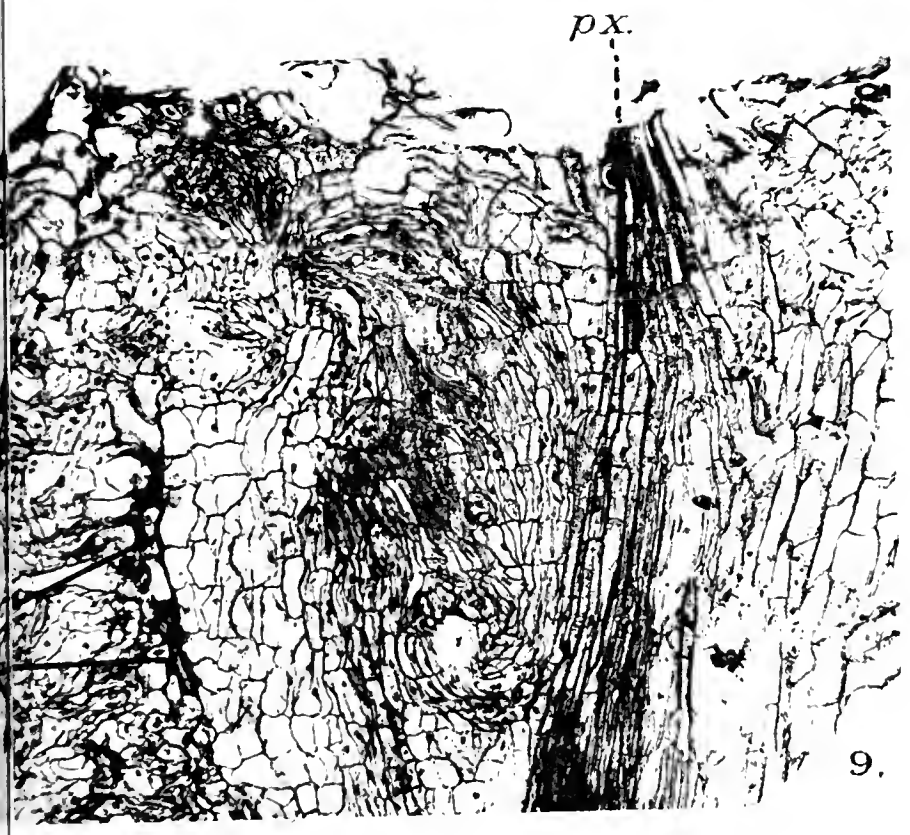

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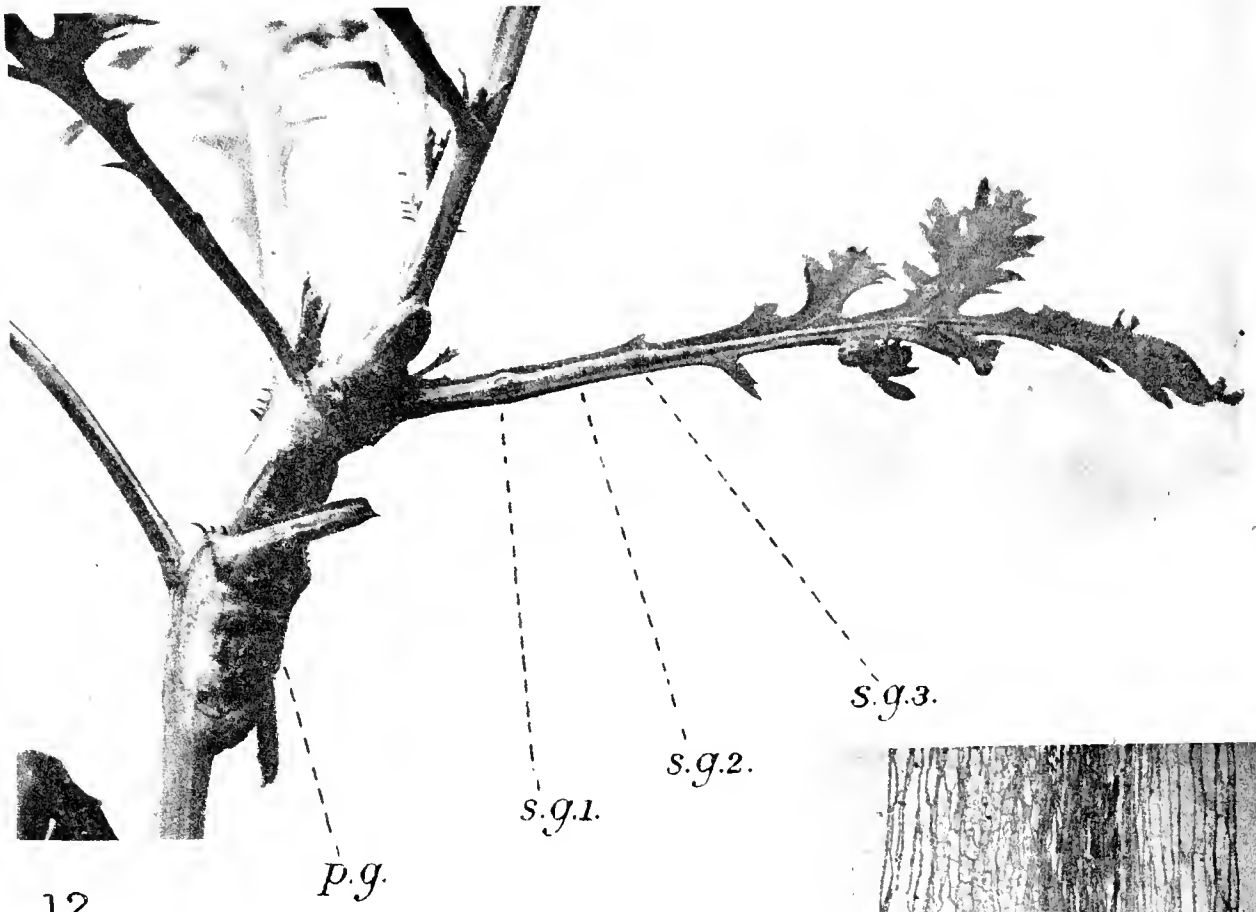

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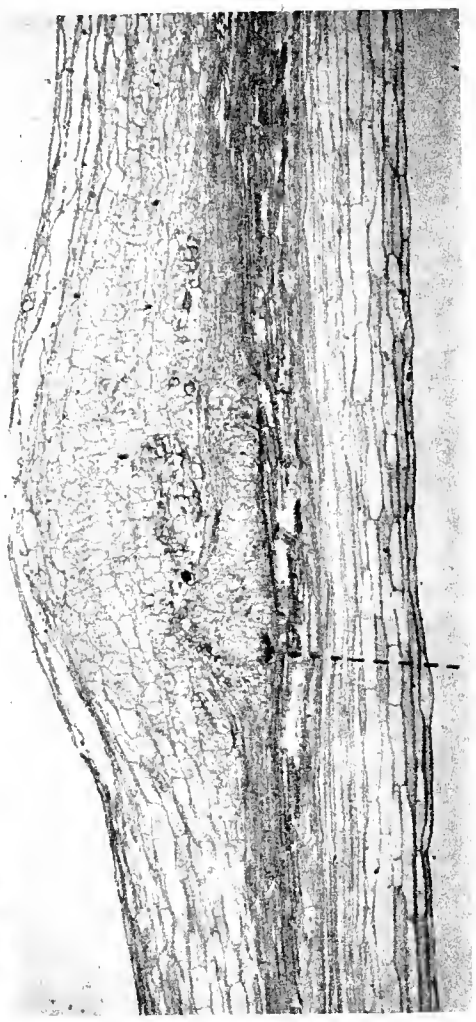

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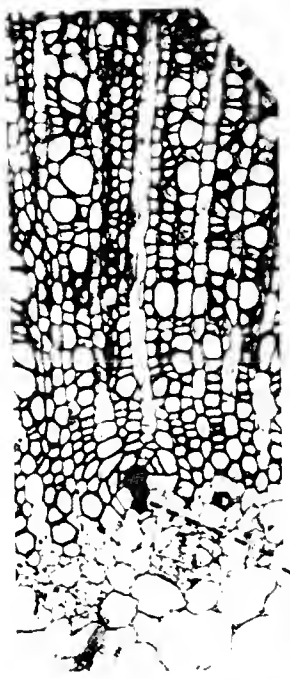

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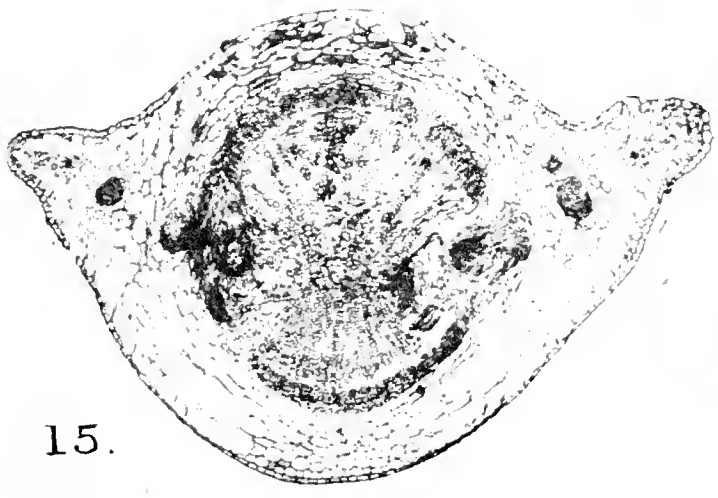

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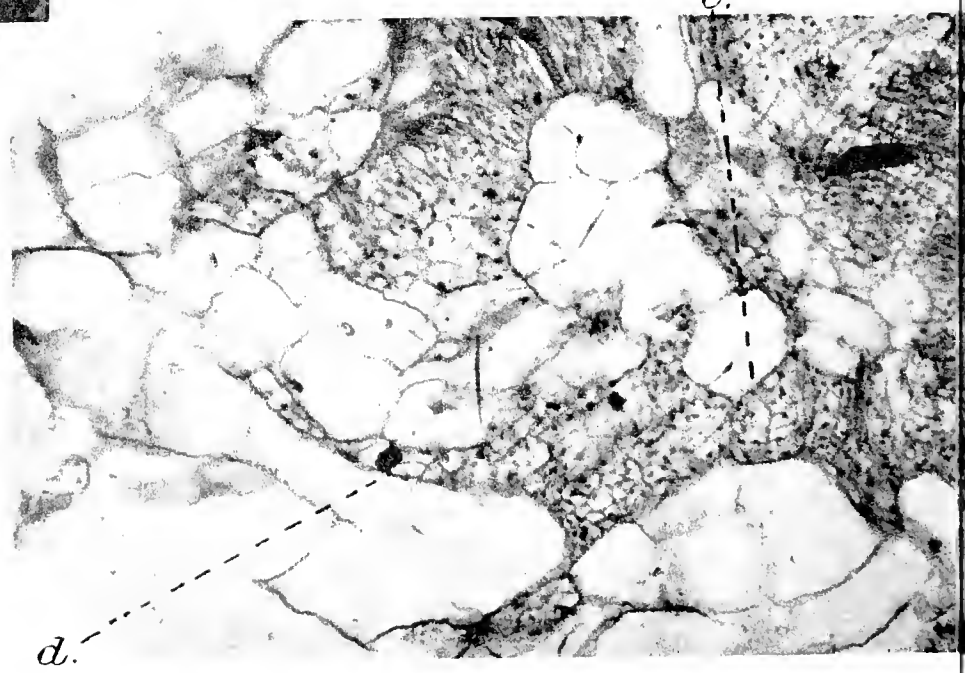

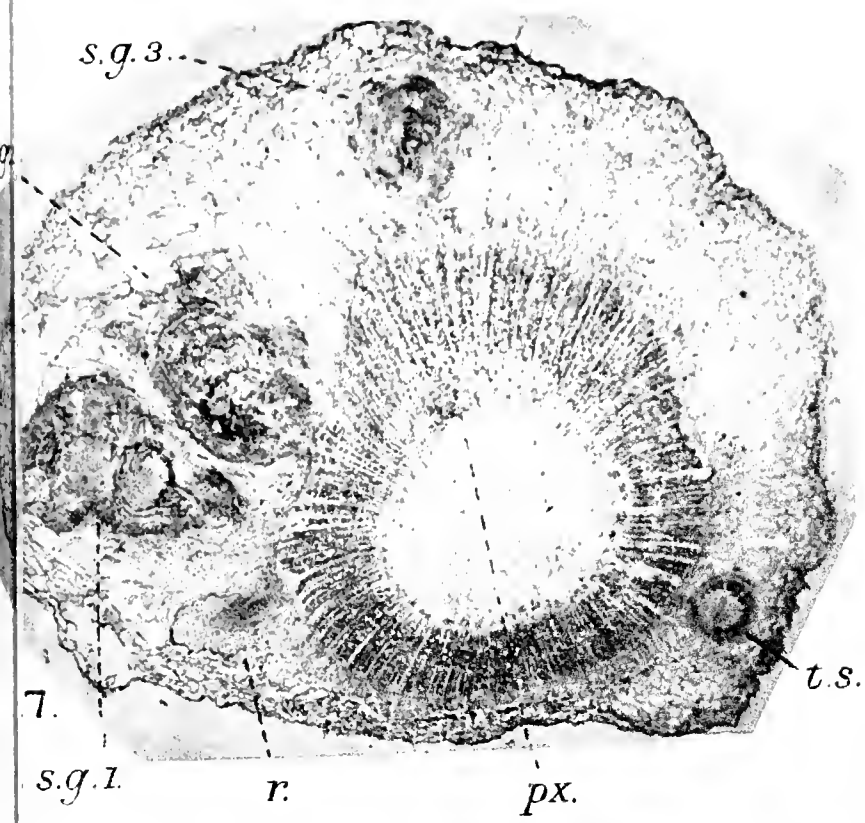

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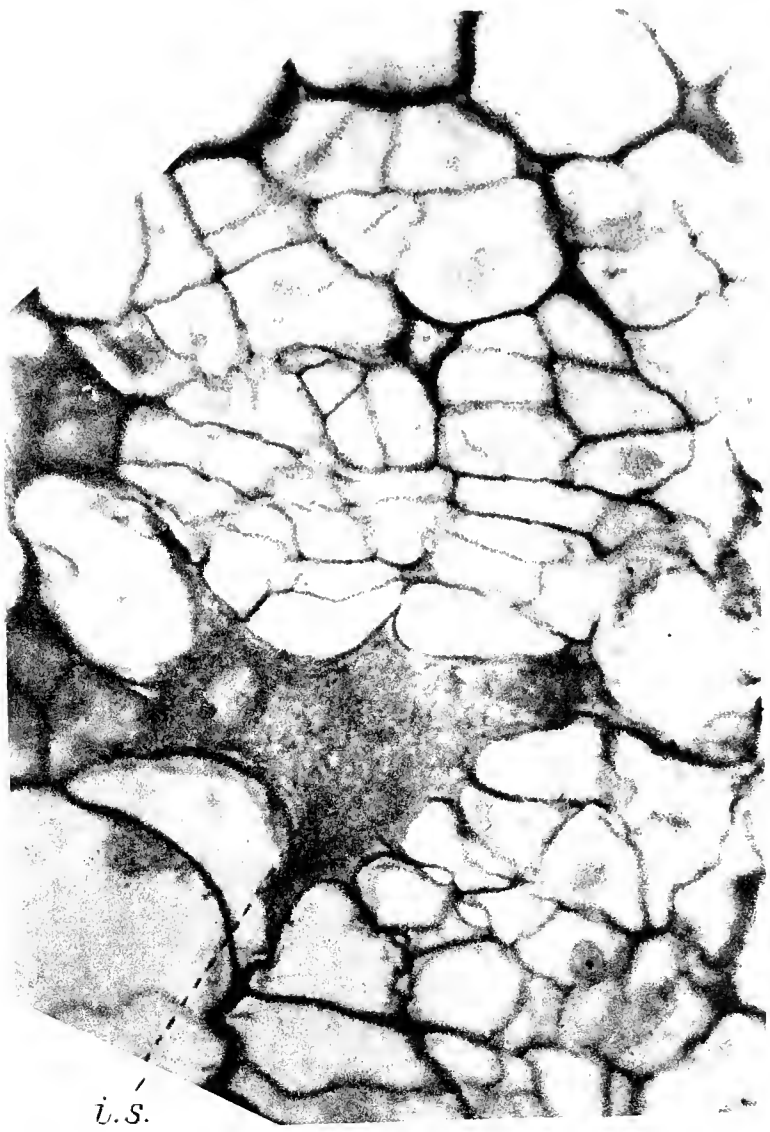

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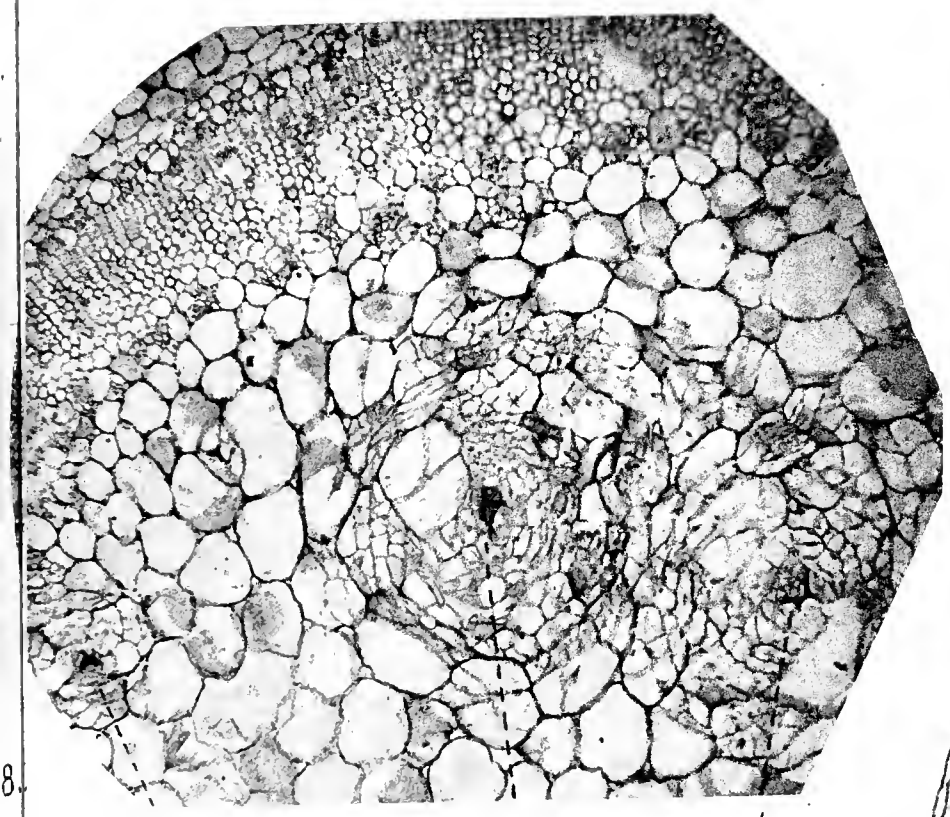

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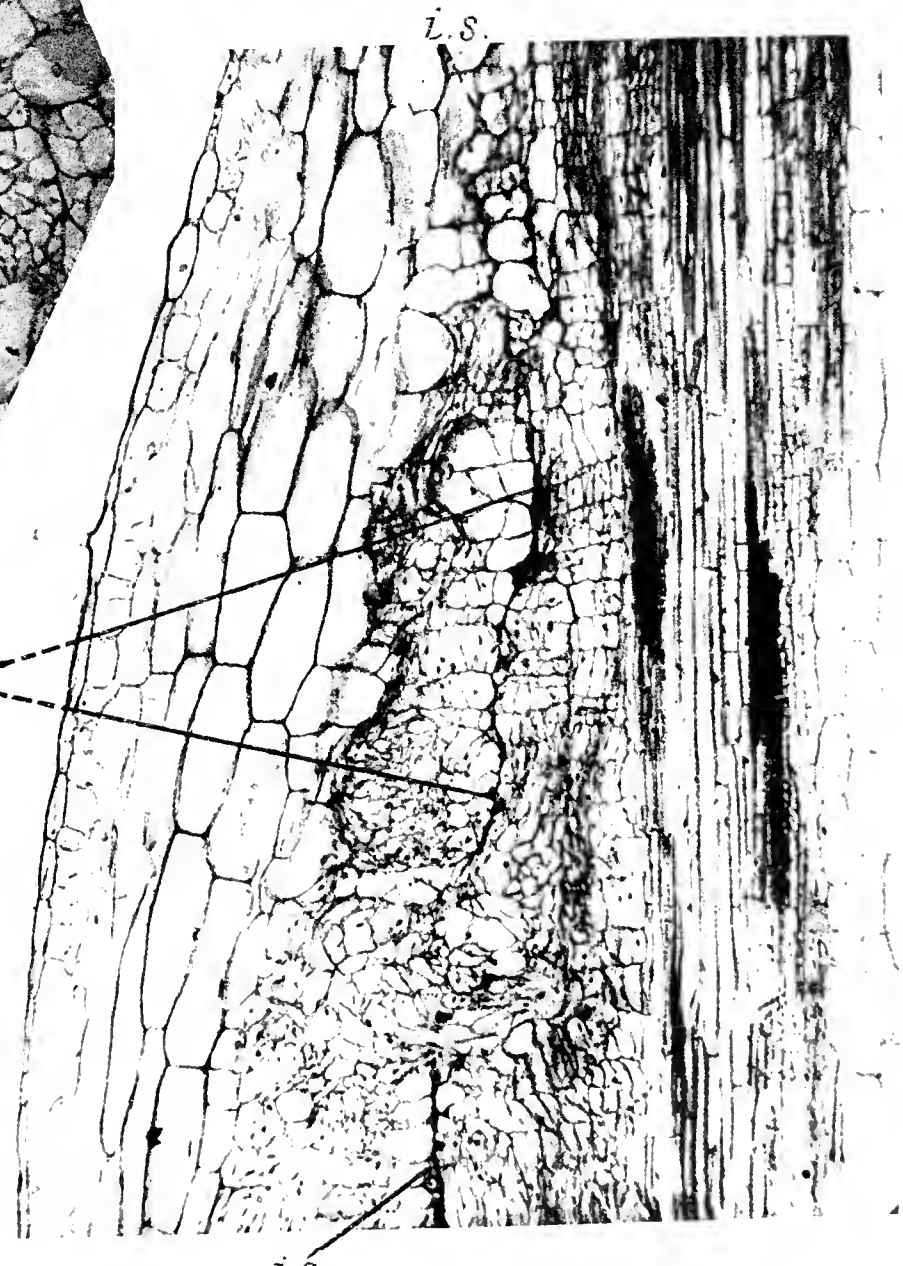

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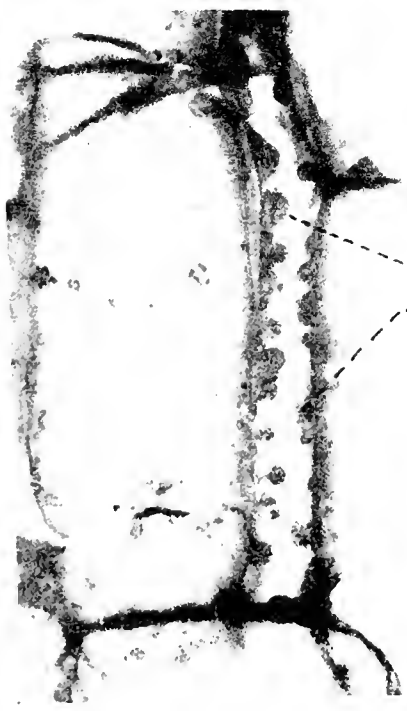

22.

The Development of the Flower of the Dipsacaceae.[1]

BY

Z. SZABÓ,

Budapest, Hungary.

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

THE development of the flower of the Dipsacaceae, according to the publications of Buchenau (1), Payer (6), van Tieghem (8), and my own former investigations (7), seems to be clear. There are, however, several questions not yet solved. Some more exact microtechnical work and the precise observation of the development are required to answer them.

These unsolved questions are as follows: 1. How does the carpel appear during the successive development of the different parts of the flower, and how is it a participant in the formation of the ovary? 2. Is the wall of the ovary an axile or a carpellary formation? 3. What is the arrangement of the vascular tissues of the flower-parts?

I wish to answer these questions, discussing the successive development of the flower-parts and the arrangement of their vascular bundles after having given a general description of the flower?

I. The Flower.

According to van Tieghem's precise definition, *the flower of the Dipsacaceae* is nothing but a single-flowered capitulum, which appears in the axil of a bract closely surrounded with an involucel. The stalk of this partial capitulum usually fails to develop appreciably; it elongates only in cases of proliferation. The involucel originates in four joined bracts, as is a general rule in the family of the Dipsacaceae. These four bracts, in prolific examples, may also form separate diverging involucels; even the number of the bracts may increase, as has been observed in several irregular cases. The situation of the four leaves of a normal involucel is median transverse, and it surrounds the inferior ovary, resembling a closed tube. After fertilization it grows longer, enclosing the genuine fruit, and as it is a closed integument, it falls off together with the fruit. The flower itself is hermaphrodite; its single leaf-zones are situated at the top of the inferior

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ovary, forming three whorls (calyx, corolla, stamens). The zone of the sympetalous corolla in the genera *Knautia*, *Dipsacus*, *Succisa*, *Succisella*, and *Cephalaria* is four-membered. The four episepalous diagonal stamens with their filaments grow on the top of the corolla. The calyx is situated on the spindle-shaped ovary and has a various number of members: in the tetramerous genera ordinarily four, in the pentamerous genera five. The inferior cupular ovary has only one cavity with a single anatropous pendent ovule. The situation of the latter is epitropous introrse. The stigma is double in the groups Knautiae and Scabiosae (van Tieghem's 'Knautiées et Scabiosées'), but it is simple in *Dipsacus* and *Cephalaria*.

To complete my former investigations of the flower of the Knautiae, which have two stigmas and no bract, I chose the flower of *Cephalaria* as the subject of my present study. This flower is completely tetramerous, with a single stigma and bract. The flower with a single stigma shows the greatest reduction, and the presence of a bract is necessary for any conclusions possible as to orientation.

II. The Development of the Flower.

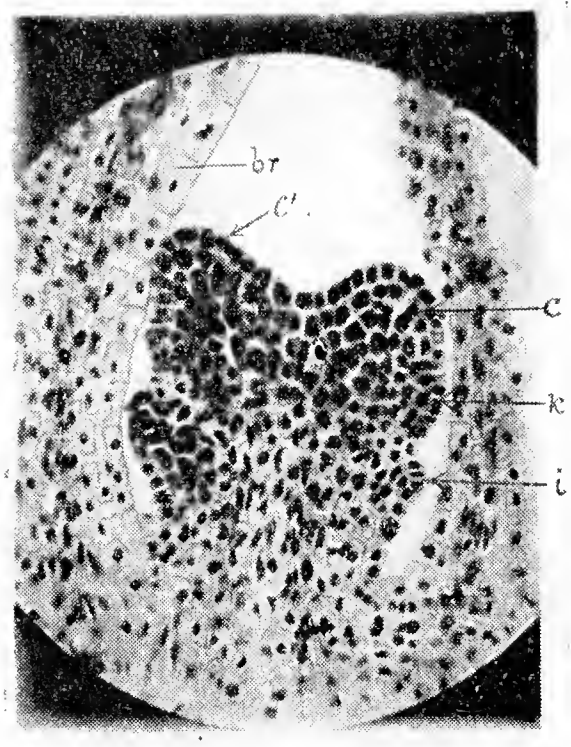
All authors, since Payer's investigations (6), agree that *the succession of the development of the different flower-parts* is uniform throughout the family. The four protuberances of the involucel appear first. According to Gjurašin (4) the two median protuberances appear first, and the two transverse ones later (Pl. VII, Figs. 1, 2). The flower-protuberance is still closely covered by the bract. Alternately with the four protuberances of the involucel arise the four protuberances of the calyx (Pl. VII, Fig. 3), and these are alternate with those of the corolla, which develop still later. During further growth, the development of the zone of the gamosepalous calyx (*k*) is slower than that of the gamopetalous corolla and of the involucel. The development of the corolla (Pl. VII, Fig. 5) is the most rapid. Alternating with the median-transverse protuberances of the latter, the protuberances of the stamens appear. The common basal part of the staminal and corolla whorls elongates to become the tube of the corolla (see Text-fig. 2), therefore the stamens and the tube of the corolla have grown together at their lower part. On Pl. VII, Fig. 6, there is shown the stage in which the four median-transverse protuberances of the involucel have become strong, and, what is more, there appears between them a protuberance diagonally situated. The teeth of the calyx situated diagonally are the strongest. Among the four protuberances of the corolla, the one in the front is a little larger; its situation is median, near the bract (*b*). The figures of Pl. VII show nearly the same gradations as Payer's Figs. 1, 2 (relating to *Dipsacus*). It is a pity, however, that in Payer's figures no

orientation is shown, and the parts of the calyx are not shown except in the more developed state.

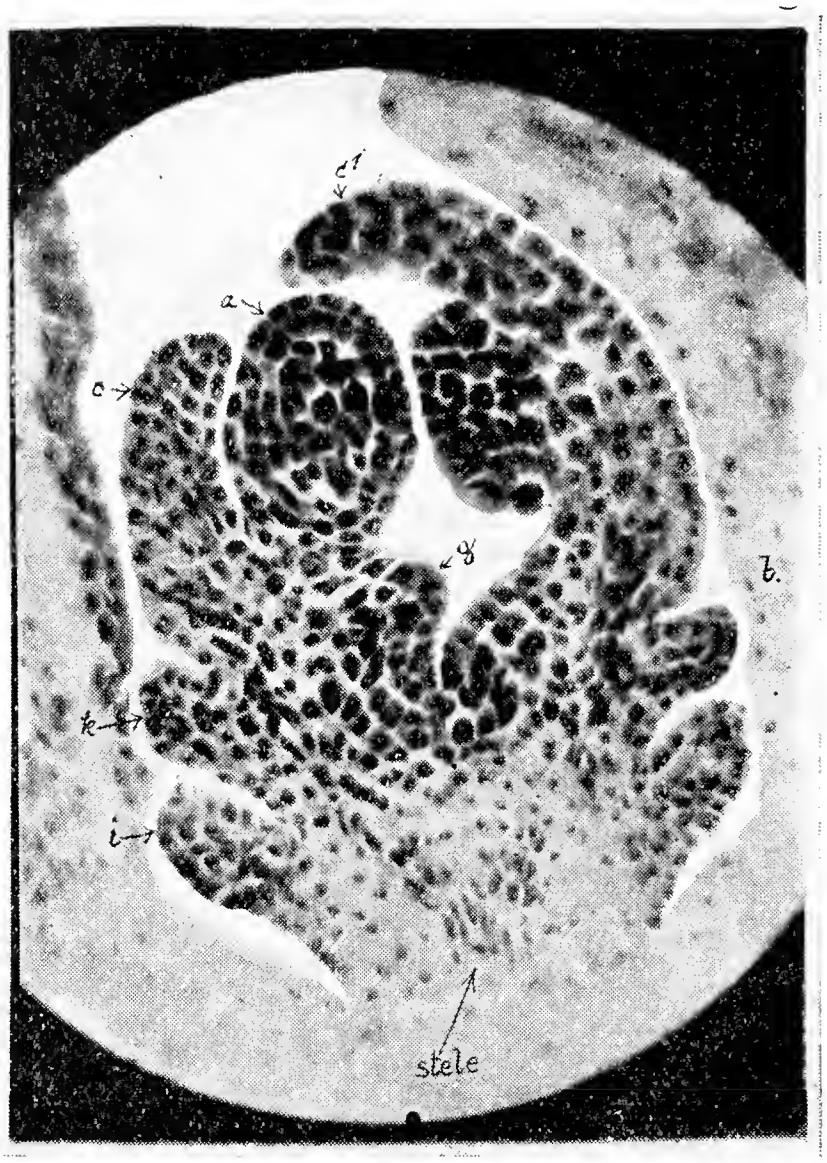
During the further development there are to be seen other and more important differences, when the ovary is in the course of formation. According to Payer the development of the ovary of *Dipsacus* (with one stigma) takes place so that at the edge of the carpel cavity there appear not two antero-posterior protuberances, but a single one (l. c., p. 630), which elongates and becomes the style. The situation of this horseshoe-shaped protuberance is transverse on the right in Payer's drawing (l. c., Pl. 131, Figs. 12, 13). The ovule begins to develop from the transverse (left) side (l. c., Pl. 131, Figs. 15, 16). Van Tieghem comments on this observation and drawing of Payer's, supposing that Payer sees the origin both of the ovary and of the style in the same anterior carpellary protuberance, while the posterior carpel is assumed to be totally missing. Eichler's and van Tieghem's statements are not based upon actual observation of the development, but are deductions from the adult condition. It has been already stated by Buchenau (1) that the pistil originates with the median-dorsal (posterior) carpel. According to Eichler the median-posterior ovule appears on the suture of the carpel, and therefore this carpel must have a median-anterior situation. The papillose, furrowed part of the stigma looks forward. This situation may be explained by the posterior carpel; van Tieghem assented to this opinion too, concluding that the carpel of the Scabiosae with two stigmas originates in two protuberances, but that only one of them takes part in the formation of the ovary. On the other hand, from the median-anterior protuberance of the Dipsacaceae and Cephalariae with one stigma, the ovary only develops; the style and the stigma originate from the posterior protuberance.

The results of my own investigations do not support these views; I find that on the one-stigmated Cephalariae there appears only one carpel-protuberance, the situation of which is median-posterior, or it is protruded in the direction of the diagonal. *From this protuberance develop the style and the ovule, but the ovary is a cupular (axial) formation.* This protuberance is a rudiment of four carpels, as I shall show later on when discussing the formation of the tissues. This result may be proved by the following phenomena: In the early stage of the development of the flower-bud the conditions are just the same as in the genera with two stigmas. The top of the flower-protuberance is concave. This cavity is already visible when the protuberances of the involucel and those of the calyx and corolla appear. (See Text-fig. 1.) This cavity becomes more and more deep, just as in the case of the Knautiae. In Text-fig. 2, which is a nearly median, longitudinal section of the stage shown in Pl. VII, Fig. 6, there is a protuberance to be seen (*g*) which is the carpel protuberance. This, together with the basal part of the opposite median-anterior petal outgrowth (*c*), borders a depres-

sion which may be called the *ovary-groove*. The situation of the protuberance may be seen also in Pl. VII, Figs. 7, 8 (*g*). This protuberance is horseshoe-shaped. The flexure of the 'horseshoe' is lying on a higher level; its situation is median posterior or diagonal; its two extremities become gradually lower and they border a slit which opens into the ovary-groove. The shape of the ovary-groove may be seen in Pl. VII, Fig. 8. This state of development is in accordance with Payer's (6) drawings on Pl. 131, Figs. 13–16. The horseshoe-shaped protuberance, however, is shown as trans



TEXT-FIG. 1. Median longitudinal section of the flower-protuberance of *Cephalaria elata* (from a microphotograph). *br*, bract; *c* and *c'*, the median-posterior and the median-anterior petal outgrowth; *i*, involucel outgrowth; *k*, calyx outgrowth. × 80.



TEXT-FIG. 2. Median longitudinal section of the flower-protuberance of *Cephalaria elata* in a more developed stage. *a*, stamen attached to the basal part of the corolla; *b*, bract; *c* and *c'*, the median-posterior and the median-anterior lobe of the corolla; *g*, the beginning of the development of the carpel outgrowth; *i*, involucel; *k*, calyx; *stele*, the formation of the procambial tissue in the stalk. × 16.

verse and the depth of the ovary-groove has been wrongly figured. At this stage its depth is never greater than that shown in Pl. VII, Fig. 8, i.e. the bottom of the ovary-groove is at the most at the level of the union of the calyx and corolla; it only becomes deeper later.

The growth of the horseshoe-shaped protuberance of the carpel and the formation of the ovary-groove may be traced gradually in Pl. VII, Figs. 9–13. The development of the style is figured in Pl. VII, Figs. 14–19,

and Pl. VIII, Fig. 20. The rapid growth of the horseshoe-shaped protuberance begins at its median-posterior part at the flexure, so that the 'horseshoe', which is at first low-lying, rises more and more and the slit becomes oblong (Pl. VII, Figs. 17, 18). After this follows the growth of the basis of the horseshoe, in consequence of which the horseshoe becomes a ring. From this ring develops the papillose part of the stigma; the stylar part originates in the basal part of the ring. The stigma extends itself and, as may be seen in Pl. VIII, Fig. 20, it acts a part as receiver of the pollen (*p*). It is simple, not bifurcated as in the Knautiae.

Together with the development of the style and its stigma described above, the bud also grows in the longitudinal direction and thickens. Changes set in also in the ovary-groove. It is thin, resembling a slit (Pl. VII, Fig. 11). The style (*s*) with the stigma (*st*) is situated at the top, and in its broader part below there appears a protuberance (*o*). This protuberance grows rapidly to form the ovule (Pl. VII, Fig. 13). The part below the junction of the ovule (shaded in Pl. VII, Figs. 9–13) grows just as described by Goebel (5) for *Valeriana*, and by me for the Knautiae (7). This part is the part of the axis between the protuberances of the involucel (*i*) and the protuberances of the calyx (*k*), i.e. the receptacle of the flower which finally surrounds the ovary-groove completely. The introrse ovule is hanging in the ovary with an anterior suture and a micropyle looping backwards and upwards (Baillon, 'Hist. des plantes', vii, 1880, p. 520), just the opposite to that described by Payer for *Dipsacus* (see van Tieghem (8), p. 185. This situation, however, is not uniform, and it varies in accordance with the position of the flower in the inflorescence. The longitudinal symmetry-plane of the ovule may decline from the median to the transverse direction in a different degree.

During the further development of the flower (Pl. VIII, Figs. 21–9) on the development of the cupular ovary follows the development of the involucel (*i*). The growth of the calyx (*k*) is limited. As a general rule in the flowers of Dipsacaceae the median-anterior petal-lobe covers the two transversally situated lobes and the posterior petal-lobe is covered by the transverse lobes. At the time of blossoming, in accordance with the proterandry, the stamens are ripe earlier than the pistil, their bent filaments become straight, and in the succession the two posterior stamens rise earlier than the two anterior ones (Pl. VIII, Figs. 25–7). The difference in the behaviour of the two anterior stamens is already to be observed at an early period of the development; it is probable that the phenomenon is a compensatory one, as the cause of it may be the powerful growth of the median-anterior petal-lobe which is in connexion with this pair of stamens. On the other hand, the retardation of the growth of the median-posterior petal-lobe assists the quick development of the two posterior stamens, with which it is in connexion. A considerable elongation of the style takes place after pollination

when the development of the cupular ovary of the ovule and of the involucel has been completed (Pl. VIII, Fig. 29).

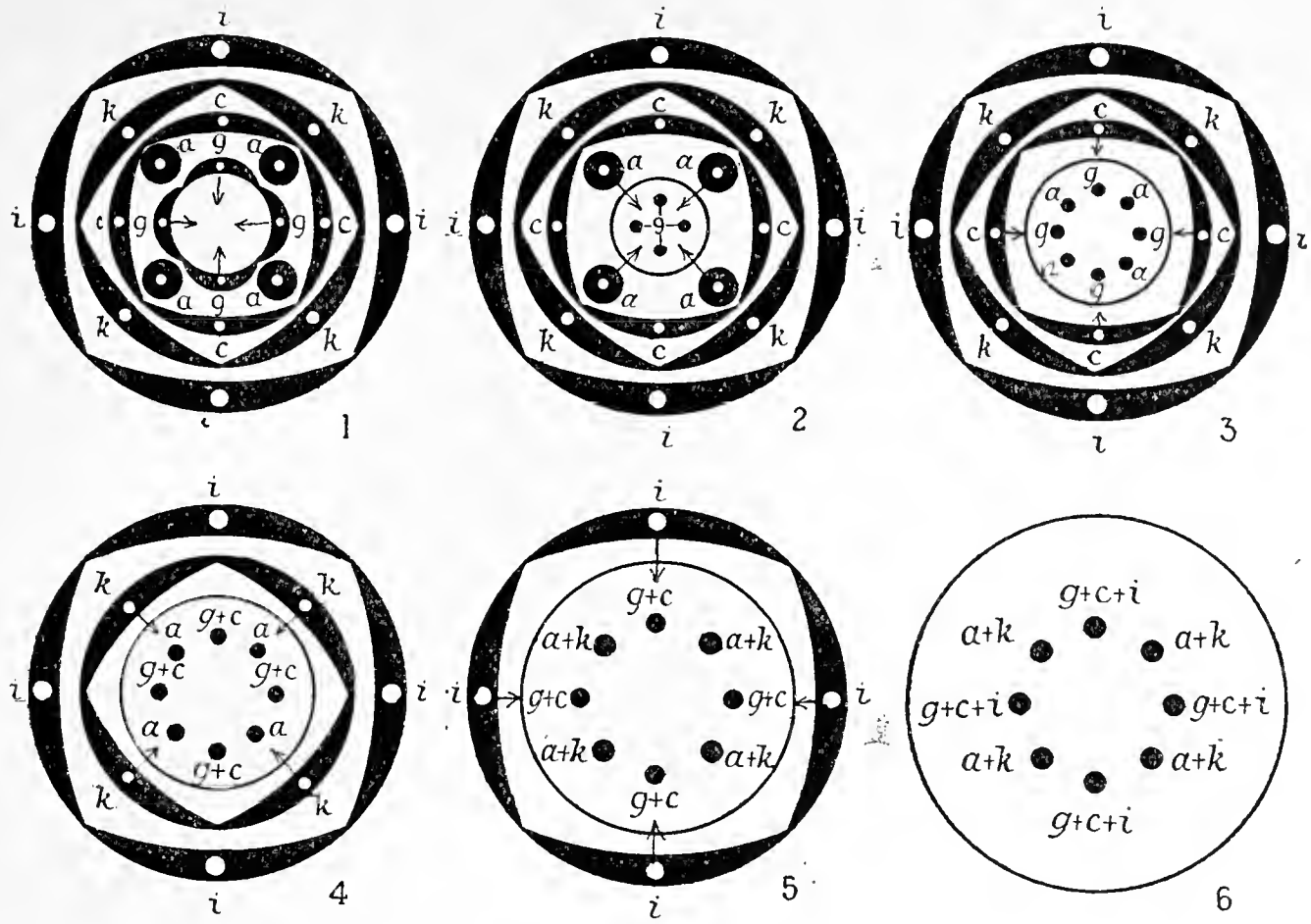
During the development of the flower the bract plays an important part as a protector, thoroughly covering and surrounding the little bud in its boat-shaped cavity (Pl. VIII, Fig. 23). While the bud is growing, the basal part of the bract grows too; its end bends over the top of the bud (Pl. VIII, Fig. 24). The outer side of the petal-lobe, covering the bud and also the outer side of the bract, is generally thickly hairy; or, if the latter is bare, then it has a red colour caused by anthocyan. During the blossoming the tip of the bract is bent back, but the lower part still surrounds the involucel.

III. The Arrangement of the Vascular Bundles.

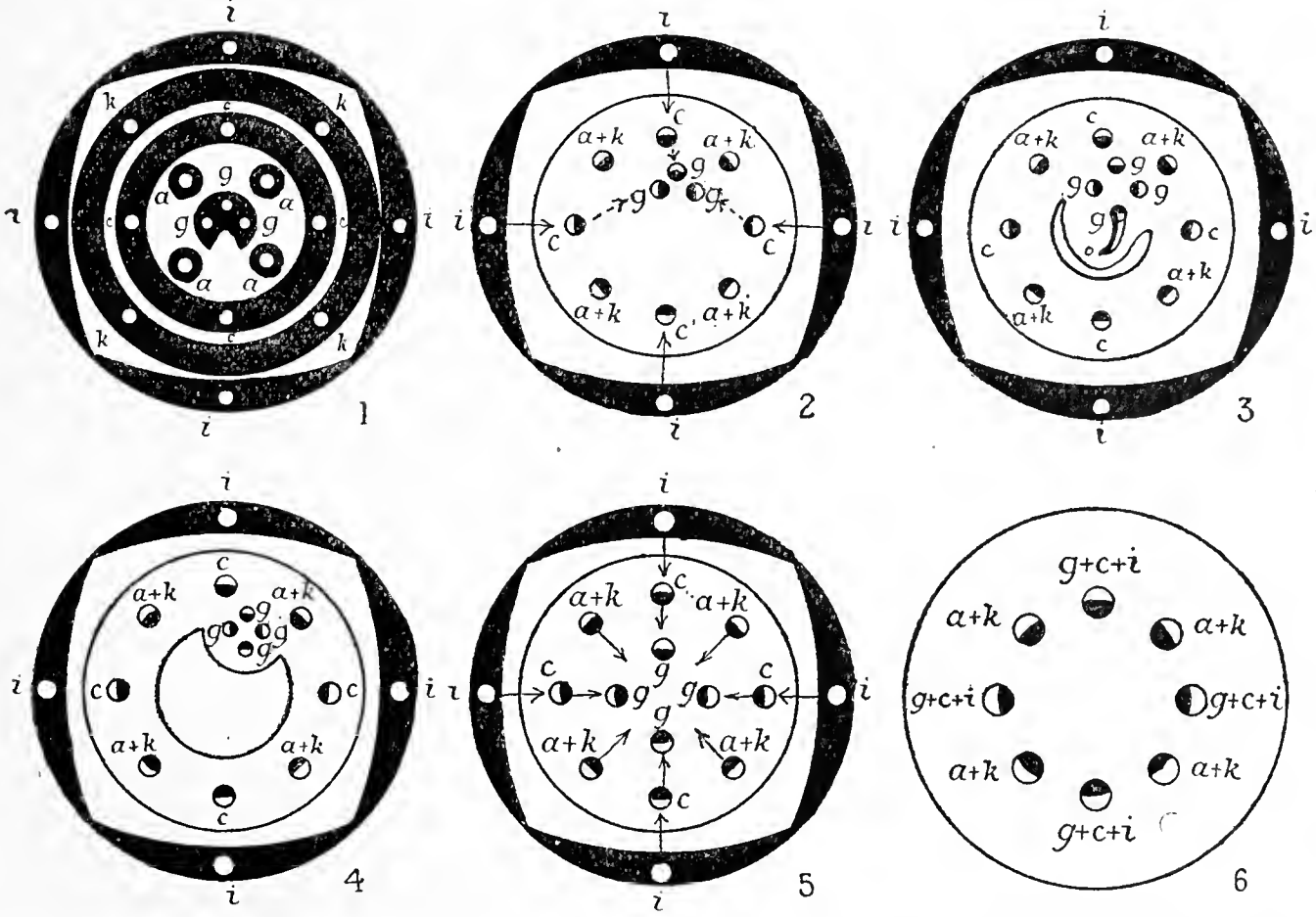
Only the arrangement of the vascular bundles in the development of the flower, which are of importance in connexion with the morphological value of the ovary, will be considered. The joining of the vascular bundles has been determined by means of a series of longitudinal and cross-sections made with the microtome. The relations observed in this way have been compared with the situation of the vascular bundles in a typical tetramerous flower.

For comparison I have figured the joining of the vascular bundles of a pentacyclic tetramerous flower with a superior ovary (Text-fig. 3). In Text-fig. 3, 1 the four leaves of the involucel, the four leaves of the calyx, the four petals, the four stamens, and the four carpels, situated alternately, are seen. The primary median vascular bundle of each has been indicated with a white circle. It is seen that the vascular bundles of the involucel, those of the petals, and those of the carpel are situated in the median-transverse line. The position of the vascular bundles of the calyx and of the stamens is diagonal. In the later drawings the flower-leaf whorls are seen to have been gradually inserted in the axis. In the axis first appear the four median-transverse carpel bundles (g); the four bundles of the stamens (a) will soon be inserted between them diagonally (3). There are now eight vascular bundles in the cross-section of the axis and they have their woody portions directed inwards forming a 'eustele'. To the eight bundles of this eustele join the vascular bundles of the petals in the median-transverse line ($g+c$), those of the calyx in the diagonal line ($a+k$), finally the bundles of the involucel in the median-transverse line ($g+c+i$). The result is that in the axis there are also eight procambial bundles forming a eustele. Later on, however, these eight bundles join to form a ring. This might be the course of formation of the procambial joining of the vascular bundles of a tetramerous flower theoretically, based upon the observations made on the Dipsacaceae.

In the flower of the Dipsacaceae, however, the manner of union of the



TEXT-FIG. 3. Theoretical ground-plan of the junction of the vascular bundles of a tetracyclic tetramerous epigynous flower. *i*, the whorl of the involucel; *k*, the whorl of the calyx; *c*, the whorl of the corolla; *a*, the whorl of stamens; *g*, the whorl of the ovary. The white and black discs indicate the situation of the vascular bundles.

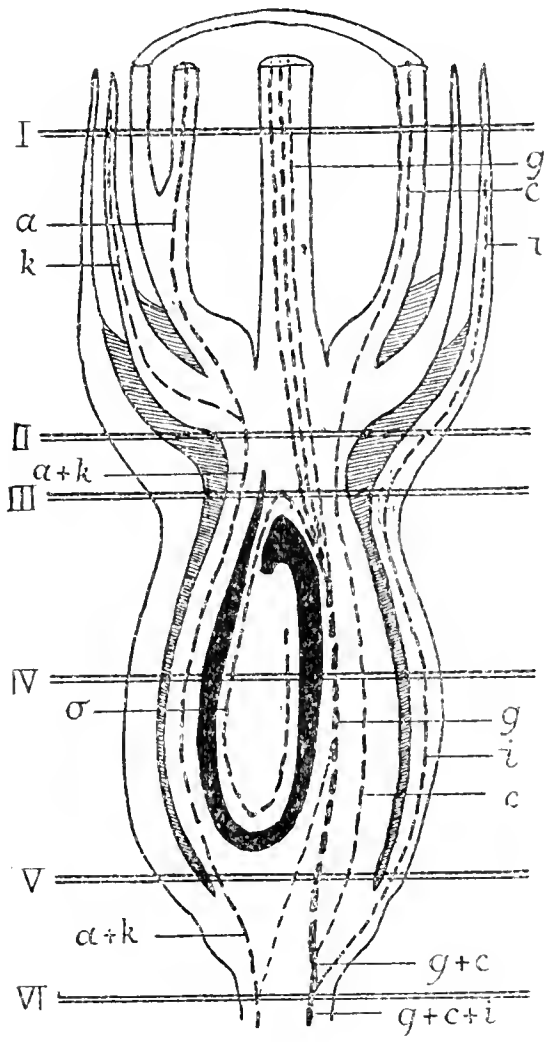


TEXT-FIG. 4. Ground-plan of the flower and the junction of the vascular bundles in the genus *Cephalaria*. 1–6 are ground-plans of the levels indicated in Text-fig. 5 by horizontal double lines and by corresponding roman letters; lettering as in Text-fig. 3.

vascular bundles has been altered by the fact that the situation of the ovary is inferior owing to the reduction of the carpel zone. Text-fig. 4 shows the arrangement of bundles in the Cephalariae, in the same way as in Text-

fig. 3. The single figures of drawing 4 show different levels, the situation of which is indicated by numbered lines in Text-fig. 5.

Text-fig. 4, 1, shows the ground-plan of the flower at the level of the stigma where the stamens branch off. The involucel is shown as lengthened as far as this level. The involucel is gamophyllous with four median-transverse lobes; this is followed by the united calyx and alternately by the corolla, and the middle bundle has also been figured in each of them. Now it is obvious that the carpel zone, following on the four diagonally situated stamens (indicated by the four circles), apparently consists of a single carpel only, but it has three vascular bundles, one of them median-posterior and two transverse, the median-anterior being missing.



TEXT-FIG. 5. A sketch of the longitudinal section of the flower of *Cephalaria*, showing the union of the vascular bundles. The double lines I–VI indicate the different levels at which the diagrams of Text-fig. 4 were taken. The right half of the drawing represents the median-posterior plane, the left half shows the diagonal plane; *a*, the vascular bundles of the stamens; *c*, those of the corolla; *g*, those of the ovary; *i*, those of the involucel; *k*, those of the calyx; *σ*, those of the ovule, which have been indicated by interrupted lines.

Text-fig. 4, 2, shows the insertion of the calyx on the top of the inferior ovary. At this level there are to be seen all eight vascular bundles of the 'eustele': the four median-transverse petal-bundles (*c*) and the four diagonal bundles of the stamens and calyx ($a + k$). Three bundles which originate in the style do not join the bundles *g*, but three of the *g*-bundles form a separate eustele which has been thrust away from the centre in a backwards or diagonal direction. This change of position is intelligible when we consider that the middle of the axis has been occupied by the cavity which opens into the inner part of the ovary, which is relatively important at the beginning of development (Pl. VII, Figs. 10–13). At a little lower level the funicular bundle *o* of the pendent ovule enters, just at the point where the missing fourth bundle, *g*, would be situated (Text-fig. 4, 3 and 4).

The distribution of the vascular bundles shown in Text-fig. 4, 4, above, is characteristic for the whole ovary. Throughout the whole length of the wall of the ovary, all the eight vascular bundles are to be found situated in the median-transverse and diagonal planes, there being bundles of the whorls of the calyx, corolla, and stamens. Further, in the posterior-median or diagonal region the 'ninth vascular bundle',

as this stele (*g*) has been called by van Tieghem (8), is to be seen. Van Tieghem did not know the structure and origin of this bundle; he has only distinguished it as a bundle much larger than the others and running into the ovule. I described it in like manner in dealing with the Knautiae (7), and Fodor (3) has done the same in his work on the Cephalariae. The structure of this vascular bundle, however, shows its hadrocentric nature; it is not a collateral bundle as are all the eight others; while tracing its development it is possible to determine that it originates in four collateral bundles with their woody portions turned inwards; they actually form the central stele of the axis. The eight peripheric bundles join this stele (Text-fig. 4, 5) at the bottom of the ovary. The monocyclic eustele (Text-fig. 4, 6) is seen restored in the stalk and shows an arrangement identical with that figured in Text-fig. 3, 6. The final result is a collateral ectophloic siphonostele. If the *g*-bundles would go in the wall of the ovary, surrounding its cavity in median-transverse direction, before the *c*-bundles, then one might suppose that the tissue which lines the inside of the ovary has a carpellary origin. But it is not so; the carpels do not take part in the building of the wall of the ovary. On the other hand, the independence of the *g*-stele, even the fact that it often parts from the wall of the ovary, shows directly that this stele must be a rudiment of the separating wall of the ovary, which is a general formation in the series of the Rubiales. Its connexion with the wall of the single cavity of the ovary may be explained by the fact that other cavities do not develop at all. It might be concluded from this circumstance that the *g*-stele is a direct lengthening of the top of the axis.

IV. Summary.

From the investigation of the development of the floral leaves it may be concluded that the wall of the ovary as a whole is an axile structure, originating in that part of the axis which corresponds with the internode between the insertions of the calyx and corolla. In the wall of the ovary as in an axis there are the eight vascular bundles of the flower whorls, the bundles of the gynoecium still forming for a while a separate stele. From the construction and development of this separate stele one may conclude that it is a rudiment of the ancestral diaphragm (partition wall) or centric column, and it actually shows its ancestral tetramerous structure. Of the four original carpels the median-anterior alone forms the ovule; the other three (the one median-posterior and the two diagonal) form the simple style. The change in the situation of the stele, which is pushed backwards in the median plane, makes it appear that there is only one median-posterior carpel which gave origin to both the style and the ovule.

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EXPLANATION OF FIGURES IN PLATES VII AND VIII.

Illustrating Professor Szabó's paper on the Development of the Flower of the Dipsacaceae.

PLATE VII.

Figs. 1–8. The early stages of development of the flower-of *Cephalaria elata*. *a*, stamens; *b*, bract; *c*, corolla; *g*, the protuberance of the carpel; *i*, involucel; *k*, calyx.

Figs. 9–13. Further stages of development of the flower. *a*, anthers; *b*, bract; *c*, corolla; *c'*, median-anterior lobe of the corolla; *i*, involucel; *k*, calyx; *o*, ovule; *s*, stigma; *st*, style.

Figs. 14–19. The development of the stigma and style. 1–3, the horseshoe-shaped protuberance of the carpel; *a*, stamens; *c*, corolla; *i*, involucel; *k*, calyx; *p*, pollen.

PLATE VIII.

Fig. 20. The fully developed stigma.

Figs. 21–29. The development of the bud and flower of *Cephalaria clata*. 21. Left side view. 22. Median-posterior view. 23–4. Side view with the bract. 25. Emergence of the two stamens. 26. Emergence of two other stamens; the involucel has been removed. 27. The male (staminal) stage without the ovary. 28. At the time of elongation of the style; half of the corolla has been removed. 29. The later (female) stage. *i*, involucel; *k*, calyx; *o*, ovary; *p*, bract.

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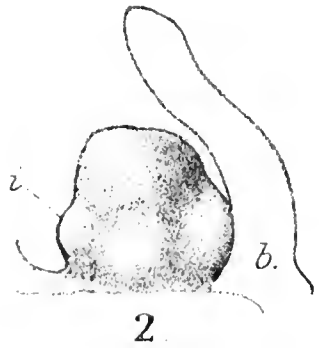

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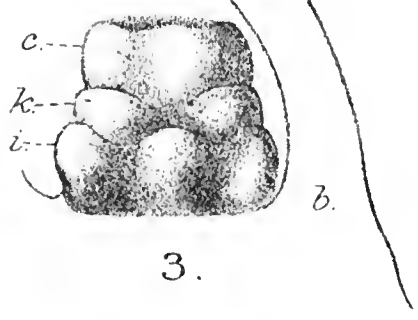

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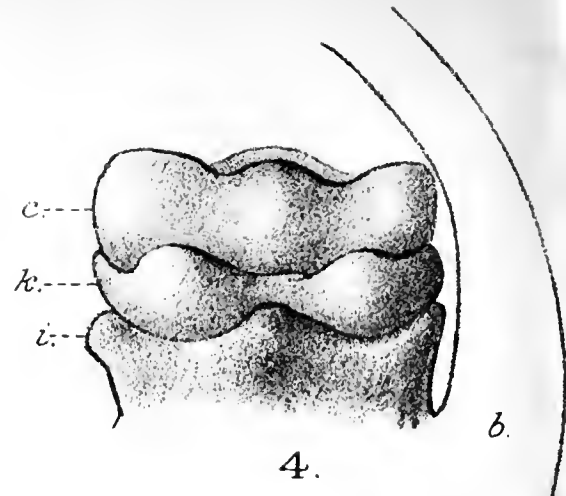

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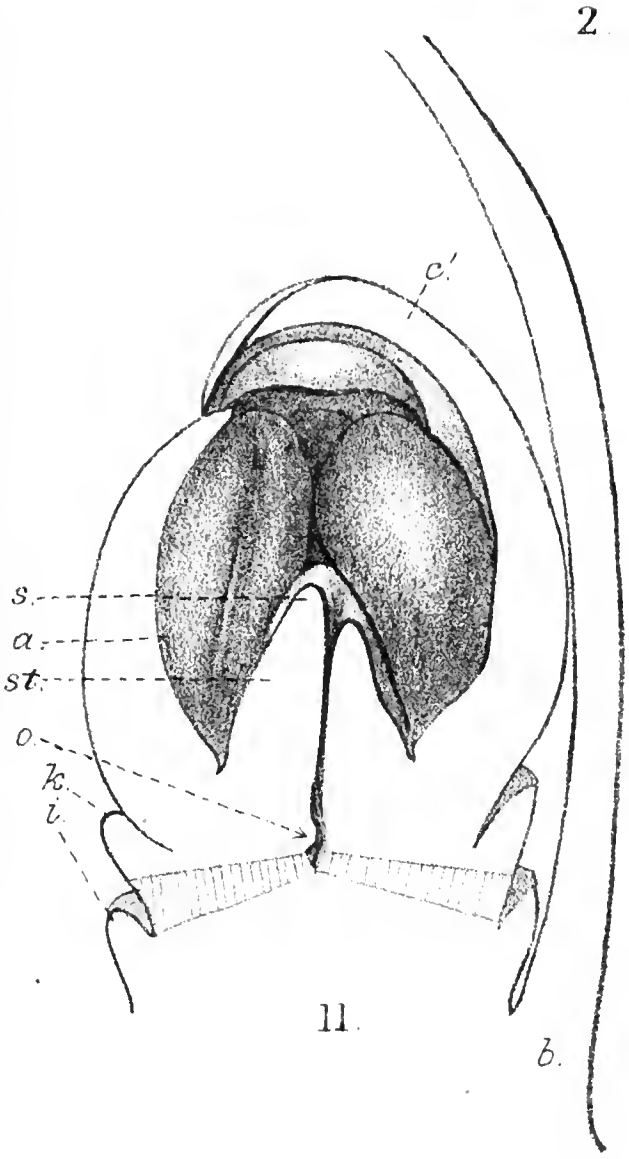

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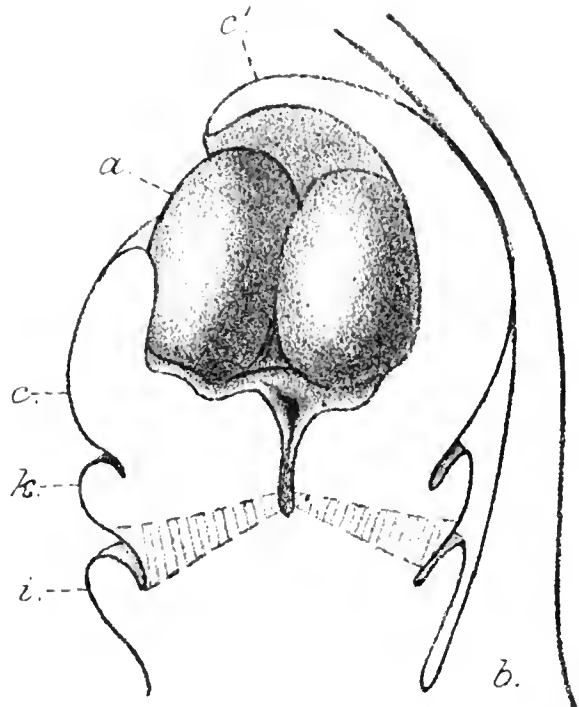

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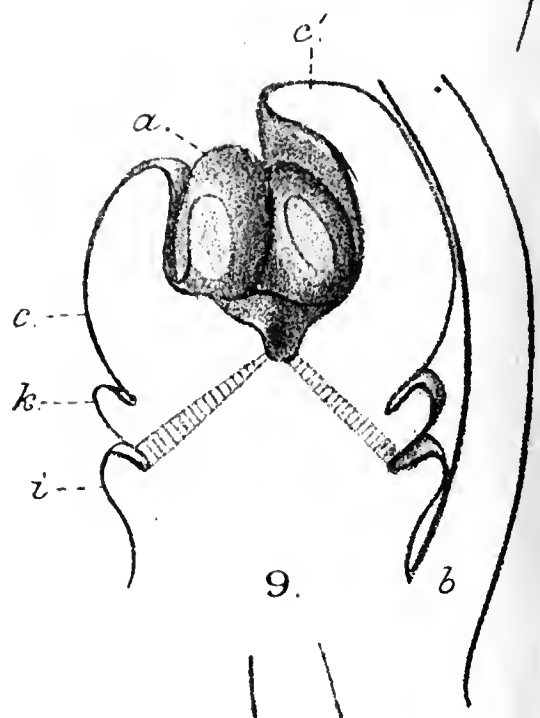

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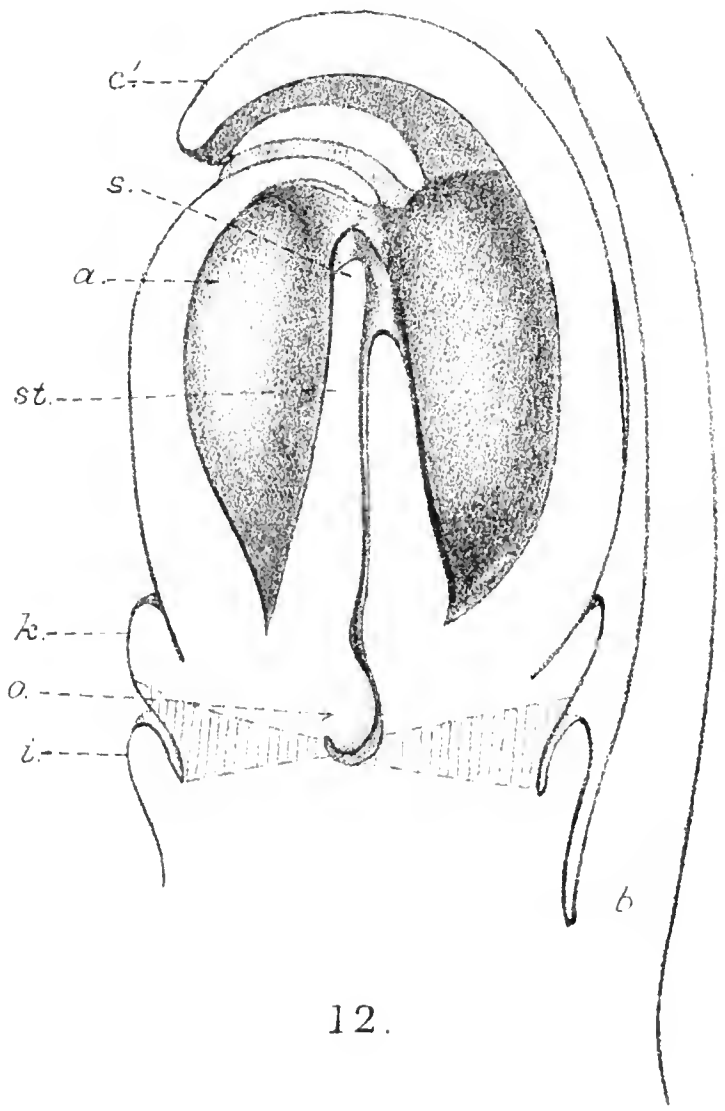

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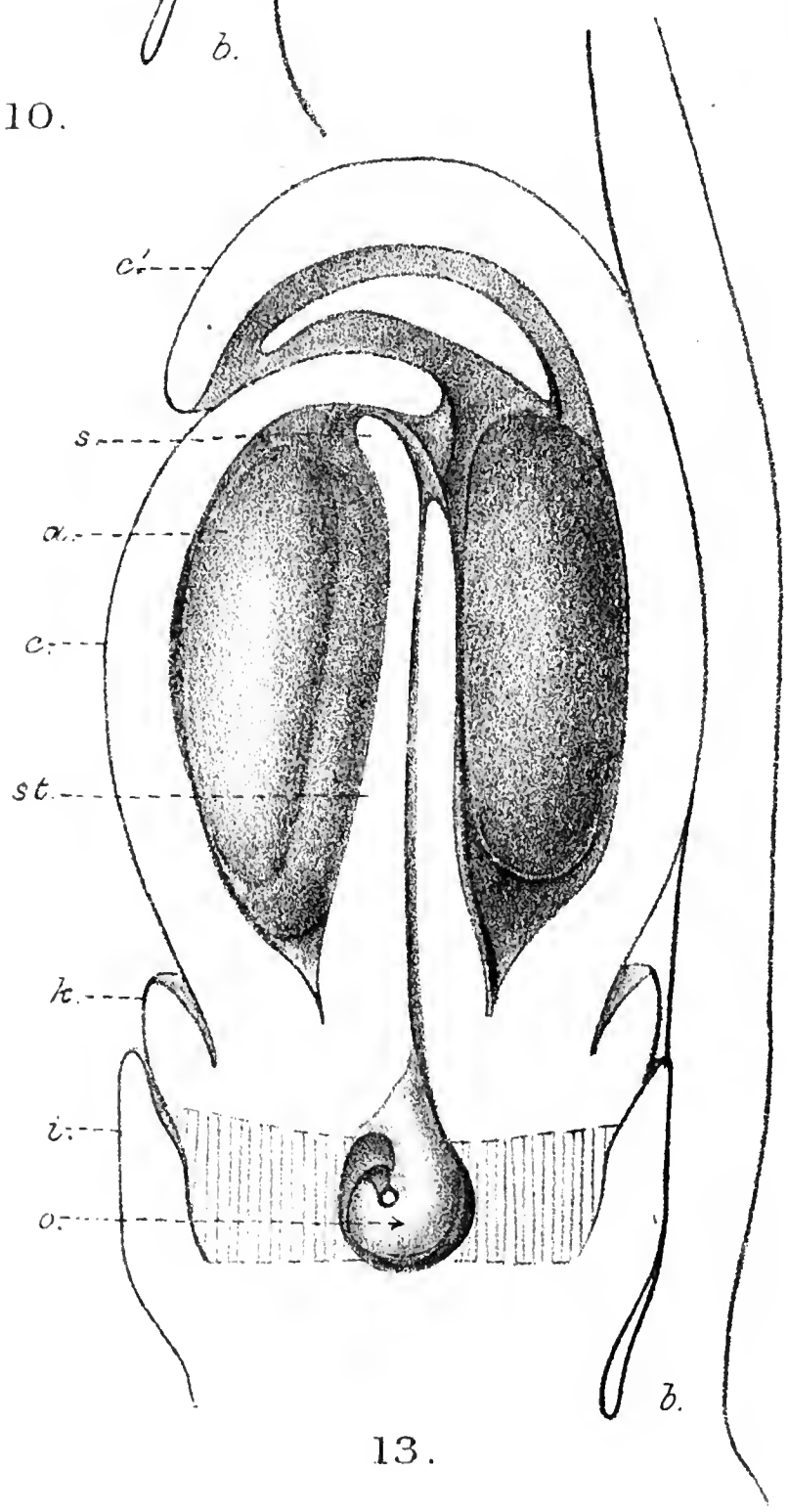

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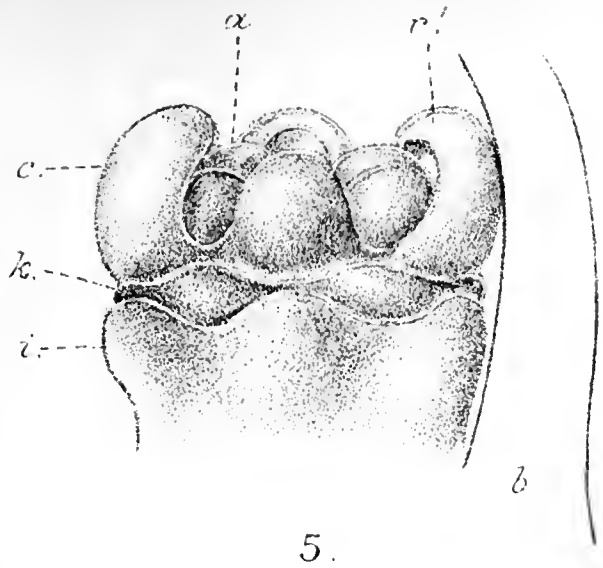
SZABÓ – FLOWER OF DIPSACEAE.



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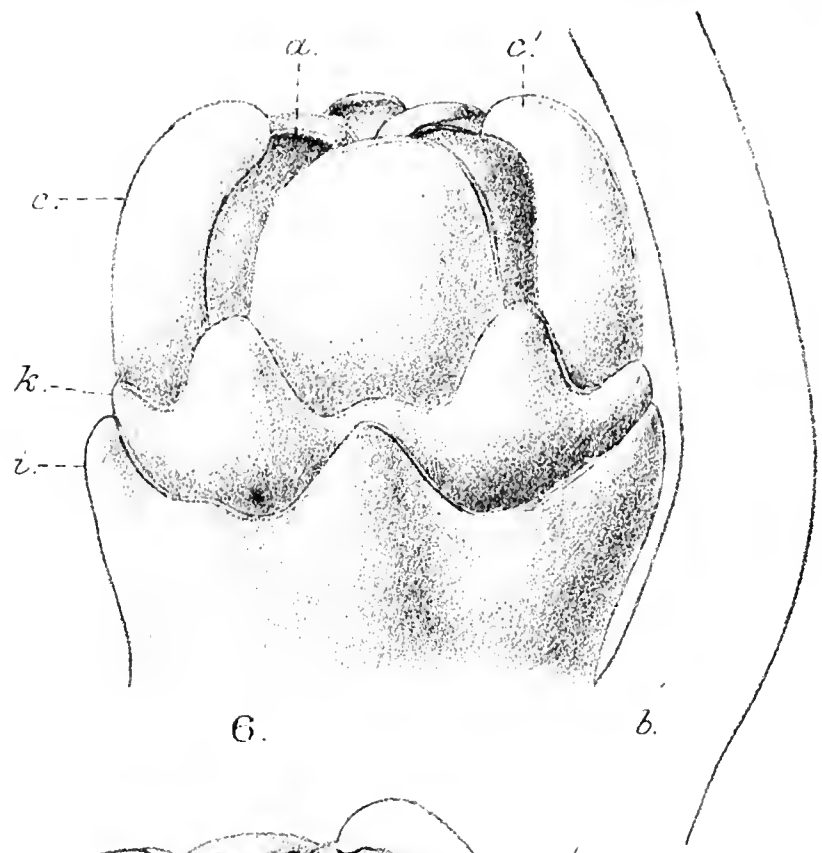

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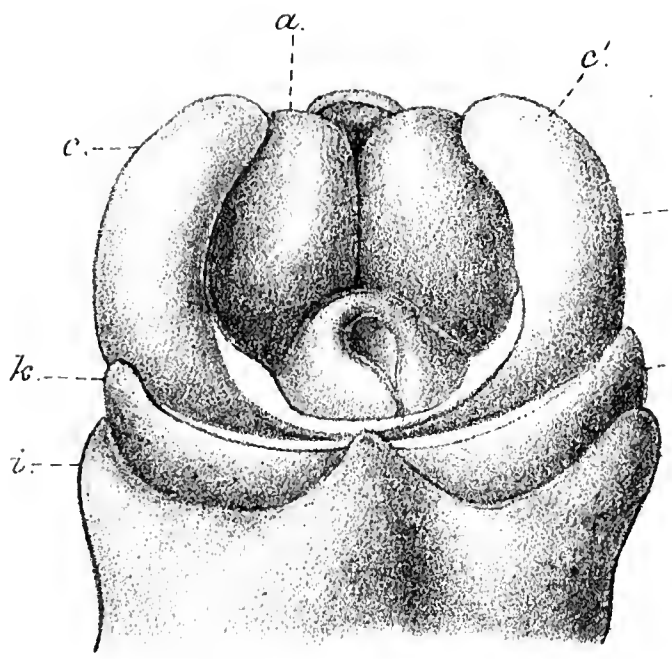

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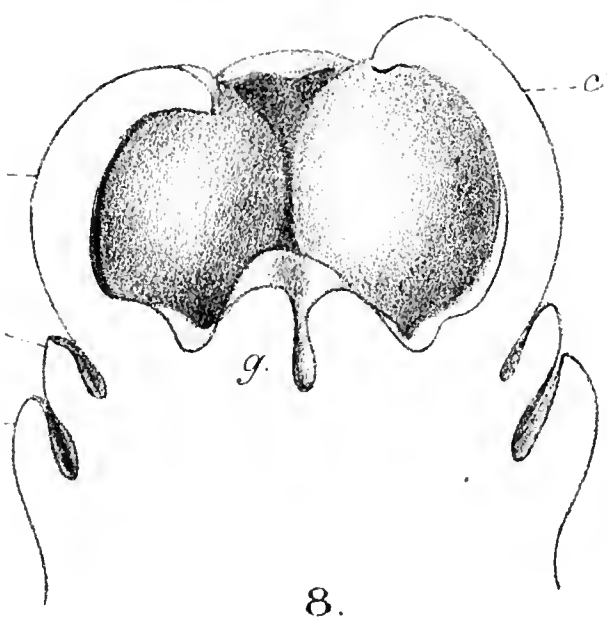

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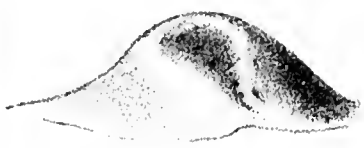

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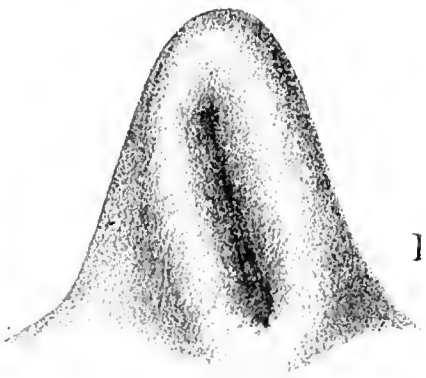

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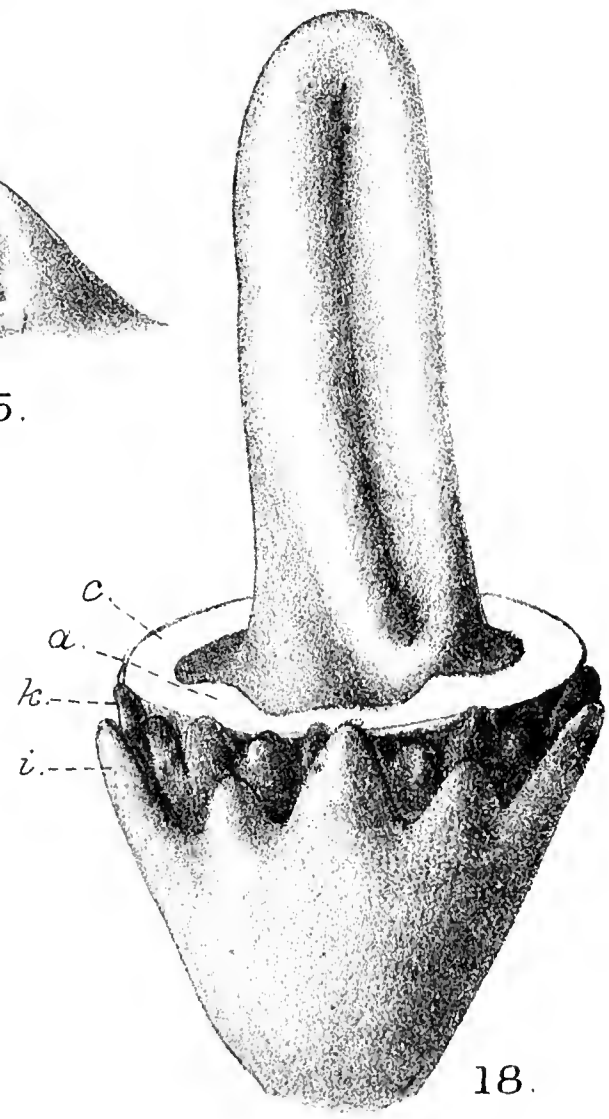

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SZABÓ — FLOWER OF DIPSACEAE.

PROFESSOR SIR ISAAC BAYLEY BALFOUR

ISAAC BAYLEY BALFOUR,

K.B.E., D.Sc., M.D., LL.D., F.R.S.

Emeritus Professor of Botany, University of Edinburgh.

BOTANICAL science has sustained a severe loss through the death of Sir Isaac Bayley Balfour towards the close of last year. His life was one of great achievement, and his work has been fruitful in lasting results extending in many directions.

He was born on March 31, 1853, and his early life was spent amid surroundings well fitted to encourage the full development of those fine qualities of mind with which nature had so richly endowed him. His father, John Hutton Balfour, was Professor of Botany in the University of Edinburgh and Regius Keeper of the Royal Botanic Gardens. His connexion with the University on his mother's side goes back to a much earlier period, as his maternal great-grandfather, the Very Rev. George H. Baird, was Principal in the eighteenth century. He was educated as a boy at the Edinburgh Academy, a school which counts so many distinguished men amongst its alumni. He graduated in science and medicine at the University, and pursued botanical study and research at Würzburg and Strassburg. He devoted himself to botany from his early days, and those opportunities for gaining first-hand practical knowledge of horticulture which the Garden at Inverleith provided were utilized to the full, and the foundations were thus laid of that profound knowledge of the horticultural side of botany which was to bear such rich fruit in after years, when he became not only a leader in the botanical world, but a recognized master of the craft of horticulture. No one who has read his masterly exposition of 'Problems in Propagation', which formed the subject of the Masters Lectures in 1912, can fail to be impressed by the breadth of his outlook combined with an astonishing knowledge of the details of horticultural practice, a knowledge which is only too rare amongst the botanists of the present day.

At the age of twenty-one he was chosen to accompany the Transit of Venus Expedition to Rodriguez, and a letter written by him from that island was judged to contain so much interesting and important matter that it was communicated by Sir Joseph Hooker to the Linnean Society. The full memoir, dealing with the flora of Rodriguez, was published in the Philosophical Transactions of the Royal Society in 1879, and it established his position as a systematic botanist of the highest promise.

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Soon after his return from this expedition he went to Germany, and it was at Strassburg that he came under the influence of de Bary, for whom he always entertained a great esteem. In 1879 he was appointed to the Regius Professorship of Botany in the University of Glasgow, and in the following year he visited the island of Socotra. The results of this expedition proved to be of great scientific value, and were published about eight years later by the Royal Society of Edinburgh. Although his tenure of the Glasgow Chair was a brief one (about five years) it was sufficient to give proof of his organizing capacity. His successor, Professor Bower, in an admirable tribute to his memory, published in 'The Glasgow Herald' (December 5, 1922), bearing witness to his constructive powers, draws a comparison between the conditions as Balfour found them and as he left them, and he adds: 'When I succeeded him in 1885 I found the machinery in working order, and it only needed to be kept running.' In 1884 he resigned the Glasgow Chair on being elected to the Sherardian Professorship at Oxford, a post which carried with it a Fellowship at Magdalen College. It was whilst he was at Oxford that the idea of founding the periodical which has taken shape as the 'Annals of Botany' was conceived. He threw himself into the project with all his heart, and it was very largely to his inspiring tenacity of purpose, coupled with his sagacity in steering the enterprise through the many dangers that threatened it during its pre-natal incubation period, that success was finally assured. Fortunately much of the correspondence has been preserved, and this material (which it is hoped may some day find an appropriate home in the Library of the Oxford Botanic Garden) not only serves to show just how this journal came into being, but it also sheds interesting sidelights on the condition of botany in this country at that time. A feeling had arisen, and was growing stronger amongst the younger men of high standing, that all was not well with botany in England. Chief amongst the protagonists were Balfour, Thiselton-Dyer, and Vines. They were fully aware of the great advances that were being made on the Continent, and had realized that changes were coming over the science which were destined profoundly to affect and extend its relations to other branches of knowledge. It was perhaps natural that the older men should view the new movement with some apprehension, but it is a remarkable fact that two of the leaders of the forward movement were themselves distinguished systematists. Against opposition, and in the face of well-meant discouragement, the view was maintained that a new journal was wanted to meet the new conditions, and that when started it would justify its inception by the increased output of research which would follow on new facilities for publication of the results. How well that prescience has been justified the pages of the 'Annals of Botany' have abundantly proved. It is not a little interesting to find that even so experienced and far-seeing a man as Sir Joseph Hooker,

at that time Director of Kew, regarded the new venture with misgiving, and indeed he warned Balfour of the risk he was running of finding all his energies absorbed in thankless administration. But he altered his attitude as time went on, and it was finally to his (Hooker's) suggestion that this periodical owes the name it has always borne. Balfour, at Oxford, was in a favourable position to arrange matters with the Clarendon Press, with the result that in 1887 the 'Annals' made its first appearance, and it has never looked back. Balfour was naturally associated with the editing from the first, and he also assumed the responsibility for its financial direction.

From this short sketch—necessarily very imperfect—it will be seen that he took a very leading share in promoting a movement which, more perhaps than any other, has served to stimulate scientific research amongst the younger generation of the British-speaking botanists.

It was a happy circumstance that the hearty sympathy and active co-operation of American colleagues was enlisted from the start. The leading botanists of that country gave their support, and the mutual interest thus aroused and continued has undoubtedly helped to cement further the ties that naturally link together colleagues on both sides of the Atlantic. In yet another direction Balfour did good service to the cause of botany amongst those who claim English as their mother-tongue by extending the series of translations of the most important German works on the subject. Thiselton-Dyer, Vines, Bower and Scott had already made a beginning with Sachs's Text-book and de Bary's 'Comparative Anatomy'. Balfour was fortunate in securing able collaborators, especially the Rev. H. E. Garnsey. He edited the translations himself, and his wide knowledge added greatly to the value of the series. None of us who are able to look back to those earlier days can fail to realize how rapid was the growth of the 'new botany' during that period, or that much of it was owing to the splendid energy and influence of Balfour himself.

But it was not only in the study and in the laboratory that Balfour made his strong personality felt, during the brief period that he occupied the Oxford Chair. The Botanic Garden was reorganized under his direction, and in this task his Edinburgh and Glasgow experience served him in good stead. But whilst he immensely improved its value from a scientific point of view, his natural artistic feeling enabled him to preserve its best features and its old-world charm. The place was transformed, but the work was directed by a master hand. The present writer well recalls how carefully every detail was thought out, and how every alteration fell naturally into its proper place as part of a well-conceived plan.

In 1888, on the death of Alexander Dickson, Balfour resigned the Oxford Chair to assume the duties of Regius Keeper of the Royal Botanic Garden, Edinburgh, together with the Professorship of Botany in the University and the office of Queen's Botanist in Scotland. Here he settled

down to thirty-four years of strenuous and fruitful work. His genius found ample scope, and the great institution as it now exists at Inverleith may be said to be his creation. Struggles there were against local prejudice, and against powerful opposition elsewhere. Strong feeling was aroused when the walls dividing the arboretum from the rest of the Garden were demolished in the early nineties, and a storm of angry protest attended the throwing open of the Garden to the public on Sundays. But time healed all the sores, and he lived to see his work applauded at home, and the Garden for which he had done so much take its place as one of the greatest institutions of its kind in the world.

No account, however brief, of his work in the Edinburgh Garden can omit reference to the rock garden which forms one of its greatest and most interesting features. Planned on large lines, no expense or trouble was spared to make it worthy of its great setting. No one who is interested in alpine plants can afford to miss making its acquaintance, and many who have walked with him through this fine collection must recall the stimulating presence of the man who had called it into existence, and who knew things of interest about every plant that was growing in it. Space fails for more than a mention of the great collection of Rhododendrons and Primulas, which Balfour got together and cultivated so successfully—in spite of climatic and other difficulties. The grouping of trees in the Garden also demands a word, for wherever it appeared desirable, whether for picturesque or other reasons, there a mature specimen or a group has been moved and planted. Of course the expense was considerable, but the result is its complete justification. Doubtless the open soil, with water moving through it at a level not far removed from the surface, contributed to the success of so serious an undertaking, but it was characteristic of the Regius Keeper to assume large responsibilities with a very accurate knowledge of the factors necessary for success.

The reorganization of the plant-houses was another task which was ably carried through, and the luxuriant way in which the plants growing in open soil thrive under the glass testifies to the skill and knowledge with which the whole work has been carried out and maintained. Indeed the Garden as a whole is what a botanic garden should be: of great scientific value, full of beauty, and abounding in suggestive hints of how the cultivation of a vast range of plants can best be carried on. If he had achieved nothing else in Edinburgh, Balfour's claim to fame would have been sufficiently established, but although the Garden, and all it stood for, perhaps held the chief place in his interest, it was by no means the only one. He designed new laboratories for study and research which are second to none in the country. Furthermore, he was himself a great teacher. Not only was his outlook over his science wide and philosophical, but his immense store of knowledge and the readiness with which he could draw on his large

reserves combined to make his lectures singularly attractive and stimulating. No trouble was spared in their preparation, and the almost prodigal wealth of magnificent material drawn from the Garden served to render them still more unique and impressive.

The 'Notes from the Royal Botanic Garden, Edinburgh', was established by Professor Balfour as an official publication, and although obstacles were put in its path at first it has survived all red-tape discouragement. Unlike many official publications, it pays its way, and some sixty-five parts, making thirteen volumes, have already appeared in the twenty-three years of its existence. It is issued at irregular intervals, depending on the rate of accumulation of suitable matter, and in this connexion it may be of interest to know that when the 'Annals of Botany' was first projected a similar form of publication was strongly advocated in some quarters.

Valuable memoirs on the Primulas and Rhododendrons, towards the elucidation of which Balfour has contributed so much, have appeared in the 'Notes' and also in the Transactions of the Botanical Society of Edinburgh.

Failing health caused him to resign his appointments at Edinburgh, and in 1921 he came south to spend the evening of his life, not in leisure but in further work. Almost to the last he was engaged on Asiatic collections of Primulas and Rhododendrons, groups in which he was recognized as the greatest living authority. The hopes entertained by his friends that relief from official cares and duties might result in convalescence were not destined to be fulfilled, and in spite of every effort, and the devoted care of his wife, the end came on November 30, 1922.

Balfour was a fine botanist, an exceptionally able administrator, and a man of wide intellectual culture. Besides all these he was a staunch friend and a wise counsellor, and no one having enjoyed the privilege of knowing him intimately can ever forget him. Really great men are very rare, and Isaac Bayley Balfour was one of them. J. B. F.

NOTES.

ON THE APICAL GROWTH OF FUNGAL HYPHAE.—It is usually stated in the text-books (e.g. Gwynne-Vaughan (1), p. 1) that the growth of fungal hyphae is apical, and it is obvious that the greater part of the elongation does take place at the tip. One might, however, reasonably expect that some extension of the more recently formed segments also occurs, and that growth, while mainly apical, is also to some extent intercalary. I have been unable to find any published account of detailed observations maintained for any considerable period of time, and, as the question is of importance in connexion with rates of growth, a brief record of some experiments *ad hoc* may not be superfluous.

Spores or, in some cases, fragments of mycelium were sown on the surface of miniature agar plates, formed by pouring agar on cover-slips,[1] which were then inverted over van Tieghem cells and the preparations incubated at 24°–25° C. After germination had occurred and the hypha had reached a convenient size, the lengths of the segments already formed were measured, at varying intervals of time, over a period extending in different cases from five to fifty-six hours. Where septa were absent or difficult to distinguish, the intervals between successive branches were determined. The agar used was a batch of clear prune agar, and the observations were made at room temperature. Measurements were made with a Bausch and Lomb screw-micrometer eyepiece and Zeiss C objective.

It is unnecessary to give a detailed account of the observations with the different organisms examined, but abbreviated records of two experiments may be given as examples.

TABLE I.

Fusarium No. 18. (*Prune agar: measurements in μ; times in minutes from the first measurement.*)

Time	0	100	161	249	323	407	489	1468
Segments 1–7	494·9	496·6	499·6	497·6	497·6	499·3	498·6	496·7
Segments 8		23·3	23·3	23·3	23·3	23·3	23·3	23·3
Segments 9–11		143·3	140·9	141·3	141·7	143·3	140·4	138·3
,, 12–14			138·7	138·4	137·3	135·9	137·7	300·7
,, 15–17				162·3	161·7	158·7	161·6	
,, 18–21					163·0	162·7	163·3	163·6
,, 22–25						305·9	306·9	305·6
To tip	186·7	206·7	190·0	200·0	183·3	70·0	235·0	2334·0
Whole hypha	681·6	869·9	992·5	1162·9	1307·9	1499·1	1704·5	3762·2

Table I shows the result of an experiment with *Fusarium* No. 18. When first measured the hypha showed seven segments or cells, which together measured 494·9 μ, and the length between the tip and the nearest septum was 186·7 μ. As the

[1] This may be done very conveniently by laying on a cover-slip a glass ring, such as is used in a van Tieghem cell (it must not be too small—1·7 cm. is a convenient size), and pouring into it melted agar to any desired depth. When the agar has set the ring is removed and leaves a circular platform of agar, which after inoculation is laid on the cell in the usual way. The growing organism is easily observed through the agar.

hypha grew, new septa were laid down, forming new segments. Every segment was measured separately, but, to economize space, adjacent segments are grouped together in the table.

It will be seen that during the period of observation no change takes place in the length of the segments after they are once formed. The measurements vary a little owing to the difficulty of every time bringing the comparatively broad cross-line of the micrometer exactly in the same place over the septum, and also owing to the fact that the hypha does not grow perfectly straight. These discrepancies are experimental, and would have been reduced had the camera lucida been employed. There is no evidence of increase in the length of a segment, once it is formed, although the whole hypha had grown over 3,000 μ.

The average length of the segments is 58·8 μ, and the distance from the nearest visible septum to the tip varied at the different observations from 70 to 260 μ, with an average of 191·5 μ, the length of about $3\frac{1}{2}$ segments. Each septum is no doubt laid down some time before it becomes visible: how much before it is not possible to tell exactly. The whole hypha elongated at a mean rate of 2·1 μ per minute during the first eight hours, so that it would require about ninety-five minutes to increase by the length of $3\frac{1}{2}$ segments. The elongation must occur in the portion between the tip and the nearest septum. It is, however, occasionally possible to get a measuring point much nearer the tip than the nearest visible septum. Branches may, and often do, arise from segments long after they are first formed; but sometimes a branch is begun immediately behind the tip, and its bud is perceptible very close to the extreme tip. In such cases the interval between the point of origin of the branch and the nearest septum then visible remains constant, showing that elongation is taking place only at the apex, and not at all in formed portions of the hypha.

A similarly abbreviated record of an experiment with *Pyronema confluens* is also given: see Table II. Here also the growth is entirely apical, the apparent small extension of the segments being probably due to errors of measurement, and in any case so very slight as to be negligible in comparison with the growth at the tip.

TABLE II.

Pyronema confluens.

Time	0	66	152	263	330	417	476	1469
Segments 1 and 2	25·1	26·2	25·8	26·2	26·9	27·3	27·0	25·1
Segment 3	15·7	16·4	16·4	16·8	15·0	14·2	16·1	16·5
Segments 4–6				73·4	75·3	76·0	74·9	75·7
Segment 7						47·2	48·0	47·9
,, 8								48·0
Segments 9–19								518·6
To tip	109·2	137·2	149·6	127·5	177·3	190·5	239·2	420·0
Whole hypha	150·0	179·8	191·8	244·1	294·5	355·2	405·2	1151·8

The following organisms have been examined similarly, and all show the same thing, that growth in length is exclusively apical. When septa were difficult to see, the intervals between successive branches were measured.

Phytophthora parasitica (Strain: Egg-plant, Nat. Coll. Type Cultures No. 1187).

No change in intervals between branches in 22 hours; hypha grew 389 μ.

Aspergillus niger (Nat. Coll. Type Cultures No. 594). No change in branch intervals in $7\frac{1}{2}$ hours; hypha grew 250 μ.

Penicillium expansum (Washington 4189; Nat. Coll. Type Cultures No. 593). No change in branch intervals in 47 hours; four hyphae observed, of which one grew 719 μ in $9\frac{1}{2}$ hours.

Pyronema confluens (Birkbeck: Nat. Coll. Type Cultures No. 1245). No change in segments in 24 hours; two hyphae observed; mean length of segments, 39·7 μ; mean length from tip to nearest septum, 136·9 μ; one hypha grew 1,001 μ.

Rhizoctonia solani (Jersey potato; Nat. Coll. Type Cultures No. 1007). No change in segments in 56 hours; two hyphae and five branches measured; mean length segments, 151·7 μ; mean length tip to nearest septum, 179·6 μ; one hypha grew 979 μ.

Rhizopus nigricans (Roth. No. 13). No change in identifiable portions of hypha in 44 hours; hypha grew 4,149 μ.

Botrytis cinerea. No change in segments in $10\frac{1}{2}$ hours; mean length segments (ten hyphae), 51·6 μ; tip to nearest septum, 133 μ; one hypha grew 1,710 μ. With this organism the growth of aerial hyphae was also investigated, growth occurring also only at the apex.

Fusarium (three species).

1. No. 10 A *a* 4. No change in segments in 5 hours (two hyphae); mean length segments, 31·1 μ; tip to nearest septum, 138·5 μ; one hypha grew 176 μ.
2. No. 13 *c* B *b*. No change in segments in $6\frac{1}{2}$ hours; mean length segments, 65·7 μ; tip to nearest septum, 179 μ; one hypha grew 1,288 μ.
3. No. 18. No change in segments in 24 hours; mean length segments, 58·8 μ; tip to nearest septum, 191·5 μ.

Summary.

These fungi are representatives of widely separated genera, and in all it is found that the growth in length is purely apical, no appreciable elongation occurring in any part of the hypha other than the tip. This then would seem to be the general rule for fungi, though exceptions may perhaps occur. It may be contrasted with what occurs in filamentous bacteria, where each of the segments expands equally at the same rate (Marshall Ward (2)), and in algae, where both types of growth occur, the purely apical and the intercalary (West (3)).

J. HENDERSON SMITH.

Mycological Department,
Rothamsted Experimental Station.

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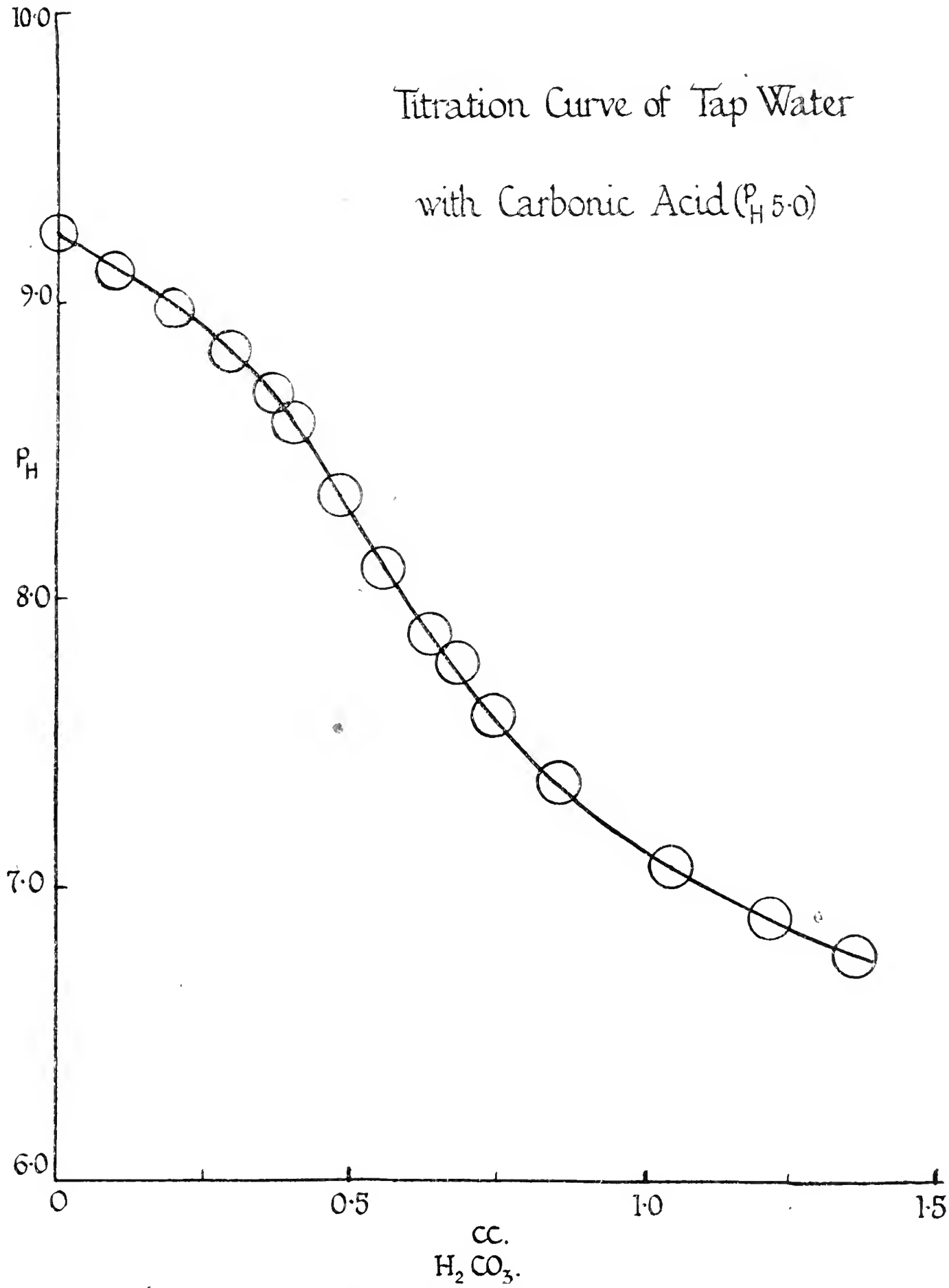
BUFFER EFFECTS OF TAP-WATER IN THE ESTIMATION OF CARBON DIOXIDE BY CHANGE IN HYDROGEN-ION CONCENTRATION.—The hydrogen-ion concentration method of measuring respiration, as described by Haas (1), involves a consideration of the buffer effects of the reagents employed. In this method the respiring material is placed in water containing an indicator, and the time taken to change the medium from one known P_H value to another is noted. The P_H is determined by comparison with standard buffer solutions having the same concentration of indicator and contained in tubes of the same dimensions as the tube with the respiring material. After several closely agreeing determinations have been made, a solution of the reagent is substituted for the water and the effect noted. In this way the times taken to do the same amount of work are compared: that is, a measure of the reaction velocities is obtained. The normal rate of respiration is taken as the reciprocal of the time required to effect the standard change of P_H in water alone, and the rate under the influence of the reagent is expressed as per cent. of the normal.

Owing to the known injurious effect upon organisms of prolonged exposure to distilled water, tap-water is frequently used in these experiments. As a preliminary to a study of the effect of the hydrogen-ion concentration of the medium on the respiration of Wheat seedlings, the writer has made an investigation of the buffer effects of tap-water which brings out some points of interest.

Method. Water from the Edinburgh main supply was boiled in a 'Resistance' glass flask until free from carbon dioxide. The flask was closed with a clean stopper while still boiling, and allowed to cool to room temperature before being used. The temperature at which the experiments were performed was 16° c. ± 1. This boiled water was titrated with a standard solution of carbon dioxide, prepared as follows: distilled water was charged with carbon dioxide under pressure in a 'Sparklet' siphon; 10 c.c. of this solution were mixed with 30 c.c. distilled water. This standard solution was approximately saturated (a saturated solution of CO_2 at 25° C. has a P_H of 4·8) and showed a P_H of 5 ± 0·05 with methyl red, and did not vary appreciably in four hours' exposure to the laboratory air in an open beaker. As each determination occupied about twelve minutes, the CO_2 solution may be assumed to be constant for that period. With a pipette of fine bore, graduated to 0·01 c.c., and provided with a rubber bulb, it was possible to drop accurately 0·05 c.c.

10 c.c. of the boiled tap-water were taken in a Jena glass test-tube ($4'' \times \frac{1}{2}''$) fitted with rubber tubing at the mouth to enable it to be clamped off easily. Three drops each of phenol red (1 in 10,000) and thymol blue (1 in 1,000) were added, and the resulting colour matched with a set of borate buffers made up according to Palitzsch's directions (2). These gave a range of P_H values from 9·24 to 6·77, and with the above mixture of indicators a well-graded and easily read series of colours was obtained. The boiled tap-water gave a very constant upper limit of P_H 9·24. One drop of the CO_2 solution was added, and the tube at once clamped off and quickly inverted. A small bubble of air included acted as stirrer. (Any error introduced in this way was a constant one.) This was repeated until the next (more acid) buffer standard in the series was matched, and the procedure continued until P_H 6·77 was reached

In order to discount as far as possible variations in the salt-content of the tap-water due to dilution by rain, &c., sets of experiments were made over a period of four weeks. There appeared to be no substantial variation from this cause.



Titration curve of boiled tap-water with carbonic acid of P_H 5 ± 0·05. The curve represents the mean of nine experiments. Probable error of the mean, less than 2 per cent. of the mean, except for points corresponding to P_H 8·69 and 8·08, where it is 5 per cent. of the mean. Indicators: thymol blue (1 in 1,000) and phenol red (1 in 10,000), three drops of each to 10 c.c. water. Temperature 16° C. ± 1.

The results may be expressed by the above curve, which is constructed from the mean of nine experiments. It will be seen that the buffer action is least

between P_H 8·98–P_H 7·88, and that the portions of the curve between P_H 9·24–8·98 and P_H 7·88–6·77 have the same slope. It follows that if the same P_H interval is used as standard in all measurements of respiration by this method the buffer action can be disregarded, provided that the same water is used for making up all the reagents: in which case only are all the experiments strictly comparable. If, however, it is intended to test the effect of varying hydrogen-ion concentrations on respiration, care must be taken to choose P_H intervals which represent equal amounts of work done (that is, CO_2 evolved). These can readily be determined from the titration curve. The particular curve presented was developed to fill a practical need, and cannot be regarded as directly applicable to tap-water in other towns, but the method described is offered as simple, practical, and reasonably accurate.

It is obvious that the same method can be used to determine the additional buffer action of reagents whose effect on respiration is being studied. In cases where there is marked buffer action it is necessary to adjust the standard interval to be used in determining the normal, so that the amount of CO_2 required to produce an appreciable change in the P_H of the reagent shall be the same as the normal amount. For example, if it requires 0·05 c.c. of the standard CO_2 solution to change the P^H of 10 c.c. of tap-water from 7·60 to 7·36, and 0·5 c.c. CO_2 solution to change the P_H of 10 c.c. of the reagent by the same amount, then the standard must be taken as the change produced in 10 c.c. tap-water by 0·5 c.c. CO_2 solution (in this particular case, P_H 8·51–7·24).

Precautions. All glass used must be of the best quality. Pyrex glass is the best for all indicator work, but where unobtainable Jena glass makes a reliable substitute. All the glass used in these experiments was carefully cleaned with nitric and chromic acids, then boiled in three changes of distilled water, and finally rinsed with alcohol. The rubber tubing used for closing the titration tube was boiled repeatedly and sealed on to the glass with fresh unused paraffin wax (m.p. 52°). The colours were matched by daylight (north light) against an unglazed white paper.

Conclusions. Tap-water shows a decided buffer action when titrated against a standard solution of carbonic acid.

That this relation can be expressed in the form of a titration curve from which the change in hydrogen-ion concentration produced by equal amounts of CO_2 (produced in respiration or otherwise), at any point on the curve, can be deduced.

The writer wishes to express her thanks to Professor W. Wright Smith, and to Dr. R. J. D. Graham, of the Royal Botanic Garden, for their kindness in providing facilities for these experiments.

EDITH PHILIP SMITH.

THE ROYAL BOTANIC GARDEN,
EDINBURGH.

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THE WATER-MOULD THRAUSTOTHECA FOUND IN FORMOSA.— Although the Saprolegniaceae have been collected extensively, the genus *Thraustotheca*, comprising the single species *T. clavata*, (de B.) Humph. (= *Dictyuchus clavatus*, de B.), has been found but rarely. After being discovered by de Bary near Strasburg, Germany, in 1880, the fungus was reported from Hamburg by von Minden in 1911, and later was found in the United States in North Carolina in 1911 by Coker and Hyman, and in Massachusetts in 1914 by the writer. In a discussion of its history, the writer [1] listed these as the only recorded findings of *Thraustotheca*. Later, however, he encountered an article on 'Paddy Seedling Decay in Formosa' by Kaneyoshi Sawada, written in Japanese, but indicating by Latin references to *Dictyuchus clavatus* (*Thraustotheca*) and by figures of the unmistakable sporangia that the species had been found in Formosa also. This paper, a detailed report of eighty-four pages with ten excellent plates, appeared in 1912 as Special Bulletin 3 of the Agricultural Experiment Station of the Government of Formosa. Unfortunately, however, it remained unnoticed outside of Japan, because it was written entirely in Japanese with no summary or abstract in a more generally understood language. Mr. Sawada, however, has been kind enough to furnish the writer with a somewhat abridged English translation of his interesting article, and from this, supplemented by additional translations of critical points made by Japanese friends, the following information on *Thraustotheca* in Formosa is derived. During his detailed study of *Achlya prolifera*, (Nees) de Bary, as the cause of a serious decay of rice seedlings in Formosa, Mr. Sawada encountered other water-fungi, most of them growing as saprophytes on organic remains in the inundated seed-beds. Among these he found *Thraustotheca* growing on dead shrimp in the water of a seed-bed in the nursery at Daimokku. The material thus collected lacked oogonia or oospores, but showed the characteristic clavate to obovate, sympodially renewed sporangia which Sawada describes (p. 74) and figures (Plate X, Figs. 15–22). He over-emphasizes somewhat the delicacy of the sporangium wall as 'very thin compared to other species', and his statements, first that the 'sporangium is rounded at the tip, lacking an apical papilla', and later that 'sometimes the spores are protruded slowly from the apical opening of the sporangium as in *Saprolegnia* and *Achlya*', leave one in doubt as to whether he was quite clear on this important point. Nevertheless, from his statement that 'when ripe the spore mass is protruded at any place through the sporangium wall, separates gradually and scatters in the water' it is

[1] Ann. Bot., 1918, vol. xxxii, pp. 155–73, Pls. IV and V. The writer wishes to take the opportunity of correcting a most unfortunate error in this paper. On p. 170 the first sentence of the summary should read 'spore limitation' not 'spore liberation'. The uncorrected sentence is, of course, in direct contradiction to that of the next paragraph of the summary and to the description in the body of the paper.

obvious that he must have witnessed this interesting process, a method of sporangium dehiscence which is unique among the Saprolegniales, and which the writer, two years later, described in detail for the first time. Although Sawada's account of *Thraustotheca* is merely a one-page note incidental to his more intensive study of *Achlya prolifera*, it is none the less of great interest to mycologists as the first report of this rare fungus from the Orient, a region that will inevitably yield many remarkable water-moulds when more intensively studied.

WILLIAM H. WESTON, Jr.

Department of Cryptogamic Botany,
Harvard University.

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PROFESSOR OF PLANT PHYSIOLOGY AND PATHOLOGY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

AND

R. THAXTER, M.A., PH.D.

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PROFESSOR OF BOTANY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

F. W. OLIVER, M.A., D.Sc., F.R.S.

QUAIN PROFESSOR OF BOTANY, UNIVERSITY COLLEGE, LONDON

AND OTHER BOTANISTS

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Studies in the Phylogeny of the Filicales.

VIII. On Loxsoma and Loxsomopsis.

BY

F. O. BOWER.

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BOTANICAL

With six Figures in the Text.

LOXSOMA Cunninghamii, R. Br., is a Fern which has always commanded attention from the time of its first discovery by Allen Cunningham.[1] Its endemic occurrence in New Zealand, together with its peculiarities of structure, marked it out for over half a century as the sole living representative of a distinct family of the Loxsomaceae, and indicated for it a problematic position in the system. It appeared to be a solitary surviving, synthetic type. A relatively primitive position was indicated for it in some degree by its habit, and also by its anatomy with solenostelic rhizome and undivided leaf-trace, now fully known by the work of Gwynne-Vaughan[2] and of McLean Thompson;[3] by its investiture of hairs, without any flattened scales: but more particularly by the position and characters of its sori and sporangia.

The sorus is strictly marginal in position, with a cup-like indusium surrounding the cylindrical receptacle, which bears the sporangia in basipetal sequence. These characters suggested a relationship to the Hymenophyllaceae; but on the other hand the facts would also countenance comparison with *Thyrsopteris*. The sporangia are, however, distinctive from either, for they alone among those of gradate Ferns dehisce along a median plane, while only a fraction of the oblique annulus is indurated; the rest of it consists of thin-walled cells, which nevertheless are still to be recognized as a continuous oblique ring. This endemic New Zealand species remained thus for nearly sixty years an isolated type.

In 1904 a closely related Fern, discovered by Werckle and Brune in Costa Rica, was described by Christ under the name of *Loxsomopsis costaricensis*, Christ.[4] This discovery was quickly followed by others, viz. *L. Lehmannii*, Hier,[5] from Ecuador, and *L. notabilis*, Slosson, collected in

[1] Hooker: Comp. to Bot. Mag., 366, 1836.
[2] Ann. of Bot., vol. xv, p. 71. [3] Trans. Roy. Soc., Edin., vol. lii, p. 715.
[4] Bull. l'Herbier Boissier, ii, 4, p. 399, tom. 1. [5] Engler's Jahrb., vol. xxxiv, p. 435, 1904.

[Annals of Botany, Vol. XXXVII. No. CXLVII. July, 1923.]

1902 near Apolo in Bolivia at a level of 6,000 feet by Mr. R. S. Williams, and described by Miss Slosson.[1] Thus the new genus *Loxsomopsis* appears to be widely spread in Central America, a habitat far removed from its nearest congener *Loxsoma*. Small portions of a dry specimen of the last-named species, together with photographs, having been most kindly sent to me by the officials of the Smithsonian Institution, Washington, B.C., I am able to contribute some notes on this interesting Fern from personal observation. These, assisted by the description and drawings already published by Miss Slosson, may form a basis for further discussion of the relations of the genera *Loxsoma* and *Loxsomopsis*.

L. notabilis is a stately Fern of Bracken-like habit, its rather slim fronds rising to a height of as much as eight feet: this is a much greater height than that of *Loxsoma*, though the diameter of the rhizome is less. The general characters of the leaf resemble those of *Loxsoma*, but with minor differences of outline, surface, and vestiture. The sori are constructed on the same gradate plan. They are marginal on the ends of anadromic vein-branches, and are curved strongly downwards. When ripe the sporangia project from the cup-like indusium, owing, as in *Loxsoma*, to intercalary lengthening of the base of the receptacle (Fig. 1).

The hairs are characteristic. The rhizome is closely invested by stiff bristles. The upper part of each is formed of a simple chain of cells. Passing downwards, this widens conically towards its base, with numerous cell-divisions, both longitudinal and transverse. A turgid insertion is thus produced. It is not a flattened scale, but the base of each hair stands out from the surface as a pear-shaped boss. The size may vary even in closely grouped hairs (Fig. 2). This is essentially what is found also in *Loxsoma*. The lower surface of the lamina, on the other hand, bears numerous soft curved hairs without any basal swelling (Fig. 3).

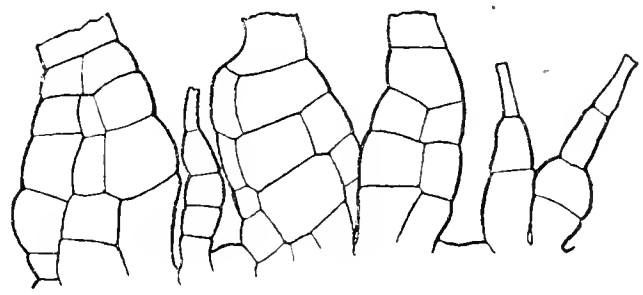
The rhizome is cylindrical and rather thin, being about 3·5 mm. in diameter, which is odd, seeing that the frond is so tall. The cortex is much narrower than in *Loxsoma*, and surrounds a solenostele in all essentials resembling that typical example of solenostely. The departure of the leaf-trace was not observed, but sections of the rachis above the second pair of pinnae show a structure like that observed by Gwynne-Vaughan in *Loxsoma*.[2] It is an undivided meristele, and presumably the leaf-trace itself is also undivided. Thus the similarity of the vegetative region of *Loxsomopsis* in form and structure to that of *Loxsoma* amply bears out the close relationship of the two genera recognized by Christ, and indicates for both a relatively primitive position.

This is further established by comparison of the sori as a whole, as may be seen from Fig. 1. The elongating receptacle bears the sporangia in

[1] Bull. Torrey Club, vol. xxxix, p. 285, Pl. XXIII, 1904.
[2] Ann. of Bot., vol. xv, Pl. III, Figs. 8 and 7 *d*.

gradate sequence interspersed with curved hairs of the same nature as those seen in *Loxsoma*.[1] The general likeness of the sori makes only the more striking the difference of their sporangia both in form and as regards dehiscence. Their form in *Loxsomopsis* is pear-shaped, though less markedly so than in *Loxsoma*. The stalk is short, and consists of about six rows of cells (Fig. 4). The basal face is here less convex and smaller in proportion, but the distal face is larger and flatter than in *Loxsoma*. Accord-

1



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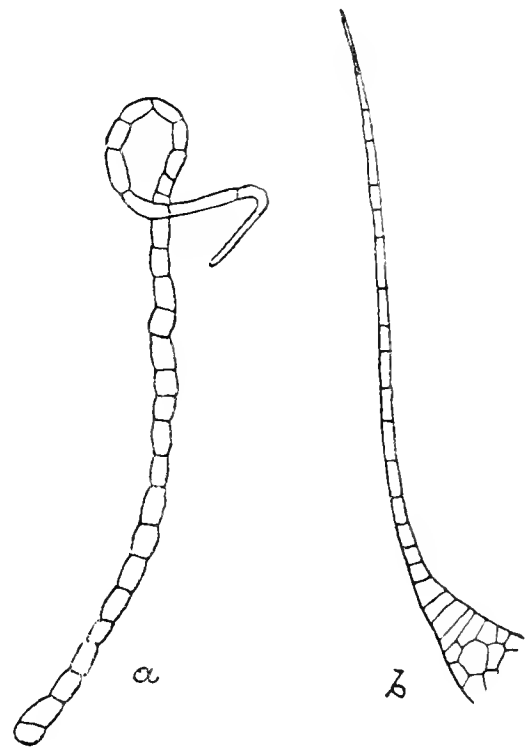


3

FIG. 1. Part of pinna of *Loxsomopsis notabilis* seen from below, showing two pinnules each with a sorus arising from an anadromous vein: in position and structure they closely resemble those of *Loxsoma*. × 10.

FIG. 2. Bases of hairs of *Loxsomopsis notabilis* of different structure, in juxtaposition on surface of rhizome. The ends of the hairs were broken off short in preparation. × 100.

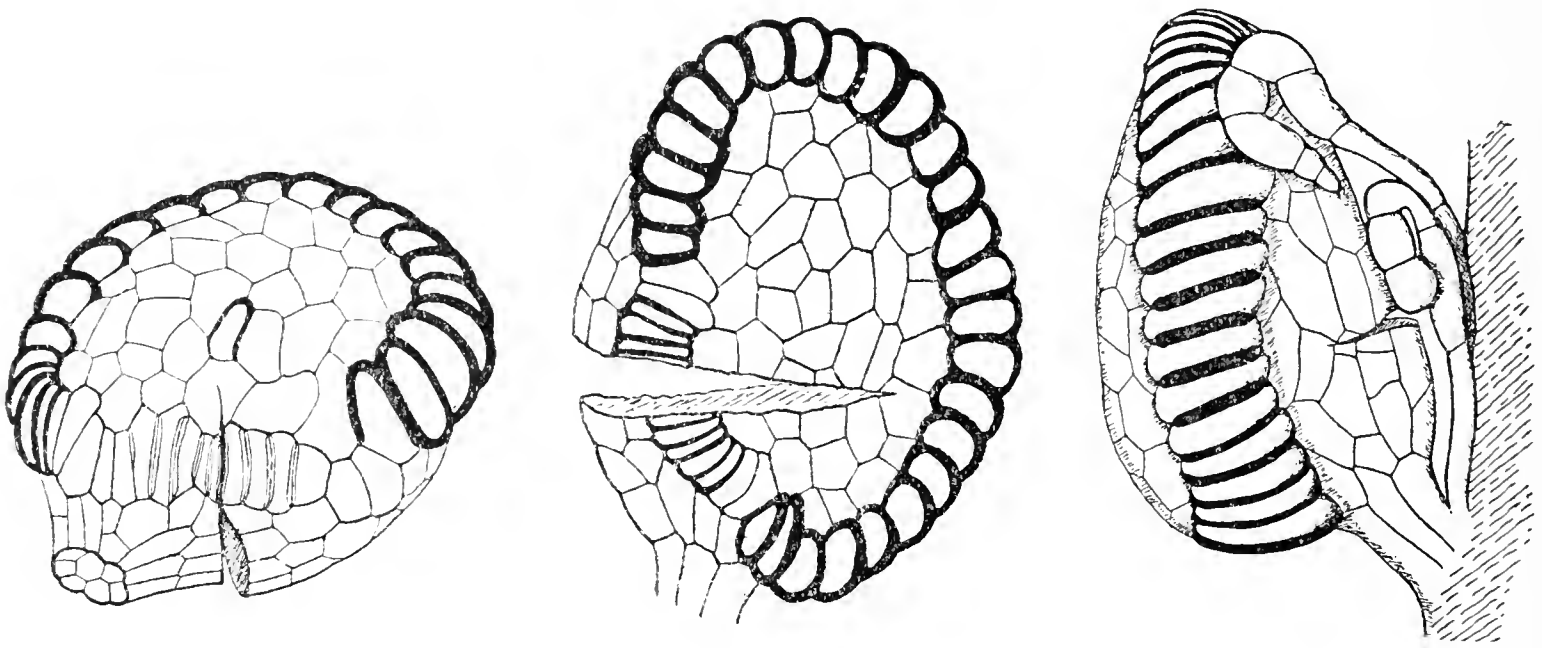
FIG. 3. *a* = soft hair from leaf. *b* = stiff bristle with enlarged base from the rhizome. After M. Slosson.

ingly the annulus, which here also may be recognized as a complete ring, appears nearly vertical. Its cells are indurated round fully three-quarters of the whole ring. The number of its cells is about 40, or less, and about twelve of these in an obliquely lateral position are relatively narrow and thin walled. The actual rupture takes place about the middle of a group of eight cells which constitute the stomium.

The position of the stomium and of the slit of dehiscence is lateral.

[1] See Bower: The Ferns, Cambridge Press, vol. i, 1923, Fig. 206.

lying obliquely below the equator of the sporangium ; but, as shown by comparison of Figs. 4, 5, 6, it may be either right or left of the stalk. I do not remember any previously recorded example of this. The fact has an interest in the general theory of the annulus, and of its relation to the whole sporangium. Clearly where the annulus is vertical, as in the Polypodiaceae, the distal face of the sporangium can only be distinguished from the proximal by a careful analysis. In the sporangia of *Dryopteris* this is possible, but in most of the Polypodiaceae it would be at least extremely difficult. Where as in the Gradatae the annulus is oblique, and consequently the distal and proximal faces are really distinguished, this difficulty does not arise. Nevertheless, there is in them, I think, no record hitherto of the



4 5 6

FIG. 4. Sporangium of *Loxsomopsis notabilis*, presenting its distal face, in the middle of which one cell appears indurated. × 80.

FIG. 5. Sporangium with stalk of several rows probably as in *Dicksonia*, but short conical in form as in *Loxsoma*. 39 cells of annulus. *Loxsoma* has approximately same number. × 80.

FIG. 6. *Loxsomopsis notabilis*. Sporangium attached to the receptacle, with two hairs. The distal face of the sporangium is to the left, the proximal to the right. The stomium and slit of dehiscence have been on the side remote from the observer. × 80.

fact that the dehiscence may be either right or left in the same Fern. Comparison of Figs. 4, 5, 6, puts the fact beyond doubt. In Figs. 5 and 6 the dehiscence is on the left-hand side of the distal face as it would be seen in surface view ; in Fig. 4 it is on the right. This variability of position will be found interesting in the detailed comparison of the sporangia of *Loxsoma* and *Loxsomopsis*. It is difficult to make correct spore-enumerations from dry material. But such observations as have been possible in *L. costaricensis* and in *L. notabilis* point in both species to a number of not less than sixty-four in each normal sporangium.

Comparing the sporangia of *Loxsomopsis* with the well-known structure of those of *Loxsoma*, the general type is the same, with a relatively thick stalk, and an oblique annulus forming a complete ring which marks off the distal or peripheral face (to the left in Fig. 6) from the proximal or central

face (right in Fig. 6). There is, however, a difference in outline, those of *Loxsomopsis* being more flattened, so that the annulus, though continuous as a complete ring, appears to form a margin to the almost discoid capsule. The degree of induration is limited in *Loxsoma* to some twenty cells or less at the side of the ring remote from the stalk, the rest making up about half of the ring not being indurated: in this they are reduced, and appear as a vestigial tract of the annulus. No distinct stomial group is seen in *Loxsoma*.[1] In *Loxsomopsis* nearly three-quarters of the ring is indurated, and an obliquely lateral stomium is well defined. There are two ways in which the sporangium of *Loxsoma* may be interpreted in relation to that of *Loxsomopsis* on the one hand, and that of the Schizaeaceae on the other. The current interpretation is founded on the fact that the sporangium of *Loxsoma* appears to share with the Schizaeaceae and Gleicheniaceae the dehiscence in the median plane, and accordingly it is held to be in that respect relatively primitive. But the state now observed in *Loxsomopsis* suggests another possibility: viz. that *Loxsoma* itself may have been derived from a type with the features now seen in *Loxsomopsis*, and that its median dehiscence may have originated secondarily by rupture of the indurated distal portion of the annulus. That rupture actually occurs in the position which would be most convenient for the shedding of the spores in a sporangium in which half of the annulus is mechanically ineffective. The facts appear insufficient to form a basis for a settled opinion on this point: if the latter view be found correct, one of the most interesting points of *Loxsoma* for comparison with the Simplices which have median dehiscence would fall away.

Such considerations leave still unresolved the difficulty of transition from the types of sporangium with complete annulus and median dehiscence, as seen in the Gleicheniaceae and Schizaeaceae, to those which also have an oblique annulus, but lateral dehiscence. The biological advantage of the change is patent in any crowded sorus. The sporangial type of *Gleichenia* or *Schizaea* demands elbow-room laterally for effective shedding of the spores: room which could not be afforded where the parts are closely packed. The lateral dehiscence, whether by means of an oblique or a vertical annulus, allows of the distal part of the ring being everted and the spores being shed distally. This is the natural method for gradate and mixed sori. It may be held that in these the annulus is the correlative of the annulus in the higher Simplices. What we require is evidence of how the transition came about. It seems that the comparison of the Simplices on the one hand with *Loxsoma*, and on the other with *Loxsomopsis*, does not even yet yield decisive evidence. But all that would be required to carry out the change would be the establishment of a region of imperfect induration of the cells of the ring of a more perfect sort than that seen in the Simplices, and a swing of its position to one side or the other.

[1] Compare Ann. of Bot., vol. xxvii, Pl. XXXIV, Figs. 26 *a*, *c*.

On this last point the fact is significant that in *Loxsomopsis notabilis* the stomium may lie either right or left of the stalk (Figs. 4, 6). If such a difference as this be found between the individual sporangia of a single plant, it suggests that there is in them just that sort of plasticity of development of the annulus which would be required to effect the transition from median to lateral dehiscence. It is probable that such a transition has actually occurred more than once in the evolution of the Ferns, and the plasticity above suggested would support this view. A very clear instance is to be found among the Superficiales in *Lophosoria quadripinnata*, Gmel.[1] Here the annulus is of essentially the same type as in *Loxsomopsis*, though with a less highly specialized stomium. It appears probable that while *Loxsoma* and *Loxsomopsis* illustrate the result of transition from the median to a lateral dehiscence in the Marginales, *Lophosoria*, and in the more exact degree *Metaxya*, show it among the Superficiales. The former instance might be traced to such a source as the living Schizaeaceae still illustrate: the latter clearly owe their origin to some such source as the Gleicheniaceae. These considerations appear to me to strengthen the view previously expressed[2] that the Leptosporangiate Ferns progressed from very early times along two parallel lines, the one characterized by marginal, the other by a superficial position of their sori. As already shown elsewhere,[3] the distal position was probably general in the first instance for all Ferns. But as the leaf-blade expanded the marginal sorus slid to the lower surface, and it is probable that this has happened along many distinct phyletic lines, sometimes early, sometimes late in their evolution. In the Superficiales we may believe that it happened early: in the Marginales the marginal position was long retained; but ultimately the transition occurred, as indeed it is foreshadowed in the Schizaeaceae, and was carried out more fully in the Pteroideae.

The effect of the observations on *Loxsomopsis*, imperfect though they are, is to cast a doubt upon the validity of the median dehiscence of the sporangium of *Loxsoma* as a point of comparison with the Schizaeaceae. But nevertheless these two related genera will retain their interest as synthetic types of clearly primitive nature. They occupy in the marginal series of Ferns a position between the Schizaeaceae and the Dicksonioid-Davallioid-Pterid sequence: and this is comparable to that occupied by *Lophosoria* and *Metaxya* in the superficial series, between the Gleicheniaceae and the Cyatheoid-Dipterid-Dryopterid sequence.

[1] Ann. of Bot., vol. xxvi, Pl. XXXV, Figs. 17–20.

[2] Ibid., vol. xxvii, p. 470.

[3] The Ferns, Cambridge Press, vol. i, 1923, pp. 216–25.

Influence of Ammonium Sulphate on Plant Growth in Nutrient Solutions and its Effect on Hydrogen-ion Concentration and Iron Availability.[1]

BY

LINUS H. JONES AND JOHN W. SHIVE.

With six Figures in the Text.

INTRODUCTION.

THE subject-matter reported in this paper is the result of a careful investigation of the use of ammonium sulphate in a complete nutrient solution as a source of nitrogen for soy beans during the early stages of growth. In so far as possible particular attention has been given to the effect of ammonium sulphate on the growth of this plant, the effect on the solutions in contact with the plant roots, and the influence which this compound has on the availability to the plants of different forms of iron. A previous study with wheat (20) along similar lines has been carried out, though not so completely. The methods of solution culture followed were those of Tottingham (37) and Shive (36), and these need not be considered here except as found necessary to explain the methods and conditions under which the various experiments have been performed.

The ammonium sulphate was substituted for potassium nitrate in twenty representative solutions of the eighty-four comprising the Tottingham series, and run in parallel with the same solutions unmodified, all having a total osmotic concentration value of one atmosphere. At the end of each interval between two successive solution renewals the hydrogen-ion concentrations were determined, and it was found that during the early stages of growth the plant roots in contact with the solutions containing ammonium sulphate cause these solutions to become more acid in reaction; other solutions become less acid, as many investigators have previously reported.

Greater differences in plant growth are to be found with different forms of iron supplied to the plants in these solutions than with any other factor.

[1] Paper No. 115 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology. This paper will appear in Rutgers College Studies, vol. i.

Iron supplied in small quantities, 0·83 milligram per litre, in the form of ferric phosphate, does not appear to be available for the plants growing in the Tottingham solutions, but is sufficient to meet the requirements of the plants growing in the solutions containing ammonium sulphate. Ferrous sulphate, on the other hand, produces excellent growth in the Tottingham solutions, but when it is introduced into a solution containing ammonium sulphate it brings about a condition which is very toxic to plants. It was therefore necessary to work out the proper form and amounts of iron to be supplied to each series of cultures so that this element could not become a limiting factor for growth. As this was not done for each separate solution used, only general conclusions may be drawn. It must be emphasized that the results presented in this paper for soy beans may not wholly agree with those for wheat previously reported, since these are two very different types of indicator plants.

A review of the literature shows that the substitution of ammonium sulphate, either partially or wholly, for nitrate nitrogen is not a new procedure. Thus Lehmann (27) concluded that some plants require nitrate nitrogen for their normal development, others require ammonium nitrogen during the first half of their growth period and nitrate nitrogen during the last half of the life cycle.

Hutchinson and Miller (17, 18) obtained good growth with wheat and pea plants grown in a nutrient solution with ammonium sulphate as the source of nitrogen in such a way that nitrification could not occur, showing that the nitrogen was derived from the ammonium sulphate as ammonium and not as nitrate. They conclude that 'agricultural plants of various kinds can produce normal growth when supplied with nitrogen in the form of ammonium salts under conditions which exclude the possibility of nitrification'.

Other investigators interpret their data to the effect that the ammonium-ion is directly absorbed by the plant, but the possibilities of nitrification were not entirely removed.

There is one crop which apparently requires the ammonium-ion, and this is rice. Kellner (23) found that rice in nutrient solutions did better in the early stages with its nitrogen supplied in the ammonium form. Later on nitrate is better, but it does best with a combination of the two forms of nitrogen. Nagaoka (29) recognized the superiority of ammonium salts over the nitrate forms for rice. Krauss (25, 26), Daikuhara (4), Kelley (21, 22), Trelease (39) with his co-workers Paulino (40) and Jurado (41) are other investigators who have found ammonium salts the best source of nitrogen for rice.

Wolkoff (43) has successfully used ammonium sulphate with nitrate in a four-salt solution, as have Espino (6) and Jones and Shive (20).

The following experiments were undertaken for the purpose of studying

more closely and under controlled conditions the effect of the ammonium-ion on the plants, on the reaction of the nutrient medium, and on the availability of other nutrient elements, especially that of iron.

Methods of Procedure.

The experimental work of this study was carried out with nutrient solutions as the culture media. Two series of solutions were used, each comprising twenty cultures. In the first series twenty representative solutions were chosen from the eighty-four of Tottingham's (37) complete series. The twenty solutions selected are uniformly distributed throughout the series, and are designated by the culture numbers referring to the positions which they occupy on the four-co-ordinate diagrammatic scheme employed by Tottingham. The second series was like the first in every respect, except that ammonium sulphate in equal osmotic concentrations was substituted for the potassium nitrate in the Tottingham solutions of the first series. Two control solutions serving as standards for comparison were added to each experiment. These consisted of Tottingham's best solution for wheat, number $T_3R_1C_4$ with a total osmotic concentration of 2·5 atmospheres, and Shive's (36) best solution for wheat, number R_5C_2 with a total osmotic concentration of 1·75 atmospheres. The latter contains no potassium nitrate. Thus any marked differences in the response of the plants towards the nutrient media in the corresponding cultures of the two series compared with the controls could be attributed to the influence of the ammonium sulphate upon the plants either directly or indirectly, assuming the cultures to be subjected alike to all other experimental conditions.

Baker's analysed salts were used to prepare the half-molecular stock solutions from which the culture solutions were made up. Table I gives the numbers of the cultures, which correspond to the numbers designating the solutions selected from the Tottingham series, and the partial volume-molecular concentrations of the salts as they occurred in the solutions of the two series to give a calculated total osmotic concentration value of one atmosphere. Cryoscopic determinations showed that this concentration was closely approximated in the solutions.

The essential element iron was supplied to all the cultures in equivalent amounts either in the form of the so-called insoluble ferric phosphate or the soluble ferrous sulphate. These two salts contain no anion different from those provided by the four main salts which are contained in each solution. These two sources of iron were used to determine which was the more efficient form of iron in the solutions of the two types. The ferric phosphate was supplied as a suspension in water from a stock supply prepared as described in a previous publication (19), and the ferrous sulphate was added in the form of a freshly prepared aqueous solution. The latter does not precipitate so rapidly nor so completely from the culture solutions here used

as do other forms of soluble iron. By direct qualitative tests it was found that the iron added to the nutrient solutions in this soluble form was not all precipitated seven days after it had been added. The precipitation of this soluble iron is attended with a slight increase in the H-ion concentration of the culture solution.

TABLE I.

Description of solutions used.

Solution. No.	*Partial volume-molecular concentrations.*[1] *Tottingham series (A).*				*Ammonium-sulphate series (B).*
	KNO_3.	KH_2PO_4.	$Ca(NO_3)_2$.	$MgSO_4$.	$(NH_4)_2SO_4$.
$T_1R_1C_1$	0.0020	0.00211	0.00146	0.01659	0.0014
C_3	0.0020	0.00211	0.00438	0.01185	0.0014
C_5	0.0020	0.00211	0.00730	0.00711	0.0014
C_7	0.0020	0.00211	0.01022	0.00237	0.0014
R_3C_1	0.0060	0.00211	0.00146	0.01185	0.0042
C_3	0.0060	0.00211	0.00438	0.00711	0.0042
C_5	0.0060	0.00211	0.00730	0.00237	0.0042
R_5C_1	0.0100	0.00211	0.00146	0.00711	0.0070
C_3	0.0100	0.00211	0.00438	0.00237	0.0070
R_7C_1	0.0140	0.00211	0.00146	0.00237	0.0098
$T_3R_1C_1$	0.0020	0.00633	0.00146	0.01185	0.0014
C_3	0.0020	0.00633	0.00438	0.00711	0.0014
C_5	0.0020	0.00633	0.00730	0.00237	0.0014
R_3C_1	0.0060	0.00633	0.00146	0.00711	0.0042
C_3	0.0060	0.00633	0.00438	0.00237	0.0042
R_5C_1	0.0100	0.00633	0.00146	0.00237	0.0070
$T_5R_1C_1$	0.0020	0.01055	0.00146	0.00711	0.0014
C_3	0.0020	0.01055	0.00438	0.00237	0.0014
R_3C_1	0.0060	0.01055	0.00146	0.00237	0.0042
$T_7R_1C_1$	0.0020	0.01477	0.00146	0.00237	0.0014

The Edna variety of soy bean, *Soja max*, was used throughout the different experiments. The seeds were germinated either in washed sand or sphagnum moss. When germinated in the sand it was necessary to incinerate the roots to obtain their dry weights, the loss of weight upon incineration being taken as the weight of the roots. Uniform seedlings were selected when the cotyledons were opened sufficiently to determine the presence and uniformity of the unopened plumule. Three seedlings were comprised in each culture and were mounted in the double-piece paraffined cork stoppers as devised by Tottingham. These were of proper size to fit the quart fruit jars of colourless glass which were used as solution containers. Manila paper cylindrical shells, black within and light on the outside like those described by Shive (36), were used to exclude light from the cultures and prevent heat absorption.

The solutions were renewed regularly at intervals of three and one-half to four days. After each interval the solutions which had been used were tested for their hydrogen-ion concentrations. This was done by

[1] Total osmotic concentration value of each solution, 1 atmosphere.

means of the colorimetric method, using the indicators recommended by Clark and Lubs (2), the double-tube colour standards of Gillespie (10), and the apparatus devised by Van Alstine (42), and these concentrations were recorded in terms of pH values.

The plants were grown in the culture solutions during a period of five or six weeks. At the end of the growth periods of the different experiments the dry weights of the tops and roots were separately obtained by the usual method.

The non-solution environment was made uniform for all the cultures by employing the rotating table (36). Daily records were kept, in so far as this was possible, of the measurements characterizing the aerial conditions.

Experimental Results.

The detailed results of the first two experiments are purposely omitted, as their inclusion would only be indicative of the more conclusive data that are here presented. The first of these experiments consisted in growing the plants for a five-week period in the various solutions of Table I, with iron supplied in the form of an aqueous suspension of ferric phosphate in the amount of 0·83 milligram of iron per litre of nutrient solution. In general appearance the plants of many of the cultures of the Tottingham series (series A) soon showed the chlorotic condition which is characteristic of plants suffering from lack of iron. The plants in the series containing ammonium sulphate (series B) did not present a chlorotic appearance. However, a yellow mottling was present later in both series, obscuring, if present, the chlorotic symptoms of lack of iron. The dry weights of the series containing ammonium sulphate were slightly superior to those of the Tottingham series, and the fact that the solutions containing ammonium sulphate grew more acid in contact with plant roots, while the Tottingham solutions grew less acid, suggests that this slight advantage of the series containing ammonium sulphate is due to the greater availability of the iron from the insoluble ferric phosphate in these solutions which have a tendency to become more acid.

In the second experiment the same amount of iron was supplied in the form of ferrous sulphate in a fresh aqueous solution. There was no chlorosis apparent in either series. About two weeks after the experiment was started many of the plants in both series exhibited a peculiar brown specking, especially on the unfolding new leaves. Later on this was succeeded by a general mottling of the leaves. That this was due to the ferrous sulphate was experimentally determined by setting up a supplementary experiment in which both ferrous sulphate and ferric phosphate were used as sources of iron. The specking and mottling persisted in the ferrous sulphate cultures, but were absent in those cultures receiving iron in the form of ferric phosphate.

The dry weight yield values of this second experiment showed that the cultures of the Tottingham series (series A) were much superior to those of the series containing ammonium sulphate (series B). With the changing of one factor, that of the source of iron, a reversal of the superiority of the two types of solutions was brought about. When ferric phosphate is used as a source of iron, the solutions containing ammonium sulphate can make soluble enough iron from the amounts added to meet the requirements of the plants, but when ferrous sulphate is used in the solution containing ammonium sulphate it gives evidence of a toxic character not present in the Tottingham solutions.

Thus the indications are that the choice of a source of iron for plants in a nutrient solution must be very carefully made, with respect to the chemical composition of such a solution, in order to avoid iron toxicity and still supply sufficient available iron to prevent chlorosis in the plants.

Gris (11) in 1844 was the first to show the necessity of iron for the formation of chlorophyll in plants. The exact manner in which the plant utilizes iron, the most efficient form, and the proper amount to be supplied under a given set of experimental conditions, are yet to be determined. Tottingham and Beck (38) have shown that the response to iron during the early stages of growth by plants is dependent upon the amount of iron stored in the seed. Corson and Bakke (3), employing both ferrous and ferric forms of phosphate as sources of iron in nutrient solutions, found differences in their efficiency, and also differences in the response of different plants.

Jones and Shive (19) with Shive's three-salt solution R_5C_2 (36), and employing different increments of iron in the forms of ferrous sulphate and ferric phosphate, have shown that the insoluble ferric phosphate is not suitable for use with spring wheat in this culture solution. Ferrous sulphate, on the other hand, gave excellent results when supplied in quantities of 0·75 to 3·0 milligrams of iron per litre of nutrient solution. In a later publication (20) it was shown that in a solution containing ammonium sulphate the ferrous form of iron was quite toxic, and the ferric form sufficiently available to prevent chlorosis in the plants.

Gile and Carrero (8, 9), in solution cultures, have shown that the reaction, concentration of the solution, and amount of iron used have a marked influence upon the availability of iron for rice plants. Mazé (28), in a complete nutrient solution with ferrous sulphate (100 milligrams per litre) as a source of iron, found that the presence of ammonium salts of 500 milligrams per litre produced a toxic condition for maize. Hartwell and Pember (13) found ferrous sulphate toxic to barley and rye seedlings in Knop's solution in quantities of five parts per million of iron or over, while Ruprecht (35) in another nutrient solution found ferrous sulphate to be toxic to clover seedlings with four parts per million.

To demonstrate more definitely the effect of these two forms of iron,

ferric phosphate and ferrous sulphate, on the plants grown in the two types of solutions, varying amounts of iron in the two forms were used in an experiment comprising two series of twenty cultures each and two controls. Throughout each series the same solution was used for all the cultures, the cultures of each series differing only in the amounts of iron added. To represent one type Tottingham's solution $T_1R_1C_5$, as given in Table I, was chosen, and, as a representative of the other type, this solution was modified by substituting ammonium sulphate for the potassium nitrate as previously explained (solution $T_1R_1C_5$ of the ammonium sulphate series, Table I). The series in which the Tottingham solution was used will be designated series C, and that in which the modified Tottingham solution was used will be designated series D. Ferric phosphate was supplied to half the cultures in each series in amounts varying from 0·01 mg. to 5·0 mg. of iron per litre of nutrient solution, and to the other half of the cultures in each series iron was supplied in corresponding amounts in the form of ferrous sulphate, but no iron was added to the controls. The culture methods pursued throughout were precisely the same as those previously described. The cultures were conducted during a growth period of thirty-five days.

In the cultures of the Tottingham series C containing iron in the form of ferric phosphate a chlorotic condition appeared within five days after the experiment was started. In this series at harvest time those cultures receiving one milligram of iron or more had recovered. The cultures of the ammonium-sulphate series D receiving the same form of iron (ferric phosphate) were all green and healthy except the one culture receiving the smallest amount of iron (0·01 mg.), which was slightly chlorotic.

In the cultures supplied with ferrous sulphate, specking of the leaves became apparent within ten days, appearing in the Tottingham series C in cultures receiving 0·75 milligram of iron or more, and in the ammonium-sulphate series D in cultures receiving 0·10 milligram of iron or more. No specking was observed in cultures grown in solutions supplied with ferric phosphate. At the time of harvest the plants in the Tottingham solutions supplied with ferrous sulphate were healthy and in a vigorous condition, but in the solutions containing ammonium sulphate supplied with ferrous sulphate as the source of iron, the plants presented a very poor appearance, with yellow, dying, or dead leaves, and this sickly appearance increased in intensity with increase of iron above 0·25 milligram per litre of solution.

At the end of the growth period of thirty-five days the dry weights of the tops and roots were obtained in the usual way. The yields of the cultures of the two series, together with the averages of the hydrogen-ion concentrations in terms of pH values of the solutions obtained at the end of the various growth intervals, are given in Table II. The yield values of tops and roots from the cultures supplied with ferric phosphate as the source of iron are represented graphically in Fig. 1, the upper set of graphs

representing the yields of tops and the lower set the root yields. The dry-weight values as ordinates are here plotted against the amounts of iron in milligrams per litre of solution as abscissas.

TABLE II.

Hydrogen-ion concentrations and dry-weight yields of soy bean tops and roots grown in two types of nutrient solutions supplied with varying amounts of iron in the form of ferric phosphate and ferrous sulphate.

Source of iron.	*Amounts of iron mg. per litre.*	*Series C. (Tottingham solution $T_1R_1C_5$)*			*Series D. (Modified Tottingham solution $T_1R_1C_5$).*		
		Dry weights.		*H-ion concentrations.*	*Dry weights.*		*H-ion concentrations.*
		Tops.	*Roots.*		*Tops.*	*Roots.*	
	Mg.	Grm.	Grm.	pH [1]	Grm.	Grm.	pH
$FePO_4$	0.00	1.6053	0.2795	5.75	3.7769	0.4920	4.53
	0.01	1.7109	0.3561	5.80	4.2117	0.5428	4.53
	0.10	2.4170	0.4715	5.87	4.5662	0.6320	4.44
	0.25	2.2737	0.3734	5.89	4.3901	0.7113	4.64
	0.50	2.6455	0.4768	5.94	4.3715	0.7157	4.67
	0.75	2.6365	0.4188	5.98	4.0922	0.7309	4.67
	1.00	3.5942	0.6101	6.03	4.4193	0.6973	4.73
	1.50	4.0236	0.6963	6.09	4.2285	0.7157	4.62
	2.00	4.3687	0.7880	6.13	4.5789	0.7041	4.75
	3.00	4.3458	0.6555	6.19	4.4721	0.7079	4.59
	5.00	3.7965	0.6611	6.03	4.1796	0.6328	4.63
$FeSO_4$	0.00	1.6053	0.2795	5.75	3.7769	0.4920	4.53
	0.01	2.2793	0.4510	5.85	4.6748	0.7386	4.53
	0.10	4.1461	0.7517	6.24	4.8910	0.6485	4.49
	0.25	4.3252	0.7253	6.23	3.9627	0.4429	4.49
	0.50	4.2039	0.6045	6.20	3.1440	0.3168	4.50
	0.75	3.4741	0.4942	6.15	2.7560	0.3619	4.56
	1.00	3.9358	0.5178	6.14	2.8513	0.3485	4.57
	1.50	3.9546	0.5487	6.13	2.1728	0.2753	4.57
	2.00	3.7229	0.4334	6.08	2.1432	0.2725	4.54
	3.00	3.6916	0.4878	6.07	1.8180	0.2417	4.58
	5.00	3.3245	0.4025	6.01	1.9621	0.3149	4.57

As indicated by the graphs of Fig. 1 the varying amounts of iron in the form of ferric phosphate have a very great influence on the increase of the dry-weight values of the tops and roots in the Tottingham series C. The graphs strikingly show that a maximum growth can be obtained in the ammonium-sulphate series D with a very small amount of iron in this form, and that increasing the amount does not increase the yield. In the case of the Tottingham series C the yields of the cultures do not begin to approach the yields of the ammonium-sulphate series D until 2 milligrams or more of iron are employed. From the results of this experiment it may be stated in general that the presence of ammonium sulphate in a solution, either through its influence on the hydrogen-ion concentration or the possible

[1] These values represent the averages of all the determinations made for each solution at the end of the growth intervals throughout the experiment period. The initial pH value of all the solutions in each series was approximately 4.8.

effect it may have on permeability, makes the iron phosphate so available that the iron requirements of the plants are satisfied with a very small amount of iron in this form. With the Tottingham solutions greater amounts of the ferric phosphate must be added to the solutions to obtain maximum yields. The fact must not be overlooked, however, that different indicator plants may have different iron requirements, that a growth period of thirty-five days is required to bring soy bean plants to the flowering stage, and that during the reproductive and the later growth phases the iron requirements

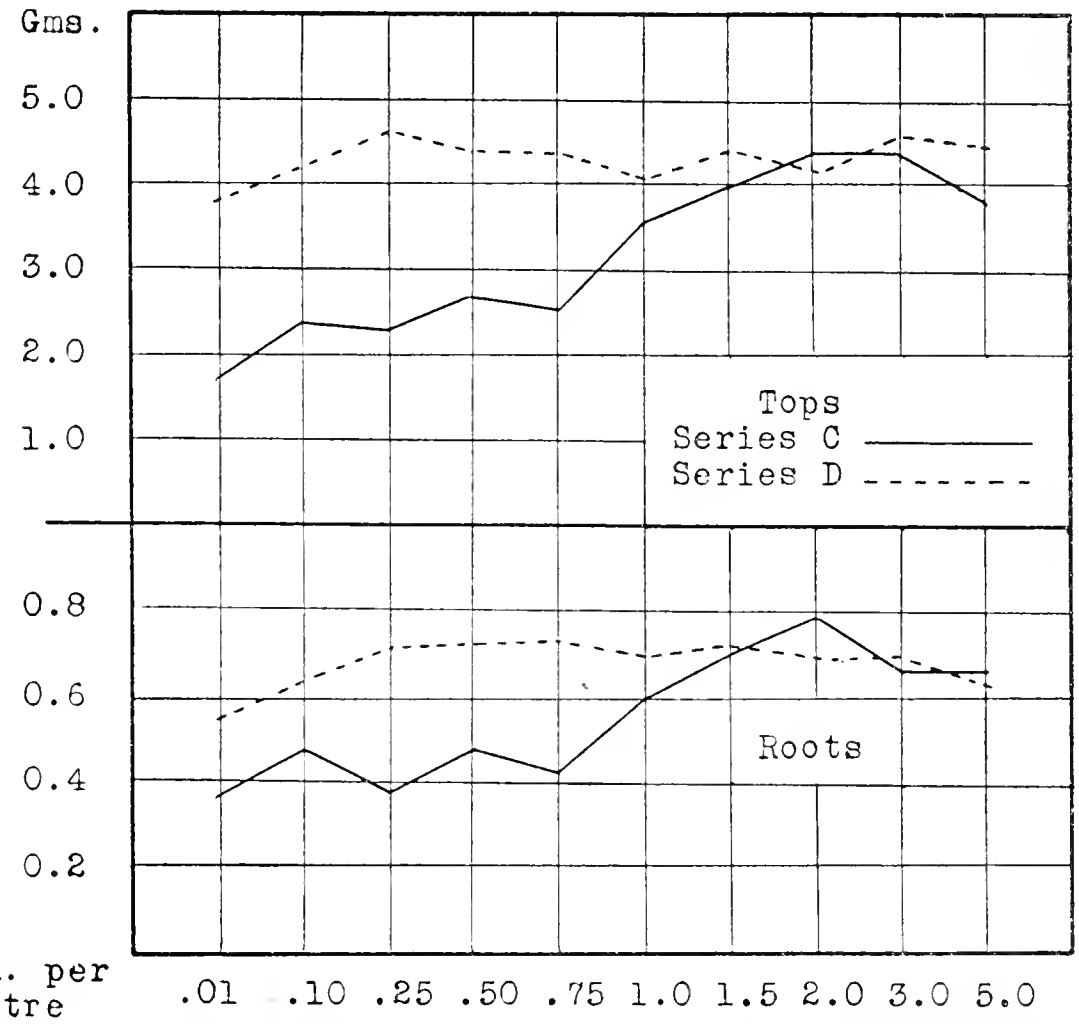


FIG. 1. Graphs of actual yield values of soy bean tops and roots grown in Tottingham's solution $T_1R_1C_5$ (series C), and in the ammonium-sulphate modification of this solution (series D), supplied with varying amounts of iron in the form of ferric phosphate.

of these plants may be entirely different from the demands during the vegetative phases of growth.

The dry-weight yield values from the cultures supplied with ferrous sulphate, as given in Table II, are graphically represented in Fig. 2. It will be observed from this figure and from the data of Table III that ferrous sulphate has a very depressing effect upon the dry weight yields of both tops and roots in the ammonium-sulphate series D. That this is a toxic effect is very evident from the general appearance of the plants, and from the fact that the yields are very much lower than the control in which no iron was supplied. On the other hand, the soluble ferrous sulphate is a very good source of iron for the plants in the solutions of the Tottingham series C, even in very small amounts.

The toxicity which occurs with plants grown in solutions containing

ammonium sulphate when supplied with iron in the form of ferrous sulphate, may be indirectly related to the H-ion concentration of the solutions. As has been shown, the H-ion concentration of the solutions containing ammonium sulphate is usually increased and maintained at a relatively high level during contact with the roots of growing plants, while the H-ion concentration of the Tottingham solutions under similar conditions is rapidly decreased. Thus the solubility and therefore the availability of iron is high in the former and relatively low in the latter. This, together with the possible influence of the ammonium salt on the permeability of the plant

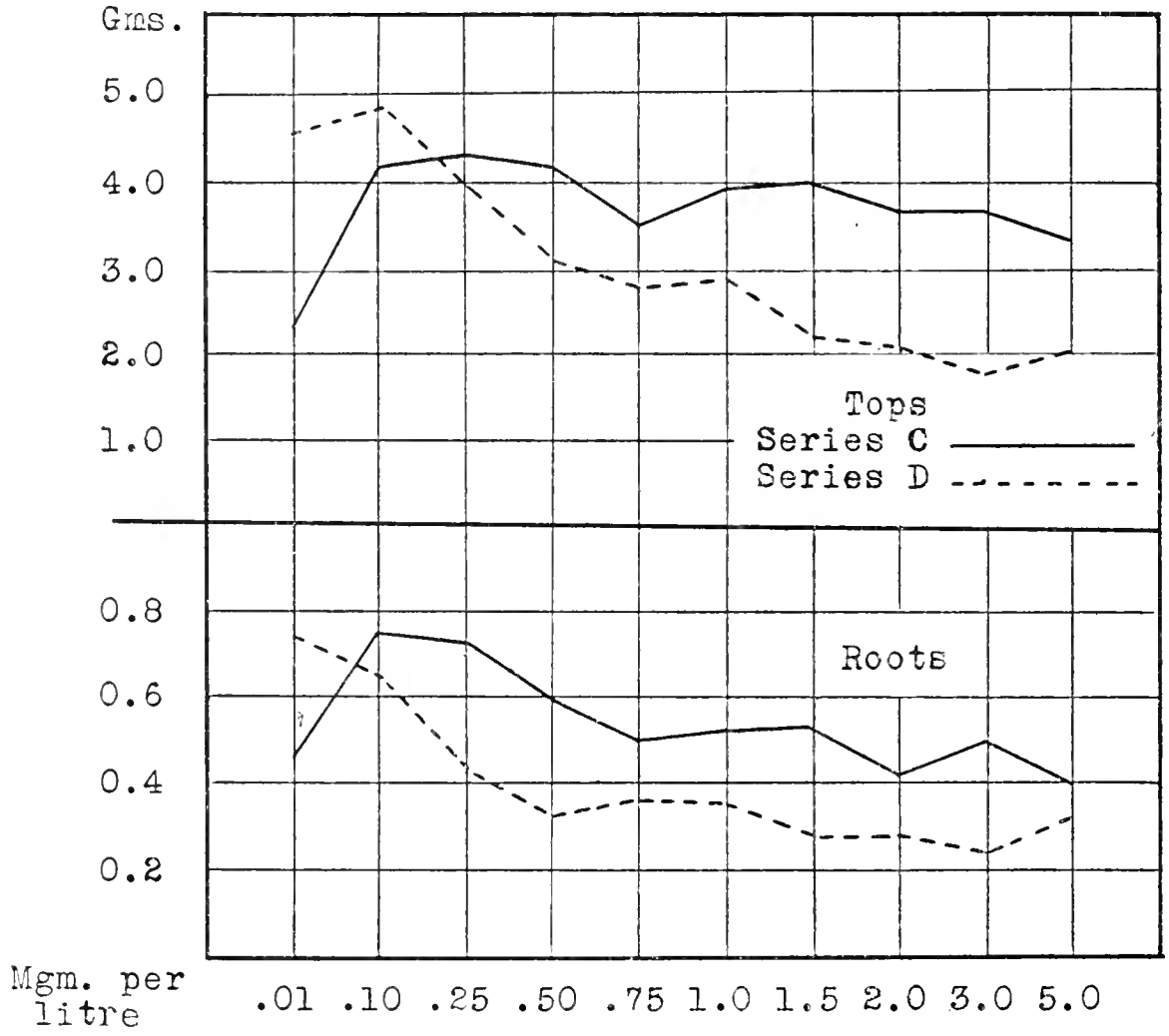


FIG. 2. Graphs of actual yield values of soy bean tops and roots grown in Tottingham's solution $T_1R_1C_5$ (series C), and in the ammonium-sulphate modification of this solution (series D), supplied with the varying amounts of iron in the form of ferrous sulphate.

cells for iron, may readily account for the difference in the behaviour of the plants towards iron in the two types of solutions, and for the toxic effects produced when ferrous sulphate in any but exceedingly low concentrations is used as the source of iron for plants in solutions containing ammonium sulphate.

From a consideration of the data presented in the above experiments it is evident that the form and quantity of iron in a medium for plant growth is a very important factor to be considered in connexion with the growth of plants in solution cultures.

Influence of Salt Proportions on the Yields of Tops and Roots.

This experiment was carried out for the purpose of determining the effect of salt proportions on the growth of soy bean plants in the Tottingham

solutions, and in the modified Tottingham solutions as described in Table I, when supplied with suitable forms of inorganic iron in concentrations which were found in the preceding experiments to be approximately optimal for the growth of these plants. Three series of cultures were conducted simultaneously. The nutrient media used with two of these series, which will be designated series E and series F, consisted of the Tottingham solutions (Table I) supplied with iron in the form of ferric phosphate and ferrous sulphate, respectively, corresponding solutions of the two series being alike in every respect except in the form and amounts of iron used. The third series, which will be designated the ammonium-sulphate series G, was carried out with the modified Tottingham solutions previously described (Table I) Ferric phosphate in quantities of 2 mg. per litre of nutrient solution was added to each solution of series E and series G whenever these were prepared. At the time of each solution renewal, freshly prepared ferrous sulphate in solution form was added to each of the nutrient solutions of series F in very small quantities, sufficiently large, however, to prevent chlorosis in the plants, but not enough to produce serious specking of the leaves in any culture. In the course of the nine solution renewals which were made during the growth period, each culture of series F received a total of approximately 2·5 mg. of iron in the form of ferrous sulphate, while each culture of series E and F received during the same time 18 mg. of iron in the form of ferric phosphate. The record indicating the nature of the environmental conditions to which the cultures were exposed during the growth period is given in Table III.

Table III.

Maximum and minimum temperatures, average daily water loss by evaporation from standard white and black spherical atmometers, and character of days for the experimental period.

Experimental period 1921.		*Air temperature.*		*Average daily evaporation.*[1]			*Radio-evaporation.*[2]			*Number of days.*		
Beginning.	Ending.	Maximum.	Minimum.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Clear.	Partly cloudy.	Cloudy.
		°C.	°C.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.			
July 24	Aug. 29	42·0	13·5	26·1	2·2	16·0	6·1	0·2	3·6	22	9	5

In general, all the cultures appeared quite healthy on the harvest date.

[1] Evaporation was measured by means of the Livingston standard spherical atmometers. Livingston, B. E.: Atmometry and the Porous Cup Atmometer. Plant World, 18, 21–30, 51–74, 95–111, 143–49, 1915. Reprinted, Tucson, 1915.

[2] The values given for radio-evaporation represent the average daily excess of water loss from the standard black spherical atmometer over that from the white.

Slight specking occurred on the leaves of the plants in some of the Tottingham cultures of series F supplied with ferrous sulphate, but this was not at all serious, and after two weeks from the time the cultures were started it did not increase appreciably.

The hydrogen-ion concentrations of the culture solutions were determined at the end of each growth interval between two successive solution renewals. In Table IV are given the initial pH values of the culture solutions, the highest and lowest values obtained during the growth periods, and the average of all the values for each solution of the three series.

TABLE IV.

pH *values of the culture solutions of the Tottingham series (E and F) and the ammonium-sulphate series (G) supplied with iron in the proper form and in sufficient amounts to prevent chlorosis.*

Culture No.	*Tottingham series (E). Source of iron* $FePO_4$. pH *values.*				*Tottingham series (F). Source of iron* $FeSO_4$. pH *values.*				*Ammonium-sulphate series (G). Source of iron* $FePO_4$. pH *values.*			
	Initial.	*Highest.*	*Lowest.*	*Average.*	*Initial.*	*Highest.*	*Lowest.*	*Average.*	*Initial.*	*Highest.*	*Lowest.*	*Average.*
$T_1R_1C_1$	4·9	6·2	5·1	5·75	4·9	6·4	5·1	5·84	4·9	6·0	4·3	5·05
C_3	4·9	6·6	5·2	5·96	4·9	6·6	5·2	5·99	4·9	5·5	4·3	4·75
C_5	4·9	6·6	5·2	6·02	4·9	6·6	5·3	6·21	4·9	5·0	4·3	4·59
C_7	4·9	6·2	5·3	5·75	4·9	6·3	5·3	6·01	4·9	4·7	4·2	4·43
R_3C_1	4·9	6·2	5·3	5·86	4·9	6·4	5·1	5·79	4·9	5·3	4·2	4·58
C_3	4·9	6·6	5·3	5·99	4·9	6·4	5·2	6·05	4·9	5·1	4·1	4·50
C_5	4·9	6·1	5·3	5·78	4·9	6·4	5·3	6·06	4·9	4·6	3·8	4·24
R_5C_1	4·9	6·2	5·2	5·85	4·9	6·5	5·3	5·88	4·9	4·6	4·2	4·33
C_3	4·9	6·2	5·3	5·83	4·9	6·5	5·3	6·08	4·9	4·6	3·8	4·18
R_7C_1	4·9	6·2	5·3	5·80	4·9	6·4	5·3	5·88	4·9	4·6	3·9	4·34
$T_3R_1C_1$	4·8	6·0	4·9	5·54	4·8	6·0	4·9	5·51	4·8	4·6	4·4	4·52
C_3	4·8	6·1	4·9	5·61	4·8	6·0	4·9	5·62	4·8	4·6	4·4	4·53
C_5	4·8	5·8	4·9	5·51	4·8	5·9	4·9	5·59	4·8	4·7	4·1	4·42
R_3C_1	4·8	5·8	4·9	5·50	4·8	6·1	4·9	5·56	4·8	4·7	4·1	4·42
C_3	4·8	5·9	4·9	5·56	4·8	6·2	4·9	5·66	4·8	4·6	3·9	4·31
R_5C_1	4·8	5·9	4·9	5·46	4·8	6·0	4·9	5·55	4·8	4·6	4·0	4·40
$T_5R_1C_1$	4·7	5·8	4·9	5·42	4·7	5·7	4·9	5·41	4·7	4·6	4·4	4·52
C_3	4·7	5·8	4·7	5·42	4·7	5·7	4·9	5·45	4·7	4·6	4·3	4·49
R_3C_1	4·7	5·8	4·9	5·44	4·7	5·7	4·9	5·42	4·7	4·6	4·3	4·47
$T_7R_1C_1$	4·7	5·7	4·9	5·38	4·7	5·6	4·9	5·36	4·7	4·6	4·4	4·55
Shive's R_5C_2					4·5	5·7	4·6	5·30				
Tottingham's $T_3R_1C_4$					4·6	5·7	4·7	5·29				

To bring out clearly the magnitude and direction of the reaction change of the solutions brought about by the action of the plants during the intervals of contact throughout the growth period, the pH values given in the columns of averages in Table IV for each of the three series were plotted to form the graphs of Fig. 3. The data represented by these graphs were plotted according to the descending order of values in series E. These

graphs show clearly the marked increase in the average pH values of the unmodified Tottingham solutions of series E and F, and a considerable decrease in these values for all but one of the solutions containing ammonium sulphate (series G), thus indicating that the effect of the growing plants upon reaction change is directly opposite in the two types of solutions here used. The graphs of series E and F further show that the greatest reaction change occurred in the solutions with low proportions of the mono-potassium phosphate, and that the reaction change was correspondingly less as the concen-

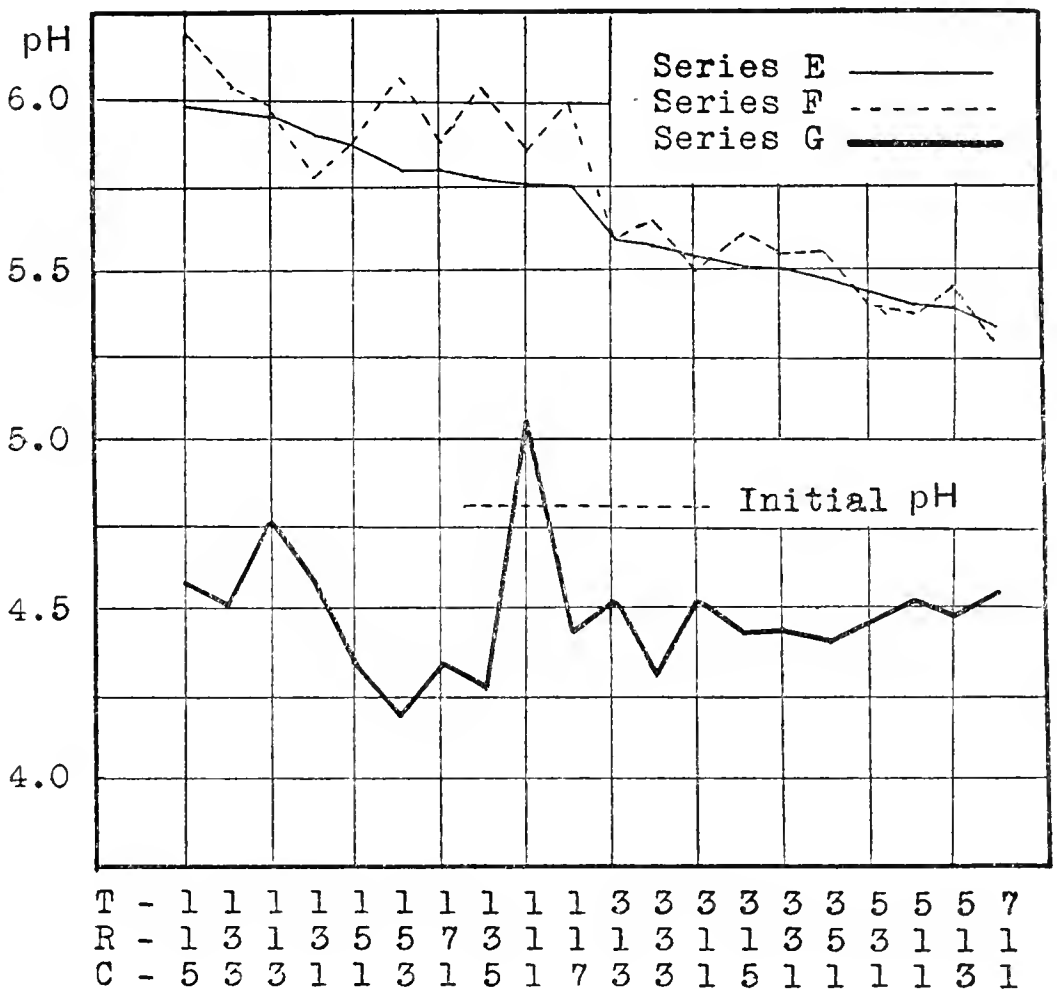


FIG. 3. Graphs of pH values of culture solutions after contact with plant roots during the growth intervals between solution renewals; averages of all tests made during the growth period.

tration of this salt increased. On the other hand, no such relation is shown for the solutions containing ammonium sulphate (series G).

It has been found by a number of investigators that one of the effects of plants upon a nutrient medium containing an ammonium compound is to increase the acidity of the medium. As far back as 1860 this was recognized by Knop (24) in solution cultures, and shortly afterwards was mentioned by Rautenberg and Kühn (34). Among many other workers who have confirmed this observation may be mentioned Mazé (28), Nathansohn (30), Ehrenberg (5), Prianischnikow (33), Nikitinsky (31), Jones and Shive (20), and others. Pantanelli (32) in a series of studies with single salt solutions has shown how the ratio of the anion to the kation changes as one ion is removed by the plant more rapidly than the other. In a similar way Breazeale and Le Clerc (1) showed that this differential ion absorption by plant roots occurs in solutions of potassium chloride and potassium sulphate.

Hall, Miller, and Gimingham (12) ascribe the acidity of the acid soils of the Rothamstead grass plots that have been treated with ammonium salts to the action of various micro-fungi which are able to remove the ammonium-ion from a solution of its salts and set free the acids with which it was combined.

Hoagland (14) made some studies in changes of reaction in nutrient solutions by barley seedlings, and says: 'In general it was found that alkaline solutions decreased markedly in OH-ion concentration, acid solutions decreased slightly in H-ion concentration, while neutral solutions remained practically constant. This must be the result either of the secretion of neutralizing substances by the plant, of chemical reaction with the material of the roots, or of selective absorption of specific ions.' Later (15) with an ammonium chloride solution he found this solution increased in acidity after contact with plant roots. On continuing his studies (16), and applying quantitative chemical methods to the solutions before and after contact with plant roots, he concludes that 'in a complete nutrient solution it is impossible to say exactly what ions and undissociated salts are present before and after absorption by the plant. Such a system, with its various hydrolysable salts, is very complex. The resultant reaction is due to the particular state of equilibrium existing among all these constituents; and while we may determine the H-ion concentration with considerable accuracy, the data at present available do not enable us to determine the exact relations between the different components of the system.'

The plants were harvested, and the dry-weight values of tops and of roots were obtained in the usual way. The numerical data of yields of tops and of roots for each of the three series are presented in Table V in terms of the yields from culture $T_1R_1C_1$ in the respective series taken as unity, but the actual dry-weight yields of this culture are given in parentheses in grammes. The highest five yield values in each series (upper one-fourth) are indicated by bold-face type.

From the data of Table V it will be observed that a large range in relative yield values occurs within each series, which may be attributed in the main to the variations in the relative salt proportions from culture to culture in the respective series, but since the low and medium yields have little that might be of interest or value in this connexion they will not here be considered.

The relative yield values for the five cultures producing high yields of tops and of roots in each series as given in Table V were plotted on the tetrahedral diagrams like that employed by Tottingham (37), but here presented in perspective in somewhat the same manner as was done by Espino (6).

The high yield values of series E, F, and G are represented separately on the diagrams of Figs. 4, 5, and 6 respectively, areas of tops and of roots

of the same series being represented on a single diagram and distinguished by differences in shading. The yields of tops are represented by dotted areas, and those of roots by stippled areas.

TABLE V.

Relative dry-weight yields of soy bean tops and roots from the Tottingham solutions of series E and F and from the modified Tottingham solutions of series G, supplied with suitable forms of iron and in sufficient amounts to prevent chlorosis.

Culture number.	*Series E, source of iron $FePO_4$.*		*Series F, source of iron $FeSO_4$.*		*Series G, source of iron $FePO_4$.*	
	Tops.	*Roots.*	*Tops.*	*Roots.*	*Tops.*	*Roots.*
$T_1R_1C_1$	1·00 (3·1911)	1·00 (0·4561)	1·00 (3·5431)	**1·00** (0·5891)	**1.00** (5·1786)	**1·00** (0·9828)
C_3	**1·39**	**1·23**	**1·30**	**0·96**	**0·87**	**0·62**
C_5	**1·52**	**1·33**	**1·47**	**0·99**	**0·93**	**0·51**
C_7	0·73	0·65	1·07	0·45	0·59	0·20
R_3C_1	0·96	0·98	0·88	0·69	0·59	0·40
C_3	**1·59**	**1·32**	1·10	0·81	0·70	0·35
C_5	0·94	0·76	**1·22**	0·53	0·65	0·23
R_5C_1	0·88	0·86	0·87	0·67	0·50	0·30
C_3	0·91	0·71	0·97	0·50	0·50	0·18
R_7C_1	0·65	0·46	0·65	0·52	0·33	0·37
$T_3R_1C_1$	1·15	0·94	0·93	0·76	0·67	**0·49**
C_3	**1·48**	**1·37**	**1·38**	**1·28**	**0·91**	**0·52**
C_5	0·97	0·64	**1·21**	0·58	**0·75**	0·31
R_3C_1	0·93	0·80	0·99	**0·91**	0.57	0·42
C_3	0·86	0·64	0·98	0·47	0·66	0·23
R_5C_1	0·68	0·56	0·76	0·58	0·44	0·25
$T_5R_1C_1$	1·09	**1·09**	0·84	0·65	0·52	0·42
C_3	**1·17**	0·99	0·96	0·51	0·68	0·26
R_3C_1	1·03	0·92	0·73	0·58	0·45	0·29
$T_7R_1C_1$	1·02	0·95	0·81	0·65	0·47	0·34
Shive's R_5C_2			1·40	1·07		
Tottingham's $T_3R_1C_4$			1·28	1·26		

Examination of the three diagrams graphically representing the high yields from the three series here considered shows that there is much overlapping of the areas of top yields and root yields in each of the three series. In some instances, as in series E, Fig. 4, the areas are practically superimposed. This indicates a very close agreement between tops and roots with respect to the proportions of the salts required to produce high yields.

Comparing the diagrams of Figs. 4 and 5 (series E and F) it will be observed that the areas representing high yields of tops and roots occupy the same general regions at the bases of the several triangles on each diagram. This is to be expected, of course, since the two series are alike in every respect except in the amounts and forms of iron supplied to the cultures, ferric phosphate being the source of iron for the plants of series E and ferrous sulphate for those of series F, in such amounts as were

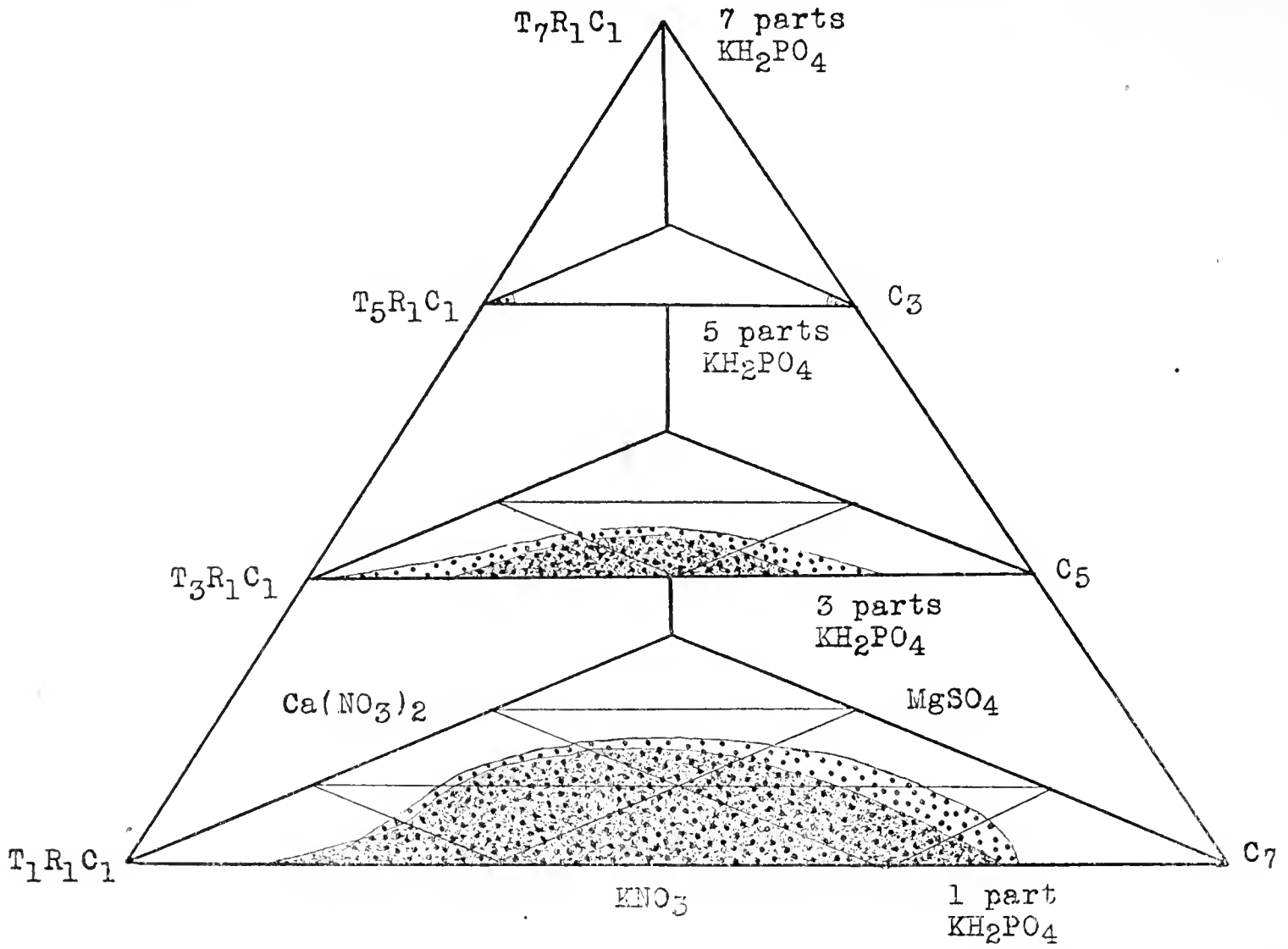


FIG. 4. Distribution of the highest five yields of soy bean tops and roots from the cultures of series E, supplied with ferric phosphate as the source of iron for the plants. Yields of tops represented by dotted areas, those of roots by stippled areas.

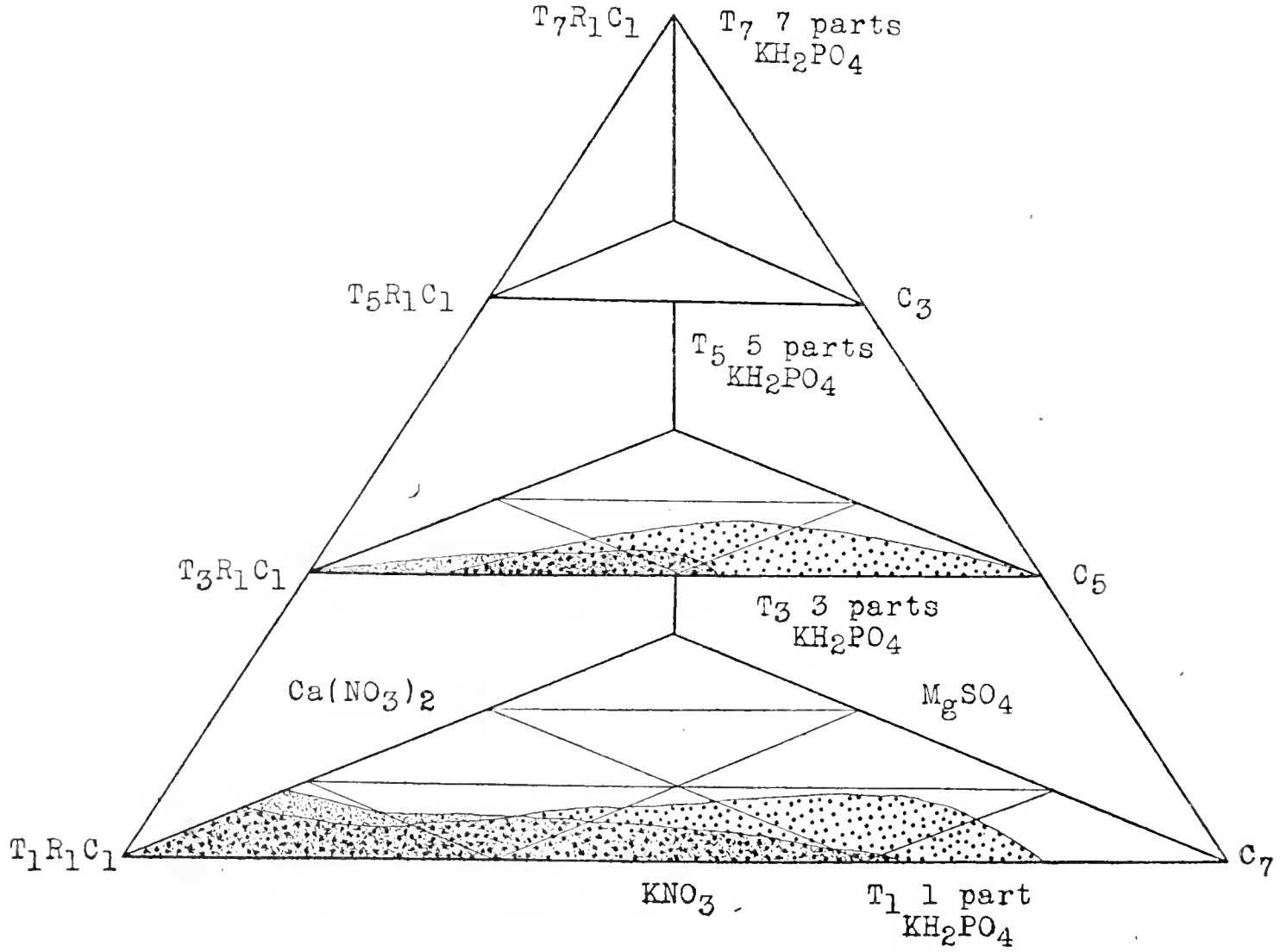


FIG. 5. Distribution of the highest five yields of soy bean tops and roots from the cultures of series F, supplied with ferrous sulphate as the source of iron for the plants. Yields of tops represented by dotted areas, those of roots by stippled areas.

previously found to be very efficient in maintaining the plants in a healthy condition without chlorosis in these solutions. Three cultures of the five in each of these two series producing high yields of tops and three producing high yields of roots are corresponding cultures, and are included in the areas marking high yields in both series. The maximum yield of tops was produced by culture $T_1R_3C_3$ in series E and by culture $T_1R_1C_5$ in series F. The maximum yield of roots in each of the two series was produced by culture $T_3R_1C_3$.

A comparison of the diagrams of Fig. 6 (series G, modified Tottingham

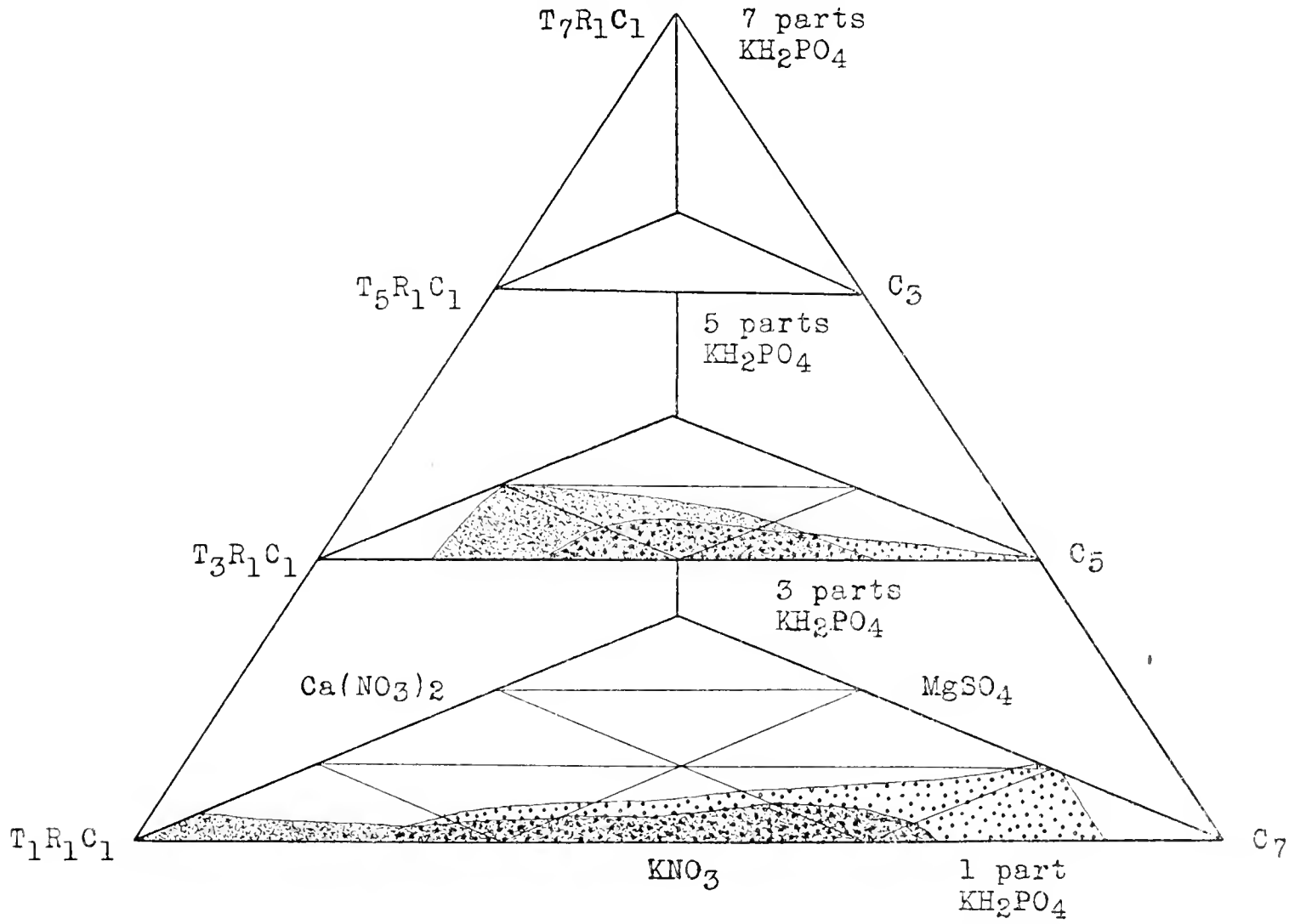


FIG. 6. Distribution of the highest five yields of soy bean tops and roots from the cultures of series G, supplied with ferric phosphate as the source of iron for the plants. Yields of tops represented by dotted areas, those of roots by stippled areas.

solutions) with those of Figs. 4 and 5 shows the areas of high yields of both tops and roots on all three diagrams to occupy the same general positions at the bases of the several triangles. Three of the five cultures which produced high yields of tops and three of those which gave high root yields in each series are corresponding cultures, and are included in the yield areas on each of the three diagrams. The maximum yield of both tops and roots from the cultures containing ammonium sulphate (series G, Fig. 6) was produced by culture $T_1R_1C_1$.

The volume-molecular partial concentrations of the salts, and the ranges of these for the culture solutions which produced the highest five yields of tops and roots in each series, are given in Table VI, together with the absolute dry-weight yields produced. The maximum yields from each

series are indicated by bold-face type. The data given in this table refer to the cultures comprised in the yield areas on the diagrams of Figs. 4, 5, and 6. At the bottom of the table are given the maximum and minimum partial concentrations employed with each salt, and the total ranges of these for the entire series.

Table VI.

Absolute dry-weight yields, volume-molecular partial concentrations and ranges of these for the salts in the solutions producing the highest five yields of tops and roots in the Tottingham series E and F, and in the ammonium-sulphate series G.

			Volume-molecular partial concentrations.					
		Culture number.	KNO_3.	KH_2PO_4.	$Ca(NO_3)_2$.	$MgSO_4$.	$(NH_4)_2SO_4$.	*Yields.*
Series E, source of iron $FePO_4$.	Tops	$T_1R_1C_3$	0·0020	0·00211	0·00438	0·01185		4·0346
		$T_1R_1C_5$	0·0020	0·00211	0·00730	0·00711		4·8503
		$T_1R_3C_3$	0·0060	0·00211	0·00438	0·00711		**5·0728**
		$T_3R_1C_3$	0·0020	0·00633	0·00438	0·00711		4·7242
		$T_5R_1C_3$	0·0020	0·01055	0·00438	0·00237		3·7335
		Range	0·0040	0·00844	0·00292	0·00948		
	Roots	$T_1R_1C_3$	0·0020	0·00211	0·00438	0·01185		0·5610
		$T_1R_1C_5$	0·0020	0·00211	0·00730	0·00711		0·6066
		$T_1R_3C_3$	0·0060	0·00211	0·00438	0·00711		0·6021
		$T_3R_1C_3$	0·0020	0·00633	0·00438	0·00711		**0·6247**
		$T_5R_1C_1$	0·0020	0·01055	0·00146	0·00711		0·4970
		Range	0·0040	0·00844	0·00584	0·00474		
Series F, source of iron $FeSO_4$.	Tops	$T_1R_1C_3$	0·0020	0·00211	0·00438	0·01185		4·6059
		$T_1R_1C_5$	0·0020	0·00211	0·00730	0·00711		**5·2082**
		$T_1R_3C_5$	0·0060	0·00211	0·00730	0·00237		4·3225
		$T_3R_1C_3$	0·0020	0·00633	0·00438	0·00711		4·8893
		$T_3R_1C_5$	0·0020	0·00633	0·00730	0·00237		4·2870
		Range	0·0040	0·00422	0·00292	0·00948		
	Roots	$T_1R_1C_1$	0·0020	0·00211	0·00146	0·01659		0·5891
		$T_1R_1C_3$	0·0020	0·00211	0·00438	0·01185		0·5704
		$T_1R_1C_5$	0·0020	0·00211	0·00730	0·00711		0·5831
		$T_3R_1C_3$	0·0020	0·00633	0·00438	0·00711		**0·7489**
		$T_3R_3C_1$	0·0060	0·00633	0·00146	0·00711		0·5360
		Range	0·0040	0·00422	0·00584	0·00948		
Series G, source of iron $FePO_4$.	Tops	$T_1R_1C_1$		0·00211	0·00146	0·01659	0·0014	**5·1786**
		$T_1R_1C_3$		0·00211	0·00438	0·01185	0·0014	4·5029
		$T_1R_1C_5$		0·00211	0·00730	0·00711	0·0014	4·8755
		$T_3R_1C_3$		0·00633	0·00438	0·00711	0·0014	4·7120
		$T_3R_1C_5$		0·00633	0·00730	0·00237	0·0014	3·8835
		Range		0·00422	0·00584	0·01422	----	
	Roots	$T_1R_1C_1$		0·00211	0·00146	0·01659	0·0014	**0·9828**
		$T_1R_1C_3$		0·00211	0·00438	0·01185	0·0014	0·6093
		T_1R_1C		0·00211	0·00730	0·00711	0·0014	0·5012
		$T_3R_1C_1$		0·00633	0·00146	0·01185	0·0014	0·4816
		$T_3R_1C_3$		0·00633	0·00438	0·00711	0·0014	0·5111
		Range		0·00422	0·00584	0·00948	----	
Entire series								
		Maximum	0·0140	0·01477	0·01022	0·01659	0·0098	
		Minimum	0·0020	0·00211	0·00146	0·00237	0·0014	
		Range	0·0120	0·01266	0·00876	0·01422	0·0084	

From the data of Table VI and from the distribution of the areas representing high yields on the diagrams of Figs. 4, 5, and 6 it is at once apparent that good growth of soy bean tops in the Tottingham series E and F is correlated with only narrow ranges in the proportions of potassium nitrate and calcium nitrate, but with relatively wide ranges in the proportions of the other two salts. For the cultures producing the highest five yields of roots in the two series, relatively wide ranges are shown in the proportions of all the salts except potassium nitrate. However, the ranges in the salt proportions correlated with high yields of both tops and roots are always less extensive than the corresponding total ranges employed, and with only one exception do these ranges include the highest proportions of any salt used, this exception being indicated for magnesium sulphate in culture $T_1R_1C_1$, which produced a high yield of roots in series F. On the other hand, the ranges in the salt proportions associated with high yields of tops in these series include the lowest proportions of potassium nitrate, mono-potassium phosphate, and magnesium sulphate, and likewise those correlated with high yields of roots include the lowest proportions of potassium nitrate, mono-potassium phosphate, and calcium nitrate.

The modified Tottingham solutions of series G which produced the highest five yields of both tops and roots are limited to those solutions of the series which comprise the lowest proportions of ammonium sulphate only. These solutions are further characterized by a low range in the proportions of mono-potassium phosphate and high ranges in the proportions of calcium nitrate and magnesium sulphate, the range in the proportions of the latter for the cultures producing the highest five yields of tops being co-extensive with the corresponding total range for the entire series.

Comparing now the absolute dry-weight yields of series E with those of series F as given in Table VI, it will be observed that the maximum yield of tops from the series in which iron was supplied to the cultures in the form of ferrous sulphate (series F) is slightly superior to the corresponding yield from series E, in which the source of iron was ferric phosphate. The difference in these values, however, is scarcely large enough to be significant, although the average of the highest five yields from series F shows a pronounced superiority over the corresponding average from series E. The average of the highest five yields of roots, as well as the maximum yield from series F, show a marked superiority over the corresponding yield from series E. It thus appears that ferrous sulphate when supplied in the proper concentrations is a better source of iron for soy bean plants grown in the Tottingham solutions than is ferric phosphate under similar conditions.

The maximum yield and the average of the highest five yields of tops from series G are approximately the same in value as the corresponding yields from series F and slightly superior to those from series E, but the maximum and average yields of roots from series G are much superior

to those from the other two series. On the whole, it appears that the modified Tottingham solutions of series G, in which nitrogen is available for the plants in the forms of both nitrate and ammonium, are somewhat more efficient than are the unmodified Tottingham solutions for the growth of soy bean plants during the early phases of development, but any of the solutions whose formulae appear in Table VI may be expected to produce good growth of this species if supplied with a suitable form of iron in the proper concentrations.

Summary.

The experiments described in this paper were conducted for the purpose of studying, in a comparative way, the effects of ammonium sulphate upon the growth of soy beans in nutrient solutions during the early stages of development, and to determine the influence of this salt upon the availability for the plants of different forms of iron. A study was made also of the reaction change of the nutrient solutions induced by contact with plant roots. Two type-series of culture solutions were used. The first of these comprised twenty solutions selected from the Tottingham series of eighty-four, and the second consisted of the same solutions modified by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. All the solutions had a total osmotic concentration value of approximately one atmosphere. Ferric phosphate in an aqueous suspension and ferrous sulphate in solution form were added in stated amounts of iron per litre of nutrient solution. The culture solutions were renewed at regular intervals of three and one-half days throughout the growth period of approximately five weeks.

The main results of the experiments may be summarized as follows:

1. The plants grown in the Tottingham solutions invariably produced a marked decrease in the hydrogen-ion concentration of the solutions.

2. The plants grown in the solutions containing ammonium sulphate invariably increased the hydrogen-ion concentration of these solutions during the early stages of growth, the hydrogen-ion concentrations of these solutions being maintained at a much higher level than those of the unmodified Tottingham solutions, although the initial pH values of corresponding solutions of the two types were practically the same.

3. The nature of the salt constituents determines the direction of the reaction change of the culture solutions in contact with the roots of the growing soy bean plants.

4. Ferric phosphate in quantities of less than one milligram of iron per litre of nutrient solution was not sufficiently available in the Tottingham solutions to supply the needs of the plants for iron during the early stages

of growth. On the other hand, this form of iron in quantities of less than one-half milligram of iron per litre of solution was ample to supply the needs of the plants for this element in the solutions containing ammonium sulphate. The maintenance by the plants of a higher level in the hydrogen-ion concentration of the solutions containing ammonium sulphate, and the possible influence of this salt upon the permeability of the plant cells towards iron, undoubtedly account for the greater efficiency of iron in these solutions.

5. Ferrous sulphate in quantities of from 0·25 to 0·50 milligram of iron per litre of nutrient solution was sufficiently available in the Tottingham solutions to satisfy the needs of the plants for iron. However, ferrous sulphate in the solution containing ammonium sulphate produces a condition very toxic to the plants, the degree of toxicity increasing with increase in the amounts of iron from 0·25 to 5·00 milligrams per litre of nutrient solution.

6. Ferrous sulphate when used in too high concentrations produces on the leaves of soy bean plants in both series a characteristic brown specking which is more pronounced in the solutions containing ammonium sulphate than in the unmodified Tottingham solutions.

7. The availability for the plants of a given iron compound and its efficiency appear to be determined in large measure by the composition of the nutrient solution and by the nature of the reaction change induced by contact with the plant roots.

8. With the solutions containing the salts KNO_3, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ (Tottingham solutions) the maximum yield of soy bean tops was obtained when these salts were present in the volume-molecular proportions 0·0020, 0·00211, 0·00730, and 0·00711, respectively, with ferrous sulphate as the source of iron. The maximum yield of roots was obtained with a solution containing these salts in the volume-molecular proportions 0·0020, 0·00633, 0·00438, and 0·00711, respectively, with ferrous sulphate as the source of iron. Good yields of tops and roots were obtained with only a narrow range in the proportions of potassium nitrate, but with relatively wide ranges in the proportions of the other salts.

9. With the solutions containing $(NH_4)_2SO_4$, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ the maximum yield of both tops and roots was obtained when these salts were present in the volume-molecular proportions 0·0014, 0·00211, 0·00146, and 0·01659, respectively, with ferric phosphate as the source of iron. Good yields of tops and roots were obtained with only the lowest proportions of ammonium sulphate but with relatively wide ranges in the proportions of the other salts.

10. In general, high yields of tops were associated with high yields of roots.

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On a New Method of investigating Fossil Plant Impressions or Incrustations.[1]

BY

JOHN WALTON, M.A.,

Junior Demonstrator in Botany, Cambridge.

With Plate IX and one Figure in the Text.

Introductory.

UNTIL comparatively recently in descriptions of fossil impressions, or incrustations as they are more correctly called, authors have generally confined themselves to descriptions of the external morphology of the plant as it is exposed on the surface of the rock. The source of information is thus limited to one surface of the plant. Refinements have been used in later years. The 'collodion film method'[2] suggested by Nathorst has enabled us to investigate with greater facility the finer details of surface markings. Nathorst's method consists in taking a thin transparent cast of the surface features by applying a drop of a solution of collodion in ether to the surface of the fossil. A tough film of collodion is formed on evaporation of the solvent; this can be stripped off and examined by transmitted light. The collodion method is of great assistance, as it is very difficult to examine the surface of a rock under the higher powers of a microscope owing to the necessary use of reflected light. In some instances, however, use may be made of one of the improved forms of vertical illuminator.[3] A $\frac{1}{6}$-in. objective can be used conveniently with this instrument. The preservation must be good and the surface of the plant must not be pitted by contact with coarse particles in the matrix in which it has been embedded.

I have made use of the vertical illuminator for the examination of

[1] Examples of fossil plants prepared by this method were exhibited by the writer in Section K (Botany), British Association, Hull, 1922.

[2] Nathorst, A. G. (1907). For a description in English see Bather, F. A. (1907).

[3] The writer is indebted to Mr. S. M. Wadham, who suggested that the Leitz-Wetzler vertical illuminator might be used in the examination of fossil plants.

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fractured surfaces of blocks of petrified wood. Sometimes the wood has been rendered so transparent and homogeneous in the processes of petrification that no structure can be made out in thin sections of the material. This is not infrequent in silicified wood. In other specimens the wood is quite opaque, when, for example, its structure is preserved in pyrites. In both these types of fossil, examination of a radial fracture will often afford valuable data concerning the structure of the secondary wood: such features as the medullary rays and the pitting on the tracheides show up clearly.

In a specimen of *Rhexoxylon africanum*, Bancroft, a fossil wood from South Africa of Triassic Age, the pores on the bordered pits showed up very definitely. Similar perfection of detail was observed in a portion of a jasper tree (Triassic) from Arizona in America. In both these specimens the preservation is such that practically no structure is visible in thin sections on examination with transmitted light. A piece of pyritized wood from the Lower Estuarine beds of the Jurassic of the Yorkshire coast showed the features of the pitting very distinctly.

In a few exceptional cases the fossil plant incrustation may become detached from the rock and then both surfaces can be examined. Hamshaw Thomas[1] described specimens of *Thinnfeldia* fronds from the Middle Estuarine of Yorkshire that can be stripped with ease from the rock. Sometimes such fossils are transparent and then more information is available. Miss Wills[2] describes cuticles of Carboniferous plants found in clayey shales in the Upper and Middle Coal Measures which could be removed from the shale by soaking. The fact that coherent samples of the specimens can be obtained is largely due to the excellent state of preservation of the cuticles of the upper and lower surfaces of the leaves. A. G. Nathorst[3] described Tertiary coniferous twigs from Ellesmere Land, with the leaves attached, which could be similarly removed from the rock.

There are also, of course, intermediate examples in which, by exercising considerable care, small fragments of plants may be detached; but opportunities such as these are rare, and it is not often that we are able to examine specimens which, as Hamshaw Thomas says,[4] may be regarded as true examples from the 'Herbarium Diluvianum'.

With the realization that a considerable amount of the original plant substance was still preserved in fossil plants, and that the cuticle still exists in an almost unchanged condition, a great advance in the study of these plant impressions was made possible. Schulze[5] in 1855 introduced a method of isolating portions of the cuticles of such fossils by chemical treatment, and Nathorst employed the same method in many of his researches. In this country Hamshaw Thomas and several other palaeo-

[1] Thomas, H. Hamshaw (1913).
[2] Wills, Lucy (1914).
[3] Nathorst, A. G. (1915).
[4] Thomas, H. Hamshaw (1915).
[5] Schulze, F. (1855).

botanists have used this method extensively. So far Schulze's method has been used almost exclusively in the study of Mesozoic plants; Huth,[1] however, has shown that it can be employed in the investigation of Carboniferous incrustations.

Now most fossil plants of this encrusted type occur in rocks which have been formed by deposition of sediment in some lake or estuary and have a layered structure, or, in other words, exhibit planes of bedding. The rock splits or cleaves more readily in a direction parallel to these planes than in any other direction.

As the plant fragment is drifted and sinks to the bottom it will generally, if it is a leaf, tend to lie in the plane of the bedding, and that is what is usually found. Occasionally, however, it may settle down and be fixed in a position oblique to the plane of bedding. The presence of a leaf or frond often determines the plane along which the rock cleaves; there seems to be a surface of weakness due to the discontinuity at the surface of the fossil. Very often when a leaf occurs embedded at an angle with the plane of the bedding the rock cleaves along the surface of the fossil and not along the bedding plane of the rock. The split will occur over either the upper or lower surface of the plant, whichever offers least resistance to cleavage; but, other conditions being equal, it will occur over the most even of the two surfaces.

On the whole the upper surface of a leaf, in the mature state usually convex, is the most uniform. The under surface may have prominent veins, hairs, or scales, and the majority of the stomata. On this supposition, therefore, we should expect to find that in the majority of specimens the upper surface of the leaf is exposed on cleavage of the rock in which it is embedded. On the whole this seems to be borne out after examination of a large number of such fossils. If the plane of cleavage did by any chance pass over the lower surface of a leaf which had hairs, scales, or other appendages, these would probably be shorn off and remain embedded in the other half of the block, and would not be represented on the surface of the specimen except by very minute scars, or possibly depressions, so that some of the lower surface features would escape notice even though the lower surface was exposed.

By a method of transferring the fossil on to a transparent base I have been able to examine both surfaces.

There is a very common fern-like frond occurring in Carboniferous strata, *Dactylotheca plumosa,* Artis sp., the specific name having been given to it presumably on account of the feathery appearance of the multipinnate frond. In the commonest type of specimen of this plant the surfaces of the pinnae and pinnules are convex and smooth. On examining the under surface by the transfer method I found that it was covered with long hairs

[1] Huth, W. (1913).

consisting of branching filaments (Pl. IX, Fig. 1). The hairs arose principally from the surface of the veins. To check this observation, specimens from different localities were examined and the same features were again apparent. I found one specimen in which the surface of the fossil was concave and had not the smooth appearance so characteristic of the majority. On examination of the unexposed surface by the transfer method I found it to be smooth and free from emergences. The dull appearance of the specimen was due no doubt to the fact that the hairs detached by the splitting of the block left irregularities on the surface, which was in this example the under surface of the frond.

Method of Transfer.[1]

1. A block or chip bearing a representative portion of the fossil is taken from the specimen by chipping or cutting and roughly trimmed. If the surface of the sample on which the fossil occurs is very uneven it should be levelled as much as possible by scraping or cutting without injuring the plant.

2. A glass slide of suitable size is chosen, and sufficient balsam of the consistency of treacle is put on at one end of the slide. The slide is then placed on a metal plate heated by a Bunsen burner, and the balsam is slowly 'cooked' without being allowed to boil, to remove volatile constituents, until a sample drawn out between the points of a pair of forceps is brittle when cool. The balsam is better overcooked than undercooked.

3. The sample is then placed face downwards on the hot plate for a moment, so that the surface showing the fossil may be heated. It is then placed face downwards on the cooked balsam at one end of the slide, and the slide removed to a cooler portion of the plate. It may be found that bubbles of air have formed between the fossil and the slide, and it will require manipulation to get rid of them.

4. The preparation is cooled in air; rapid cooling in water causes the balsam to crack. The balsam should set hard and brittle.

5. The rough excess of rock is then ground off from the back of the sample on a glass plate with some abrasive such as carborundum, care being taken not to grind too near the fossil.

6. The slide is dried, the exposed surface of the rock moistened with water, and the whole slide dipped into melted paraffin wax. It is then cooled in water and a second and third coat put on in the same way. By cutting round the edge of the rock with a knife the wax can be removed completely from the back of the rock, as it does not stick to the wet surface.

7. The slide, with all the glass portion thus covered with wax, is then

[1] In certain details this method resembles that used by Wiman in the investigation of *Graptolites*. See Wiman (1895).

put into an etching bath of hydrofluoric acid (HF) and the rock etched away. If there is any effervescence on immersion in the HF the preparation should be removed at once, as effervescence indicates the presence of a carbonate, probably of calcium, in the rock. If the reaction were allowed to proceed insoluble calcium fluoride (CaF_2) would be produced. If calcium carbonate ($CaCO_3$) is present dilute hydrochloric acid (HCl) must be used as a mordant until all the $CaCO_3$ is removed. The preparation must then be washed thoroughly to remove all traces of soluble calcium salts, and if any matrix insoluble in HCl is left it will probably be silica or silicates, which may then be removed by treatment in the HF bath.

The etching is continued until the matrix is dissolved or is loosened sufficiently to be washed away by a gentle stream of water. The preparation should not be left too long in the HF as the balsam is acted on very slightly.

It is often instructive to watch the process of etching by taking the slide out of the bath from time to time, washing, and then examining under the microscope, as portions of other plants embedded in the rock may be seen which are removed in the course of the etching.

8. The preparation is washed in water.

9. The wax is cut away and any excess of balsam on the slide trimmed off if necessary.

10. It is sometimes advisable to warm up the preparation on the top of a steam oven until the balsam is soft; this ensures that the plant is firmly fixed. Before it is warmed every trace of wax must be scraped away or it may flow over the surface of the balsam and spoil the preparation.

11. The resulting preparation may be covered with hot fused balsam transferred to it on a cover-slip, but there is danger of the plant moving and cracking up if this is attempted. It is generally sufficient to keep the preparation free from dust, and for examination under the microscope glycerine in water can be put on the surface, or some other liquid with a refractive index as near as possible to that of balsam, but which will not dissolve or react chemically with it.

Examples.

In order to demonstrate the possibilities of this method of preparation in the elucidation of fossil plant structure I give a series of examples of different types of preservation of carbonized impressions from horizons ranging from the Devonian to the Cretaceous. In conjunction with Schulze's method of isolating cuticles the investigation of Coal Measure plants is rendered easier; for after the plant has been transferred the balsam may be dissolved away subsequently and large coherent portions obtained for cuticle preparations. The more uncertain procedure of chipping off small pieces is thus avoided.

I. *Psilophyton princeps*. Dawson.

Locality: Callendar, Perthshire. *Horizon*: Lower Old Red Sandstone.

The transfer preparation of a specimen of *Psilophyton princeps* figured in Pl. IX, Fig. 12, at once reveals the presence of a considerable amount of the original organic substance of the plant. It will be seen on examining the figure that the stem is studded with small spine-like emergences which are somewhat irregularly placed over the surface of the plant exposed by this method of preparation. It is possible that some have been knocked off, and that they are therefore not completely represented. It is hoped that further preparations may give more reliable information as to their arrangement on the stem. The general form of the emergences can be made out at the top of Fig. 12. They are somewhat expanded at the base and are flattened in a radial plane (with reference to the axis of the plant). On examination by transmitted light they were found to be transparent and brown in colour and probably represent the cuticularized portions of the original emergences.

It is worth noticing that these spinous structures seem to be better preserved than the rest of the plant represented in the fossil. This is probably due to the greater proportion of cuticularized material in them, and seems to me to suggest that they were probably not structures representing an extension of the area of photosynthetic tissue, and that if they had any function at all it was of a mechanical nature. We know from the researches of Kidston and Lang[1] that the Rhynie plants which are included in the same group (Psilophytales) as *Psilophyton* had well-developed cuticles on the stems.

II. *Mariopteris*, cf. *muricata*, Schloth., sp.[2]

Horizon: Carboniferous.

I am indebted to Mr. Hamshaw Thomas for calling my attention to the work of Huth[3] on the foliar epidermis of *Mariopteris muricata*, in which air pores ('Atemporen') comparable in structure to those of *Marchantia* are described. Mr. Thomas suggested that a structure which occurred on the epidermis of a specimen of *Sphenopteris nummularia* (to be described later) resembled the air-pores described by Huth very closely. Huth mentions that Haberlandt, objecting to his theory of the nature of the Atemporen, suggested that they might be hair-bases. As stomata of quite usual type were found on *Sphenopteris nummularia*, the suggestion that these 'Atemporen' might really be the points of insertion of hairs or emergences of the epidermis seemed possible. More recently Gothan[4]

[1] Kidston and Lang (1917), p. 769.
[2] Dr. Kidston very kindly identified this specimen.
[3] Huth, W. (1913), Fig. 3, p. 16.
[4] Gothan, W. (1915).

investigated the cuticle of the rachis of *Mariopteris muricata*, and discovered structures which he concluded were the bases of epidermal emergences.

Some time later I obtained material of *Mariopteris*, cf. *muricata*, Schloth., and made some transfer preparations. The under surface was found to be regularly studded with capitate glandular hairs[1] (Pl. IX, Fig. 2, *h*, and Fig. 5). These glands occurred also on the veins and revolute margins of the pinnules. Portions of the pinnules which became loosened in the etching process were used to make cuticle preparations by Schulze's method.[2] I found that the cuticles of the upper and under surfaces were easily separable. The upper cuticle (Pl. IX, Fig. 3) was apparently identical with that figured by Huth,[3] and showed structures (Pl. IX, Fig. 3, *h.*, *h.'*) which corresponded exactly to the 'Atemporen' in form and number. The lower cuticle (Pl. IX, Fig. 4) was of quite a different nature: it was much thinner than the upper, and showed the outlines of the epidermal cells only very faintly. Glandular hairs were frequent, and occasionally I found the cuticularized portions of the supporting cells of a hair, which corresponded so closely with the structures seen on the upper cuticle as to leave no doubt of their similar nature. There was one very interesting fact observed which throws light on the nature of these glands. In many instances numbers of microspores of various types were found attached to the stalks of the glands and to the epidermal cells surrounding them. These spores were not loosened even by prolonged treatment in Schulze's macerating fluid. They were also found scattered in large numbers over the surfaces of the prepared cuticles (Pl. IX, Figs. 3, 3 *a*, and 5). There were few spores in the matrix surrounding the plant, as could be verified by examination of the transfer preparations, for any spores lying in the bedding plane of the frond would have remained fixed to the balsam. We may therefore conclude with considerable confidence that the spores were sticking to the plant when it fell into the lake or river in the bed of which it was subsequently silted up, and that the glands secreted some sticky substance during the life of the plant.

In addition to these glandular structures, groups of two to six small papillae occurred at frequent regular intervals over the surface of the cuticle of the abaxial surface in areas which corresponded to the spaces between the veins (Pl. IX, Fig. 3). The papillae in these groups surrounded a small area which in some examples was occupied by a small stoma of quite the usual type, consisting of two curved guard cells surrounding a narrow elliptical pore (Pl. IX, Fig. 6). The whole structure was very much smaller than the hair-bases. The papillae may almost certainly be

[1] Cf. glands of *Lagenostoma lomaxi*. Oliver and Scott (1904), Pl. VI, Figs. 20, 21, and Pl. VIII, Figs. 17, 18.

[2] Schulze, F. (1855).

[3] Huth, W. (1913).

considered as projections from subsidiary cells surrounding the stoma, which was slightly sunk below the surface. There were at least seventy-five stomata per sq. mm. As an argument against considering the 'Atemporen' as hair-bases, as Haberlandt suggested, Huth writes: 'Ich habe eine recht grosse Anzahl von *Mariopteris muricata*-Resten in der Hand gehabt, und habe nie, weder mit blossem Auge, noch mit der Lupe, noch mit dem Binokularmikroskop jemals Härchen entdeckt.' This example serves to stress the fact that the exposed surface of a fossil plant has almost always been stripped, by the splitting of the rock, of any emergences it may have borne.

III. *Cladotheca undans*, (Halle) Lind. and Hutt. sp. Jurassic Estuarine Beds, Gristhorpe Bay, Yorkshire.

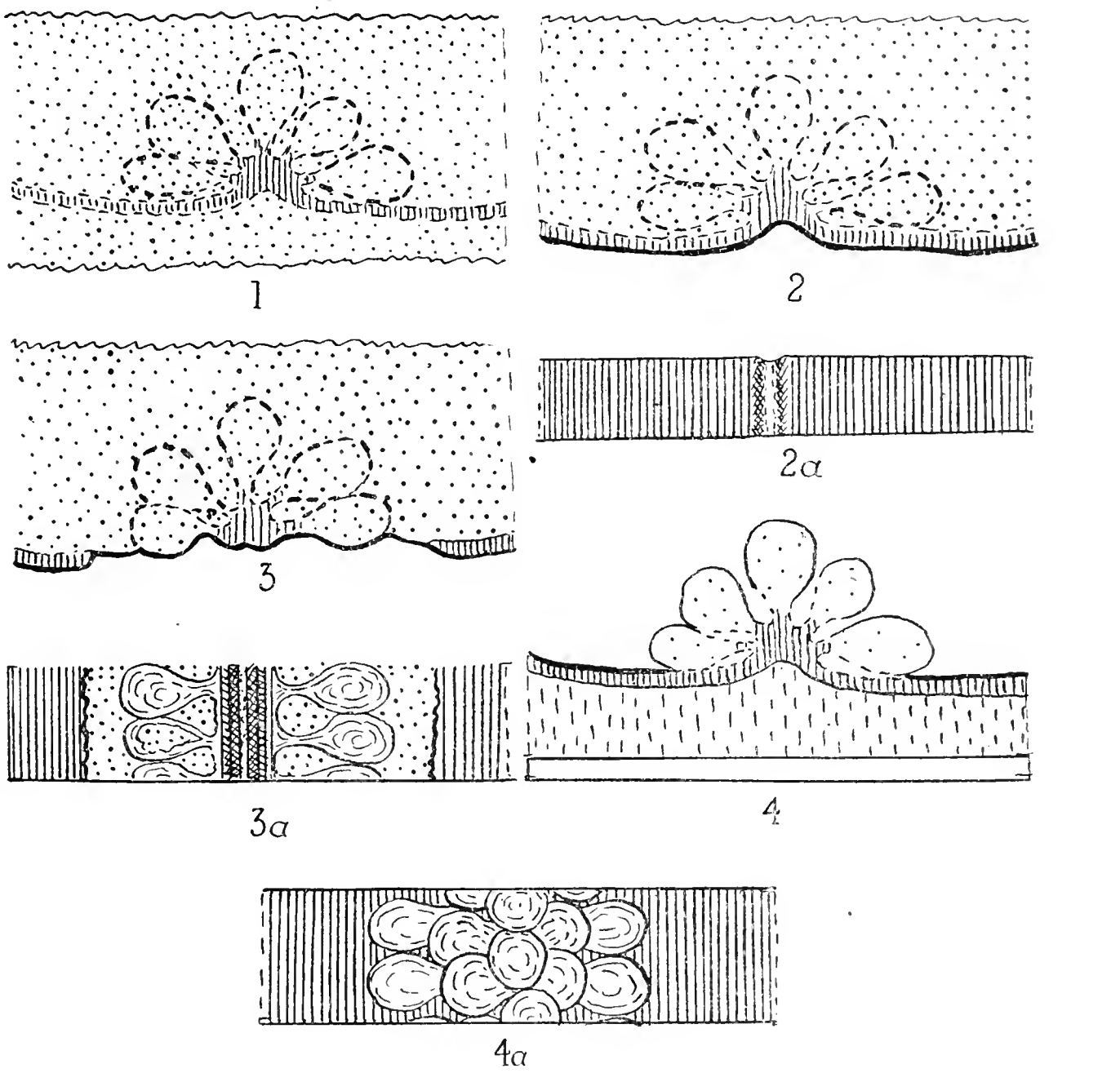
As an example of the way in which this method can be employed in the investigation of fertile fronds, I give the results obtained by examination of a specimen of the above fern. Halle[1] gives a schematic figure of the disposition of the sporangia on the specimens he investigated. As both his specimens had been exposed by splitting open the shale in which they had been embedded, an inaccurate idea of the character of the sorus is given. The sporangia are described as forming two rows, one on each side of a linear placenta, which was probably superimposed over a lateral vein of the pinnule. This apparent arrangement is due to the fact that only the sporangia which lay next to the surface of the lamina are visible (Text-fig., 3 and 3 *a*). Halle says: 'In places where the coal' (presumably the material of the lamina) 'has been removed, both specimens show fairly clearly the arrangement and structure of the sporangia.'

The split in the shale has occurred over the upper surface of the leaf (Text-fig., 2), and where the material of the lamina has disappeared (Text-fig., 3 and 3 *a*) the sporangia, which were actually in contact with its under surface, can be seen (Halle,[2] Pl. II, Fig. 2), but those sporangia which arose from the placenta at right angles to the under surface of the leaf are embedded out of view in the matrix.

When a transfer preparation (Pl. IX, Fig. 7) was made from a specimen collected in 1922 at Gristhorpe, the sori were found to be superposed on the lateral veins of the pinnule (Text-fig., 4), extending from close to the main vein of the pinnule to near the lobed margin of the latter. The sporangia formed a dense covering (Text-fig., 4 *a*), and only in places where they had presumably fallen off was it possible to see the underlying sorus or vein. It has been already mentioned that fossil plants when detached from the rock may be partially transparent. This type of fossilization is quite common, although in the majority of examples the plants are too fragile to be detached wholesale by mechanical means, such as were

[1] Halle, T. G. (1911), p. 6, Fig. 1.
[2] Ibid. (1913), loc. cit. Cf. Pl. IX, Fig. 2.

employed with the above-mentioned leaves of *Eretmophyllum* and *Thinnfeldia*. This type of preservation may generally be detected by examination of the fossil on the rock. Such a fossil shows, particularly when wet, a characteristic brownish coloration due to reflection from the surface of the rock through the semi-transparent plant substance. It is possible that this type of fossil was produced by the decay and dissolution of the mesophyll



TEXT-FIG. *Cladotheca undans* (Halle): 1, transverse section of sorus embedded in the rock; 2, ditto, upper surface of lamina exposed; 2 *a*, surface view of 2; 3, transverse section of sorus with portion of lamina removed, exposing some of the underlying sporangia; 3*a*, surface view of 3, showing a row of sporangia on each side of the vein; 4, transverse section of sorus in a transfer preparation; 4 *a*, surface view of 4, showing the sporangia covering the underlying vein. The matrix is represented by dots and the coaly material of the fossil by vertical lines. In 4 the balsam is represented by short vertical strokes.

or softer tissues prior to the plant becoming buried in sediment under conditions in which further dissolution was inhibited. In the larger number of fossil leaves the mesophyll is represented by a black coaly substance which has to be removed before any structure can be seen in the cuticles between which it lies. In Schulze's method [1] of isolating cuticles the black matter is removed in solution by certain reagents.

[1] Schulze, loc. cit.

The following examples are given of plants which yielded transparent transfer preparations:

IV. *Sphenopteris nummularia*, Andr.

This specimen has exceptionally tough, well-preserved cuticular surfaces. The mesophyll has decayed, and no black substances are left. The lamina is thus quite transparent (Pl. IX, Fig. 8). The conducting tissue of the rachis and pinnae shows up as dark bands. Other dark lines due to folding of the cuticles also occur, and must not be confused with the conducting system. In places the scalariform thickening of the walls of the tracheides in the rachis can be seen. A few small hairs occur on the under surfaces of the pinnae. The outlines of the epidermal cells are clearly defined, and in one or two cases stomata (Pl. IX, Fig. 9) are visible. The ends of the pinnae and pinnules have a markedly revolute margin. Owing to its toughness, the plant figured in Pl. IX, Fig. 8, was flattened out in soft balsam under a cover-slip.

V. *Oligocarpia gutbieri*, Göpp.

Gresford Colliery, Wrexham. Carboniferous. (Middle Coal Measures.)

The genus *Oligocarpia* was instituted for a group of Carboniferous ferns, of which the sporangia and the soral arrangement were more or less known. I made several transfer preparations of this plant, as a considerable quantity of material was available, and many interesting details of structure were revealed by them. Sterile and fertile pinnules were observed (Pl. IX, Fig. 10). The lamina in each case was transparent and the venation showed up clearly. The rachis was furnished with scattered hairs, consisting of a filament of cells tapering off to a fine point. In one portion of the surface stomata of the common type could be seen, but it was impossible to tell on which surface the individual stomata occurred. The cuticles of the lamina are not well preserved. The sori are situated over the lateral veins on the fertile pinnules and have two to even sporangia. Some of these sporangia (Pl. IX, Fig. 11) are semi-transparent. The annulus appears to be uniseriate in most of those examined, but it is possible that it might be double in a few examples. The sporangia appear to be almost sessile; the stalks must be very short.

VI. Fungus. Cf. Dematiaceae. Carboniferous.

In several of the transfer preparations made in the course of these investigations, fungal hyphae are found in association with the remains of the vascular plants. Sometimes the hyphae are found on the surfaces of the plants, at others they are found traversing the matrix between them, suggesting that the fungi are found in their place of growth.

In a few preparations spore-forms have been observed. In one pre-

paration of *Gleichenia* sp. from the Cretaceous of Greenland spores closely resembling the modern genus *Helminthosporium* were found in association with the frond. In another preparation a spore-form was found, which is figured in Pl. IX, Fig. 13. It is seen to consist of a mass of septate spores resembling forms found in the recent fungi included in the Dematiaceae, such as *Septosporium*, *Cladosporium*, &c. Although there is in these examples no very conclusive evidence, the nature of the spores strongly suggests that they were developed in subaerial conditions. If this is so, then we must suppose that the plants were rotting on the ground, and were subsequently inundated and covered with the silt.

Some General Conclusions.

1. It is contended that the study of the usual type of fossil plant impression or incrustation gives insufficient data for an accurate description of the plant, and that it is necessary to study the other surface of the plant as well. A method is described by which this may be effected.

2. That in view of the uncertainty which exists at the present day as to the relations between form and function in plants, it is better to avoid trying to reach a definite conclusion as to the ecological conditions under which Carboniferous plants lived by the examination of a few types only, and that the study of the Coal Measures stratigraphically[1] gives us at present more reliable information as to the nature of the habitat than observations based on separate types of plants found in them.

Summary.

1. A method of examining the 'other side' of fossil plant impressions or incrustations is described. *Dactylotheca plumosa* is quoted as an example of a plant in which the characters of the under surface have escaped notice owing to the fact that, when the matrix is split open and a fossil plant exposed, all hairs or other easily detached projections are removed, and an inaccurate representation of the plant is given.

2. Suggestions are given of the kind of problem which the method may assist to solve. Examples are quoted:

I. *Psilophyton princeps.* A description is given of the spine-like emergences on the axis.

II. *Mariopteris*, cf. *muricata.* Epidermal structures (glands, &c.) are described and figured. The form of the stomata is shown to be of the usual type, and the structures previously described as air-pores of the *Marchantia* type are shown to be glandular hair-bases. Figures are given.

III. *Cladotheca undans.* The arrangement of the sporangia in the sorus is described.

[1] Kendal, P. F. (1922).

IV. *Sphenopteris nummularia.* A portion of a frond is described in which nothing but the transparent cuticle and traces of the lignified tracheides in the vascular strands are preserved. Stomata of the usual type are described.

V. *Oliocarpia gutbieri.* Preparations showing sterile and fertile pinnae are figured, also sporangia exhibiting an apparently uniseriate annulus. Stomata of the usual type are recorded.

VI. A fungus (spore-form) is figured. It is suggested that, by a careful study of the position of the hyphae of such fungi, it may be possible to deduce something about the vicissitudes through which the plant fragment has passed before fossilization.

In conclusion, I have to thank Prof. Seward for a great deal of helpful criticism given in the course of this work, and Mr. H. Hamshaw Thomas for invaluable advice in matters of technique and for many of the specimens, some of which he kindly identified.

I am indebted to several members of the staff of the Sedgewick Museum, Cambridge, for the loan of specimens and for references to literature.

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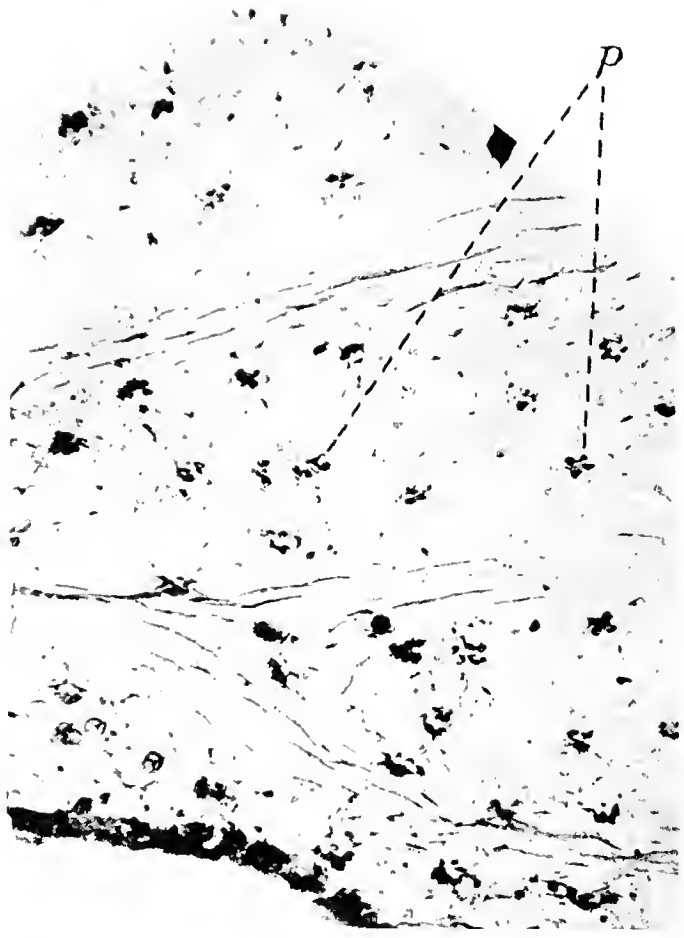

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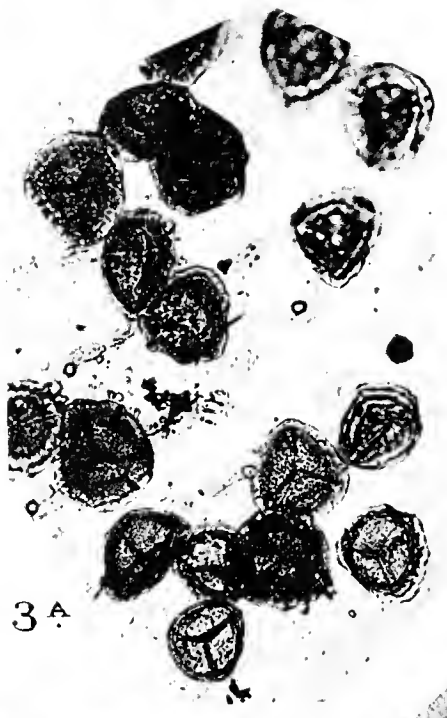

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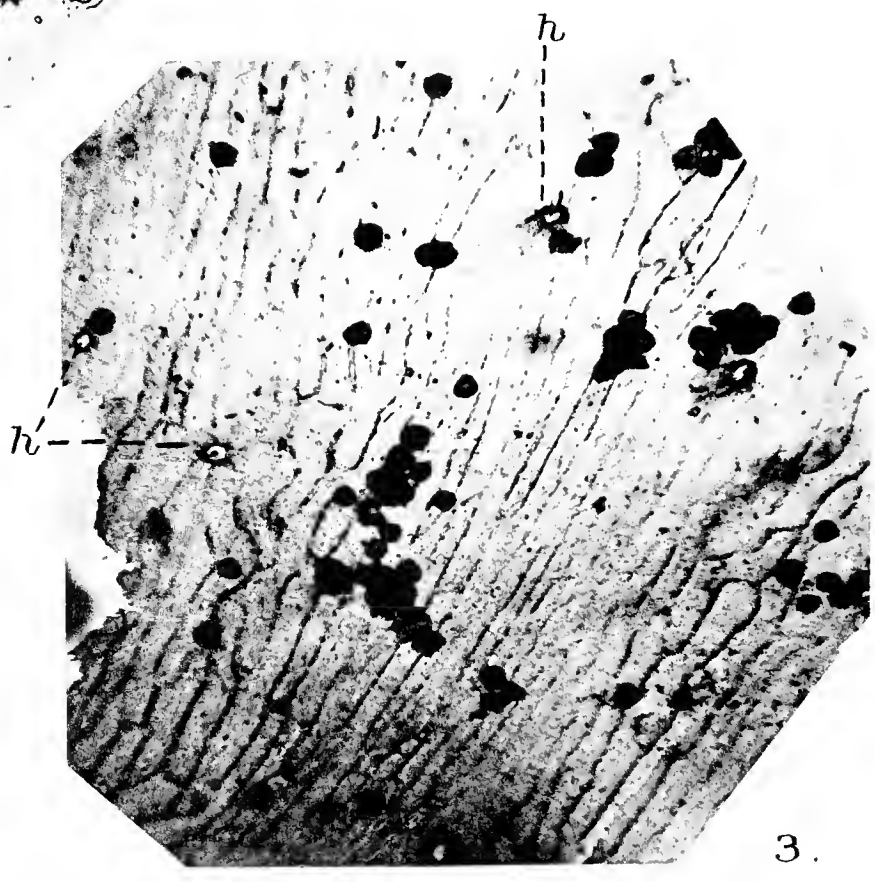

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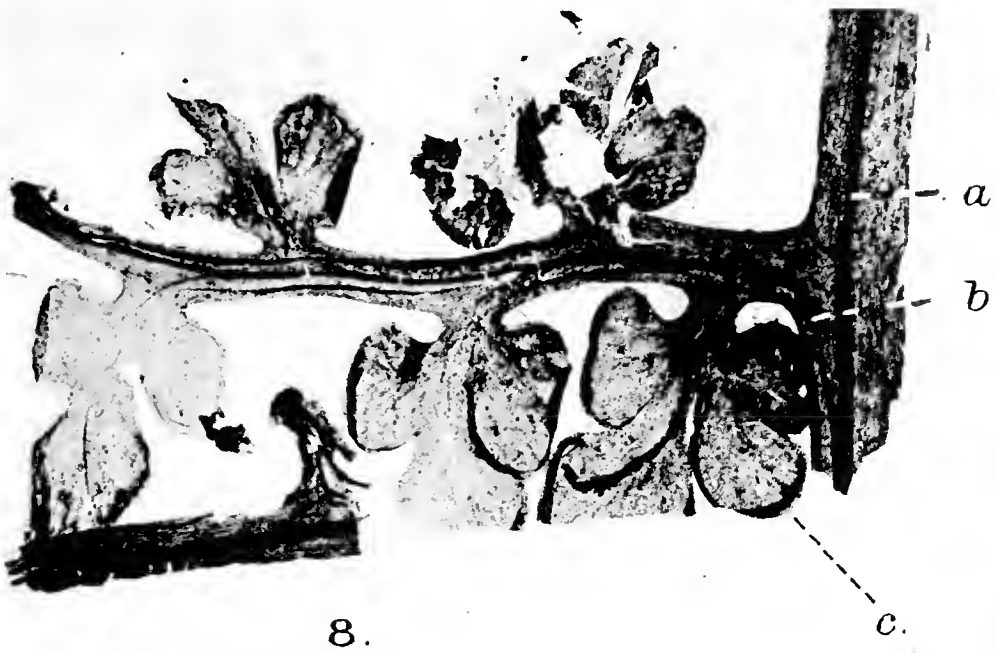
WALTON — FOSSIL PLANT 'IMPRESSIONS'.



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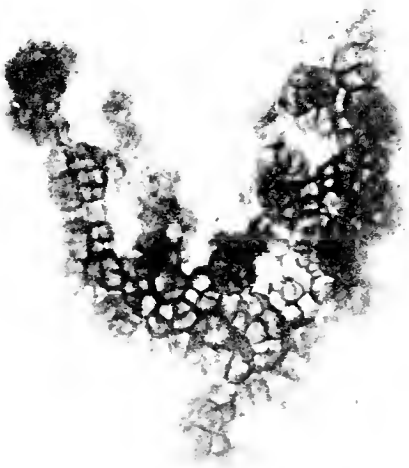

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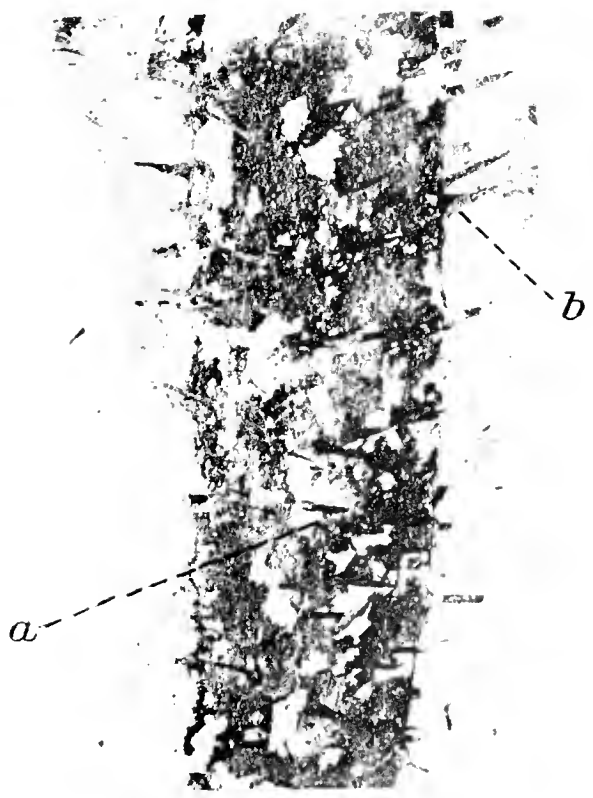

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———————: (ii) Sonder-Abdruck aus Bull. of the Geol. Inst. of Upsala, No. 4, vol. ii, pt. ii.

EXPLANATION OF FIGURES IN PLATE IX.

Illustrating Mr. Walton's paper on Fossil Plant Impressions or Incrustations.

Dactylotheca plumosa, Artis sp.

Fig. 1. Photograph by reflected light of a transfer preparation, showing the hairy under surface of the frond. The hairs can be seen most clearly at *a*. × 3·1.

Mariopteris, cf. *muricata*, Schloth.

Fig. 2. Photograph by reflected light of a transfer preparation. The shale (white in the photograph) has not been etched away completely in order that the glandular hairs, *h*, may be shown projecting through it. × 2.

3. Photograph of upper cuticle. The outlines of the epidermal cells can be seen. Glandular hair-bases at *h* and *h'*. Microspores can be seen sticking to the surface. × 60.

Fig. 3 *a*. Photograph of upper cuticle with adherent microspores. × 230.

Fig. 4. Photograph of lower cuticle. *v*, small folds in the cuticle marking the courses of the veins; *p*, groups of papillae on the epidermal cells surrounding each stoma. × 60.

Fig. 5. Photograph of a glandular hair situated at the edge of a fold in the cuticle. It is thus seen in profile. Note the microspores sticking to the cuticle near the hair. × 83.

Fig. 6. Photograph of one of the stomata on the under surface. Note the papillae, in this example five in number, which surround the stomatal depression in the epidermis. The guard-cells of the stoma itself are at a slightly lower level and can be seen within the ring formed by the papillae. × 440.

Cladotheca undans, (Halle) Lind. and Hutt. sp.

Fig. 7. Photograph by reflected light of a transfer preparation showing the fertile lower surface of a portion of a frond. The linear sori with densely packed sporangia can be distinguished. × 2·4.

Sphenopteris nummularia, Andr.

Fig. 8. Photograph by transmitted light of a transfer preparation of a portion of a frond. *a*, the vascular strand of the rachis; *b*, the vascular strand passing out to the lateral; *c*, incurved margin of pinnule. × 3·3.

Fig. 9. Photomicrograph of a stoma with the outlines of the surrounding epidermal cells. × 400.

Oligocarpia gutbieri, Göpp.

Fig. 10. Photograph by transmitted light of a transfer preparation showing sterile (*a*) and fertile (*bb*) pinnules. × 3·2.

Fig. 11. Photomicrograph of three sori. *a*, annulus; *v*, vein to sorus; *w*, main vein of pinnule. × 60.

Psilophyton princeps, Dawson.

Fig. 12. Photograph, partly by transmitted, partly by reflected light, of a transfer preparation. *a*, one of the spines on the surface of the preparation; *b*, spine exhibiting the basal expanded portion. × 3.

Fungus (spore-form, cf. Dematiaceae).

Fig. 13. Photomicrograph of a transfer preparation showing mass of septate spores. × 250.

The Morphology and Physiology of the Genus Eidamia.

BY

A. S. HORNE, D.Sc., and H. S. WILLIAMSON, B.Sc.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology.*)

With twenty-three Figures in the Text.

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I. Introduction.

THE genus *Eidamia* was founded by Lindau (11) to include fungi which bear a general resemblance to *Aspergillus*, but differ from it in possessing not only conidia but also spores of a second type. These spores are larger than the conidia and are borne singly on lateral branches as in certain species of *Monosporium*. This genus has hitherto consisted of one species only; *Eidamia acremonioides*, Harz, originally described as *Monosporium acremonioides* by Harz in 1871, and as *Papulaspora aspergilliformis* by Eidam (6) in 1883.

Comparatively recently the writers, working concurrently on different problems, obtained two fungi which, although differing from one another and from *Eidamia acremonioides* somewhat widely, nevertheless agree with *Eidamia* in possessing spores of the *Monosporium* type in addition to their

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conidial fructifications. The two new species differ from *E. acremonioides* chiefly in the form of the conidiophores, which are irregularly branched and not typically swollen at the apex, and in the fact that the second type of spore is uncoloured, whereas it is brown in *E. acremonioides.* Since the two species agree more closely with *Eidamia acremonioides* than with any allied fungus, they have been named *Eidamia viridescens*, n. sp., and *Eidamia catenulata*, n. sp., respectively.

II. Morphological Characters.

1. *Eidamia acremonioides* ; syn. *Monosporium acremonioides*, Harz, *Papulaspora aspergilliformis*, Eidam.

A culture on rice was obtained from the fungus collection at Amsterdam. The growth on rice was filmy and coloured brown, due to the number of large brown spores produced. Single spore cultures were obtained from gelatine dilutions and used to subculture on a variety of media.

On potato mush agar at 20° C. a filmy growth of mycelium was obtained producing macrospores (spores of the *Monosporium* type). The colour of the culture gradually changed from snuff-brown (see Ridgeway's 'Colour Standards' for nomenclature adopted) to Vandyke brown as the spores matured. These spores are large, brown, and ovoid, resembling those described for *Monosporium* by Delacroix. They are borne singly on lateral branches of variable length, and these are themselves branched often at a right or obtuse angle. These spores are thick-walled and vary in size from 12 $\mu \times 10\ \mu$ to 34 $\mu \times 28\ \mu$, or even reach 40 $\mu \times 34\ \mu$ on potato glucose agar. Fig. 1 shows the typical branching and the macrospores of *Eidamia acremonioides.* Macrospores were the only spores produced when the fungus was grown on rice at 20° C., potato slab at 25° C., and potato mush agar at 30° C.

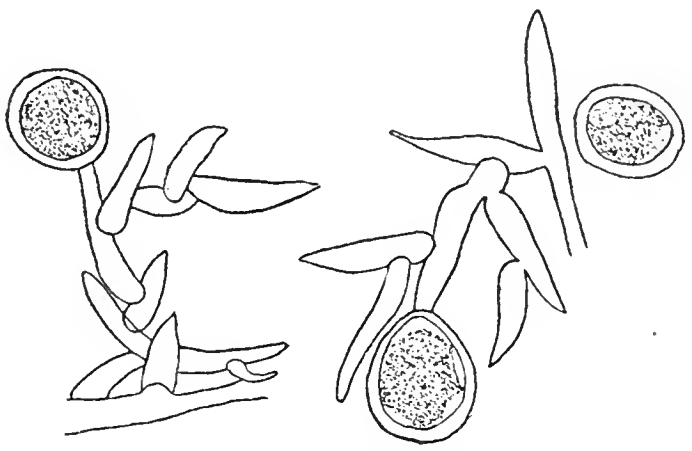

Fig. 1. *E. acremonioides.* Mycelium, showing typical branching and macrospores. × 390.

The conidiophores are long, averaging 140 μ in length, terminating in a swollen head of about 24 μ in diameter on which are borne the sterigmata and conidia. The sterigmata are swollen towards the base and pointed at the apex, being 6 μ in length and 4 μ in basal width. On these the conidia arise typically in short chains, though often they appear to cling to the pointed ends of the sterigmata in groups (Fig. 2). The conidia are hyaline, circular, or egg-shaped, being 1·5 μ to 2 μ in diameter.

The aspergilliform head of conidia is typical for this fungus, but on potato extract agar every stage between this form and sterigmata borne singly on a lateral hypha occurred (Fig. 3). This variation was noted by Bainier (1) in his description of *Papulaspora aspergilliformis*, and by Eidam (6) in his description of *Helicosporangium parasiticum*, the figures of which markedly resemble those found by the writers in *Eidamia acremonioides* on potato extract agar.

The peculiar growths (bulbils) figured by Rabenhorst for *E. acremonioides* were not observed in the culture media employed in this investigation.

Growth was obtained on potato extract agar, potato mush agar, potato glucose agar, rice, in a 2 per cent. solution of peptone (where many irregular

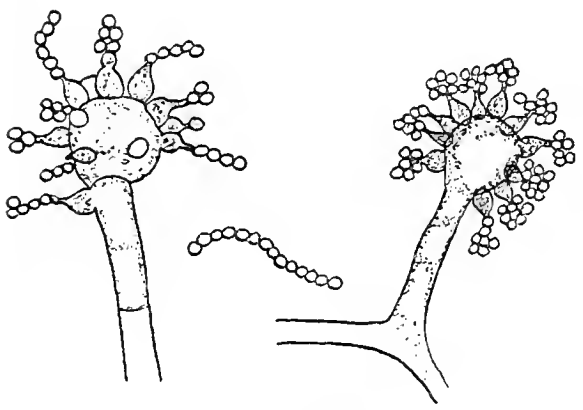

FIG. 2. *E. acremonioides.* Conidiophores showing chains and groups of conidia. × 390.

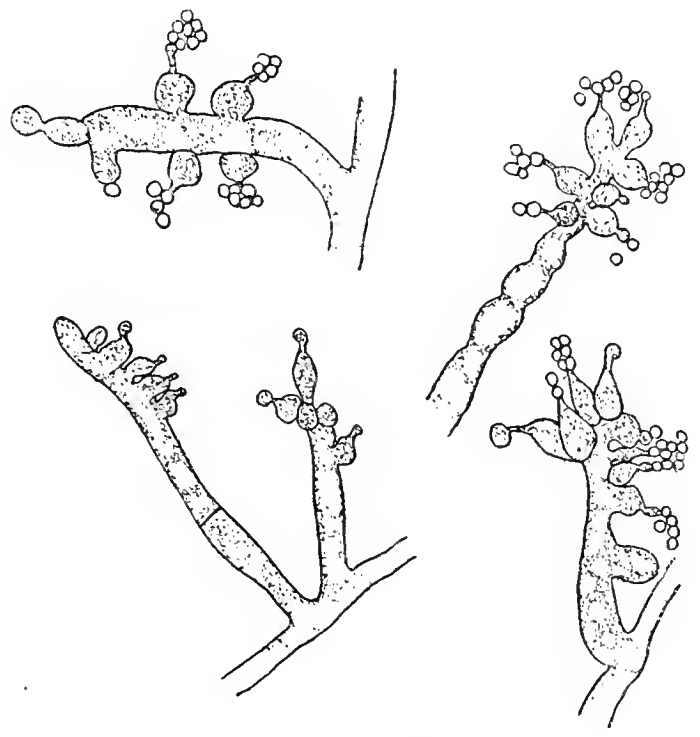

FIG. 3. *E. acremonioides.* Types of conidiophores produced when grown on potato extract agar at 20° C. for four days. × 390.

short branches occurred on the mycelium, probably representing incipient conidiophores, and the majority of the macrospores were hyaline), and 2 per cent. peptone containing a 4 per cent. concentration of glucose. Slight growth also was visible in solutions of sucrose and maltose and a synthetic nutrient agar. With potato extract agar and certain percentages of citric acid the growth was tufted and exhibited considerable sporulation with macrospores at the higher concentrations; whilst on neutralized potato extract a thick scum-like growth was produced, dotted with brown macrospores and conidia in chains and groups.

The colour of the growth produced in various media varied with the age of the culture and temperature conditions. At 20° C. the initial colour was light brown, changing to snuff-brown and finally Vandyke brown, whereas at 25° C. the initial colour was, as a rule, pinkish buff or pinkish cinnamon; and where growth occurred at 30° C. the colour was usually ochraceous salmon.

Optimum growth in all the culture media employed was obtained at 20° C., with very little growth at 30° C. On synthetic nutrient agar no growth occurred at 25° C. or 30° C.

The production of conidia and the colour of the macrospores are affected by temperature. At 30° C. no conidia were observed and the majority of the macrospores were hyaline, whilst at 20° and 25° C. numerous conidia and brown macrospores were formed.

2. *Eidamia viridescens*, n. sp.

This fungus was isolated from rotting apples and single spore cultures obtained. The mycelium consists of colourless septate branched hyphae, which may be as much as 7 μ to 11 μ in width. The character of the mycelium varies according to the medium used. It may have a gelatinous consistency, as when the fungus is grown in 2 per cent. peptone, neutralized potato extract, and potato extract with hydrochloric acid in N/100 or N/1000 concentrations. The mycelium is filmy in appearance in 1 per cent. concentrations of wheaten starch in agar and in potato extract agar, containing various concentrations of malic, citric, tartaric, gallic, tannic, or hydrochloric acid or in solutions of various sugars. A flocculent woolly appearance occurs in potato extract solutions containing gallic or malic acid in 1·5 or 2 per cent. concentrations.

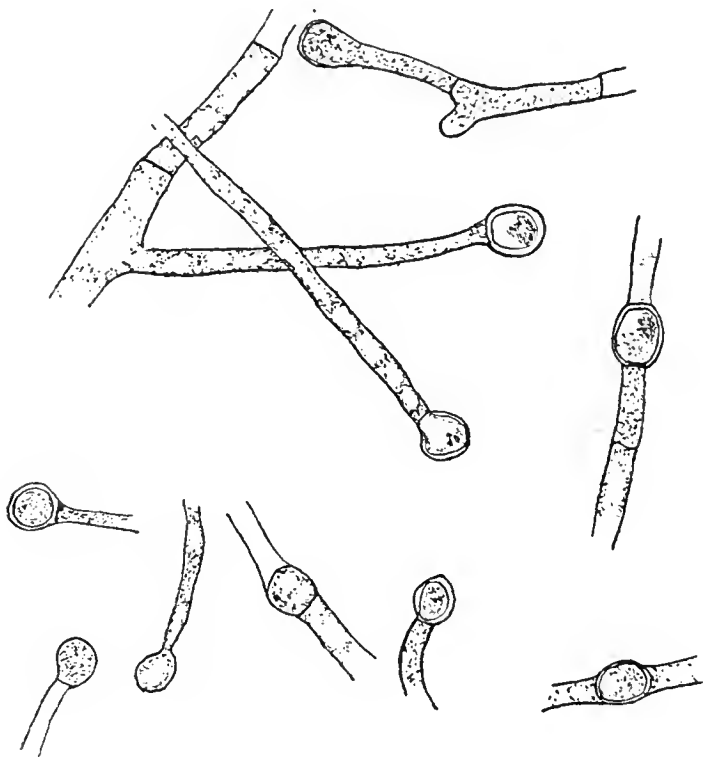

FIG. 4. *E. viridescens*. Terminal and intercalary macrospores on potato extract agar at 25° C. × 390.

Two kinds of spores are produced, hyaline macrospores and conidia. The macrospores are thick-walled, circular, or ovoid, varying in size from 11 μ × 8 μ, 13 μ × 9 μ, to 12 μ in diameter. They are borne singly at the end of lateral branches or may be intercalary (Fig. 4). These are developed in from 3 to 8 days after inoculation according to the medium employed. Macrospores were found in all the culture media employed except prune agar at 20° C. and synthetic nutrient agar at 20° C., though a few were present on synthetic nutrient agar at 25° C. after eight days' growth.

The conidiophores are, as a rule, branched structures with the sterigmata borne either in groups or singly along the branches (Figs. 5 and 6). The sterigmata resemble those of *E. acremonioides* in structure, being broad

at the base and pointed at the apex. They vary in size from 8 μ to 20 μ in length and 1·5 μ to 3·5 μ in basal width. The conidia are borne on the sterigmata in small groups, though occasionally small chains have been observed. They are slightly ovoid or ellipsoid, measuring 4 $\mu \times 5$ μ or 2·5 $\mu \times 4$ μ, or they may be spherical, and in diameter 4·5 μ. On potato mush agar conidia reached the size of 6 $\mu \times 4$ μ.

The colour of a single conidium is difficult to determine. When present in masses on the white mycelium they are often intensely coloured and pass through a series of colour changes varying according to the composition of the culture medium, temperature, and the age of the growth. These masses, which are usually evident three days after inoculation, are at first white, changing to cream, mustard yellow, dull yellow green, cinnamon buff,

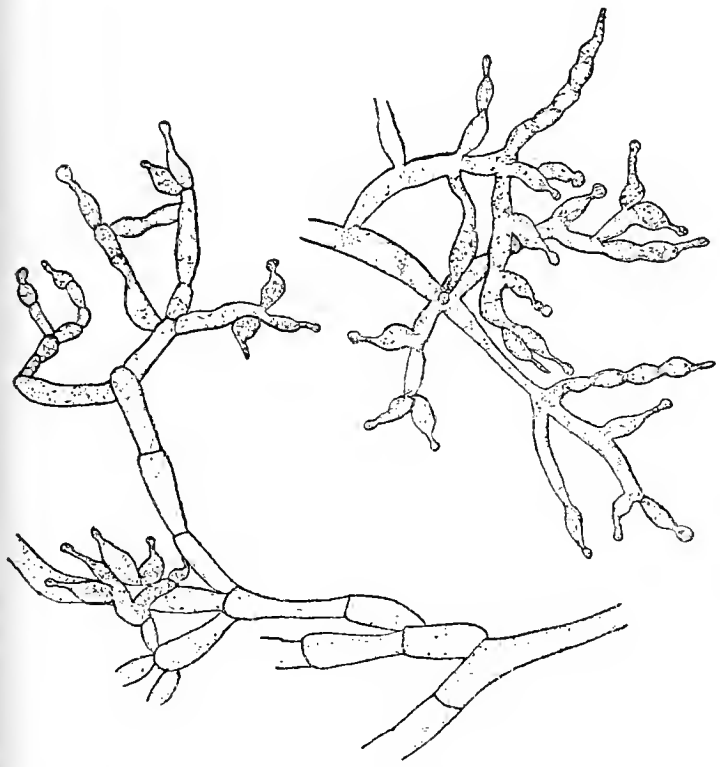

FIG. 5. *E. viridescens.* Conidiophores on synthetic medium plus agar at 20° C. × 390.

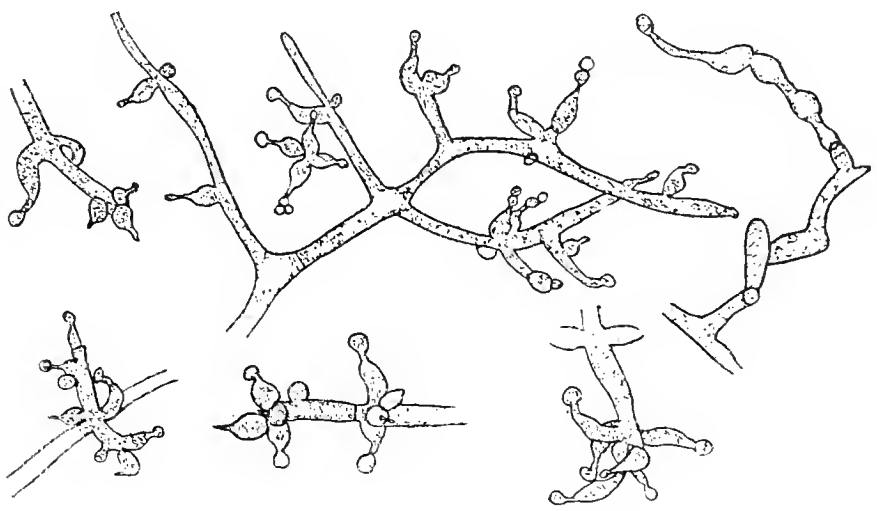

FIG. 6. *E. viridescens.* Conidiophores from a culture on potato extract agar at 20° C. × 390.

tawny olive, and elm green in eight days on potato mush agar (20° C.). At 25° C. on the same medium and during the same length of time the sequence of colour changes is massicot yellow, mustard yellow, light bice green, light elm green, dark olive buff, and buffy brown. On synthetic nutrient agar after eight days the final colour was dark cress green, and a very characteristic grass-green colour occurred when the fungus was grown in an alkaline medium. Characteristic changes in colour occur from day to day on the different media employed. The colour is insoluble in alcohol, ether, or chloroform. Spores were shaken up well in ether, and the coloured suspension of spores sank quickly to the base of the test-tube, leaving uncoloured ether above. The same experiment performed with chloroform showed the coloured conidia remaining suspended for a time and then gradually rising to the surface, leaving the uncoloured liquid below. These experiments give an indication of the specific gravity of the conidia.

Fragmentation and the production of swollen cells occurred in N/100 and N/1000 HCl, in solutions of sugars, and in soluble pectin. The optimum temperature for growth lies between 20° and 25° C., no growth being obtained at 30° C.

Bramley's seedling apples were inoculated with *E. viridescens* kept at 20° C. and examined after eight days. Very little surface growth was visible, but definite growth into the apple occurred, showing that the fungus is capable of parasitizing the living cell.

3. *Eidamia catenulata*, n. sp.

This fungus was isolated from a seasoned specimen of the heartwood of *Quercus robur*. Single spore cultures were obtained and all sub-cultures made from them.

The mycelium consists of colourless septate branched hyphae varying from 3 μ to 11 μ in width. These hyphae are usually thin-walled, but thick-walled hyphae do occur when the fungus is grown on certain media, such as potato glucose agar or prune agar. The mycelium usually forms a close thin felt, though it may be filmy, when grown on gallic or citric acid agar and sugars such as sucrose, lactose, and maltose; or flocculent, when grown on 1·5 and 2 per cent. tannic or gallic acid agar, and potato extract with various concentrations of malic acid; or of a sub-leathery consistency, when grown on 2 per cent. peptone with 4 per cent. glucose. In potato extract containing N/10, N/100, or N/1000 concentrations of HCl the hyphae show many swollen bulbous cells, some of which produce sterigmata and conidia (Fig. 7).

Two kinds of spores are produced, hyaline macrospores and conidia. The macrospores are borne directly on the hyphae or at the end of a short branch, or may be intercalary. They often occur in pairs and are thick-walled, round, or pear-shaped, with well-marked contents (Fig. 8). They vary in size from 7·5 $\mu \times$ 8·5 μ to 14 $\mu \times$ 10 μ, or may be even 18 μ in diameter. These hyaline spores occur on all the culture media employed when kept at 20° or 25° C.: they are less numerous or absent at 30° C.

The conidia are borne in long chains (containing up to 100 spores in each) on sterigmata arising directly from an aerial hypha, or on short branches from such a hypha, or on a more complicated conidiophore where a branch from the mycelium has again branched several times, forming one to several sterigmata on each branch (Fig. 9). Occasionally, several sterigmata arise from a slightly swollen short branch (Fig. 10), and sometimes a *Penicillium* type of conidiophore may occur (Fig. 11). The sterigmata are slender, slightly swollen at the base, and vary in size from 8 μ to 16 μ in length and 1 μ to 2·5 μ in width at the base.

The conidia are yellow in colour, narrow to broadly elliptical, pointed

at both ends, and vary in size from 4 $\mu \times 2$ μ to 7 $\mu \times 3{\cdot}5$ μ. Under certain conditions the conidia are disposed in groups: the conidium at the end of a chain tilts over until it rests sideways on the one below, and this process continues until all the spores are arranged in a group at the tip of

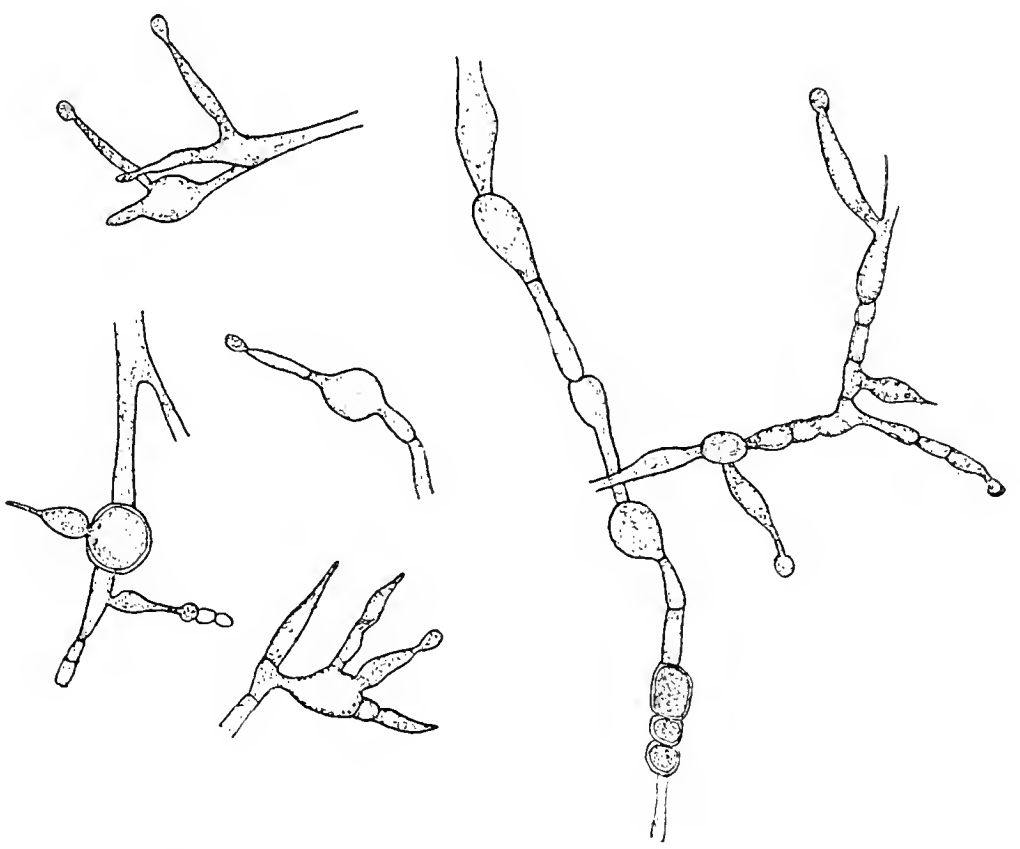

FIG. 7. *E. catenulata.* Swollen cells producing sterigmata; from a culture on N/100 HCl potato extract agar at 20° C. × 390.

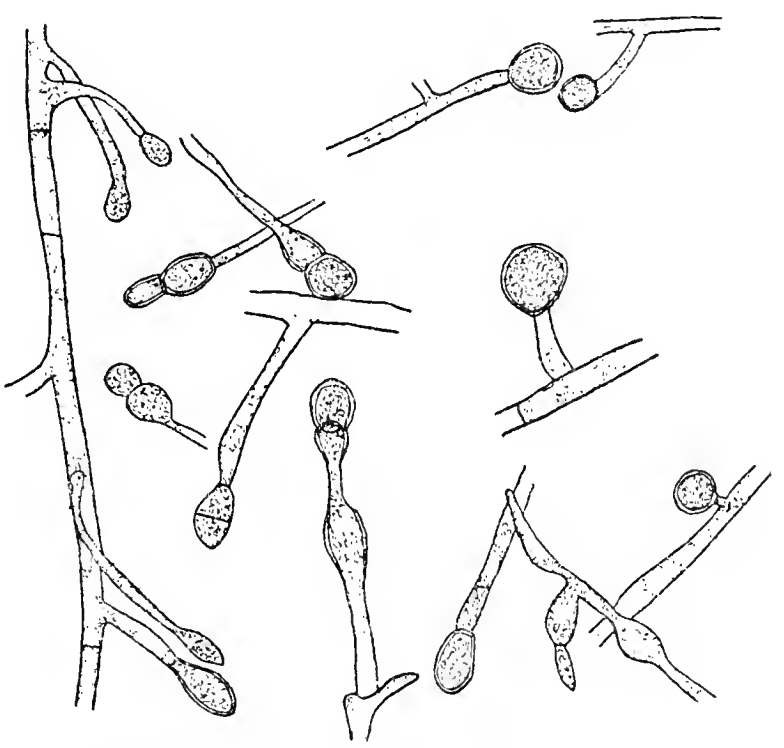

FIG. 8. *E. catenulata.* Macrospores from a four-day culture on potato extract agar at 20° C. × 390.

the sterigma (Fig. 12). This grouping, which is incidental for this species, is typical of *E. viridescens*, where chains are only occasionally seen. The disposition of conidia in groups may be easily overlooked when the conidia are mounted in liquid media, since they easily fall away from the sterigmata.

The colour exhibited in cultures of this species is due to the innumerable

coloured conidia produced on the aerial mycelium. This colour is insoluble in ether, chloroform, alcohol, and water. When the spores are suspended in ether the liquid remains uncoloured; the spores sink slowly, indicating a lower specific gravity than in the case of *E. viridescens*. When suspended

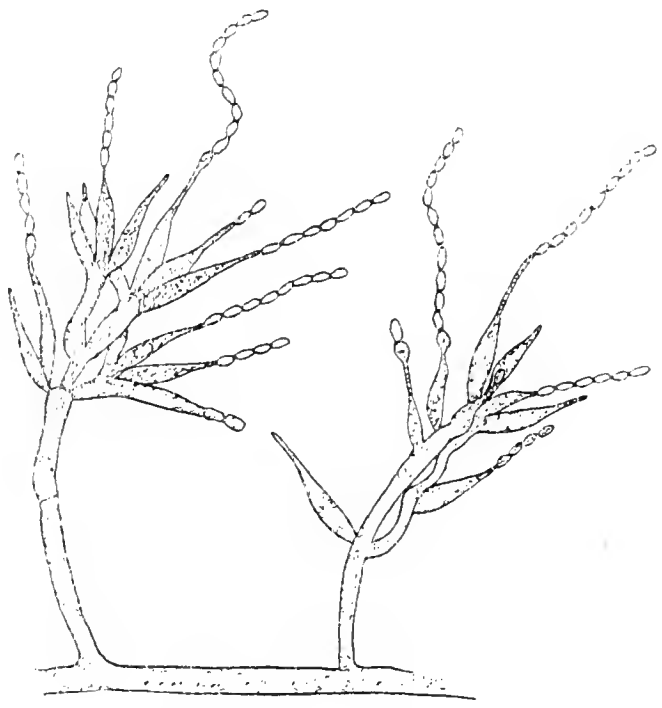

FIG. 9. *E. catenulata.* Condiophores. × 390.

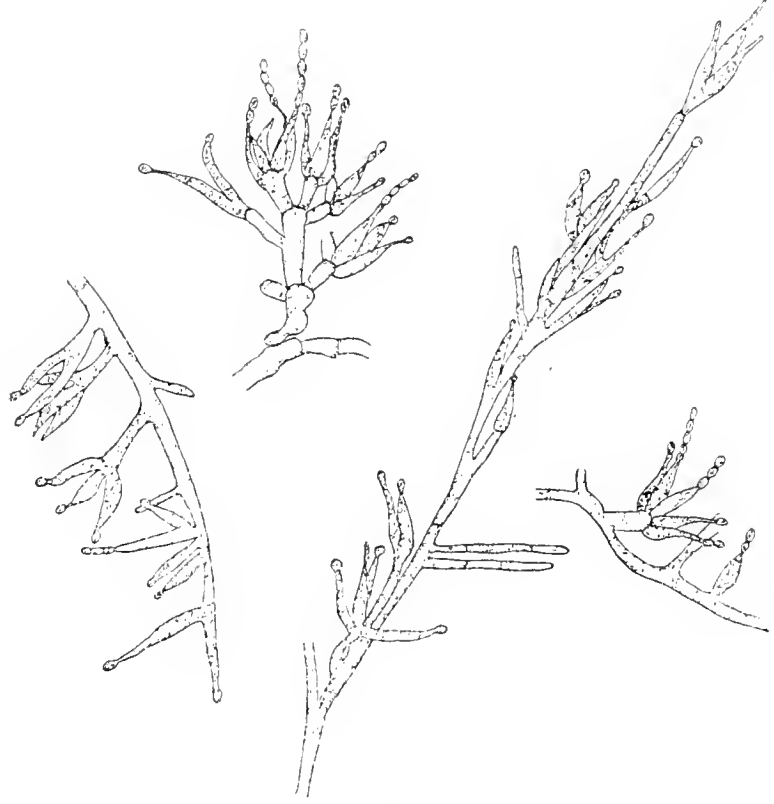

FIG. 10. *E. catenulata.* Conidiophores of various types on a synthetic medium plus agar at 20° C. × 290.

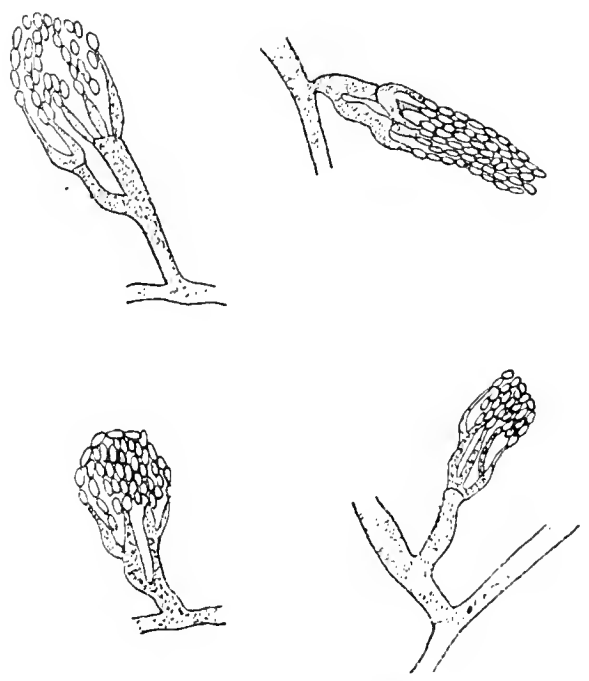

FIG. 11. *E. catenulata.* The *Penicillium* type of conidiophore on potato glucose agar at 25° C. × 390.

in chloroform the spores slowly rise to the surface, leaving the liquid uncoloured below.

The spore colour varies according to the medium (this factor had not so pronounced an effect as with *E. viridescens*), temperature, and the age of the growth. During fourteen days at 20° C. (potato extract agar) the

following sequence of colour changes was observed: pale cream, straw yellow, tawny olive to sayal brown; at 25° C. on the same medium the sequence was straw yellow, old gold, and sayal brown specked with pinkish buff; while at 30° C. the colours ranged from cream buff to honey yellow, and finally to avellaneous. Other colours produced on different media are Naples yellow, chamois, Isabella colour, russet, and cinnamon brown. The darker colours are those produced after about fourteen days' growth, and they do not show so much variation as in the case of *E. viridescens*.

The optimum temperature for growth proved to be 30° C., moderate growth occurred at 25° C., and least at 20° C. Growth took place to

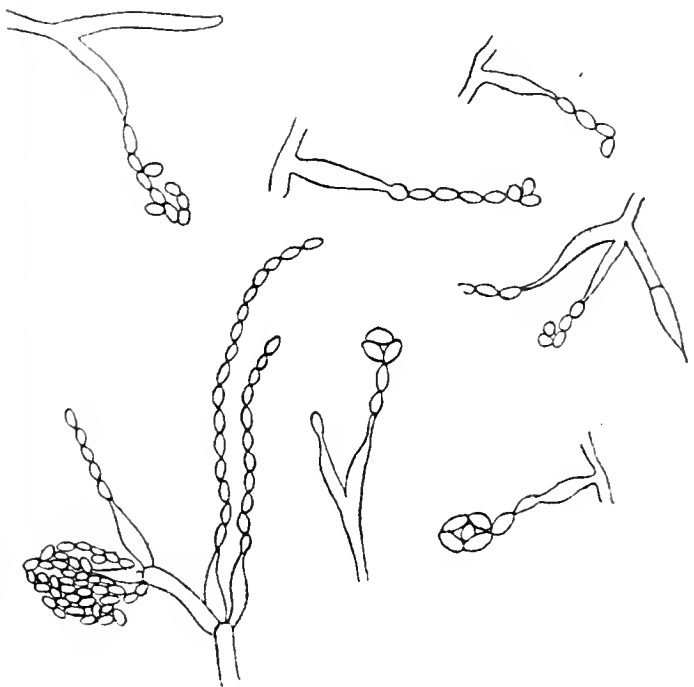

Fig. 12. *E. catenulata*. The formation of conidial group from a culture on potato extract agar at 25° C. × 390.

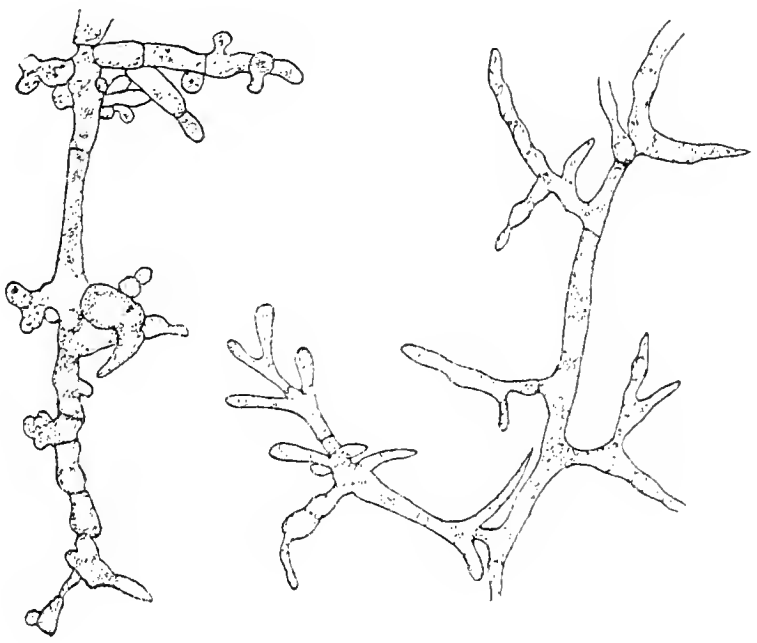

Fig. 13. *E. catenulata*. Mycelium with irregular swollen clusters of cells; from a synthetic medium plus agar at 25° C. × 390.

a greater or less extent on all the culture media employed except cellulose. On synthetic nutrient agar some variation occurred in the mycelium, where very irregular cells and small branches arose in clusters (Fig. 13) suggesting remotely the bulbils figured for *E. acremonioides*.

Bramley's seedling apples inoculated with *E. catenulata* after eight days at 20° C. showed a surface growth of mycelium and conidiophores at the place of inoculation, but no parasitism of the living cell occurred.

Growth on seasoned pine, chestnut, beech, and oak was prolific, and penetration soon took place (in the case of *E. viridescens* no growth occurred).

III. Physiological Characters.

A. *Growth in Carbohydrate, Protein, and Asparagin.*

1. *Sugars.*

Growth was studied in 4 per cent. solutions of sugars (flask cultures), sugar solutions with agar added (plate cultures), and nutrient agar containing sugar.

The sugars used include glucose, sucrose, fructose, lactose, mannose, galactose, and maltose.

For the flask cultures the sugars were dissolved in distilled water, and steamed for thirty minutes on three consecutive days in the steam sterilizer. The flasks (capacity 100 c.c. and containing 50 c.c.) were then inoculated, using small masses of spores in each case, and kept at a temperature of 20° C. *E. acremonioides* produced only slight growth in maltose with a certain amount of sporing at the surface of the liquid, but no growth whatever occurred in glucose, sucrose, and lactose.

When grown in sucrose, both *E. catenulata* and *E. viridescens* produced feeble growth with comparatively few spores after five weeks. At the end of this period appreciable precipitates were obtained on using Fehling's test for reducing sugar. Tests carried out under comparable conditions indicated that *E. catenulata* had produced relatively more invert sugar than *E. viridescens*. Control solutions kept under the same conditions for the same length of time were not inverted. All the solutions were originally, and remained, neutral to litmus. A faint smell of coco-nut oil was emitted from the cultures of *E. viridescens*.

In solutions of glucose *E. catenulata* forms much submerged growth of loose texture and a grey green powdery superficial film, owing its colour to small groups and masses of conidia. Hyaline spores (macrospores) were not observed. The initial acidity of the solution (pH 4·8 and total acidity equivalent to 1·3 c.c. N/1 NaOH per litre) remained unchanged after fourteen days' growth. *E. viridescens* also formed considerable growth and a powdery surface film, green owing to the colour of the spore masses. Intercalary and terminal hyaline spores were present. No change in the acidity occurred. The odour of coco-nut oil was moderately evident. The colour of the liquid initially pale yellow changed to bright yellow.

In lactose, *E. catenulata* formed much superficial and submerged growth after twenty days, accompanied by moderate spore formation. The acidity increased (initial acidity equivalent to 0·72 c.c. N/1 NaOH per litre: final 1 c.c.). Under the same conditions *E. viridescens* also produced much growth with dark green superficial spore masses. The liquid initially uncoloured became bright yellow green, and a strong smell of coco-nut oil was emitted. The acidity increased (final acidity equivalent to 1·2 c.c. N/1 NaOH per litre). This result was not constantly obtained; in some cases the solution became less acid (initially pH 4·9: final, after thirty days, pH 5·6), an anomaly probably due to differences in the degree of purity of the lactose used in different experiments.

Both *E. catenulata* and *E. viridescens* produce considerable growth in maltose (twenty days), the former species forming numerous spore masses, and the latter sporing moderately. The acidity in *E. catenulata* remained unchanged, but in *E. viridescens* it was slightly reduced (initial acidity

equivalent to 2·96 c.c. N/1 NaOH per litre: final 2·24 c.c.). The colour of the liquid (*E. viridescens*) changed from yellow to greenish yellow. Odour of coco-nut oil present.

E. viridescens produces moderate growth in galactose and little growth in mannose and fructose, when kept for eight weeks at 20° C.; in each case the odour of coco-nut oil is obtained on heating the solution.

From these results, it is clear that the two fungi react differently in solutions of sugars: in *E. viridescens* growth is accompanied by coloration of the liquid and emission of odour; in *E. catenulata* these characteristics are absent.

Cultures of *E. viridescens* in maltose, glucose, lactose, and galactose were tested for actual utilization of sugar by titrating with Fehling's solution after thirty days' growth. The sugar in a control flask, kept under the same conditions, was estimated at the same time. Definite evidence that sugar had been utilized was obtained in each case: for example, with glucose control flasks contained 4 per cent. of sugar, the inoculated 3·5 per cent., lactose control 3·75 per cent., inoculated 3·5 per cent.

Solutions of pure sugar made up in conductivity water should prove neutral to litmus. The acidity of the solutions used in these experiments is attributed partly to the use of ordinary distilled water and partly to impurities present in the sugars themselves. Reduction of acidity where it occurs is probably not connected in any way with the fungal action on sugar, but due to reaction to the impurities present or to actual utilization of acid by the fungus.

The series of sugars containing agar comprised molar, and fifth, tenth, fiftieth, and hundredth molar solutions of lactose, glucose, and sucrose, using distilled water. The layer of medium in the plate when poured was approximately 3 mm. thick. Two series of plates were prepared and inoculated with *E. catenulata* and *E. viridescens* respectively, and kept at 20·5° C. Control plates containing agar only were also prepared. Observations were made on the rate of growth in terms of the diameter of the colony, degree of sporulation, colour of the spore masses, and emission of odour.

The actual growth measurements made at twenty-four hours' intervals are given on p. 404.

The results do not differ in general from those obtained in liquid cultures. The odour of coco-nut oil was again evident in cultures of *E. viridescens*, whereas *E. catenulata* produced no smell. The growth-rate of *E. viridescens* is considerably greater than that of *E. catenulata* in molar solutions of all strengths, and the total growth at the end of four days is about three times as great. Since *E. catenulata* exhibits relatively slow growth as compared with *E. viridescens* under all temperature conditions, and even when cultures of these fungi are grown at their respective tempera-

ture optima, the fact that *E. catenulata* was grown at a temperature below the optimum (25° C.) would not greatly affect the comparative value of the results.

Growth in diameter (cm.) of E. viridescens in Solutions of Sugars.

Sugar.	*Strength.*	*Interval in hours.*			
		24	48	72	96
Lactose	N/5	0·8	2·5	5·5	7·7
	N/10	0·9	2·9	6·0	8·0
	N/50	0·9	2·85	5·0	7·0
	N/100	1·1	2·75	4·7	6·5
Glucose	N	0·4	1·6	3·3	4·5
	N/5	0·35	2·5	5·0	7·2
	N/10	0·8	3·3	5·5	7·9
	N/50	0·8	3·85	5·2	7·3
	N/100	0·85	2·9	4·9	7·0
Sucrose	N	0·25	1·3	2·6	4·2
	N/5	0·8	3·1	5·7	8·4
	N/10	0·2	1·9	4·9	7·2
	N/50	0·8	3·0	5·3	7·3
	N/100	0·8	2·9	4·9	7·1
Control I.		0·8	2·6	4·6	6·6
Control II.		0·9	2·65	4·65	6·55

Growth in diameter (cm.) of E. catenulata in Solutions of Sugars.

Sugar.	*Strength.*	*Interval in hours.*			
		24	48	72	96
Lactose	N/5	0·15	0·65	0·95	2·55
	N/10	0·25	0·7	1·5	2·3
	N/50	0·35	0·85	1·35	1·8
	N/100	0·35	0·8	1·2	1·7
Glucose	N	0·1	0·7	1·6	2·5
	N/5	0·2	1·0	2·0	2·85
	N/10	0·4	0·9	1·8	2·8
	N/50	0·25	1·0	1·7	2·35
	N/100	0·3	0·85	1·5	2·1
Sucrose	N	0·15	0·9	1·65	2·5
	N/5	0·2	1·1	2·0	2·9
	N/10	0·2	1·1	1·95	2·75
	N/50	0·3	0·95	1·55	2·2
	N/100	0·3	0·8	1·4	1·9
Control I.		0·25	0·75	1·25	1·55
Control II.		0·2	0·7	1·15	1·5

The results are not strictly comparable with those obtained in the liquid cultures, since in this case agar is present, and both *E. catenulata* and *E. viridescens* are capable of growth and sporulation in agar alone, producing scanty mycelium and small scattered spore masses. Since agar itself belongs to the group of carbohydrate substances it is possibly utilizable to different degrees according to the kind of sugar added to it, and especially if impurities are present in it. Thus both *E. catenulata* and *E. viridescens* exhibited poor growth in sucrose as compared with lactose in liquid cultures, but in the plate cultures the growth after four days in sucrose and lactose is nearly the same. Little comparative value is attached to the growth differences exhibited in lactose, glucose, and sucrose respectively, since these

results are likely to vary with the use of distilled water or conductivity water and according to the degree of purity of the chemicals themselves.

In both *E. catenulata* and *E. viridescens* the maximum growth occurs in N/5 or N/10 solutions. In the case of *E. viridescens* growth is considerably retarded in molar solutions, but in *E. catenulata* the retardation is not strongly marked.

In the case of *E. viridescens*, odour was present in every member of each series, being strongest in the fifth molar member. Whilst practically no difference could be detected between the glucose and lactose series the odour was appreciably less in the sucrose series. With regard to sporing, after four days no sporing was evident in the unimolar cultures: in the remaining cultures very little difference in the amount of sporing was manifest. The sporing is confined to a zone about 15 to 17 mm. wide in the peripheral region of the colony. After a longer period of time the colour of the spore masses in unimolar glucose varied from white to pale olivine, passing to dark yellowish green and dark green in N/50 and bottle-green in N/100, with the central region of the plate free from spores: in lactose the colour varied from pale olivine to French green in N/5 and N/10, changing to Empire green in N/100, whilst in sucrose the colour ranged from French green to Danube green.

In the case of *E. catenulata* there was little colour variation in the spore masses (relatively more numerous in N/5 cultures) throughout the series; the colour varied from cream-buff to chamois, whereas in agar alone it was avellaneous.

2. *Starch.*

The ability of the species of *Eidamia* to hydrolyse starch was tested by the method employed by George L. Peltier (14). Peltier applied a solution of iodine in potassium iodide to the surface of starch-containing media containing growths of *Pseudomonas citri*, and found that the colonies of *P. citri* remained yellow, whilst the surrounding medium was coloured dark blue. Each colony was surrounded by a narrow, clear, starch-depleted zone: this was followed by a reddish-brown band indicating erythrodextrin, an intermediate product, which merged into a bright blue band and finally into the dark blue colour of the starch-containing agar. This method has been in use for some time by the first-named writer as an aid to the specific determination of fungi, since fungi often show well-marked differences in the effect produced upon irrigation with iodine, related chiefly to the width of the coloured and uncoloured zones and the intensity of the colours present in the region where partial hydrolysis exists. Various starch-containing media were used for this purpose, notably potato extract agar with or without various organic acids and synthetic nutrient media of different kinds. In the case of *Eidamia acremonioides* no evidence indicating starch hydrolysis

was obtained. In *E. catenulata* (potato extract agar with a 0·25 per cent. concentration of malic acid) after six days at 20° C. the colony, after irrigation, was surrounded by a narrow clear zone with a purplish-pink border where it abutted on the starch-containing agar: the altered zone having a width of from 2 to 3 mm. The agar below the colony was uncoloured. A certain amount of variation occurred in plates containing other concentrations of malic acid: thus with a 1 per cent. concentration the purplish band coincided with the margin of the colony. Very similar results were obtained with *E. viridescens*.

Owing to the very poor growth of *E. acremonioides* obtained in the plate cultures, the failure to obtain a definite reaction by iodine irrigation could not be ascribed to the inability of the species to hydrolyse starch. Hence it was necessary to employ other methods. Solutions containing 1 per cent. peptone and 0·1 per cent. starch made up in flasks were inoculated with the three fungi and kept at 20° C. for five and a half days. Each fungus having produced a moderate amount of growth in this period, the peptone-starch solutions were tested with a weak iodine solution. With a given amount of iodine added to a fixed quantity of peptone-starch, the control (uninoculated solution) was deeply coloured; the solution containing *E. acremonioides* exhibited the same intensity of coloration as the control; the solution with *E. catenulata* was slightly coloured, whilst that containing *E. viridescens* was uncoloured. Again, *E. acremonioides* did not show any indication of action upon starch. A difference in the amount of hydrolysis, induced through the growth activities of *E. catenulata* and *E. viridescens* respectively, was apparent.

A further experiment was made using 1 per cent. wheaten starch made up with distilled water. The flasks were kept at 20° C. and tested after three weeks. At the end of this period *E. acremonioides* had made practically no growth. The solutions containing the remaining species were tested with standard iodine solution as before. In *E. viridescens* hydrolysis was nearly complete: the liquid was of a greenish colour and a pronounced smell of coco-nut oil was evident. In *E. catenulata* hydrolysis was incomplete. No acid was produced in either case.

Since *E. viridescens* is a more rapidly growing fungus than *E. catenulata*, its greater hydrolysing activity, under the conditions obtaining in these experiments, is not surprising.

3. *Soluble Pectin.*

The ability of the species of *Eidamia* to grow in soluble pectin extracted from apples was tested in connexion with a series of experiments, relating to fungal growth in pectin, carried out by the first-named author in collaboration with Miss M. H. Carré (2). Since these experiments will be

described in detail elsewhere it is unnecessary to make more than brief reference to this subject here. The growth experiments were carried out in 50 c.c. flasks containing 25 c.c. of a 1 per cent. neutral solution of practically pure pectin. The amount of pectin actually present in each flask was estimated after autoclaving. The flasks were inoculated in duplicate series and kept at 20° C. for a given period. At the end of this period final pectin estimations were made, the solutions were titrated against standard alkali and tested with Fehling's solution for the presence of reducing sugar. The following general results were obtained:

E. acremonioides. No growth.

E. catenulata. Moderate growth with utilization of pectin. Solution slightly acid. Liquid does not become green. Fehling's solution not reduced.

E. viridescens. Moderate growth. Pectin completely used up. Solution slightly acid. Liquid becomes greenish in colour. Fehling's solution reduced.

4. *Peptone.*

Flasks, containing a 2 per cent. solution of peptone with an initial pH 6·8 and total acidity equivalent to 10 c.c. N/1 NaOH per litre, were inoculated with *E. catenulata*, *E. viridescens*, and *E. acremonioides* and kept at a temperature of 20° C. After fourteen days the growth characteristics were recorded, the final pH was obtained by the indicator method, the total alkalinity estimated in terms of c.c. N/1 HCl using neutral red as indicator, and the atmosphere of the flask tested for the presence of ammonia by means of suspended litmus paper.

Result:

E. catenulata. Moderate floating and submerged growth: superficial growth with a fine, white, mealy appearance: both conidia and macrospores present. Solution alkaline to litmus and suspended red litmus paper blues slowly. pH 7·8. Alkalinity equivalent to 5·8 c.c. N/1 HCl per litre.

E. viridescens. Moderate submerged and somewhat gelatinous growth, suspended from large white colony occupying the whole surface of the liquid, depressed in the middle with wrinkled margin: conidia not observed: macrospores present, but relatively small. Ammonia present: red litmus blues rapidly. Solution alkaline. pH 8·1. Alkalinity 6 c.c. N/1 HCl per litre.

E. acremonioides. Somewhat meagre submerged growth of loose texture: mycelium white with terminal and intercalary swollen cells: macrospores rare; ends of branches resemble appressoria, probably incipient conidiophore formation. Ammonia not detected. Solution neutral to litmus. pH 6·9. Acidity equivalent to 12 c.c. N/1 NaOH per litre.

5. *Peptone and Glucose.*

Flasks (capacity 50 c.c.), containing 25 c.c. each of a solution made up to contain 2 per cent. peptone and 4 per cent. glucose, with an initial pH 5·0 and total acidity equivalent to 34 c.c. N/1 NaOH per litre, were inoculated with the three species and kept at 20° C. Observations were made as in the case of peptone after fourteen days' growth.

Result:

E. catenulata. Considerable thick growth of a somewhat leathery consistency and very much wrinkled: colour white to grey and buff: both macrospores and conidia present, but the latter not numerous. Ammonia not detected. Solution acid to litmus; strong yeasty smell emitted. pH 4·2. Total acidity equivalent to 56 c.c. N/1 NaOH per litre.

E. viridescens. Growth similar in character to that exhibited by *E. catenulata*, but less bulky. Surface growth cream-grey. Macrospores numerous: conidia not observed. Ammonia present, but litmus blues slowly. Solution *acid* to litmus. Strong odour of coco-nut oil emitted. pH 4·6. Total acidity equivalent to 40 c.c. N/1 NaOH per litre.

E. acremonioides. Much submerged growth: surface growth meagre, thin, greyish white in colour: mycelium white, tough: incipient hyaline macrospores present; conidia not observed. Ammonia not detected. Solution acid to litmus. pH 5·1. Total acidity equivalent to 30 c.c. N/1 NaOH per litre.

6. *Asparagin.*

With *E. catenulata*, *E. viridescens*, and *E. acremonioides* very little growth occurred in 1 per cent. asparagin made up in distilled water. The growth was comparable to that in distilled water without asparagin. The growth obtained when *E. catenulata* and *E. viridescens* were grown in solutions containing 1 per cent. asparagin and 4 per cent. glucose was comparable to that obtained when these species are grown in 4 per cent. glucose without asparagin. When, however, mineral salts are present in addition to glucose and asparagin, the reactions occurring are similar to those obtained when these fungi are grown in peptone alone, viz. the solution becomes alkaline and ammonia is liberated.

B. *Growth Limitation in Relation to Hydrogen-ion Concentration.*

For the investigation of growth limitation on the acid side, N/10, N/100, and N/1000 solutions of HCl made up with distilled water, and solutions of neutralized potato extract and potato extract agar, to which the acids were added in similar concentrations, were used. The pH values of all the media thus prepared were determined, after autoclaving, by the electrical method. By this means a series of media having a wide hydrogen-ion concentration range was obtained. For these experiments the two species,

E. catenulata and *E. viridescens*, were employed. The solutions of HCl and HCl potato extract were made up in 250 c.c. flasks containing each 100 c.c. of liquid. The HCl potato extract agar was used for plate cultures, and growth measurements in terms of the diameter of the colony were made at regular intervals. The more important results are given in tabular form below :

Growth in media containing HCl of different initial hydrogen-ion concentrations at 20° C.

Medium.	*pH.*	*E. catenulata.*	*E. viridescens.*
$\frac{N}{10}$ HCl sol.	1.03	nil	nil
$\frac{N}{10}$ HCl P.E. sol.	1.16	several small colonies	nil
$\frac{N}{10}$ HCl P.E. agar	1.23	colony 5 mm.	nil
$\frac{N}{100}$ HCl sol.	2.0	numerous colonies (14 days)	very slight growth (3 days) numerous colonies (14 days)
$\frac{N}{1000}$ HCl sol.	3.0	numerous colonies (14 days)	some growth
$\frac{N}{100}$ HCl P.E. agar	4.15	colony 1.5 cm.	colony 6.2 cm.
$\frac{N}{100}$ HCl P.E. sol.	4.35	much growth, larger colonies	much growth
Distilled water	5.8	nil	nil
$\frac{N}{1000}$ HCl P.E. agar	6.4	colony 0.85 cm.	colony 3 cm.
$\frac{N}{1000}$ HCl P.E. sol.	7.88	one colony (similar to P.E. control)	much growth

N.B. The data given are those after 3 days' growth unless stated otherwise.

The following points are evident :

1. The limit for *E. catenulata* (in N/10 HCl P.E. solution) is pH 1.16 approximately.

2. The limit for *E. viridescens* (in N/100 HCl) is approximately pH 2. It will be noted that growth diminishes as the neutral point is approached with both species (see growth in solutions initially pH 4.15 and pH 6.4 respectively).

In the case of *E. acremonioides* no growth was obtained in nutrient media containing more than one per cent. concentration of malic or tartaric acid : the hydrogen-ion concentration of these media would be less than that indicated by pH 3.

In order to obtain growth limits on the alkaline side a boric acid buffer solution was employed. The solutions used were prepared as follows :

A. Buffer solution only, in flasks.

(*a*) 50 c.c. H_3BO_3, KCl (12.4048 grm. H_3BO_3 + 14.912 grm. KCl to

1,000 c.c. distilled water) + 10 c.c. N/5 NaOH diluted to 200 c.c. with distilled water. pH 8·7.

(*b*) 50 c.c. H_3BO_3, KCl + 25 c.c. N/5 NaOH diluted to 200 c.c. pH 9·3.

(*c*) 50 c.c. H_3BO_3, KCl + 45 c.c. N/5 NaOH diluted to 200 c.c. pH 10.

(*d*) Distilled water.

B. A similar series in which the dilutions were made with neutralized potato extract instead of distilled water.

(*a*) pH 8·5: (*b*) pH 8·9: (*c*) pH 9·8: (*d*) neutralized potato extract, pH 8.

C. A similar series in which the dilutions were made with neutralized potato extract agar (1·5 per cent.).

(*a*) pH 8 to 8·2: (*b*) pH 8·5: (*c*) pH 9·1: (*d*) neutralized potato extract agar, pH 6 approximately.

The pH values were obtained by the indicator method after the media had been autoclaved. Owing to the fact that the agar is coloured to a certain extent, the exact value was difficult to obtain in media containing agar.

The media were made up in flasks containing 50 c.c. each, autoclaved, subsequently inoculated, using the three species of *Eidamia*, and kept at 20° C. The results obtained are given below:

Growth at 20° C. in boric acid buffer solutions and media containing boric acid buffer solutions, of different hydrogen-ion concentrations.

Medium.	*pH.*	*E. catenulata.*	*E. viridescens.*	*E. acremonioides.*
P.E. agar (C. *d.*)	6	moderate	moderate	moderate
P.E. (B. *d.*)	8	numerous colonies	solid gelatinous mass	scum-like growth
P.E. agar with buffer (C. *a.*)	8–8·2	several colonies	slight	nil
Various	8·5–10	nil	nil	nil

N.B. At the end of 15 days the hydrogen-ion concentration of the liquid in neutralized potato extract containing *E. catenulata* was pH 8·2, of that containing *E. viridescens* pH 8·4, and of that containing *E. acremonioides* pH 7·6.

Additional experiments were made with *E. catenulata* and *E. viridescens* using buffer solutions grading from pH 6·4 to 7·6, hydrogen-ion concentrations at which moderate growth occurs in other media (not containing boric acid), but the growth produced was of a feeble character in each case. A species of *Aposphaeria*, on the other hand, formed numerous well-defined colonies in boric acid buffer solutions, containing no other source of nutrient, ranging from pH 7·8 to pH 10, and *Pleospora pomorum* was capable of growth at pH 8·7. From these results it seems probable that the boric acid exerts an unfavourable effect on growth in so far as the species of *Eidamia*

are concerned, and hence the respective limits obtained for the species, viz. *E. catenulata* and *E. viridescens*, pH 8·2, and *E. acremoniodes*, pH 8, should be regarded as very approximate.

C. *Utilization of Acid.*

Since it was found that both *E. catenulata* and *E. viridescens* were capable of growth in solutions of acids made up in distilled water, in the absence of other source of nutrient, several experiments were made by titration with standard alkali, with a view to ascertaining whether the acid was actually utilized. For the purpose of the experiments flasks containing 50 c.c. of 1 per cent. solution of acid in distilled water were prepared. The flasks were set up in duplicate series and inoculated with *E. catenulata* and *E. viridescens* respectively. Control flasks were prepared, and flasks inoculated with *Penicillium glaucum* set up for comparison. In a particular experiment tartaric (pH 1·9), malic (pH 2·2), and citric (pH 2·1) acids were used. The cultures were kept at 25° C. for three months. At the end of this period the original volume of the liquid in the flasks had been somewhat reduced owing to evaporation; accordingly in the case of each acid the volume in the inoculated flasks was made up to those of its respective control, using conductivity water (neutral). The results are given below in terms of c.c. N/5 NaOH required to neutralize 10 c.c. of acid solution.

Amount of growth and acid utilized in 1 *per cent. solutions of certain organic acids.*

Acid.	*c.c. N/5 NaOH*	*Growth.*
Tartaric control	8·15	—
E. catenulata	7·7	poor
E. viridescens	7·5	poor
P. glaucum	8·1	nil
Malic control	8·975	—
E. catenulata	7·95	moderate
E. viridescens	8·05	moderate
P. glaucum	8·0	moderate
Citric control	8·65	—
E. catenulata	7·95	poor
E. viridescens	8·3	poor
P. glaucum	8·6	nil

The amount of growth corresponded fairly closely to the quantity of acid used in each case.

P. glaucum differed from the species of *Eidamia* in producing no growth in 1 per cent. tartaric and citric acids. It was at first thought that the action upon acid might be due to an avidity for one or other of the stereoisomeric modifications of the organic acids employed. Accordingly, flasks, containing 2 per cent. racemic acid obtained from Kahlbaum made up with distilled water, were inoculated with *Eidamia catenulata* and *E. viridescens* respectively. The flasks were kept at 20° C. for one month. The solutions were then tested in the polarimeter, but there was no deviation from zero in

each case. Since the specific rotation of tartaric is 15·06, the rotation for a 2 per cent. solution would be only 0·30 approximately—

$$\left(\text{specific rotation} = \frac{\text{rotation per decimetre length}}{\text{conc. in grm. per 1 c.c.}}\right);$$

therefore when small amounts of acid are utilized it would be difficult to obtain a polarimeter reading which would fall outside the limits of probable error. For this reason this method was abandoned. It was also found impractical to work with synthetic nutrient media, such as Coon's, with racemic acid added, since the angle of rotation is often greatly affected by the presence of the optically active carbohydrate or protein which these media contain. Pasteur (13) found that when some pure ammonium racemate is dissolved in water containing a small quantity of phosphates and inoculated with *Penicillium glaucum* this fungus is able to grow and sporulate: correlated with this growth the dextrorotatory modification of racemic acid disappears, leaving only levorotatory acid. Accordingly some ammonium racemate was prepared by taking 100 c.c. of 5 per cent. racemic acid and adding to it 18·4 c.c. of ammonia of strength 0·22 N. This amount of ammonia should neutralize the racemic acid. To this solution 0·148 grm. K_3PO_4 were added. This medium was then autoclaved, and flasks, containing 25 c.c. of solution each, were inoculated with *Eidamia catenulata*, *E. viridescens*, and *Penicillium glaucum*. The usual controls were set up. At the end of three months *P. glaucum* had produced numerous colonies, but no growth had taken place in the flasks containing *E. catenulata* and *E. viridescens*. This result was the opposite to that obtained when the fungi were grown in *tartaric acid* without other source of nutrient. Hence, from the evidence, neither *E. catenulata* nor *E. viridescens* appears to resemble *Penicillium glaucum* in its behaviour towards organic acids exhibiting stereo-isomerism. Where growth in these acids occurs it seems probably due to a reaction analogous to certain bacterial acid fermentations, such as the fermentation of malic acid by *B. lactis aerogenes* and citric acid by *B. cloacae*.

D. *Growth on Agar with Organic Acid in Various Concentrations.*

The general method adopted for these experiments was to prepare a number of flasks, containing a known quantity each of neutralized agar solution made up with distilled water, and an equal number of test-tubes, containing small measured quantities of acid of different known concentrations, devised with a view to obtaining, upon adding the acid to the agar, a series of media containing definite percentage concentrations of each acid.

The flasks and tubes were autoclaved and the acids rapidly added to the flasks after autoclaving and before the agar had commenced to solidify.

In the case of malic, citric, and tartaric acids, 1, 0·5, 0·25, 0·1, and 0·05

percentage concentrations were employed. Experiments were made on several occasions using different batches of similarly prepared media, and the cultures were kept at various temperatures, approximately constant for each experiment. In a particular experiment (16·5° to 18·2° C.) using *E. catenulata* the total growth at the end of ten days was as follows :

Growth in diameter (cm.) of E. catenulata on agar containing organic acid in different concentrations.

Acid.	1·0	0·5	0·25	0·1	0·05 %	*Control.*
Malic	3·9	4·1	4·0	4·4	4·2	2·3
Citric	4·1	4·0	3·9	3·9	4·2	
Tartaric	4·1	4·0	3·9	4·2	3·9	

At these relatively low acid concentrations the rate of growth is very similar throughout a given series, and in each case there is more growth than in the control plates. Although the rate of growth in tartaric is nearly equal to that in malic and citric acids, the fungus forms growth of a more meagre character and produces fewer spore masses, hence it is not surprising that the amount of tartaric acid utilized by the fungus after a long period (see section relating to utilization of acid) is less than that of malic or citric acid. Somewhat similar results were obtained for *E. viridescens*, but in the case of *E. acremonioides* no growth was obtained in the malic, citric, and tannic acid series, with the exception of 0·05 per cent. citric, where growth was of a very meagre character.

Growth in diameter (cm.) of E. catenulata on agar containing tannic acid in different concentrations.

Concentration.	*Interval in days.*					
	4	5	6	7	9	10
2·0	—	—	—	—	—	2·5 × 1
1·5	0·35	0·7	1·05	1·35	2·0	2·3
1·0	0·6	0·95	1·4	1·8	2·8	3·2
0·5	0·65	1·1	1·6	2·0	3·0	3·45
0·25	0·7	1·2	1·7	2·1	3·15	3·55
0·1	0·8	1·3	1·7	2·25	3·3	3·8
C. 1	0·55	1·05	1·45	1·9	2·55	3·05
C. 2	0·6	1·1	1·4	1·8	2·5	2·95

At the end of ten days, growth in all concentrations except 1·5 and 2 is greater than that in the controls.

Growth was considerably checked in the 2 per cent. series; slight growth of an irregular character was observed on the seventh day.

The growth-rate increased very slightly, passing from the 1·5 to 0·1 concentrations although the hydrogen-ion concentration exhibits little change (2 per cent. = pH 4·4 ; 1 per cent. = pH 4·4).

Growth in diameter (cm.) of E. viridescens on agar containing tannic acid in different concentrations.

Concentrations.	*Interval in days.*					
	4	5	6	7	9	10
2·0–0·5	nil	nil	nil	nil	nil	nil
0·25	0·35	0·35	0·4	0·4	0·5	0·5
0·1	0·25	0·5	0·85	1·3	1·8	2·3
C. 1	2·05	4·2	5·85	7·65	10·6	>11·0
C. 2	2·1	4·35	6·15	8·1	10·5	>11·0

Growth occurred only in cultures containing 0·25 and 0·1 per cent. acid with appreciable growth only in the 1 per cent. concentration, but considerably less than that in the control plates.

From observations made of growth in other media with a similar pH value, the hydrogen-ion factor should not cause growth retardation to this extent. Hence the check to growth must be ascribed to some other factor.

Growth in diameter (cm.) of E. catenulata and E. viridescens on agar containing gallic acid in different concentrations.

Concentrations.	*Interval in days.*					
	4	5	6	7	9	10
			I. *E. catenulata.*			
2·0	0·2	0·5	0·8	1·05	1·6	1·95
1·5	0·5	0·9	1·2	1·6	2·4	2·9
1·0	0·65	1·3	1·7	2·2	3·3	3·8
0·5	0·8	1·45	2·0	2·6	3·9	4·55
0·25	0·85	1·55	2·2	2·7	4·0	4·65
0·1	0·8	1·55	1·95	2·7	3·85	4·5
C. 1	0·55	1·05	1·45	1·9	2·55	3·05
C. 2	0·6	1·1	1·4	1·8	2·5	2·95
			II. *E. viridescens.*			
2·0–1·0	nil	nil	nil	nil	nil	nil
0·5	0·6	0·9 × 1·1	1·4 × 1·7	2·8 × 3·0	5·9 × 6·0	7·0 × 7·5
0·25	1·2	2·7 × 2·9	4·6 × 4·7	6·3 × 6·7	>10·0	>10·0
0·1	1·7	3·6	5·6	7·3 × 7·5	>10·0	>10·0
C. 1	2·05	4·2	5·85	7·65	10·6	>11·0
C. 2	2·1	4·35	6·15	8·1	10·5	>11·0

N.B. The pH values of the gallic agars were as follows: 2 % = pH 3·1; 1 % = pH 3·2; 0·1 % = pH 3·8.

The following are the chief points of interest:

I. *E. catenulata.*

(*a*) The total growth at the end of ten days exceeds that in the controls (agar alone) in all except the 2 and 1·5 per cent. members of the series.

(*b*) Increase in the rate of growth occurs when passing from the 2 to 0·5 per cent. concentration.

II. *E. viridescens.*

(*a*) No growth occurred in 2, 1·5, and 1 per cent. gallic agars.

(*b*) The total growth in any case does not exceed that in the controls.

(*c*) Increase in the growth-rate occurs, passing from the 0·5 to 0·1 per cent. concentrations.

In both species growth in each member of the series is greater than that in the corresponding members of the tannic agar series, although from the point of view of the hydrogen-ion factor (taking into consideration the data obtained when other media with a similar pH range are used) the reverse result might be anticipated.

It is worthy of note that practically no acceleration of growth occurs in any given member of the tannic and gallic acid series. This is probably due to the fact that there is no appreciable production of ammonia (compounds of nitrogen would not exist except as impurities), hence change in the hydrogen-ion concentration through acid neutralization would not occur.

E. *Growth on Potato Extract Agar with Organic Acid in Various Concentrations.*

Numerous experiments were made with acid agars, using neutralized potato extract, employing methods of preparing the media and obtaining growth statistics similar to those already described (section II). Since the acid agar experiments have been described in some detail, the chief results are expressed in graph form:

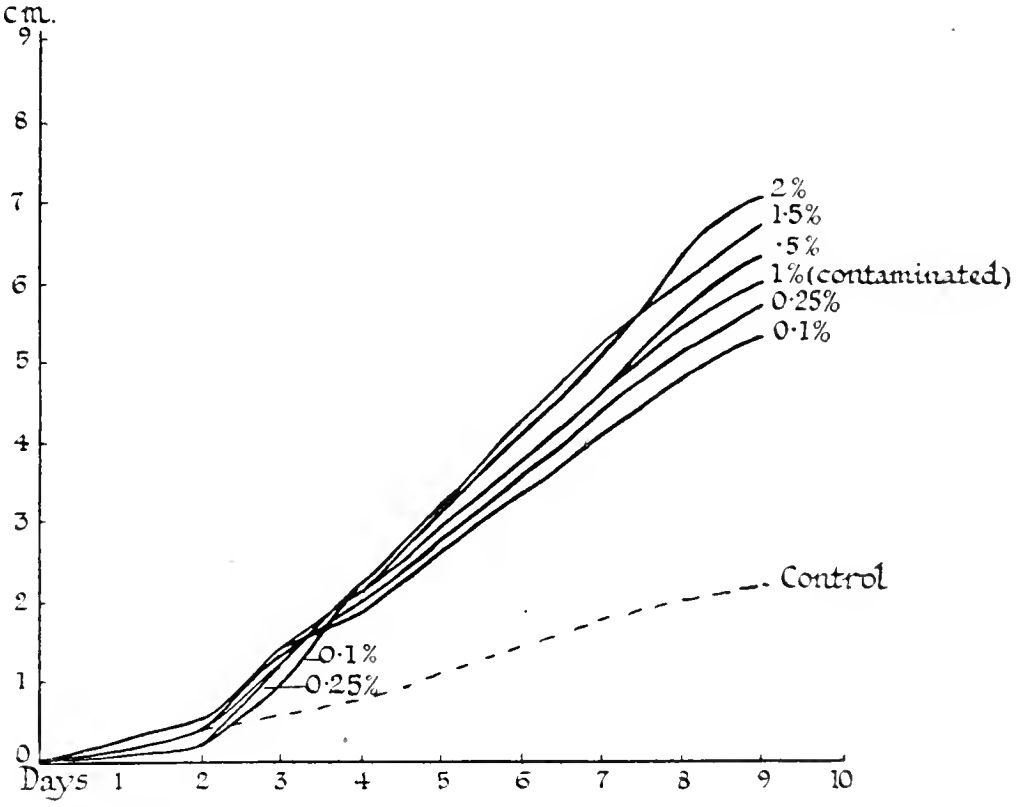

FIG. 14. *E. catenulata.* Growth on potato extract agar with various concentrations of malic acid.

I. Malic acid (Figs. 14 and 15) 2 per cent. = pH 2·4; 0·25 per cent. = pH 3.
E. catenulata (Fig. 14).

(*a*) The optimum growth takes place in the 2 and 1·5 per cent. concentrations.

(*b*) The growth-rate *decreases* progressively when passing from the 2 to 0·1 per cent. concentration.

(*c*) The growth-rate in all concentrations is considerably greater than it is in the controls (pH 7·4).

E. viridescens (Fig. 15).

(*a*) The optimum growth takes place in the 0·1 per cent. concentration (hydrogen-ion concentration less than pH 3).

(*b*) The growth-rate *increases* progressively when passing from the 2 to 0·1 per cent. concentration.

(*c*) The growth-rate is greater than it is in the control in all except the 2 per cent. concentration.

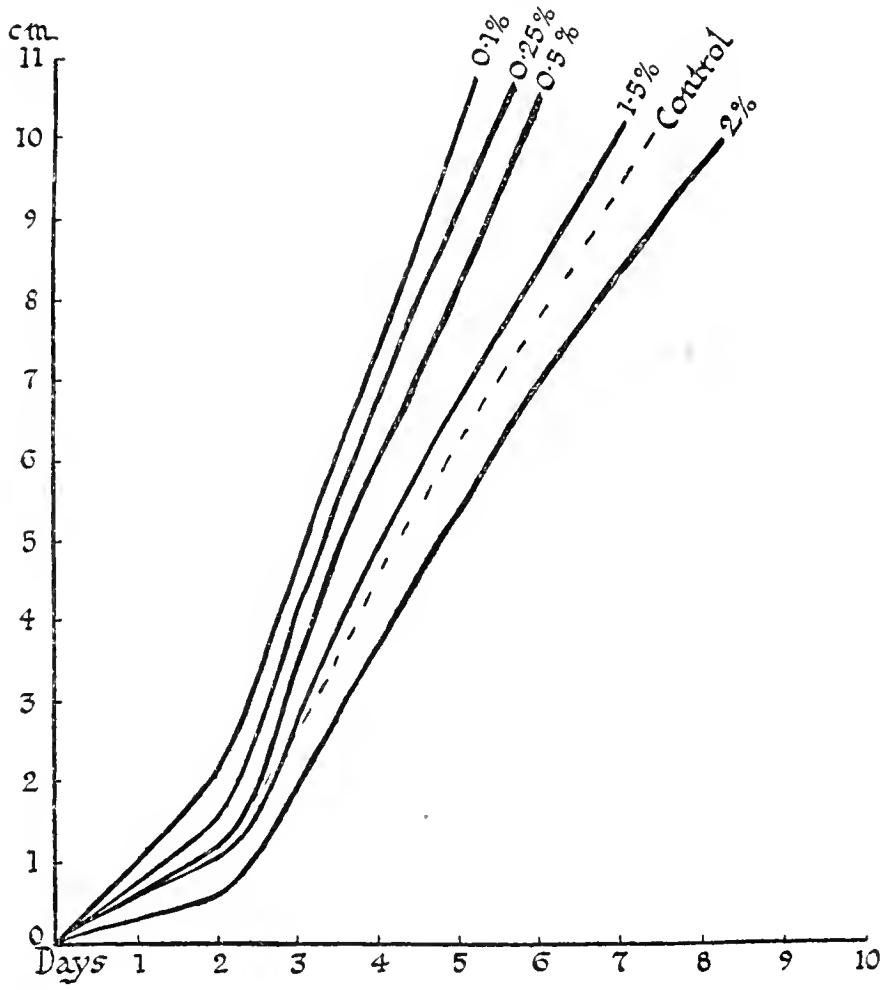

FIG. 15. *E. viridescens.* Growth on potato extract agar with various concentrations of malic acid.

II. Tartaric acid (Figs. 16 and 17) 2 per cent. = pH 2·4; 0·25 per cent. = pH 3.

E. catenulata (Fig. 16).

(*a*) The optimum growth occurs in the 1 per cent. concentration.

(*b*) The order of progressive decrease in the growth-rate is as follows: 1, 2, 0·5, and 0·1 per cent.

(*c*) The growth-rate is greater than it is in the controls in all concentrations.

(*d*) The curves illustrating growth in the 1, 0·5, and 0·1 per cent. concentrations bend after the eighth day. As growth proceeds, both the acid concentration and the hydrogen-ion concentration of the media will be lowered, owing to the evolution of ammonia through the digestion of protein present in the potato extract, in addition to the lowering caused through utilization of acid.

This change would produce progressive growth retardation, as the concentration falls away from that favoured by the fungus. Indications of this effect occur, according to expectation, first in the 0·1 and 0·5 per cent. and later in the higher percentage concentrations. A similar phenomenon is exhibited with malic acid (Fig. 14).

E. viridescens (Fig. 17).

(*a*) The optimum growth occurs at a concentration of 0·1 per cent. (less than pH 3).

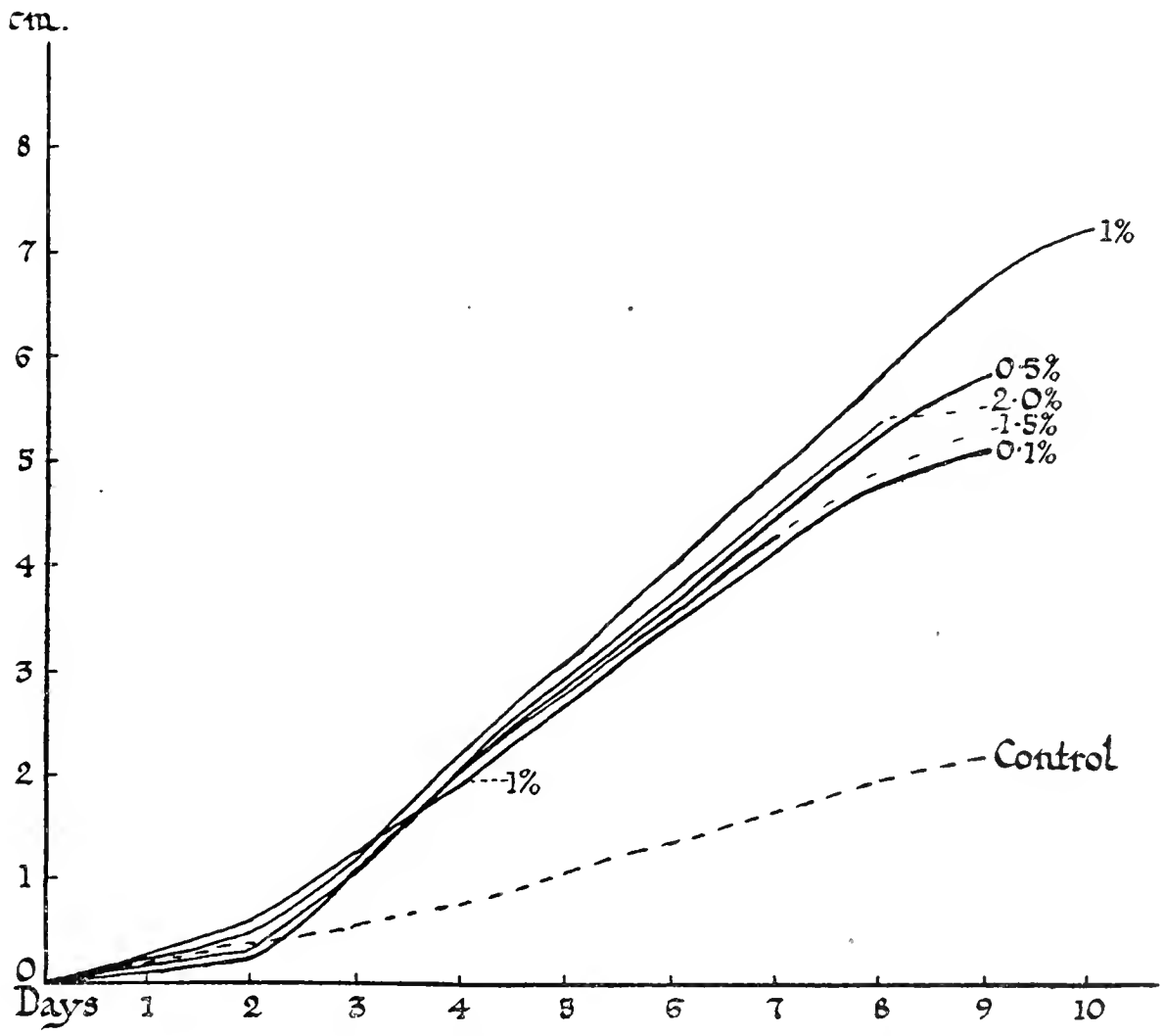

FIG. 16. *E. catenulata.* Growth on potato extract agar with various strengths of tartaric acid.

(*b*) The growth-rate progressively increases when passing from the 2 to 0·1 per cent. concentration, as with malic acid. With the exception of the 0·1 per cent. concentration, the growth-rate is less than it is in the corresponding malic acid series, and the difference is strongly marked in the 1, 1·5, and 2 per cent. concentrations.

(*c*) The growth-rate is less than it is in the controls in the 1, 1·5, and 2 per cent. concentrations.

(*d*) The curves do not bend downwards with the lowering of the concentration.

From these results it is clear that tartaric exercises a retarding influence on growth, as compared with malic acid, in media containing similar concentrations of these acids, the hydrogen-ion concentration being approxi-

mately similar in each case. Also the two species of *Eidamia* react very differently towards these acids. The hydrogen-ion concentration favouring optimum growth is not far removed from pH 2·4 (malic) for *E. catenulata*, and pH 3 (malic) for *E. viridescens*. In the latter species the region of optimum growth does not appear to be sharply marked, and might possibly be represented by a curve exhibiting a gentle gradient towards the neutral point, but with a steeper descent on the acid side.

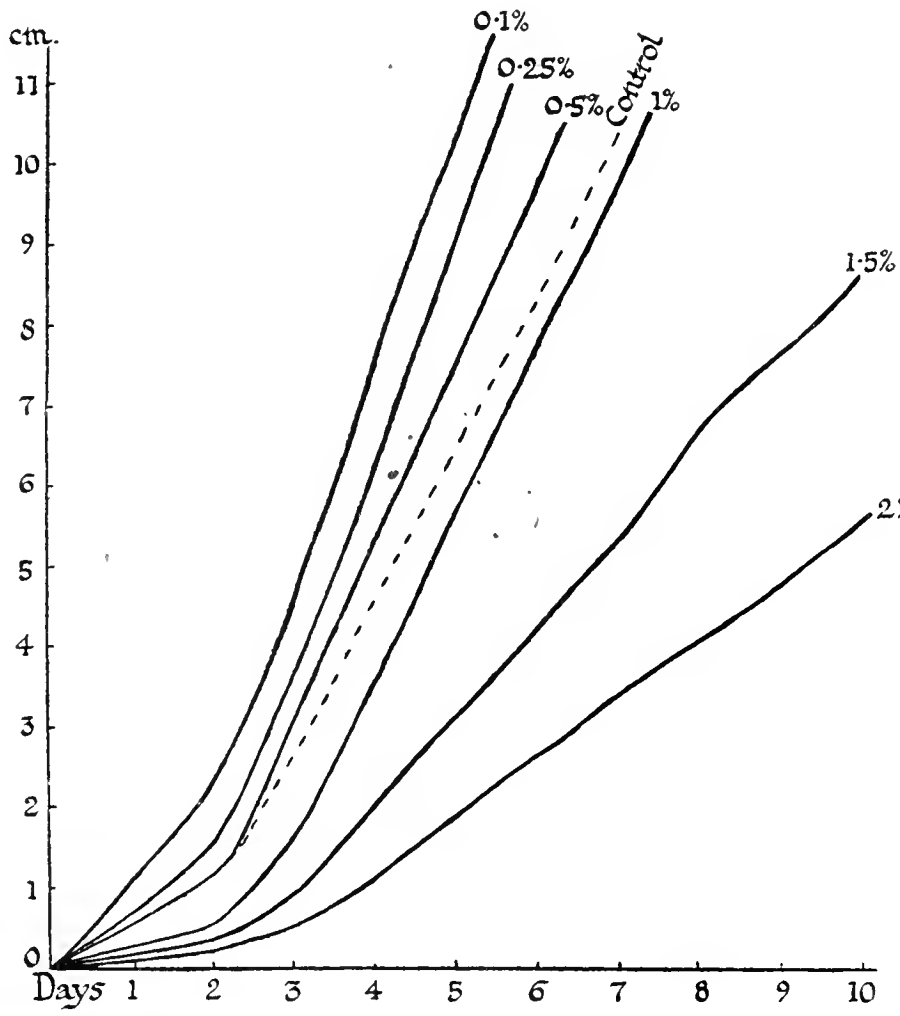

FIG. 17. *E. viridescens*. Growth on potato extract agar with various concentrations of tartaric acid.

III. Gallic acid (Figs. 18 and 19), 2 per cent. = pH 3·4–3·6; 1 per cent. = pH 3·4–3·6; 0·1 per cent. = pH 4·2.

E. catenulata (Fig. 18).

(*a*) The optimum growth occurs at 0·5 per cent., but the curves of the 0·5, 0·1, 0·25, and 1 per cent. concentrations approximate closely.

(*b*) The growth-rate progressively decreases when passing from these to a 2 per cent. concentration.

(*c*) The growth-rate is greater than it is in the controls, in all except the 1·5 and 2 per cent. concentrations.

(*d*) In this last respect the curves differ from those obtained for malic and tartaric acids, but the hydrogen-ion concentration of the 1·5 and 2 per cent. gallic acid cultures is perhaps less favourable to the growth of *E. catenulata* than that of the corresponding malic and tartaric acid series.

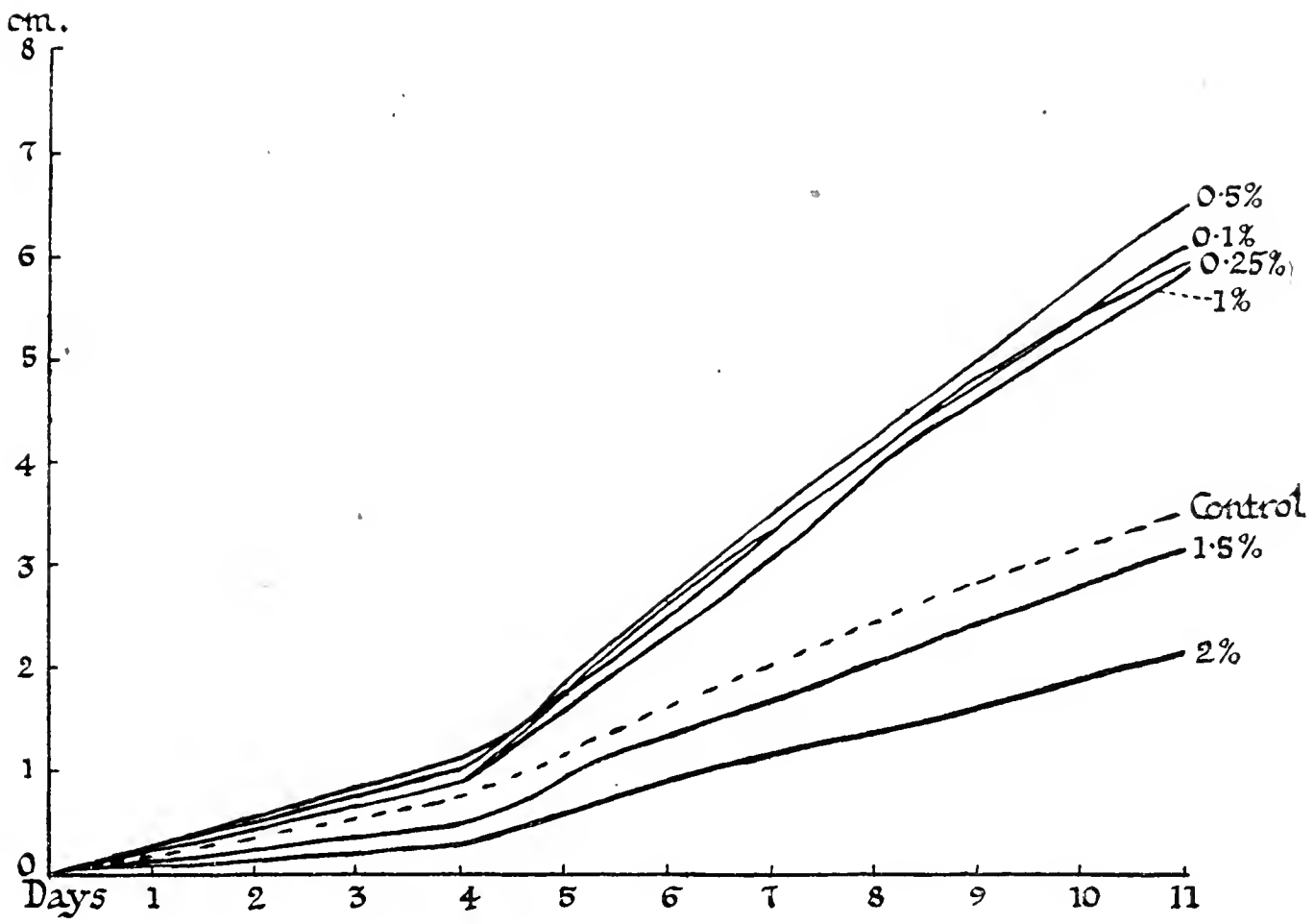

FIG. 18. *E. catenulata.* Growth on potato extract agar with gallic acid.

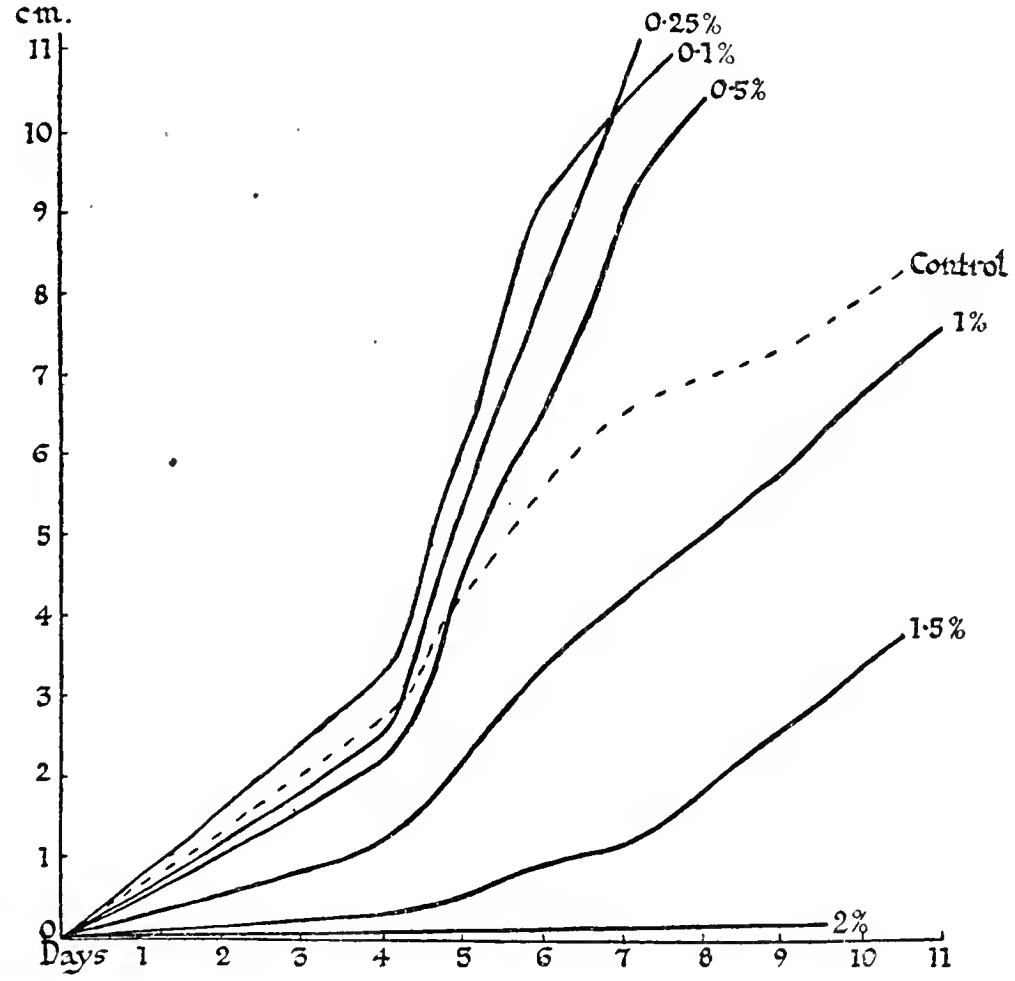

FIG. 19. *E. viridescens.* Growth on potato extract agar with gallic acid.

E. viridescens (Fig. 19).

(*a*) The optimum growth occurs at a 0·1 per cent. concentration.

(*b*) The growth-rate progressively decreases when passing from the 0·1 to 2 per cent. concentration.

(*c*) The growth-rate is greater than in the control, in all except the 1, 1·5, and 2 per cent. concentrations.

(*d*) The curves for the 1, 1·5, and 2 per cent. concentrations diverge widely ; there is practically no growth in the 2 per cent., although the hydrogen-ion concentration is not unfavourable to growth (pH 3·4–3·6). In this respect the curves differ from those obtained for both species of *Eidamia* in the case of malic and tartaric acids.

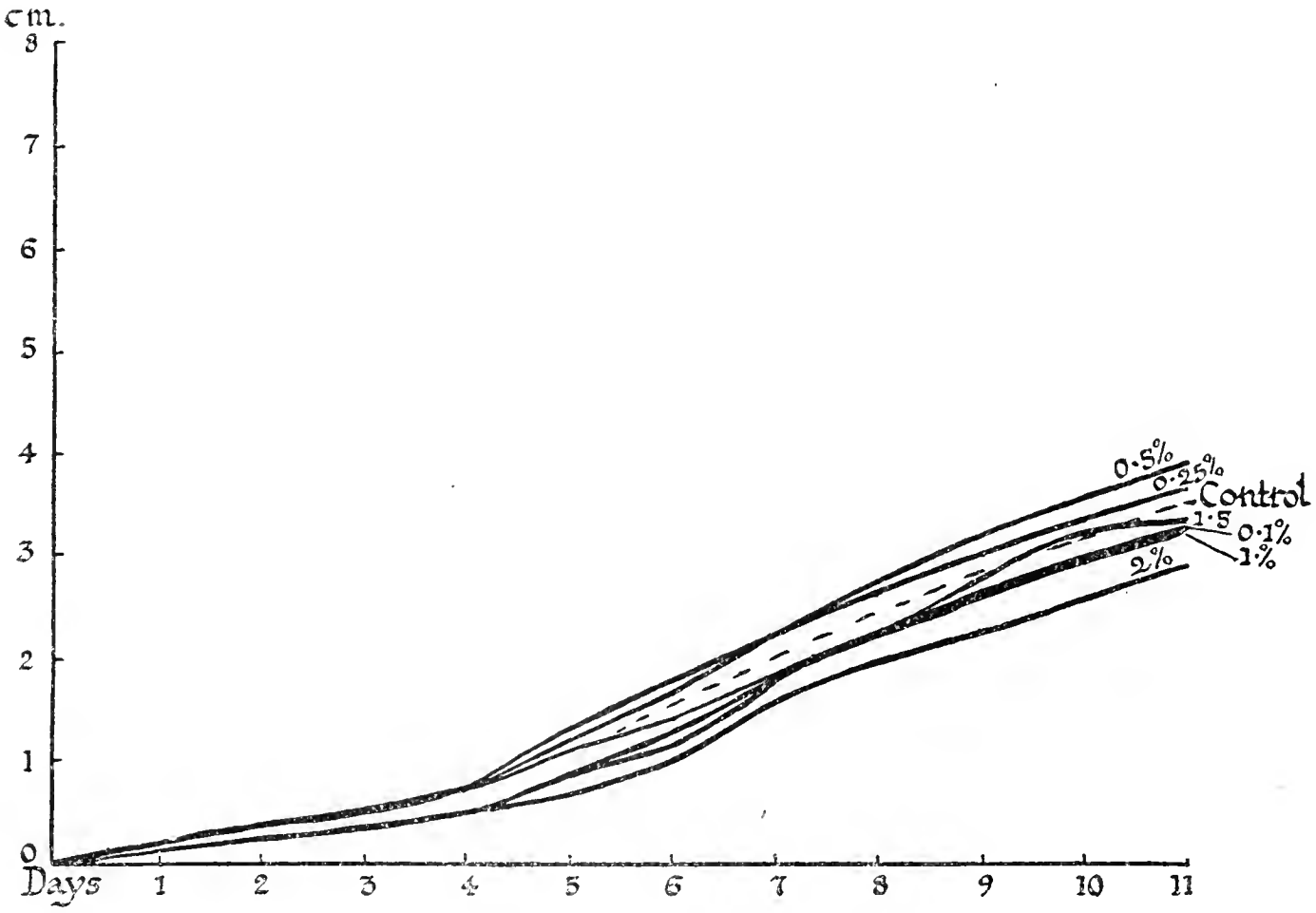

FIG. 20. *E. catenulata.* Growth on potato extract agar with tannic acid.

IV. Tannic acid (Figs. 20 and 21) 2, 1, and 0·1 per cent. = pH 4–4·4.

E. catenulata (Fig. 20).

(*a*) The optimum growth occurs at a 0·5 per cent. concentration, but growth at all the other concentrations approximates closely to this.

(*b*) The growth-rate is least in the 2 per cent. concentration.

(*c*) The curve for the control lies midway between those for the 0·5 and 2 per cent. concentrations.

E. viridescens (Fig. 21).

(*a*) The optimum growth occurs at the 0·1 per cent. concentration.

(*b*) The growth-rate is retarded in concentrations higher than 0·1 per cent., with no growth beyond 0·5 per cent.

(*c*) The growth-rate is greater than it is in the control in the 0·1 per cent. concentration after the seventh day.

Tannic acid, in the concentrations used, appears to have little effect on growth in the case of *E. catenulata*, but with *E. viridescens* an increase beyond 0·1 per cent. exercises a strongly retarding influence, the retardation being more pronounced than that exercised by gallic acid. Although *E. viridescens* exhibits these striking growth differences, the hydrogen-ion concentration of the tannic acid cultures (pH 4–4·4) is approximately the same, and not inimical to growth (see *E. viridescens*, malic acid). Again, with *E. catenulata* the growth-rate is very different in 0·1 per cent. gallic acid and tannic acid respectively, where the hydrogen-ion concentration lies between similar limits.

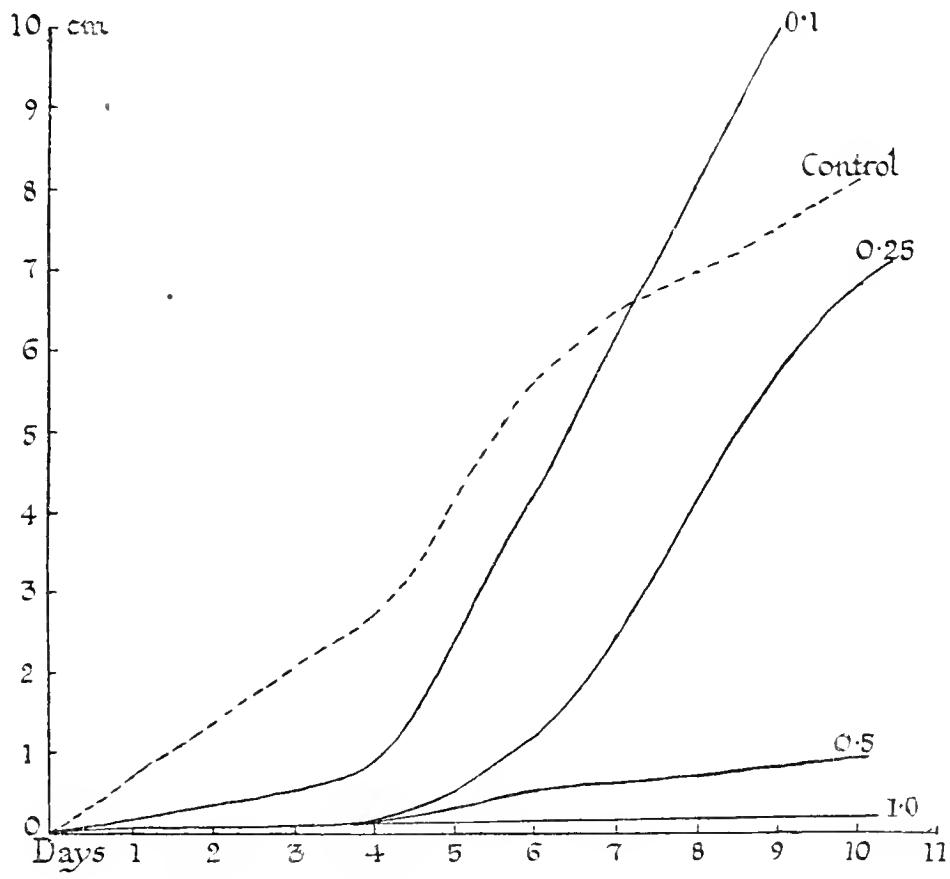

FIG. 21. *E. viridescens*. Growth on potato extract agar with tannic acid.

F. *Growth in Equimolar Solutions of Organic Acids.*

In order to assist in interpreting the experimental results obtained through growing the species of *Eidamia* in media containing organic acid in percentage concentration, some preliminary experiments were set up, using a series of media with certain acids in equimolar proportions. For this purpose potato extract agar was prepared, containing N/50 citric, malic, tartaric, and gallic acids. Four lots of 100 c.c. acid-containing media were prepared, using 80 c.c. of potato extract agar, and 20 c.c. of distilled water containing (*a*) 0·268 grm. of malic acid, (*b*) 0·3 grm. of tartaric acid, (*c*) 0·384 grm. of citric acid, and (*d*) 0·34 grm. of gallic acid respectively. The potato extract agar and the acid solutions were autoclaved separately, and the latter added to the extract afterwards. The pH values of the

media, after final preparation and immediately before inoculation, were as follows:

P.E. extract agar control			pH 7·0
,,	,,	and malic acid	,, 3·8
,,	,,	,, citric acid	,, 3·4
,,	,,	,, tartaric acid	,, 3·4
,,	,,	,, gallic acid	,, 4·2

Series of plate cultures in duplicate were inoculated with *E. catenulata* and *E. viridescens*, and kept at 25° C. and 20° C. respectively. Growth data were recorded in the usual manner.

In order to show the extent of variation exhibited among members of a duplicate series, the actual growth measurements in centimetre diameter obtained for *E. catenulata* are given:

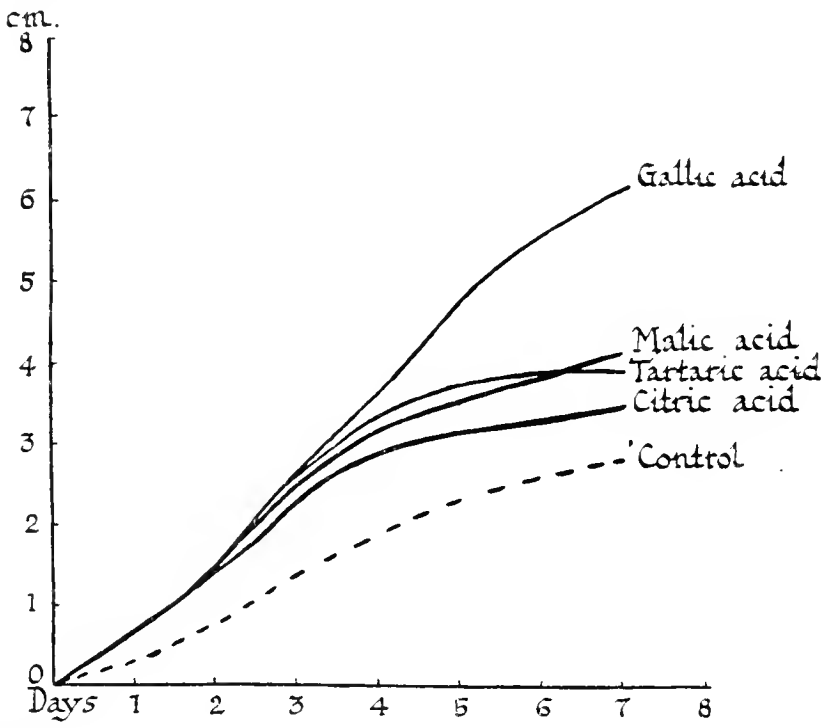

FIG. 22. *E. catenulata.* Growth on potato extract agar with N/50 solutions of various acids.

E. catenulata. Growth-rate in media containing N/50 *organic acid.*

Acid.	*Series.*	*Interval in days.*				
		2	3	4	5	7
Citric	I	1·45	2·5	2·9	3·14	3·5
	II	1·45	2·4	2·8	—	3·0 [1]
Malic	I	1·45	2·6	3·3	3·6	4·2
	II	1·5	2·5	3·1	3·45	4·1
Tartaric	I	1·55	2·65	3·3	3·55	3·9
	II	1·5	2·7	3·4	3·75	3·9
Gallic	I	1·6	2·7	3·6	4·68 [1]	6·1
	II	1·5	2·6	3·65	4·9	6·6
Control	I	0·75	1·35	1·8	2·5	2·8
	II	0·85	1·5	1·8	2·5	2·8

The general conclusions deduced are as follows:

I. *E. viridescens* (Fig. 23) (pH range of the media relatively favourable to growth).

[1] The growth-rate in these cultures was affected by the appearance of additional colonies.

(*a*) The curves for the control and acids practically coincide; the acids have exercised no appreciable effect on growth.

(*b*) After the second day, the growth-rate in each case is fairly uniform, as evidenced by the almost straight lines.

(*c*) After the fifth day the curves diverge, indicating that the growth-rate is no longer uniform, and slightly decreased in the case of malic acid, tartaric acid, and the control. The retardation experienced coincides with a lowering of the hydrogen-ion concentration, but since the control was initially pH 7, and it is unlikely that the acid-containing media would

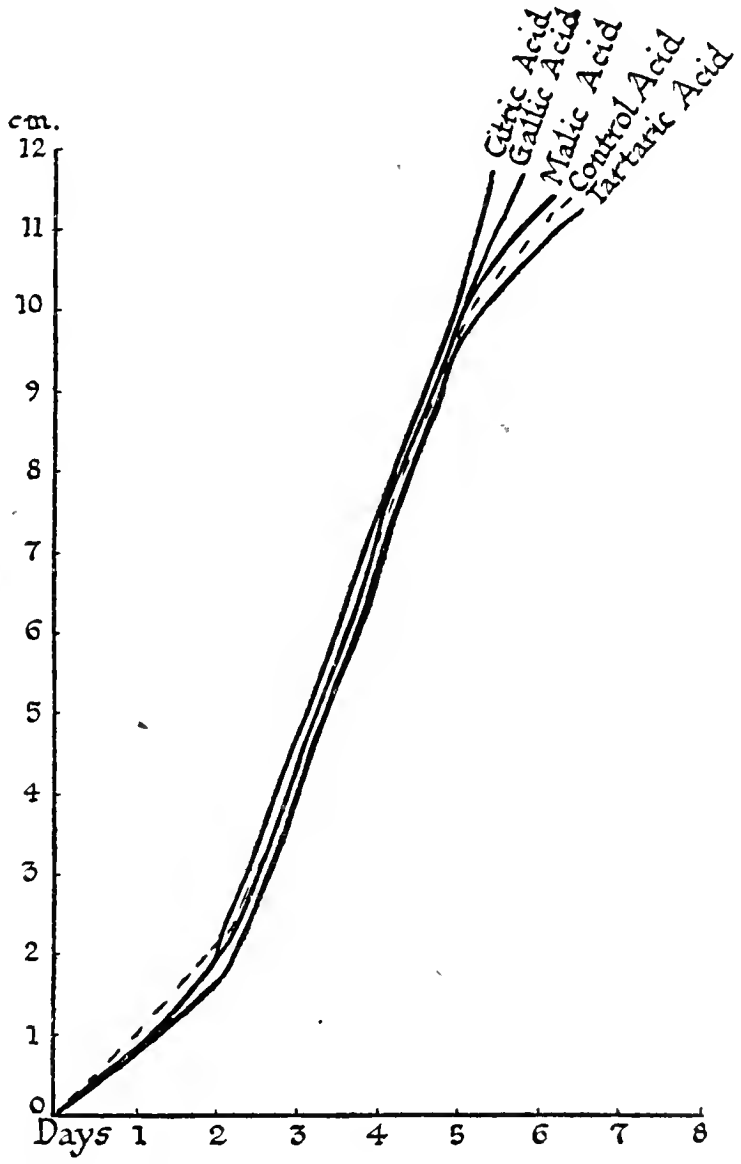

FIG. 23. *E. viridescens.* Growth on potato extract agar with N/50 solution of various acids.

exhibit greater alkalinity than the control, this retardation appears to be due to some other factor.

II. *E. catenulata* (Fig. 22) (pH range of the media relatively unfavourable to growth).

(*a*) The curve for the control (initially pH 7) falls below those for the acids.

(*b*) The curves for the acids diverge sharply after the third day, the gallic acid culture exhibiting the maximum growth-rate.

(*c*) The curves for citric and tartaric acids become depressed after the third and fourth days respectively. *E. catenulata* is highly tolerant of acid, hence the lowering of the hydrogen-ion concentration should cause growth

retardation. The *divergence* of the curves, however, is not so easily explained; the effect seems to be due to the toxicity of the acids in question.

(*d*) The growth-rate is least in the citric acid culture, the reverse of the result obtained with *E. viridescens*.

G. *Colour Production in Media containing Gallic or Tannic Acid.*

During the course of the experimental work with acids, it was observed that cultures of *E. viridescens* in potato extract agar, containing gallic or tannic acid in 0·1 and 0·25 per cent. concentrations, exhibited a deep green colour in the substratum. In work carried out concurrently coloration was obtained with numerous fungi when grown in solutions containing 0·2 per cent. gallic acid. The colour is in some cases not due, or not entirely due to acid, since certain fungi, e.g. species of *Fusarium*, produce colours in the presence of carbohydrate. Other fungi—for example, a species of *Botrytis*—produce a reddish brown substratum in gallic and tannic acid potato extract plate cultures. A similar colour, which is dark olive green when diluted with water, appears after two days when the same *Botrytis* is kept at 20° C. in solutions containing gallic acid at a 0·2 per cent. concentration, and K_3PO_4 and $MgSO_4$ (1·25 grm. and 0·75 grm. respectively per litre). In this case the oxidation of gallic acid takes place through enzymatic action. Again, solutions of gallic acid undergo oxidation when alkali is present in excess, with the ultimate production of purpurogallol, which is of a brownish olive colour (in dilute solution). Hence, if compounds of nitrogen are present in media containing gallic acid, liquid coloration might ensue through the liberation of ammonia owing to fungal activity. For these reasons, the following series of media were prepared, containing members from which compounds of nitrogen and carbohydrate respectively were omitted:

1. K_3PO_4, 1·25 grm.; $MgSO_4$, 0·75 grm.; asparagin, 2 grm.; glucose, 2 grm. per litre.
2. As in No. 1, with glucose omitted.
3. As in No. 1, with asparagin omitted.
4. As in No. 1, with both glucose and asparagin omitted.

To each of these, 10 c.c. of a 2 per cent. solution of sterile gallic acid were added after autoclaving. Flasks (capacity 250 c.c.), containing 100 c.c. of liquid (pale yellow in colour), were inoculated with the three species of *Eidamia*, and these, together with the necessary controls, were kept at 20° C.

With *E. acremonioides* no growth was obtained in solution 1 after two days and the experiment was discontinued. In the case of *E. catenulata* and *E. viridescens* colour appeared in the solutions containing asparagin.

After the appearance of colour the solution when tested with litmus paper proved to be alkaline.

The more detailed results are as follows:

E. viridescens.

Seventh day. No. 2, liquid olive green with suggestion of reddish-brown, alkaline, ammonia present. No. 1, liquid without colour change, acid. Nos. 3 and 4, little growth, liquid without colour change, acid.

Tenth day. No. 1, liquid port wine colour, alkaline, ammonia present. Nos. 3 and 4, no noteworthy change.

Thirteenth day. No. 1, liquid dark olive green. Nos. 3 and 4, no noteworthy change.

E. catenulata.

Seventh day. No. 2, liquid without colour change, acid. No. 1, liquid slightly darker in colour, slight alkalinity, ammonia not detected. Nos. 3 and 4, little growth, liquid without colour change, acid.

Tenth day. No. 1, further darkening in colour to light brown, alkaline, ammonia present. Nos. 2, 3, 4, no noteworthy change.

Fourteenth day. Nos. 1 and 2, solutions port wine colour. Nos. 3 and 4, no noteworthy change.

Controls. Liquid unchanged.

H. *General Conclusions on Growth in Relation to Acids.*

Since the behaviour of fungi in special relation to acids needs more detailed investigation than this subject has received hitherto, it is not advisable to enter into a discussion of all the factors influencing the growth of the species of *Eidamia* in acids. The present object will be better attained by expressing in summarized form the results which appear to have a general bearing on fungal work of this nature.

1. Both *E. catenulata* and *E. viridescens* are capable of utilizing certain organic acids; the power of utilization appears to be increased when the medium contains other sources of nutriment.

2. These species, unlike *Penicillium glaucum*, do not markedly utilize one only of the two stereo-isomers present in racemic acid.

3. In a general way hydrogen-ion concentration exercises a regulating influence on growth, the degree of its influence varying with the nature of the constituents of the medium employed.

4. Growth in any medium containing acid and compounds of nitrogen may be progressively affected by three factors:

(*a*) utilization of acid;

(*b*) neutralization of acid through evolution of ammonia;

(*c*) acid production through fermentation of sugar.

Where the general trend is towards alkalinity, both the molecular con-

centration of the acid and hydrogen-ion concentration of the medium will be lowered. In the case of a species highly tolerant of acid, these factors sooner or later exercise a retarding influence on growth: if the species is relatively intolerant, growth might be accelerated (*E. viridescens* is moderately tolerant).

5. In the sections of this paper relating to growth in acids many cases have been specified where fungal growth does not appear to bear any definite relation to hydrogen-ion concentration. This behaviour may be due in part to differences in molecular concentration. Tannic acid (molecular weight 1785) affects the growth of *E. viridescens* considerably more than gallic acid (188), although the hydrogen-ion concentration of the tannic acid media is more favourable to growth than that of the gallic acid. Tartaric acid (168) affects both *E. viridescens* and *E. catenulata* more than malic acid (134). Further support is obtained from the growth data determined for *E. viridescens* in the tannic extract agar series (Fig. 21); here the curves for the 0·1, 0·25, 0·5, and 1 per cent. concentrations exhibit great dissimilarity, the progressive retarding influence on growth being strikingly correlated with increased molecular concentration.

6. In media containing N/50 malic, citric, tartaric, and gallic acids respectively, the growth-rate is approximately the same for *E. viridescens*: it is the same for a few days, but differs later, in the case of *E. catenulata*. Variations of this kind in the growth-rate in equimolar concentrations of acids may be due, in part, to changes in the media owing to growth activities which may vary in character with the acid employed. On the other hand, some acids may exercise a specific toxic action, at particular molecular concentrations, on a given fungus, in which case a somewhat similar effect would be produced.

IV. Systematic Position and Specific Descriptions.

The general characteristics of the genus *Eidamia* as proposed by Lindau (11) are: hyphae branched, septate, white; conidiophore upright, branched, septate, narrow at the apex and bearing a circular swollen end cell; sterigmata arising radially on the swollen head, pointed; conidia in chains, hyaline; bulbils produced on side branches and branches similar to the conidiophores produce single-celled chlamydospores of rounded form and yellow-brown colour.

Up to the present time only one species, possessing all these characteristics, has been included in the genus, viz. *E. acremonioides*. The writers consider the inclusion of the two new fungi, *E. catenulata* and *E. viridescens*, in this genus is justifiable on the grounds of their general resemblance to *E. acremonioides* in possessing colourless branched septate hyphae producing two types of spores, namely conidia and single-celled macrospores

borne terminally on lateral branches. The differences lie in the form of the conidiophore, the appearance of the conidia in groups in the case of *E. viridescens*, and the fact that the macrospores are hyaline instead of brown in both fungi. It can be shown that these differences break down when growth takes place on different media.

The conidiophore of *E. acremonioides* is typically of the *Aspergillus* form, the globular apical portion bearing sterigmata with spores in chains. Under certain cultural conditions the sterigmata are borne, either singly or in small lateral or terminal groups, on undifferentiated branch hyphae. When Fig. 3 (*E. acremonioides*) is compared with those illustrating the conidiophores of *E. viridescens* the similarity in position of the sterigmata and their size and shape is very striking. In the case of *E. catenulata* the conidiophores are usually not apically swollen, but approximations to the types found in *E. acremonioides* do occur (Fig. 10).

In *Eidamia* the conidia are typically borne in chains, but, even in the case of *E. acremonioides*, a disposition in groups at the apices of the sterigmata is not uncommon. In *E. viridescens* grouping is the rule and catenulate conidia are rare. From the occurrence of intermediate stages (Fig. 12, *E. catenulata*) a distinction between chains and groups of spores as a criterion of specific value is of little importance in the genus *Eidamia*. The conidia in the two new fungi are coloured in contrast to the colourless state of those of *E. acremonioides*, but this difference is not of generic value.

The macrospores of *E. acremonioides* are relatively larger than those of the other two species. They are usually brown in colour as contrasted with the lack of colour in *E. catenulata* and *E. viridescens*, though occasionally hyaline spores are produced (potato mush agar at 30° C. or potato slab at 25° C.). Therefore the two new fungi cannot be excluded from the genus owing to the hyaline character of the macrospores. From the foregoing it will be seen that the differences between the three fungi are not of sufficient importance to prevent their inclusion in one genus.

The bulbils found in *E. acremonioides* (*Papulaspora aspergilliformis*) and in *Helicosporangium parasiticum* by Eidam (6) are regarded by Bainier (1) as perithecia containing an ascogonium capable of developing to form a typical perithecium, with ascospores set at liberty through an ostiole. Normally the development appears to be arrested when the bulbils contain central cells surrounded by a sheath. After resting, such a bulbil is capable of growth by the production of vegetative filaments. Moreau (12) has worked out the cytology of these structures, and his conclusion supports Eidam and Bainier in the view that the bulbils are perithecia arrested in their development, not abortive, but capable of proceeding with the normal formation of a perithecium under certain conditions. No bulbils were found in *E. acremonioides* on the media used in this investigation, nor was their presence detected in *E. catenulata* and *E. viridescens*.

These two new species show certain resemblances to *Verticillium* in form and arrangement of conidiophores, whilst *E. catenulata* sometimes produces a typical *Penicillium* form of conidiophore. They are separated from *Verticillium* and *Penicillium* by their possession of a second type of spore which is a constant feature in both of them.

The genus *Papulaspora*, originated by Preuss (16), includes many forms with bulbils which have been named without reference to the existence of any other form of reproduction; for example, *Papulaspora magnificus*, Hotson (8), was found to be an asexual stage of *Ascobolus magnificus*, and Dodge (5) has shown that sexual reproduction occurs in cultures containing two strains properly chosen. As the genus at present stands there are at least nine species of *Papulaspora* as yet unconnected with any perfect form. Moreau suggests that the nomenclature proposed by Lindau should be adopted and all fungi with bulbils of the type of *Papulaspora aspergilliformis* should be classed as *Eidamia*. *Papulaspora aspergilliformis* is synonymous with *E. acremonioides*.

The case of *Helicosporangium parasiticum* is also of interest in this connexion. A fungus was described and given this name in 1865 by Karsten (10). In 1883 Eidam (6) described a form which he called by the same name, but it is very probable that the *Helicosporangium* of Karsten and the *Helicosporangium* of Eidam are not the same fungus. Eidam describes his fungus as possessing bulbils exactly like those of *Eidamia acremonioides*. He further figures the sterigma as a single-celled flask-shaped body borne singly on a hypha or in groups on a conidiophore. The figures of these conidiophores are markedly like those represented in Fig. 3 for *E. acremonioides*. Eidam does not mention the formation of any 'chlamydospores'. Bainier (1) objects to the separation of *Helicosporangium parasiticum* from *Papulaspora aspergilliformis* (*E. acremonioides*) on the ground that it is a 'monstrous form' that the Mucedineae often present. With the facts in mind of the variation presented by *E. acremonioides* when grown on different media, the writers are inclined to agree with Bainier and to regard *Helicosporangium parasiticum* as probably synonymous with *Eidamia acremonioides*. Possibly in the case of *H. parasiticum* the macrospore was overlooked or was absent from the particular medium on which the fungus was growing.

Large brown spores were found on seeds of *Festuca pratensis* by A. L. Smith (19). These were borne singly on the ends of branches on a colourless mycelium and were named *Langloisula macrospora* by Smith on account of their resemblance to *Langloisula spinosa*, Ellis and Everhart. Smith suggested that this genus rests on too narrow a foundation and should be included with *Acremoniella*. Later Pethybridge (15) cultured the same fungus from brown spores occurring on blighted potato foliage. He noted the peculiar branching, recalling that of *Monopodium*, and sent the specimen to Paris for comparison with *M. uredopsis*, Delacroix (4).

There M. Arnaud suggested that both *Monopodium* and *Langloisula macrospora* are probably identical with *Acremoniella atra*, Corda (3). By the courtesy of the Cryptogamic Department of the British Museum (Natural History) the slides of *Langloisula macrospora* made by Smith and Pethybridge have been examined. They appear to be identical with *Eidamia acremonioides* when only the mycelium, pseudosympodial branches, and brown macrospores are present, as is the case when the fungus is grown on rice at 20° C., potato slab at 25° C., and potato mush agar at 30° C. *Acremoniella atra*, as figured by Saccardo (17), exhibits this peculiar type of branching and also apically borne brown spores, 25–28 μ long by 16–18 μ wide, resembling the macrospores of *E. acremonioides*. *Langloisula spinosa*, Ellis and Everhart, is regarded by von Höhnel (7) as belonging to the Corticiae, being only separated from *Asterostromella* by the colour of the spores. The writers have had no opportunity of examining *L. spinosa*, but consider that *L. macrospora* is certainly a Hyphomycete with a remarkable resemblance to *E. acremonioides* in the macrospore stage.

It seems probable then that *Langloisula macrospora*, A. L. Smith, *Monopodium uredopsis*, Delacroix, and *Acremoniella atra*, Corda, are identical, and may ultimately prove to be synonymous with *Eidamia acremonioides*.

The following revised description of the genus *Eidamia* is suggested :— Hyphae branched, septate, white ; conidiophore upright, branched or unbranched, septate, bearing a swollen cell with sterigmata, or the sterigmata may be borne singly or in groups on the unbranched or branched conidiophore ; conidia in chains or groups, hyaline or coloured : branches similar to the conidiophore produce single-celled macrospores of round or ovoid form, yellow brown or colourless ; infertile perithecia (bulbils) present or absent.

Eidamia acremonioides, Harz.

Syn. *Monosporium acremonioides*. Harz in Bull. Soc. Imp. Sci. Natur. Moscou, xliv, 1 (1871), p. 104, Tab. I, Fig. 3 ; Bot. Centralbl., xli (1890), p. 410 ; Sacc. Syll., iv, p. 115.
Papulaspora aspergilliformis. Eidam in Cohn's Beitr., iii (1883), p. 411, Tab. XXIII, Figs. 7 to 17 ; Sacc., Syll., ix, p. 339.
Helicosporangium parasiticum. Eidam in Cohn's Beitr., iii (1883), p. 414, Tab. XXIII.

Mycelium spreading, pseudo-sympodial branching, septate, white ; conidiophores hyaline, often branched, septate, 3–4 mm. high, mostly smaller, 10 μ broad at base by 6·5 μ at apex, often bearing swollen cell of 12–13 μ diameter; sterigmata flask-shaped, 6 μ long by 4 μ wide at base, borne on the swollen cell or singly or in groups directly on the hyphae of the conidiophore ; conidia in chains or groups, hyaline, circular or egg-shaped, 1·5–2 μ in diameter ; bulbils may occur on side branches ; macrospores formed singly

and terminally on branches, yellow brown, thick-walled, circular or ovoid, 12 by 10 μ to 40 by 34 μ.

Eidamia catenulata, n. sp.

Mycelium hyalinum, septatum, ramosum, 3–11 μ latum; conidiophora erecta, simplicia vel ramosa, septata; sterigmata gracilia, ad basim turgidiuscula, 8–16 μ longa inferne, 1–2·5 μ lata; solitaria vel gregaria tum ad hypham simpliciam tum ad apices ramorum conidiophoreorum vel gregaria ad apicem rami brevis turgidi sita; conidia catenulata (circa centena), flava anguste vel late elliptica, utrinque acuta, 4–7 × 2–3·5 μ, interdum ad apicem sterigmatis aggregata; macrosporae hyalinae, solitariae vel binae ad apicem rami brevis tum intercalaris, pachydermaticae, subglobosae, 7·5 × 8·5 μ, vel pyriformes, 14 × 10–18 μ.

Hab. in ligno exsiccato quercus.

Eidamia viridescens, n. sp.

Mycelium hyalinum, septatum e hyphis ramosis 7–11 μ latis compositum; conidiophora ramosa, septata; sterigmata lageniformia, 8–20 μ longa inferne, 1·5–3 μ lata, solitaria vel gregaria, praeter conidiophori ramos disposita; conidia aggregata vel breviter catenulata, flavida aut viridia, subovoidea, 4 × 5 μ, vel ellipsoidea, 2·5 × 4 μ, vel sphaerica, 4·5 μ diam.; macrosporae hyalinae, tum solitariae ad apices ramorum lateralium, tum intercalares, pachydermaticae, subglobosae, 11 × 8 μ, vel ovoideae, 13 × 9–12 μ.

Hab. in malis putridis.

Physiological Characters.

1. The approximate temperature optima are as follows: *E. acremonioides*, 20° C.; *E. viridescens*, 25° C.; *E. catenulata*, 30° C.

2. Whereas *E. acremonioides* produced growth of a relatively feeble character the remaining species responded freely to a wide range of nutritive conditions. The growth-rate of *E. viridescens* is much greater than that of *E. catenulata* when the species are grown at their respective temperature optima, other conditions being constant.

3. *E. catenulata* and *E. viridescens* hydrolyse starch, invert sucrose, decompose protein and asparagin with evolution of ammonia, and ferment certain sugars with acid production in the presence of protein. In these respects they differ markedly from *E. acremonioides*.

4. In the presence of carbohydrate, *E. viridescens* produces a volatile compound with an odour recalling that of coco-nut oil.

5. No growth was obtained on cellulose.

6. Unlike *E. acremonioides* the remaining species are able to utilize soluble pectin.

7. The growth-limits in relation to hydrogen-ion concentration for the media used are as follows:

E. catenulata	pH 1·16 and pH 8·2,
E. viridescens	pH 2 and pH 8·2,
E. acremonioides	pH 3 approx. and pH 8.

8. *E. catenulata* and *E. viridescens* utilize certain organic acids; the reaction is regarded as analogous to bacterial fermentations of acids.

9. Hydrogen-ion concentration exercises a regulating effect on growth: the growth reactions obtained with the species of *Eidamia* bear a definite relation to the relative degree of acid tolerance exhibited by them.

10. Growth retardation is often correlated with increased molecular concentration (acids): *E. catenulata* and *E. viridescens* differ in their response to altered molecular concentration.

11. The coloration ultimately appearing in certain cultures of *E. viridescens* and *E. catenulata* containing gallic acid and protein is due to the oxidation of the acid owing to the presence of free alkali.

12. The principle causing spore coloration in *E. catenulata* and *E. viridescens* is insoluble in ether, chloroform, and the usual solvents.

13. *E. viridescens* is parasitic on apples at 1° C., and at ordinary laboratory temperatures *E. catenulata* and *E. acremonioides* are saprophytic.

V. Summary.

The salient morphological features of three species of *Eidamia*, including two which are apparently new to science, are described. The reactions which these species exhibit when grown in various media, including sugars, soluble pectin, protein, organic acids, and various other substances, are compared and contrasted. The growth-limits of the species in relation to hydrogen-ion concentration are approximately determined.

The authors are greatly indebted to Professor V. H. Blackman for his very kind advice and criticisms, and to Mr. A. B. Manning for help in matters relating to physical chemistry; also to Mr. J. Ramsbottom of the British Museum (Natural History) for assistance in connexion with the systematics of *Eidamia* and for the specific descriptions.

This work was undertaken in connexion with investigations now being carried out for the Food Investigation Board of the Department of Scientific and Industrial Research.

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The Origin of 'Golden' Oak.

BY

HELEN STUART WILLIAMSON.

(*Imperial College of Science and Technology.*)

With Plate X and four Figures in the Text.

THE specimen of oak which was the origin of this investigation was sent to the Timber Department of the Imperial College by Dr. Butler. The wood showed yellow coloration externally and extending internally for two or three millimetres. The specimen was seasoned heartwood of *Quercus robur*. Externally there appeared to be rather fluffy mycelium, some black sporangia, and small round yellow bodies.

Methods of Isolation of the Fungus.

Three fungi were isolated from the surface of the wood, viz. a species of *Aspergillus*, *Penicillium luteum* forming yellow ascocarps, and a new fungus, with yellow conidia in long chains, which has been named *Eidamia catenulata* (4). Dilution plates of potato extract agar, inoculated from a needle drawn across the surface, gave a species of *Aspergillus*, one colony of *Penicillium luteum*, and *Eidamia catenulata* in the first dilution, with only the last-named fungus in the second and third dilution plates. This fungus is characterized by its very long chains of yellow conidia. The conidia are oval and measure 3·5 to 5 μ by 2·5 to 3·5 μ, whereas those of *Penicillium luteum* (7, 7A) are rounder and measure 2·4 to 2·8 μ by 1·6 to 2·4 μ.

In all the cultures of *Eidamia catenulata* obtained in this way, hyphae bearing hyaline spores also occurred, so single spore cultures were obtained both from the conidia and from the hyaline spores. In each case both types of spores were produced on the resulting growth, and later examination revealed the same hypha bearing both conidiophores and single hyaline spores on branches. In this way it was proved that *Eidamia catenulata* possesses two types of spores.

In order to isolate from inside the wood the fungus causing the yellow coloration, a piece three inches in length was sawn from the original board

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and soaked in running water for six hours. It was then placed for a minute in alcoholic mercuric chloride, to kill any external spores, washed in sterilized water, and placed on a glass ring in a potato dish. All the glass used had been previously treated with mercuric chloride. A little sterile water was left in the bottom of the dish to keep the air sufficiently moist. In a week to ten days a mycelium could be seen emerging from the cut surface of the wood. This was a growth from inside the wood and proved on microscopic and cultural examination to be identical with the fungus, *Eidamia catenulata*, isolated from the wood externally. The wood was left for several months in these moist conditions and the yellow colour spread throughout. No other fungus was found arising from the wood. Single spore cultures were obtained from the conidia produced on this mycelium, and subcultures of these were used in all subsequent experiments.

Artificial Production of the Golden Colour in Wood.

In order to test the effect of *Eidamia catenulata* on the heartwood of normal oak, small pieces of *Quercus robur* (about one cubic inch) were placed one above the other in wide test-tubes. These were sterilized by dry heat at 180° C. Sterile water was then poured in and the wood left to soak for some hours, after which all but 10 c.c. of the water was poured off. This left enough water at the bottom of each tube to supply a sufficiently moist atmosphere for the fungus. The degree of moisture would vary in the different blocks of wood according to the position in the tube, the moisture decreasing from the lowest block upwards. The blocks were then inoculated with spores of *E. catenulata* and left at laboratory temperature. In a week the fungus was growing well, and the oak soon had the yellow colour externally, owing to the production of many chains of yellow conidia. In four months the wood was definitely yellow, and cubes split after six months showed exactly the same golden colour seen in some of the original 'golden' oak which had been left in damp air. In these experiments a control tube of oak which had been treated in exactly the same way as the other tubes, apart from inoculation, was kept for comparison. This wood showed no change in colour or alteration in contents as far as could be seen macroscopically and microscopically.

The difference of moisture in the different blocks was not sufficient to make marked changes in the growth of the fungus and consequent colour of the wood. In this the effect of *E. catenulata* on oak is not comparable to that of the unknown fungus causing 'brown' oak. In the latter Professor Groom (2) found that the brown colour was assumed only when the heartwood contained moisture exceeding a certain minimum and falling short of a certain maximum. The blocks in the middle of each column in the tubes inoculated with the unknown fungus changed to the brown colour, and not those at the base or top of the tubes.

From the fact that *E. catenulata* is the fungus produced from the original specimen of golden oak after careful external sterilization, and that oak inoculated with this fungus becomes the same golden colour as the original specimen, it can be concluded that the golden colour in oak is caused by *Eidamia catenulata*. The other fungi found on the original specimen are growing externally only. This conclusion is confirmed by an examination of sections of the original oak and artificially infected oak.

A few other woods, such as chestnut, beech, were inoculated in the same way with this fungus. The effect on beech is to darken somewhat the colour of the wood, whereas the chestnut becomes a marked golden colour.

Morphology of *Eidamia catenulata*.

The morphology of this fungus is fully described in the paper dealing with the genus *Eidamia* (4). It may be summarized here as follows: (1) The mycelium is composed of septate branched hyphae, varying in size from 3 μ to 10 μ in width, and having thin or thick walls according to the medium supplied. (2) Two types of spores occur, conidia which are yellow in colour and hyaline spores. (3) The conidia are narrow to broadly elliptical, pointed at both ends, and vary in size from 4 by 2 μ to 7 by 3·5 μ. There may be as many as 100 in one chain, and balling of the spores may occur. (4) The conidia are borne on sterigmata which are slender, slightly swollen at the base, and vary in size from 8–16 μ in length and 1–2·5 μ in width at the base. (5) The sterigmata may be borne singly on a hypha or on a short branch or a number at the head of a slightly swollen branch, or a *Penicillium* type of conidiophore may occur. (6) The hyaline spores are borne directly on the hypha or at the end of a branch or may be intercalary. They are round or slightly pear-shaped, with fairly thick walls, and vary in size from 7·5 by 8·5 μ to 14 by 10 μ or 18 μ diameter. (7) The colour of the culture varies from pale cream, old gold, to cinnamon brown, according to the medium used, the age of growth, and the temperature of incubation.

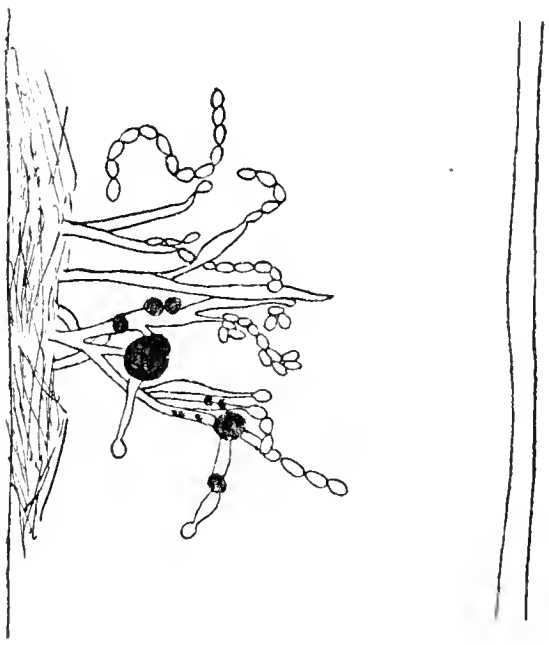
Physiological Characteristics of *Eidamia catenulata*.

Details of the experiments on the physiology of this fungus are given in the paper dealing with the genus *Eidamia*, and may be briefly summarized here. (1) *E. catenulata* is capable of hydrolysing starch, and grows on soluble pectin. (2) This fungus can grow on various sugars such as glucose, lactose, maltose, galactose, fructose, mannose, and sucrose, and it causes an appreciable inversion of cane sugar. (3) A moderate growth is obtained on peptone, which becomes considerable when glucose is added to the peptone. (4) Asparagin can be used by the fungus as a source of nitrogen only in the presence of mineral salts, and ammonia is liberated. (5) *E. catenulata* is highly tolerant of acids, being able to grow at a hydrogen-ion concentration

of 1·16. Its optimum pH value is between 2 and 3, and its minimum is 8·2. (6) There is evidence that the fungus is capable of utilizing certain acids, such as malic, tartaric, citric, and gallic. Its growth on tannic acid is practically the same as on the control nutrient medium, showing tolerance of the acid but giving no evidence of utilization. (7) The yellow colour of the conidia is insoluble in ether, chloroform, alcohol, and water. These physiological characteristics are important in the consideration of the fungus in relation to the oak.

E. CATENULATA IN THE WOOD.

The cellulose acetate method (8) was the one usually employed in order to soften the oak and obtain sections with the fungus uninjured. Small cubes of the infected oak, at different stages after inoculation, or of the original specimen after soaking, were de-aerated under water. They were left in pure acetone for two hours, and then placed in a solution of cellulose acetate in acetone. After six to ten days these could be cut on the Jung sliding microtome. This time could be about halved if the tubes containing the blocks in the cellulose acetate were placed in an incubator at 40° C. Sections were cut about 10 μ thick and placed in acetone to dissolve out the cellulose acetate, followed by alcohol to replace the acetone. After this they were stained, usually with a saturated aqueous solution of safranin followed by light green in clove oil. The fungal hyphae took up the light green very well. Another stain which proved useful was a slight variation on that recorded by E. E. Hubert (5) for staining fungi in wood. The sections were placed in Bismarck brown (2 per cent. in 70 per cent. alcohol) for about two minutes, washed with distilled water, dehydrated, and then left in a solution of methyl violet in clove oil for three minutes. The violet stain was differentiated in clove oil followed by cedar oil and Canada balsam.



TEXT-FIG. 1. Longitudinal section of wood-vessel showing hyphae with globules of secretion and chains of conidia. × 290.

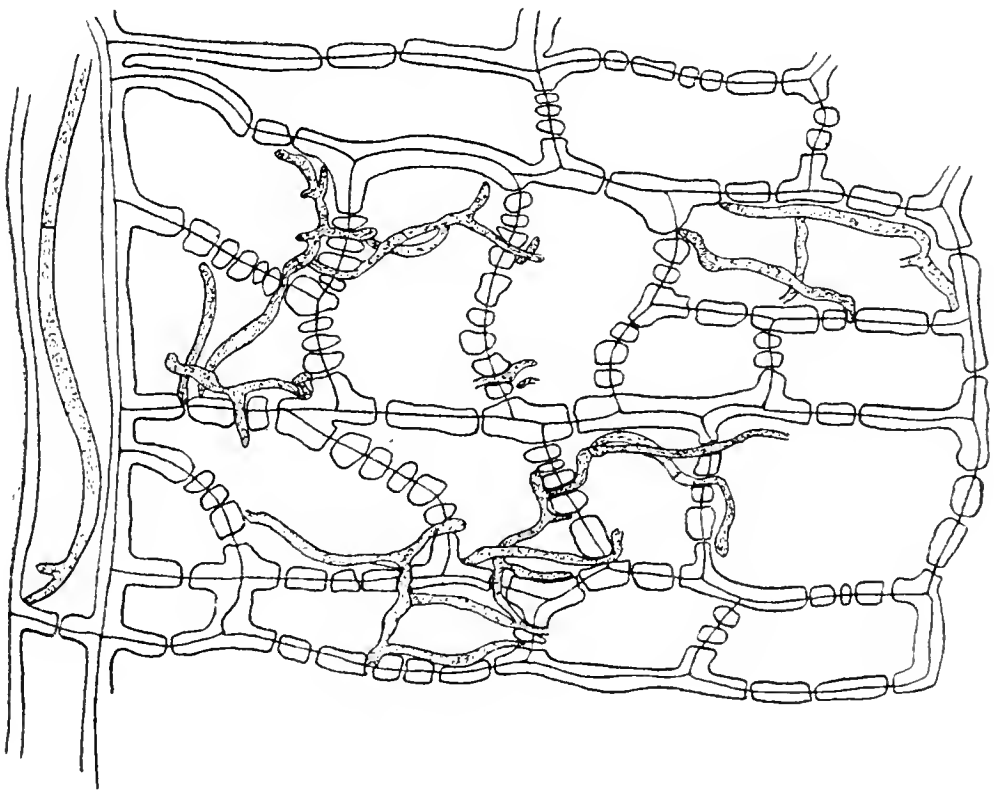
It was found possible, after the use of cellulose acetate to soften the wood, to embed in paraffin, and cut with a Cambridge rocking microtome. The small blocks were transferred from cellulose acetate to acetone for a few hours, and then placed in 95 per cent. butyl alcohol as given by Mlle Larbaud (6), after which the blocks were passed through two baths of pure butyl alcohol, to the second of which paraffin was added. Embedding followed the usual process, and sections were mounted and stained in the usual way.

Hand sections were also made from the wood before it was subjected to any softening process. These were examined in air to obtain fungal hyphae and spores in position in the large vessels. Text-fig. 1 gives an illustration of the results obtained by such sections. The wall of the vessel is lined with a mass of hyphae from which hyphae, bearing sterigmata and conidia, protrude into the lumen. This section was taken from some of the original 'golden' oak which had been soaked and left in damp air for eight months. The conidia are the shape, size, and colour of those of *Eidamia catenulata*. On the hyphae occurred glistening yellow globules of a substance exuded, in all probability, owing to the activity of the fungus, since the wood cells are dead. A substance similar in appearance also occurs in oak inoculated with spores of *E. catenulata*.

Normal heartwood of *Quercus robur* shows large vessels in the spring wood and smaller vessels in the autumn wood, with fibres, wood parenchyma, and medullary rays of the two types, multiseriate and uniseriate. Tannin is present in cells of the medullary rays and in some of the wood parenchyma. A little starch is scattered sporadically in medullary ray cells or in wood parenchyma.

Growth of the Fungus in the Wood.

By examining sections from oak at different intervals of time after inoculation with *E. catenulata* it was possible to trace its course through the

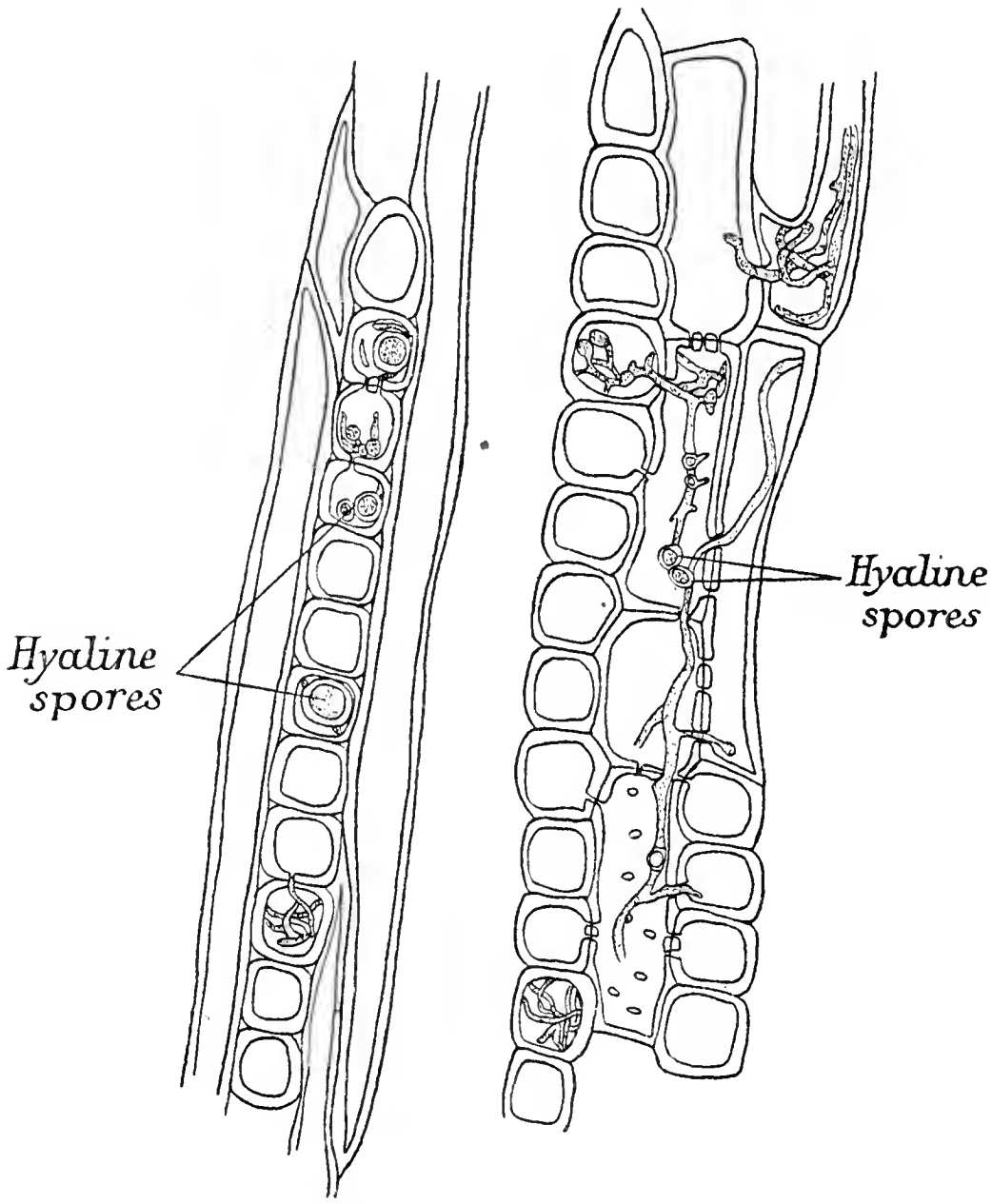


Text-fig. 2. Radial section of wood showing hyphae passing through pits in the walls of the tracheides. × 290.

wood. The hyphae advance along the medullary rays, and they may be found in ray cells when they are absent from all neighbouring cells. They run in a transversely radial direction, always passing through the pits in the

end cell-walls, with occasional branches passing to the wood parenchyma in the vicinity. The passage through the pits in radial section is illustrated in Text-fig. 2. Finally the tracheides, wood-vessels, and fibres are entered, and here the hyphae appear to travel longitudinally, in the main, though fairly numerous lateral branches into surrounding cells do occur (Pl. X, Fig. 2).

The hyphae, as a rule, are septate and branched, with occasionally a swollen irregular appearance with frequent septation, such as was found

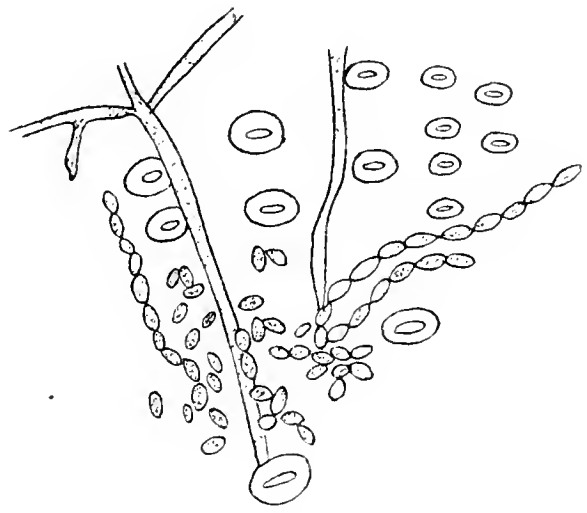


TEXT-FIG. 3. Tangential section showing hyphae and hyaline spores. × 290.

when the fungus was growing on synthetic agar at 25° C. The frequent presence of hyaline spores of all sizes was noted in the medullary ray cells and in the wood parenchyma and vessels, both in the older infections and in the original 'golden' oak. Comparisons of the dimensions of these hyaline spores and those of *E. catenulata* showed the same range of variation, though the smaller sizes were perhaps more abundant in the wood than in cultures. Text-fig. 3 shows hyaline spores in medullary ray cells and wood parenchyma in tangential sections of oak.

Conidia also occur frequently in infected oak. Text-fig. 4 shows the lumen of a vessel of the original 'golden' oak with conidia scattered and in

chains. These were measured and found to be 3–4 μ by 2–3 μ, whilst those on hyphae growing out of the wood were 3·5–5 μ by 2·5–3·5 μ. The slight difference in size may be due to the fixing and softening process the wood underwent prior to sectioning.



TEXT-FIG. 4. Lumen of wood-vessel showing pits, hyphae, and chains of conidia. × 500.

PRODUCTION OF YELLOW SUBSTANCE.

(a) *In the Wood.*

Reference has already been made to the glistening yellow globules seen on hyphae in vessels of the wood. The same phenomenon occurs in fibres, vessels, and wood parenchyma seen in microtome sections. The hyphae in a cell may be studded with roundish small masses of a substance which may retain a yellow colour or may be stained red or yellow-red with safranin. Sometimes only one hypha in a cell will have this appearance. In other stages the substances will take the form of an irregular mass completely covering a portion of the hypha. Where this substance is present in quantity the hyphae are often somewhat disorganized, and refuse to take up the light green stain taken by normal living hyphae. Instead they are often stained dark red by safranin. In one cell may be found hyphae staining light green, some disorganizing hyphae staining red, and irregular masses of a somewhat refractive substance which may or may not stain red with safranin. Finally cells are found which are completely filled with a yellow or red yellow substance in which it is sometimes possible to see broken mycelium (Pl. X, Fig. 3).

In transverse section infected oak shows refractive contents in the medullary rays, wood parenchyma, fibres, and even lining the vessels (Pl. X, Fig. 1). These refractive bodies sometimes have a crystalline appearance, in other cells they are more granular in structure, and again it may not be possible to make out any definite structure at all. They vary in colour, being yellow generally, tangerine, or red-brown to brown occasionally. In transverse section these substances appear to occur in islands of cells surrounded by areas of comparatively empty cells, and this corresponds with

the fact that the course of the hyphae is generally vertical in the wood, so that islands of vessels and tissue containing hyphae would occur.

Tests for tannin show that many of these cells contain some 'tannin body' as well as the refractive substance, often masking the yellow colour. It is possible to dissolve out the tannin and obtain cells still showing yellow or tangerine-coloured contents.

These bodies agree, in certain reactions, with the behaviour of the material known as 'wood-gum'. They are insoluble in water, alcohol, ammonia, chloroform, acetic acid, concentrated nitric, hydrochloric, and sulphuric acids. In most of these cases tannin is dissolved out and the refractive substance, yellow, light orange, or red-brown, remains. Strong potash dissolves some of the cell contents, leaving pale yellow or tangerine-coloured substances in the medullary rays and some patches of cells. Whether this 'tannin', so often present along with the yellow substances, is the same as the 'tannin' in uninfected oak it is impossible to say with the micro-chemical tests available.

The yellow colour of the wood may be due to two factors, the yellow substance exuded by the fungus and the yellow colour of the conidia. Colour may be produced in wood by fungi due to the colour of the mycelium, such as certain species of *Graphium* may produce on oak, pine, &c. Other cases have been recorded in which the fungus exudes a soluble pigment which, according to Hedgecock (3), is absorbed by the lignified cell-walls so that the wood is actually stained. Hedgecock describes *Penicillium aureum* which may produce a yellow colour in oak. This fungus exudes a soluble pigment in the form of granules and also possesses a mycelium, which may have swollen cells and bear conidiophores, consisting of a whorl of branch hyphae bearing sterigmata and long chains of oval conidia. The points of difference between this fungus and *Eidamia catenulata* are as follows :

(1) *P. aureum* is dimorphous, having a mycelium with grey-green coloured fruiting clusters and also a sterile mycelium which can be lemon-colour or orange-red according to the acidity or alkalinity of the medium. *E. catenulata* has a colourless mycelium and its fruiting clusters are yellow. (2) The swollen cells in the mycelium of *P. aureum* have no thick walls and do not separate from the hyphae, so cannot be compared with the hyaline spores of *E. catenulata*. (3) The conidia in *P. aureum* are 3–4 μ by 1·5–2 μ and contain a blue-green pigment which is soluble in hot alcohol, whereas those of *E. catenulata* measure 4–7 μ by 2–3·5 μ, and possess a yellow colour insoluble in alcohol, &c. (4) The yellow pigment exuded by the hyphae of *P. aureum* is soluble in slightly acid or alkaline water, hot alcohol, &c., and is absorbed by the lignified cell-walls. In *E. catenulata* the hyphae exude a yellow substance which is insoluble in acid or alkali ; and is stored in the cell cavities as a refractive yellow or orange 'gum', and there is no staining of the cell-walls.

Beside the yellow substance in the infected oak there occurred in certain cells, generally in small groups, dark granular masses. These were found in normal heartwood as well as in infected wood, and their distribution did not appear to be related to the hyphae at all. These patches appear to be slightly more numerous in the infected wood, but apparently their occurrence was not sufficiently great to cause any darkening in the colour of the wood. The dark substance was insoluble in hydrochloric, nitric, and acetic acids, but soluble in strong potash. Its nature was not discovered. This substance was noted in 'brown' oak by Professor Groom.

(b) *In Culture Media.*

It has been found that, when grown on prune agar, malt agar, potato glucose agar, and synthetic starch agar, the fungus produces roundish refractive yellow or brown bodies. These bodies are very noticeable among the aerial hyphae. They are larger than the hyaline spores as a rule and do not dissolve in alcohol, water, or chloroform, and do not stain with iodine, Sudan III, Scharlach red, or alkannin. In fact they behave in the same way as the yellow substance produced in wood. Cultures on potato mush agar and on a synthetic sugar medium produce globules of yellow viscid substance of somewhat the same nature.

The yellow colour does not extend to the medium in any of these cases. The only exception to this is oak-sawdust agar (4 per cent. or 10 per cent. sawdust) infected with *E. catenulata*, where yellow coloration of the medium begins to show after three to four weeks' growth. It may be that this is the only medium possessing the requisite properties that result in the production of the yellow colour in the wood.

The Action of the Fungus on the Cell-wall.

A certain amount of splitting of the middle lamella is visible in sections of infected wood, but it has not been possible to prove this to be due to fungal action. One case was noted in which a hypha was growing along such a split between two cells, but it is doubtful whether this was more than accidental. The splitting in sections may be due to some slight weakening of the tissue by the fungus, or by the process of softening, making it more susceptible to the action of the knife.

The fungus passes from cell to cell through the pits, and no case of any other method of passage was found. There is no evidence to show whether the hypha pierces the middle lamella of the pit or excretes an enzyme to dissolve it. The fungus is capable of growing on and using up soluble pectin, and may possibly have some action on the middle lamella.

Many tests were made to see if any delignification of the cell-wall by the fungus occurred, but none was observed. The wall region just round

the pits was particularly scrutinized and no cellulose reaction due to the fungus could be found. It was noted that, with the iodine-sulphuric acid test, the reaction for cellulose occurred wherever a wall had been broken or distorted or strained by the action of the knife or the pressure of the microtome holder. This made the test difficult to interpret, and great care had to be taken in observing the path of the hyphae and the walls in contact with them. The conclusion that the fungus has no power of delignification was reached.

Source of Food in the Wood.

The fungus is growing in seasoned heartwood of oak. The food available is the pectic substance of the middle lamella; the pectic bodies, glucosides, tannins, cellulose, and lignin in the walls; the tannin bodies in the medullary rays and wood parenchyma cells; any starch or waste products which may have been contained in the cells and dried in the process of seasoning. The amount of food material is necessarily small, and the chief bulk would be comprised by the lignified walls and the tannin with glucosides and other impurities in the cells. The small quantity of the food is not a deterrent to the growth of *E. catenulata*, as the fungus can grow on media with minute quantities of salts only, on starvation media suggested by Coon (1), and even on dilute hydrochloric acid in distilled water.

There is no evidence that the fungus is capable of delignifying the cell-walls, and it shows no appreciable growth on cellulose, so the lignin and cellulose are probably not utilized. Certain deductions as to its method of nutrition in the wood may be made from the study of its behaviour in various nutrient constituents, an aspect which has already been dealt with in considerable detail elsewhere.

The fungus has been shown to be capable of growth in almost pure soluble pectin derived from apples. Hence it is not improbable that the pectic bodies in the walls of the wood may supply a certain amount of nourishment. The middle lamella is not disorganized by the fungus, which seems to attack it only at the pits and that action may be physical, therefore that lamella cannot be regarded as a source of food.

Any sugar or glucoside would be readily used, as it has been demonstrated that *E. catenulata* grows freely on glucose and can invert cane sugar.

This fungus can hydrolyse starch, but the quantity available is extremely minute, being scattered sporadically in ray cells and wood parenchyma. It is able to grow to a certain extent in an acid medium containing no other source of nutriment. It is highly tolerant of certain acids (limits being pH 1·16 to pH 8·2) and is probably able to utilize malic, citric, tartaric, and gallic acids. Any trace of gallic acid in the oak can be utilized. Growth also occurs in a solution containing from 0·1 to 2 per cent.

tannic acid. A consideration of these facts makes its growth on oak more accountable, since the tannic acid present in the wood is no deterrent to its activity—though it may be the factor that inhibits the growth of *E. viridescens* on oak, since with that fungus appreciable growth was not obtained in tannic acid at higher percentages than 0·1.

The 'tannin' bodies probably contain glucosides and other impurities which act as food for the fungus. These bodies themselves do not appear to decrease, and in fact, from micro-chemical tests, would appear to be more prevalent in the infected wood. It may be that the clearing away of the impurities in the 'tannin' bodies by the fungus leaves the tannin itself more free to take up the ferric chloride, so producing the apparent increase in tannin. Without chemical analyses of the normal and infected oak it is not possible to be definite on this point.

The physiological evidence points to the source of food in the wood being any soluble pectic bodies, glucosides, starch, proteid, and any organic salts that may be present.

Summary.

Eidamia catenulata has been isolated from the specimen of 'golden' oak, both externally and internally. Since this fungus is the only one growing from inside the oak when external sterilization has been applied, and it also produces the golden colour when grown on normal oak, the conclusion has been reached that the golden colour in the specimen of oak investigated is due to the activity of *E. catenulata*.

This fungus in the infected tissue gives rise to a yellow substance as globules exuded along the hyphae. This accumulates in the cells and may finally fill them completely, with accompanying disorganization of the hyphae. This yellow substance somewhat resembles in its reactions the material termed 'wood-gum'.

The hyphae advance in the heartwood along the medullary rays in a transversely radial direction and in the wood parenchyma, fibres, and vessels longitudinally in the main. Passage from cell to cell takes place only through pits in the walls, and there appears to be no delignification or attack on the walls themselves.

The source of food for the fungus in the heartwood of oak is probably soluble pectic bodies, glucosides, any gallic acid, starch, proteids, or organic salts that may occur.

E. catenulata produces conidiophores with oval yellow conidia in long chains and also hyaline spores borne singly or in pairs, generally terminally, on lateral branches of the mycelium.

In conclusion I desire to thank Professor Groom for passing on to me the original specimen of 'golden' oak on which this investigation has been based.

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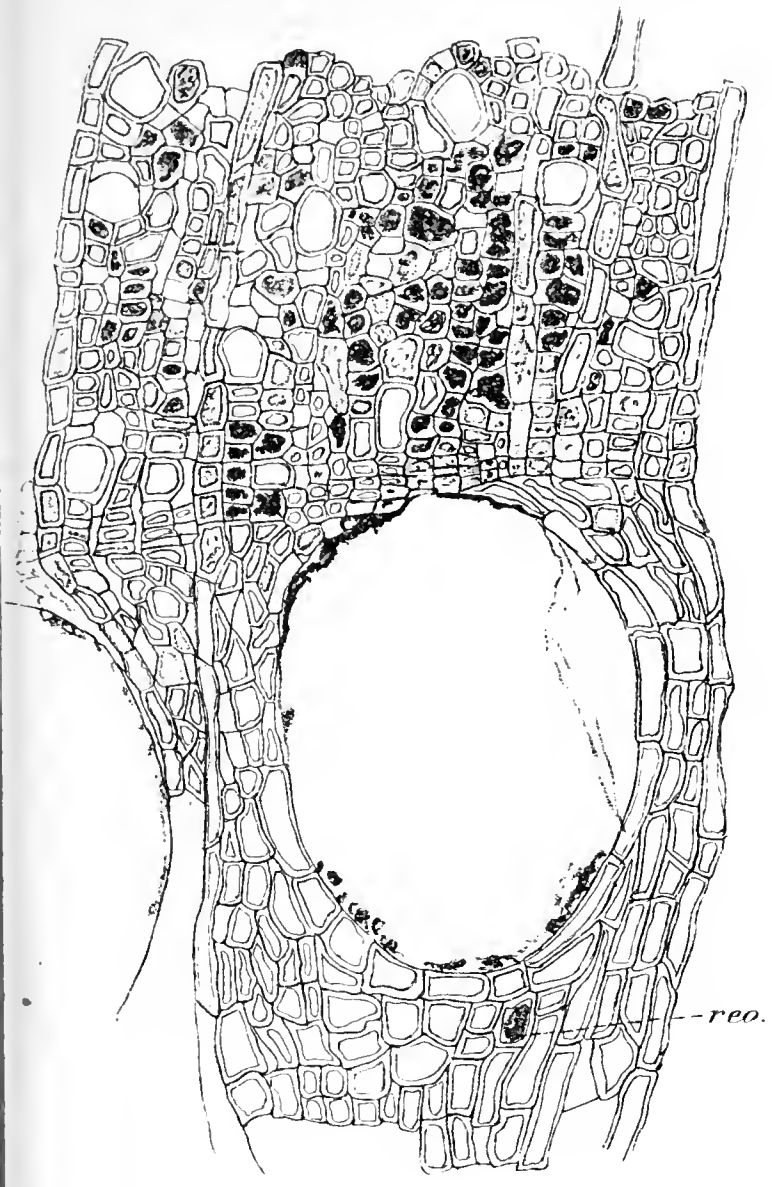
EXPLANATION OF FIGURES IN PLATE X.

Illustrating Mrs. Williamson's paper on the Origin of 'Golden' Oak.

Fig. 1. Transverse section of *Quercus robur* infected with *Eidamia catenulata*, showing islands of cells filled with a yellow or tangerine coloured, somewhat refractive substance. The large vessel also shows some of the same substance lining the wall.

Fig. 2. Tangential section of *Quercus robur* showing hyphae of *E. catenulata* running longitudinally and passing through pits in the cell-walls.

Fig. 3. Tangential section of *Quercus robur* showing excreted globules on the hyphae of the fungus. One wood parenchyma cell is full of this substance and shows remains of disorganizing hyphae.



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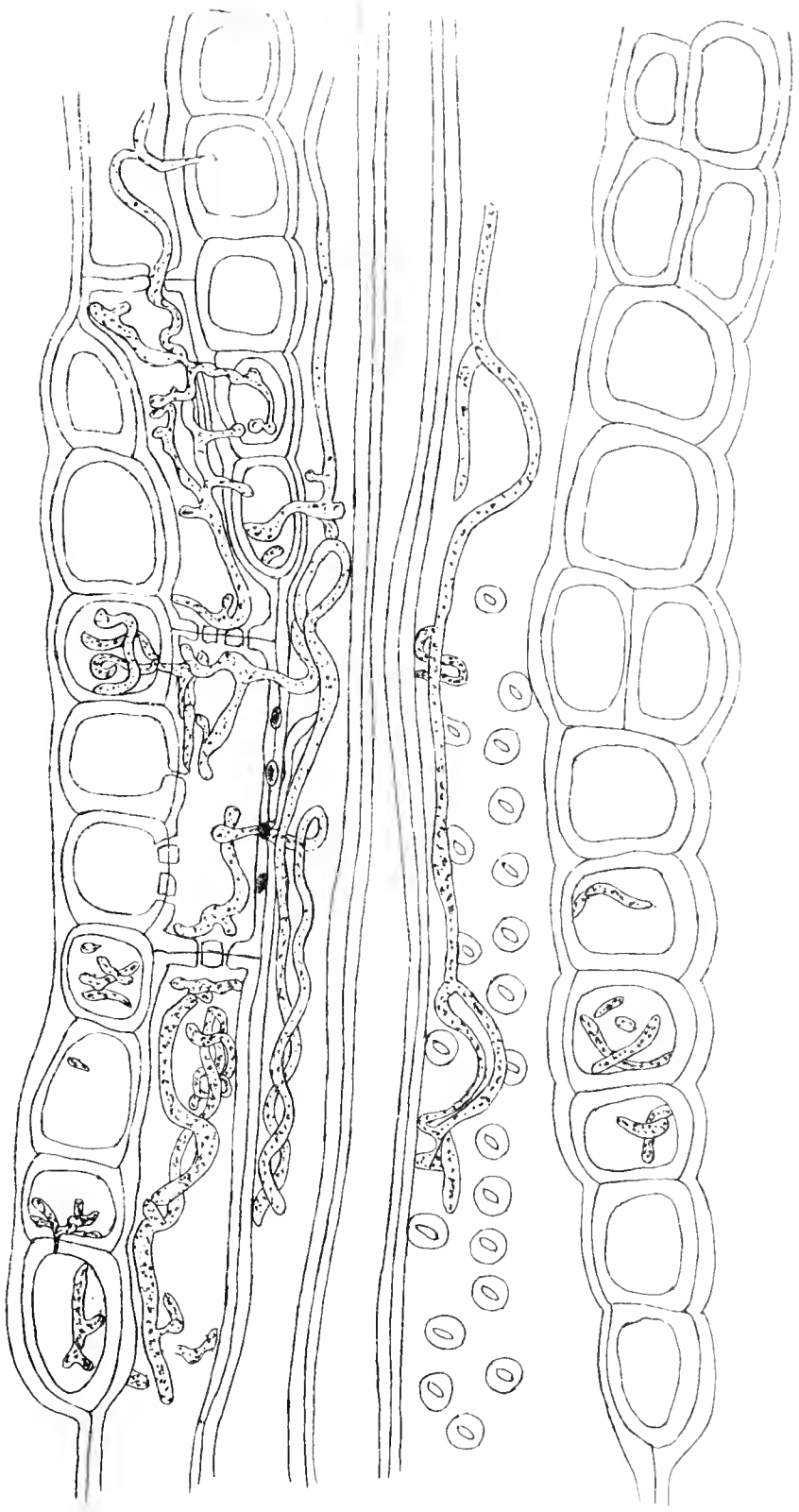

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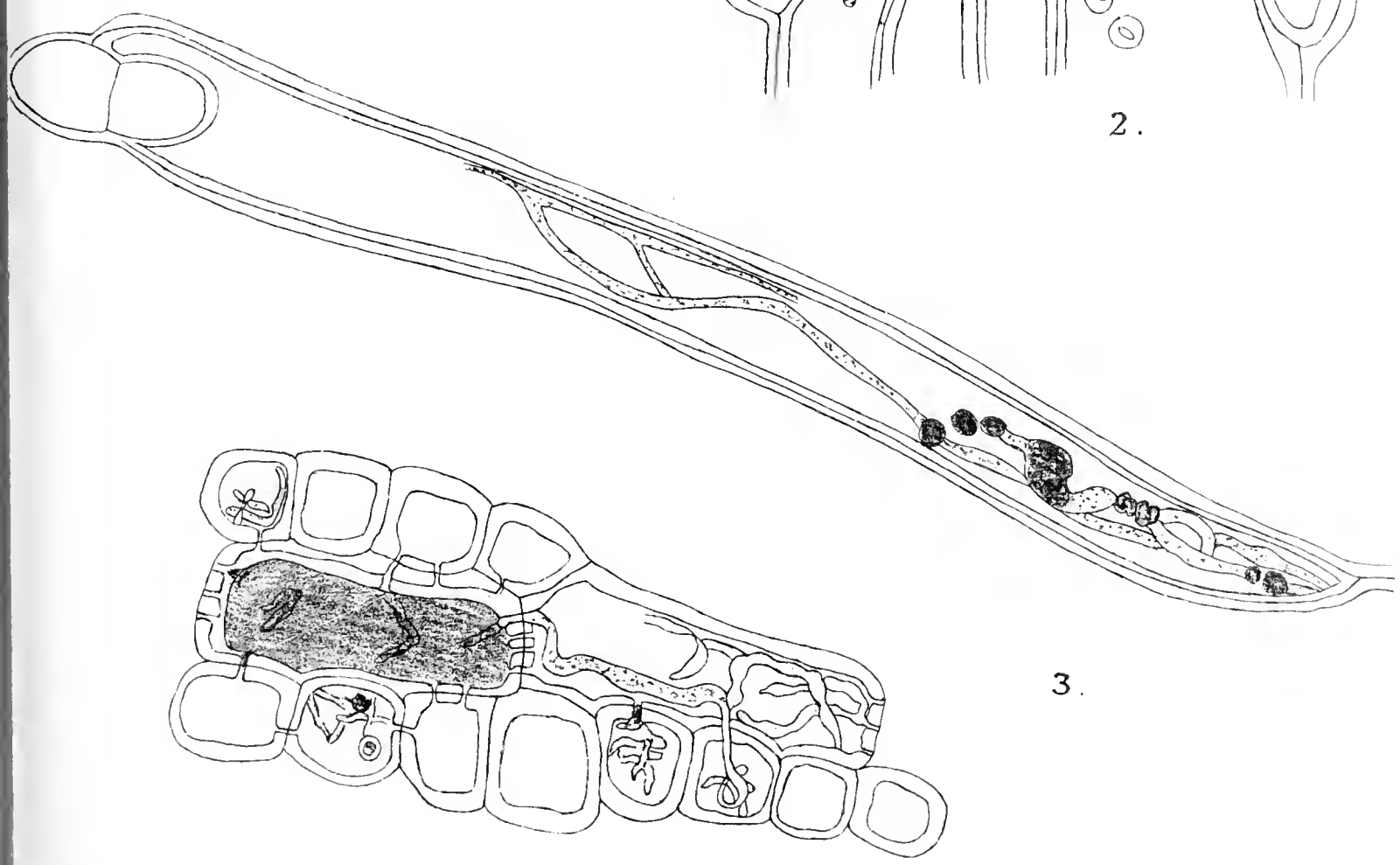

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WILLIAMSON—GOLDEN OAK.

The Replacement of the Terminal Bud in the Coco-nut Palm.

BY

T. PETCH, B.A., B.Sc.,

Botanist and Mycologist,

AND

C. H. GADD, B.Sc.,

Assistant Mycologist, Ceylon.

With three Figures in the Text.

IN the valuable contribution to our knowledge of Bud-rot in coco-nuts by Messrs. Sharples and Lambourne in this Journal, vol. xxxvi, pp. 55–70, cases are described in which, according to the observations of the authors, it appeared that the terminal bud had been destroyed and that growth had subsequently occurred from a lateral bud. Assuming that deduction to be correct, the phenomenon would be parallel to the normal condition in dicotyledonous trees, in which the loss of the leader is generally followed by the development of shoots from lateral buds, one of which shoots may ultimately form a continuation of the original main axis.

As is well known, branching does not normally occur in the coco-nut palm. On the young palm, nothing develops in the leaf axils, while on the older trees each leaf subtends an inflorescence. Rare occurrences of branched coco-nut palms have been recorded, but, in view of the normal habit and structure of the tree, it is generally assumed that such cases arise from a division, probably accidental, of the terminal growing-point. No one appears to have had an opportunity of verifying that assumption, and it is doubtful whether such opportunity could ever happen, as, owing to the slow unfolding of the leaves, many months must elapse before the branching becomes evident. Instances of virescence, i. e. the conversion of the inflorescence into an axis bearing abnormal leaves, are not uncommon, but these abnormal inflorescences die, just as they would ultimately have done had they borne flowers.

Consequently, the idea that the terminal bud of the coco-nut palm may be destroyed, and be subsequently replaced by a lateral bud, is a novel one which, in the absence of complete evidence, can only be regarded as

probable, if no other explanation of the phenomena observed is available. The object of the present note is to record a similar occurrence in Ceylon in which the origin of the apparently lateral growth was traced by dissection of the plant.

In March 1922 two specimens of diseased seedling coco-nut palms were received from the Galle district. The young unfolded leaves were withered and brown, and could easily be pulled out of the sheath. Springing from

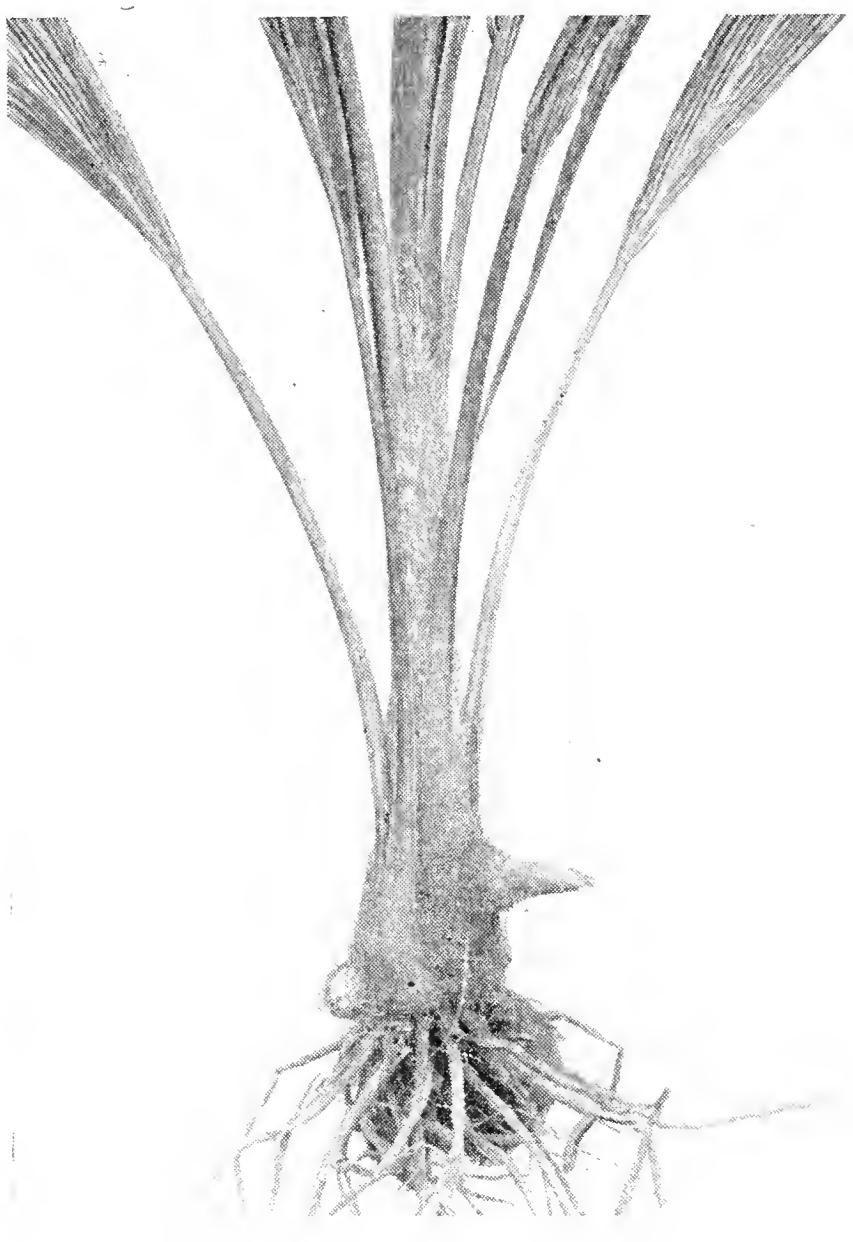

FIG. 1. Seedling coco-nut palm with a 'lateral' bud. $\times \frac{1}{12}$.

near the base of the stem, however, was what appeared to be a healthy lateral bud. A photograph of one specimen as it was received is given in Fig. 1. The withering of the central leaves and their easy withdrawal from the sheath, owing to a decay of their bases, suggested Bud-rot, but the formation of what appeared to be a new bud near the base of the palm was an abnormal condition in this disease in Ceylon.

On removing the external leaves it was found that a hole had been bored through the base of one of the young leaves as though by a large insect, possibly the rhinoceros beetle. A soft rot had occurred round the hole and had spread to the bases of the adjacent leaves. The rot at their bases had caused the withering of the leaves, and made it possible to pull

them out easily from the sheath. In one case the rot had reached the central axis, but the bud itself was not damaged. In both cases, however, the direction of growth of the bud was changed from the vertical to the horizontal, and the buds had pushed their way through the leaf-bases to the exterior. A photograph of the palm with the leaves removed from one side is given in Fig. 2. This shows the curved bud and a rotting leaf-base immediately behind it. A drawing of the longitudinal section through the bud and axis is given in Fig. 3, from which it is evident that the bud, though projecting through the leaf-bases and apparently lateral in position, is the original terminal bud.

FIG. 2. The same, with the leaves removed from one side. $\times \frac{1}{9}$.

It may be concluded that a diseased condition of the central leaves causing them to fall over does not necessarily connote the death of the palm, i. e. it is not necessarily true Bud-rot. This conclusion was also reached by Sharples and Lambourne as a result of their inoculation experiments on coco-nut palms.

Sharples and Lambourne found that, as a result of inoculating coco-nut palms with a red pigmented bacillus, the central shoots of the inoculated palms became black and decayed, and ultimately fell over, as is common in Bud-rot cases. These palms eventually recovered in a peculiar manner. The authors give a figure showing the type of recovery, of which they say, 'The central shoot has disappeared, but from the side of the bud below the remains of the central shoot a lateral shoot is pushed out. The leaves comprising this lateral shoot were strangely aborted, the leaflets being very stiff and only partially developed. Growth of this lateral shoot continues and it takes the place of the central shoot.'

The figure given shows a young palm with a developing bud projecting from the side of the stem. It is similar in many respects to that given here in Fig. 1, except that the bud is further developed, with young leaves expanded. The outer leaves of the buds of the Ceylon specimens were abnormal, having no leaf-blades and consisting solely of thick leaf-bases. It might therefore be expected that the further development of these buds

would give the palms an appearance very similar to that illustrated by Sharples and Lambourne.

Moreover, the method of inoculation used by Sharples and Lambourne, though artificial, was in many respects similar to that by which the Ceylon palms had become naturally infected. They used a small gouge with which to bore into the tender central tissues in order to admit the infecting organism, whereas in the Ceylon specimens infection had followed the natural boring of a beetle or other insect. The Ceylon specimens may be regarded as being in a similar condition to that caused by the artificial inoculations of Sharples and Lambourne.

Cases in which the spike of unfolded leaves which terminates the crown of a coco-nut palm has decayed, while the growing-point has not been destroyed, have occurred in Ceylon on full-grown palms, though they appear to be very rare. In one instance it was found on examination that the apex of the embryonic tissue was blackened superficially, and the blackened surface had cracked into numerous areolae, the cracks being about half a millimetre deep. The developing leaf rudiments were distorted, and consisted of a short stout mid-rib, with the close-packed rudimentary folded leaflets along each side, the outer ends of the leaflets being also blackened. It is clear that in such cases the decay has been arrested before the growing-point has been seriously attacked, and under such circumstances it would be expected that further growth would take place in the direction of the original main axis. It has not been possible to verify the latter supposition, because this condition has only been discovered after the palm has been felled.

FIG. 3. Longitudinal section through the stem of the same plant, almost medially through the growing-point. $\times \frac{1}{3}$.

Sharples and Lambourne claim that the bud which appeared at the side of the stem in their experiments is truly a lateral bud, and not the original terminal which has changed its direction of growth. They conclude, *inter alia*, that—

'Owing to a very definite resistance exercised by the bud tissues of mature trees against infection, such organisms [i. e. saprophytic organisms inoculated into the bud tissues] in the absence of suitable conditions will not develop beyond a certain stage, marked by the death of the central shoot. If the central shoot dies, and the bud is invested externally with the invading organism, the bud tissues have the power of pushing out

a lateral, by means of which growth is continued to take the place of the diseased central shoot' (p. 66).

'The prevailing idea that growth is no longer possible if the central shoot is killed must now be considered a fallacy, though it must be admitted that healthy growth is not immediate even if lateral shoots are produced' (p. 66).

'This cannot be regarded as any proof of the cause of the rotting of the bud tissues, no more than the death and falling of the central leaves in our experiments can be considered as proving the rotting of the central bud' (p. 68).

As regards what may be considered the most important conclusion from the purely botanical point of view, that the terminal bud of the coco-nut palm can be replaced by a lateral, evidence has already been adduced which would appear to afford another explanation of the phenomenon observed. It does not appear from their paper that Sharples and Lambourne dissected the palm in question and ascertained the origin of the supposed lateral bud. Further, as they intimate that they do not consider that the death of the central shoot in their experiments proved the rotting of the central bud, there would not appear to be any reason for supposing that the original growing-point had ceased to function.

The other conclusions depend mainly upon terminology, and on interpretations of the existing literature on the subject of Bud-rot. If the organism which causes a decay of the unfolded leaves is the same as that which causes a rot of the growing-point, the two phenomena must be regarded as different phases of the same disease, and the same name may be applied to both. But the decay of the leaf-spike only has not been regarded as 'Bud-rot' in coco-nuts in Ceylon. The statement, to which currency appears to have first been given in Ceylon, that the withering of the spike is 'the first indication of the disease' in true Bud-rot in the case of young palms cannot be interpreted as meaning that all cases of withering of the spike are cases of Bud-rot.

Sharples and Lambourne quote from Circular 15, vol. iii, of the Royal Botanic Gardens, Ceylon: 'The first indication of the disease in the case of young plants is the withering of the youngest unfolded leaf. This turns brown and can be pulled out of its sheath; it is then found to end in a soft brown mass.' But farther on in the same paragraph of that circular occur the words: 'If the dying fronds are removed and the bud exposed, there will be found, instead of the white cabbage, a pale brown semi-liquid mass which becomes dark brown with age and possesses an odour resembling that of a tan-yard. In an advanced stage this rot includes the whole of the cabbage, and stops only when the woody portion of the stem is reached. Only the soft parts are affected. The roots and stem are quite healthy, but the destruction of the terminal bud necessarily causes the death of the tree.'

Johnston, also quoted by Sharples and Lambourne, stated: 'The common name of the disease, Bud-rot, well describes its nature, for in its acute or advanced stage the bud of the tree, i. e. the growing-point in the centre of the crown, is affected by a vile-smelling soft-rot which destroys all the younger tissues.'

It will be evident that the term 'Bud' is employed in both the foregoing extracts to denote the actual growing-point; and from the third quotation from Sharples and Lambourne's paper it would appear that the latter authors use the term in the same sense. The 'fallacy' that growth is no longer possible if the *central shoot* is killed is non-existent. The opinion which is generally held is that growth is no longer possible if the *growing-point* is destroyed. Before abandoning the prevailing idea as a fallacy, further evidence must be adduced, in view of the Ceylon examples, to show that, in the condition caused by artificial inoculations, the bud is truly a lateral, and not the further growth of the original terminal after a temporary arrest caused by the invading organisms.

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A Reversionary Character in the Stock (Matthiola incana) and its Significance in regard to the Structure and Evolution of the Gynoecium in the Rhoeadales, the Orchidaceae, and other Families.

BY

EDITH R. SAUNDERS,

Fellow of Newnham College, Cambridge.

With sixty Figures in the Text.

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1. *Introduction. The Cruciferous gynoecium.*

THE composition of the Cruciferous gynoecium has been the subject of much discussion in the past, the chief points at issue being the real number of the carpels and the nature of the replum. My attention was originally directed to this question in consequence of the repeated appearance in the Stock of fruits of unusual shapes. Some were found with a longitudinal flange or wing-like structure extending the whole length and

[1] It is proposed to treat of these cases in detail in a later account.

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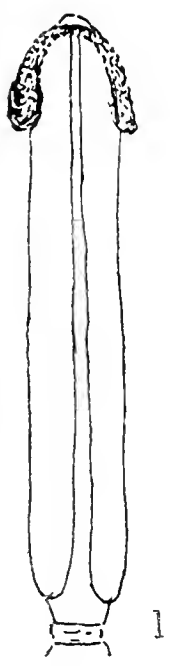
often showing deep transverse fissures as though the tissues had been torn across. Others, though exhibiting no sign of injury, were curved instead of straight, or in extreme cases coiled into a spiral like the pods of certain Leguminosae. A fuller examination of these fruits yielded the results set forth in the present account.

2. *Appearance and structure of a typical siliqua in the Stock.*

The chief external feature of the developing Stock siliqua is the outline of the valves indicating the place of separation from the replum [1] of the two laterally placed carpels when ripe. These outlines are somewhat obscured in the hoary type by the dense covering of hairs, but in the glabrous strains they are clearly defined, and for this reason these strains offer particularly favourable material for the investigation of the points above mentioned. The ovary shows the usual two valves. Between them, in the median plane, is the somewhat broad tract (commissure) regarded as formed from the conjoined carpellary edges (Fig. 1), the inward extensions of which give rise to the placentae, and ultimately become joined at their extremities and so form a complete septum. These two intervening tracts can be seen to swell out at the top of the siliqua into two large connivent knobs or horns (Fig. 2), which may eventually touch (Figs. 5, 6). The valves themselves are defined above in the developing fruit by a horizontal ridge (Figs. 3, 7), which may merge imperceptibly into the knobs or be delimited from them by a continuation of one or both of the sutural lines (Fig. 8). Above this ridge the two flat surfaces slope backwards (inwards) for a short distance and are covered at their summit by the stigmatic papillae. In the young stage the commissures are so narrow that the papillae appear to be continuous round the dividing cleft, and it may be that the ring is sometimes completed by the formation of papillae over the sutures as well as on the valves. But the fruits occasionally to be met with, in which the carpels become free from each other at the apex (Figs. 9, 10), make it clear *that the two main stigmatic areas are centred over the midrib of each lateral carpel*; that is to say, they are *not* commissural, as is stated to be the arrangement in the Cruciferae generally, but alternate with the placentae and replum. As post-fertilization development proceeds, the stigmatic bands between the swelling terminal knobs become **V**-shaped, the double **V** now bordering a shallow, bluntly 4-angled, crater-like cavity which may be considered a short stylar canal (Fig. 4). Later still this cavity becomes narrowed into a double loop or figure of 8 extended crosswise between the pincer-like knobs. Such is the outward appearance of normal fruits.

To the internal structure of the siliqua but little attention seems to have been paid by earlier observers, beyond notice of the presence or absence of

[1] The term *replum* is used throughout the present account in its wider sense to include both the frame of the placentae and the partition stretching between them.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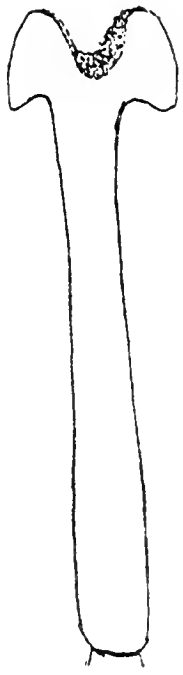
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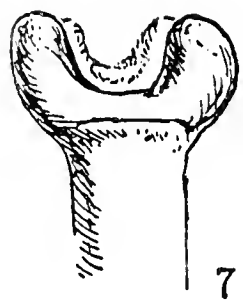
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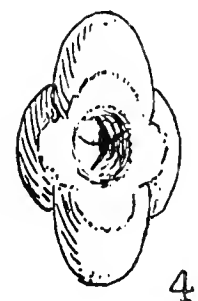
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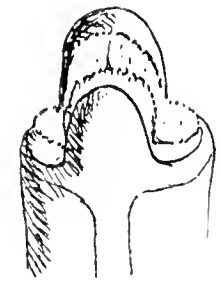
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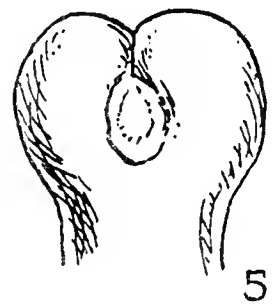
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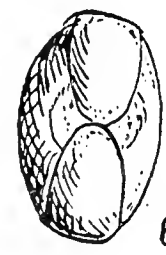
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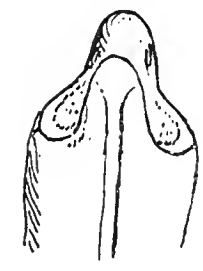
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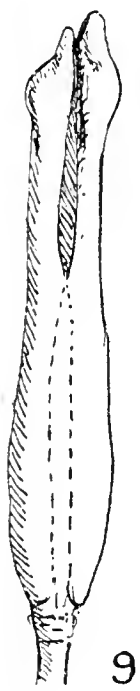
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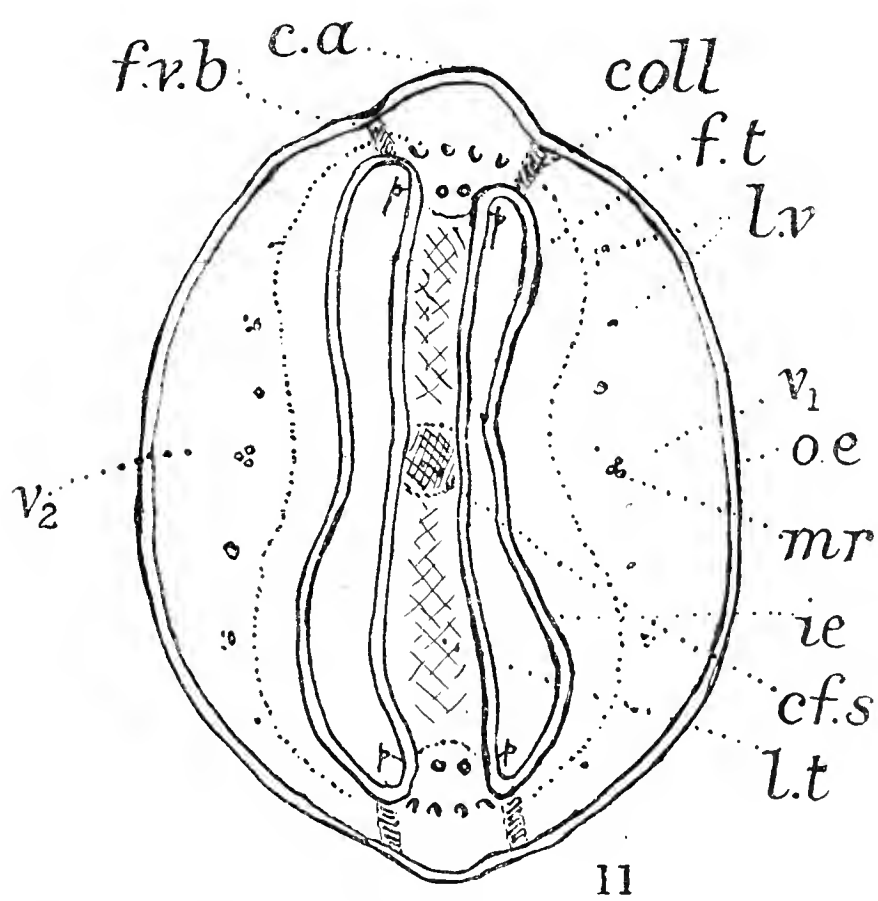

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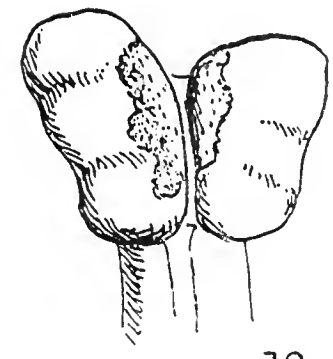
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FIGS. 1–11. *Matthiola incana.* 1. Young siliqua before fertilization viewed from the front, showing the double contour line of the suture, and crosswise to the suture the two stigmatic loops. 2. Young siliqua shortly after fertilization viewed from the side, showing one valve and development of the sutural knobs. 3. Apex of siliqua seen from the front, showing the horizontal ridge delimiting the valves. 4. The same seen from above, showing the short stylar canal. 5. A later stage seen from the side; the sutural knobs have now met. 6. The same seen from above. 7. Intermediate stage seen from the side, showing the ridge defining the valve. 8. Apex of a siliqua in which the sutural lines are continued up on to the shoulders of the knob. 9. Siliqua in which the carpels are disjoined above, seen from the front. 10. Apex of a similar siliqua seen obliquely from above and from the front. 11. Transverse section of young siliqua. *c.a.*, commissural arc; v_1 v_2, valves; *coll.*, collenchyma: *c.f.s.*, central fibrous strand; *f.t.*, fibrous tissue; *f.v.b.*, fibro-vascular bundles of the commissure; *i.e.*, inner epidermis; *l.t.*, loose tissue of the septum which later becomes lignified; *l.v*, lateral veins; *m.r.*, midrib of valve; *o.e.*, outer epidermis; *p.*, position of placenta.

a central fibrous strand in the septum, a character regarded by systematists as of diagnostic value. In *Matthiola incana*, R. Br., the main features to be noted in a transverse section of the developing siliqua are as follows (Fig. 11):

1. The outer contour, which shows two larger lateral arcs formed by the two valves, and two intervening smaller arcs (commissures) bounding the ends of the single median partition.

2. The central larger fibro-vascular bundle (midrib) of the valves, and the row of smaller bundles (lateral veins) on either side of it.

3. The broad belt of lignified cells beneath the inner (lining) epidermis of the valves, which extends along the entire side of the loculus, and may even continue round the ends. A bridge of collenchyma eventually stretches across the thickness of the ovary wall from this thick-walled tissue to the outer epidermis at the point of junction of a large and small arc, thus delimiting the assimilating tissue of the two. When ripe the valves break away through rupture of the thin-walled tissue of the smaller arc where it abuts on the collenchyma and the lignified cells at the ends of the loculus.

4. The distribution of the tissues in the smaller arcs and the connecting septum. The central region of the arc in the bud stage is occupied by a single median vascular bundle, or sometimes two equal bundles are found,[1] while at or near the level of origin of an ovule an additional smaller bundle may be seen at the angle of the loculus. The number and arrangement of the bundles bears a relation no doubt to the ovule formation, and the not unusual occurrence in this species of two main parallel strands may be connected with the considerable width of the commissure, for by this arrangement the vascular bundle of the funicle has less distance to traverse before making contact than is the case when a single median bundle is present. In *Cheiranthus Cheiri*, L., where the suture is considerably narrower,[2] a single central bundle appears to be the rule. As the fruit develops, the vascular tissue increases in amount; the several strands now become arranged more or less in a ring, the phloem of each bundle lying to the outside. A branch from the nearest strand connects with each funicle, and occasionally an anastomosis takes place round the angle of the loculus with a vein in the valve. We find in fact in the smaller arc a vascular supply quite equal to that of the valve, but compressed into a smaller area, the bundles lying on a small circle instead of an extended arc. Conversion of the neighbouring ground tissue into mechanical tissue eventually gives rise to a solid fibro-vascular cord. This cord is surrounded by thin-walled, loosely arranged cells, containing a little chlorophyll, which form the outer (basal) end of the septum. Following upon but sharply marked off from this looser

[1] The two conditions may occur at different levels in the same septum.

[2] The considerable width of the commissure in *Matthiola* as compared with *Cheiranthus* is well seen in the drawings of the cross-section of the siliqua of these two genera in Reichenbach's Icones, ii, Pl. XLV.

tissue, and forming the farther central end, are larger, colourless, elongated, irregularly shaped cells, which later also become thick-walled and lignified. The two ingrowing septa eventually meet, the lining epidermis disappears from the two contact surfaces which unite, and the partition is complete. Later the cells at the point of junction become converted into a longitudinal strand of fibres, a character of importance from the systematic point of view.

3. *Interpretation of the siliqua construction.*

The commonly accepted view of the Crucifer siliqua, as mentioned above, is that it is composed of two lateral carpels forming the two valves, and that the two smaller arcs, together with their inward prolongations, represent the incurved fertile margins of these carpels, which finally become united centrally by a later developed tissue, the origin and significance of which, on this view, remain unexplained. There are also admittedly other features which on this interpretation present a certain difficulty, the chief being firstly, that when the valves break away the small arcs, i.e. the supposed fused edges (placentae), are left behind on the partition; and secondly, that the position of the two stigma lobes, when these are distinct, is in nearly all cases over the sutures. Thirdly, there is the fact, which is clearly shown in the drawings of Payer, Eichler, and others who have investigated the early development of the gynoecium, that the placental commissures from the first moment of origin are larger than the carpels of which they are supposed to be merely the margins. In order to meet the stigma-position difficulty it was suggested by Lindley[1] (1828) and also by Kunth[2] (1831–3) that the supposed conjoined edges or placentae really represent independent carpels, the siliqua thus being composed of four members, a view adopted also by Godron.[3] Eichler,[4] though admitting that certain appearances, especially in the Papaveraceae—another family which presents the same problem—lend support to Lindley's idea, nevertheless regards it as preferable to accept R. Brown's[5] conception of the commissural stigma, and to consider the number of carpels in the Cruciferae as two. Benecke,[6] Chodat,[7] and Celakovsky,[8] surveying the arguments and evidence afresh, have severally endorsed Eichler's formula, either expressing the opinion that the construction of the Cruciferous flower is dimerous throughout (Benecke and Celakovski) or that the full gynoecium ground-

[1] Bot. Register, vol. xiv (= vol. i of 2nd series), text accompanying Pl. 1168 (*Eschscholzia californica*).

[2] Handbuch der Botanik; also Über die Blüthen- und Fruchtbildung der Cruciferen.

[3] Ann. Sci. Nat., Bot. v, 2e sér., p. 293. [4] Blüthendiagramme, ii, p. 192.

[5] Pl. Jav., note, p. 108.

[6] Zur Kenntniss des Diagramms der Papaveraceae und Rhoeadinae (Engler's Bot. Jahrb., ii, 1882).

[7] Neue Beiträge zum Diagramm der Cruciferenblüthe (Flora, lxxi, 1888).

[8] Das Reductionsgesetz der Blüthen (Sitzb. d. K. Böhm. Ges. Wiss., No. 3, 1894).

plan is never realized, two members being always suppressed (Chodat). And so G 2 it has remained. Arguments in favour of a 4-membered whorl have, it is true, been advanced by Schmitz,[1] but they are urged in favour of a *schematic* rather than an *actual* ground plan: G 4 stands, not for the siliqua as it is, but for an abstraction, the concern of the author being to realize a common generalized diagram for all Dicotyledons (!). Somewhat later Kerner,[2] regarding the placental commissures as arising *after* the valves and forming a second inner whorl, definitely ranged himself as a supporter of Lindley's view that they represent whole carpels. But this premise was directly opposed to the statements of both Payer and Eichler, and current opinion remained unchanged. A distinct advance in knowledge is, however, reached by the observations of Klein[3] upon the course of the vascular bundles. He concludes that in the case of what he calls 'open' flowers the course of the four bundles destined for the four longer stamens indicates a true diagonal position for these members, and that only in flowers with petals clawed to accommodate the nectaries do they approximate to the median plane. In accord with the conclusion that the four longer stamens represent a true tetramerous whorl, he holds that there are four carpels in the gynoecium, two of which form the replum, basing this view again on the course of the bundles and on the fact that nowhere else do the meeting *edges* of carpels develop such a considerable amount of fibro-vascular tissue as is to be found in the replum of the Cruciferae. Both arguments afford strong grounds for belief, but the outline needs to be extended and the blanks to be filled in if the picture is to be convincing.

One feature especially conspicuous in glabrous strains of *Matthiola incana*, R. Br., which has always appeared to me difficult to reconcile with the view generally held, is the considerable and often varying width of the tract of tissue (commissure) separating the valve boundaries,[4] a difficulty which does not become less after examination of the internal structure. It was, however, only after investigation of a number of fruits of the exceptional type mentioned above (p. 451) that the true nature of this tract was apprehended. It then became clear that the accepted formula G 2 did not fit all the facts observable in this species. On the other hand, these appearances, and various records by other observers of similar occurrences in other Cruciferous genera, could all be brought satisfactorily into line on the supposition *that the minimum number of carpels in the siliqua is not two but four, and that the full number in the exceptional fruits is eight*. A con-

[1] Die Familien-Diagramme der Rhoeadinen (Abh. Naturf. Ges. Halle, Bd. xiv, 1878).

[2] Pflanzenleben, ii, 1891, p. 683.

[3] Der Bau der Cruciferenblüthe auf anatomischer Grundlage (Ber. d. deut. Bot. Gesell., xii, 1894).

[4] The difficulty of regarding the commissures merely as placentae appears fully as great in *Sisymbrium pannonicum*, Jacq., where the disparity in width of valves and commissures is still less. (See Reichenbach, Icones, ii, Table LXXIV, Fig. 4406.)

sideration of the facts set forth below will show the grounds upon which this statement is based.

Variously curved and coiled fruits had been noticed in the Stock in successive seasons, but their appearance was so suggestive of the kind of unilateral injury so often caused by insects and other agencies that no close examination of them was made until their abundance in the exceptional season of 1921 attracted attention afresh. It was then seen that their asymmetric shape was not due to accidental lesion but to an unusual anatomical construction. It was furthermore found that this structural variation was sometimes, though more rarely, symmetrical, the siliqua then remaining straight. A description of some of these cases will serve to make clear the nature of these anatomical peculiarities.

4. *Exceptional forms of siliqua in the Stock.*

1. The siliqua is 4-valved, with four sometimes rounded but more often flat or concave sides and a small arc at each angle. trilocular, with two complete partitions in planes parallel to each other and to the median plane. The valve edges are *not* incurved and are *not* utilized in the formation of the septa, which are developed entirely from the small arcs.[1] Ovules arise on either side and at each end of each septum, but probably not more than three or four of these eight placentae ever mature seed. There are four stigmatic surfaces separated by four knobs, one at each corner, the stigma of each valve forming a loop, or those of the median pair remaining straight. If, as may happen, one side of the siliqua is rather more strongly developed than the other the septum joining the commissural arcs on the weaker side may not be formed. If the development is very unequal the fruit becomes spirally coiled (Figs. 12–18).

2. The siliqua is bluntly 4-angled, with the anterior and posterior surfaces often flat or concave. In the lower region there are two commissures of normal appearance, i. e. each is defined by *two* contour lines. About one-quarter up the length of the siliqua one commissure broadens out, and a small valve is interpolated which is continued up to the top. A cross-section through the lower region shows one normal convex lateral valve in contact on either side with a small arc (commissure). The one arc has the usual vascular supply; in the other *two* vascular cords are present. It is in this latter suture that the small new valve is developed higher up, between the two fibro-vascular strands which have now diverged. The

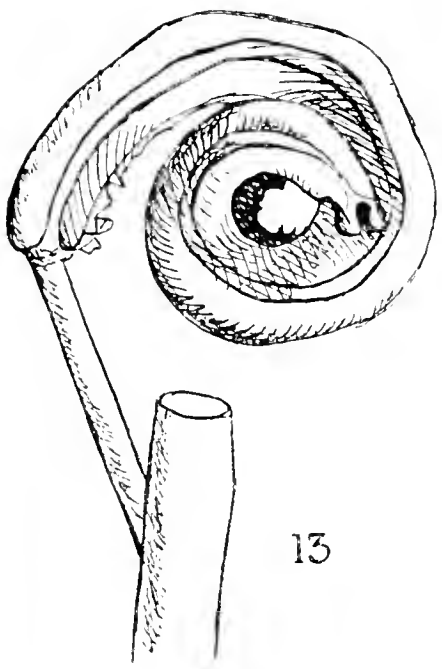
[1] In this connexion it is interesting to note that just a century ago, on grounds which he formulates, Lestiboudois expressed himself on this point thus: 'I do not believe that the edges of the valves turn in to form the septum' (Mémoire sur les Fruits siliqueux). His belief was well founded. In regard to this point the schematic diagrams for the Rhoeadales in which such incurving is invariably represented are misleading. They represent not a reality but an abstract conception which is in fact erroneous.

circumference of the section is completed by two normal-sized valves forming a blunt angle but no external suture along the line of fusion, which is without vascular elements. Thus throughout a considerable part of its length the siliqua is constructed of four valves with three intervening commissures (Fig. 19). The stigma configuration, as one would expect in these circumstances, is asymmetrical. The one commissure forms a typical large knob; of the other two on either side of the interpolated valve, the one forms a small knob, the other a barely perceptible swelling. A more symmetrical type of construction was observed by de Candolle [1] in a fruit of the Wallflower, of which he gives figures as seen whole and in cross-section. The drawings show four valve carpels, one of which is free from the others, and (apparently) only two small arcs. (The fact that the vascular strands are not represented leaves it uncertain whether the two missing arcs were suppressed wholly or in part, or whether they were exceptional in containing two vascular strands which separated as the tissue split and the one valve became free, so that each remained attached to the margin of a neighbouring valve.)

3. The siliqua is three-sided, with one unpaired valve in the lateral plane and two equal valves converging to an opposite point, most frequently curved into a half-circle, but sometimes straight or nearly so, with two normal sutures and in place of the third a sharp-angled edge becoming ragged later and often torn across.[2] Of the three valves, the lateral one is at a lower level and strongly convex on the outer surface; the other two stand at a slightly higher level and are flat or even concave. A normally formed partition usually connects the two small arcs on either side of the one lateral carpel, so as to form a fertile loculus. The other two valves often bulge so much on their inner face as to meet at the midribs and thus form a second false loculus, but on their contiguous margins they produce no placentae. These latter edges may remain confluent, forming a knife-edge; or they may prematurely separate for a longer or shorter distance, diverging so as to expose their inner face either completely, or up to the line of their coalescent midribs, their margins showing a clean split; or they may become free but remain in close juxtaposition, presenting a ragged appearance which is often further emphasized by transverse fissuring, followed by drying up of the exposed tissue. The stigmatic arrangement is in accord with this configuration. The two median commissures usually end in fair-sized knobs. On one side of these knobs is the loop of stigmatic papillae crowning the lateral valve, on the other the two parallel crests of

[1] Monstruosités végétales (in Nouveaux Mémoires de la Soc. Helvétique des Sci. Nat., v, 1841, Tab. V, Figs. 8, 13).

[2] A precisely similar appearance in the fruit of the Wallflower is depicted by Schlotterbeck (Acta Helvetica, ii, Tab. II, Fig. 16), and there is little doubt that the sickle-shaped siliqua on the Stock plant which served Besler for his drawing in Hortus Eystettensis (*Cl. Aestivalis*, fol. 33, ii) was derived from a 3-valved gynoecium.



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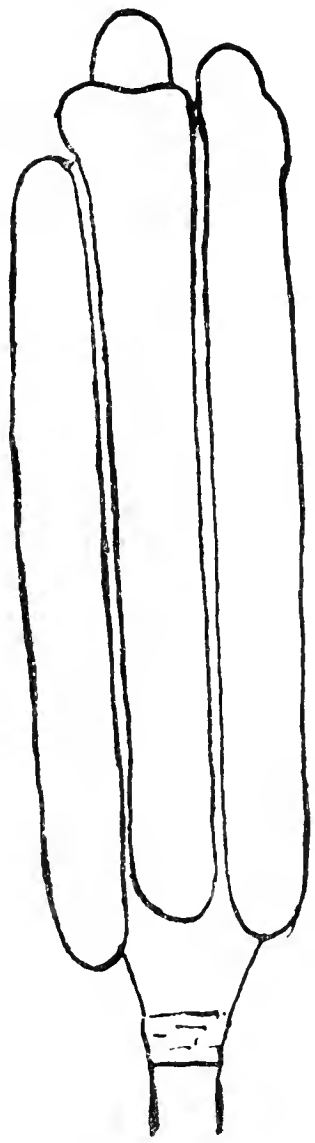

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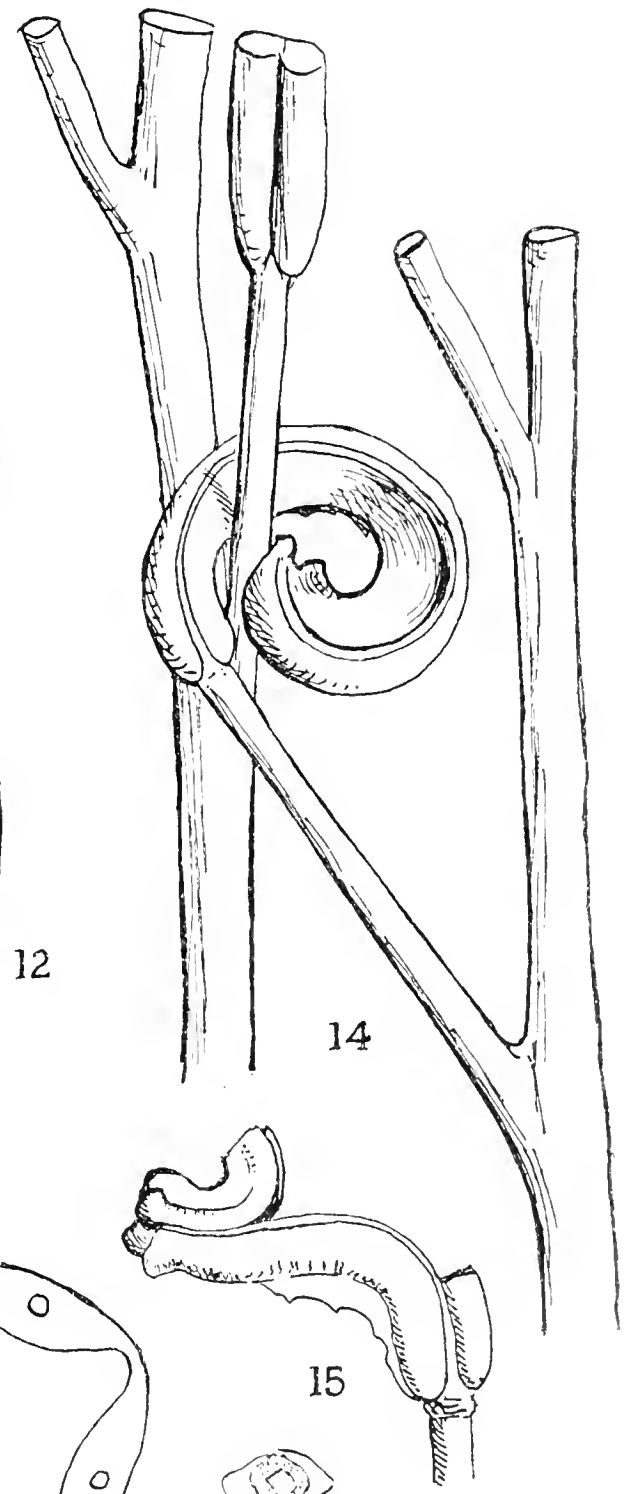

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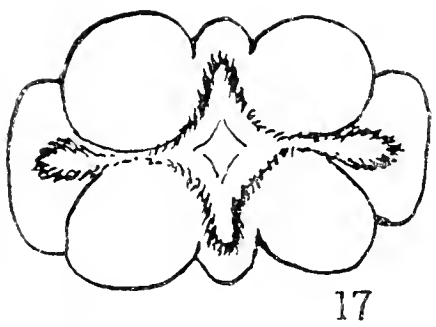

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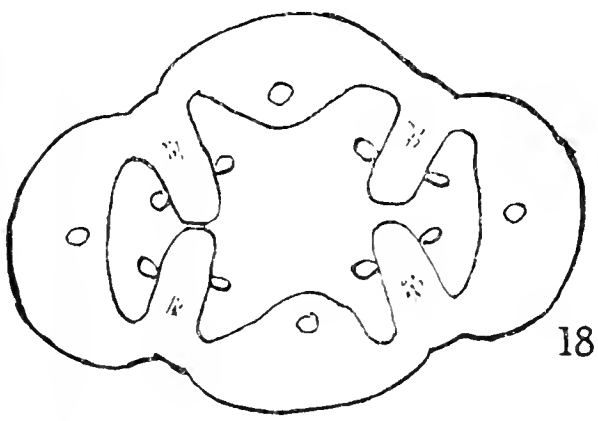

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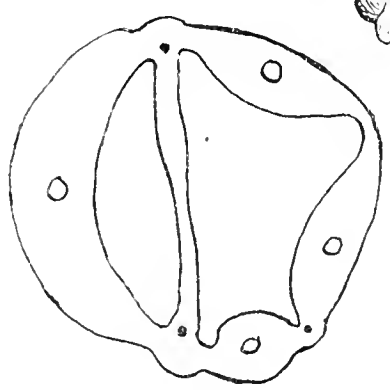

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FIG. 12–19. *Matthiola incana.* 12. A straight 4-valved siliqua slightly asymmetrical with four orthogonal valves and four diagonal commissures. 13, 14. Two coiled 4-valved fruits; the lateral valve on the one (concave) side imperfectly developed with ruptured edges, hence the spiral form of the siliqua. 15. A small 4-valved siliqua with four orthogonal valves, of which the two median show rupture of the tissues, one being torn completely across. 16. Apex of the same, showing the stigmatic area formed by three of the valves; the fourth valve ends a little below without forming a stigma. 17. (Semi-diagrammatic.) Apex of a symmetrical 4-valved siliqua, showing the four sutural knobs, the outline of the two lateral valves right and left, the central cavity, and the stigmatic surfaces forming a double figure of 8. 18. Transverse section of a young 4-valved siliqua, showing the formation of two septa by the four diagonal solid carpels. 19. Transverse section of a siliqua composed of four valves and three commissures.

the paired valves, for as no knob is developed over the knife-edge suture these latter are not compressed into loop form (Figs. 20–9).

4. The siliqua, though 2-valved, is somewhat curved, and has a projecting rib of tissue running the whole length in the middle line of one commissure, and ending above in a free tongue-like process which juts out just below the knob proper to the commissure (Figs. 30–3). A cross-section shows the usual arrangement—a single median septum, two loculi, and four placentae, but the sutural arc on the one side is larger than the other, triangular in section, and shows three fibro-vascular cords, one belonging to the projecting rib and a small one on either side. The stigmatic configuration is peculiar. Over the larger commissure with its tongue-like process we find not a knob, but a second stylar canal surrounded by stigmatic papillae.

5. The siliqua shows the interpolation of a small median valve (as much as, in one case, 3 mm.) above the level of the lateral valves (Figs. 34, 35). There are three normal commissures from this level upwards, one in the median plane opposite the small valve, and one on either side of it (i. e. in the diagonal planes). Three septa formed from the three sutural arcs meet in the centre, forming a Y-shaped partition and rendering the ovary trilocular.

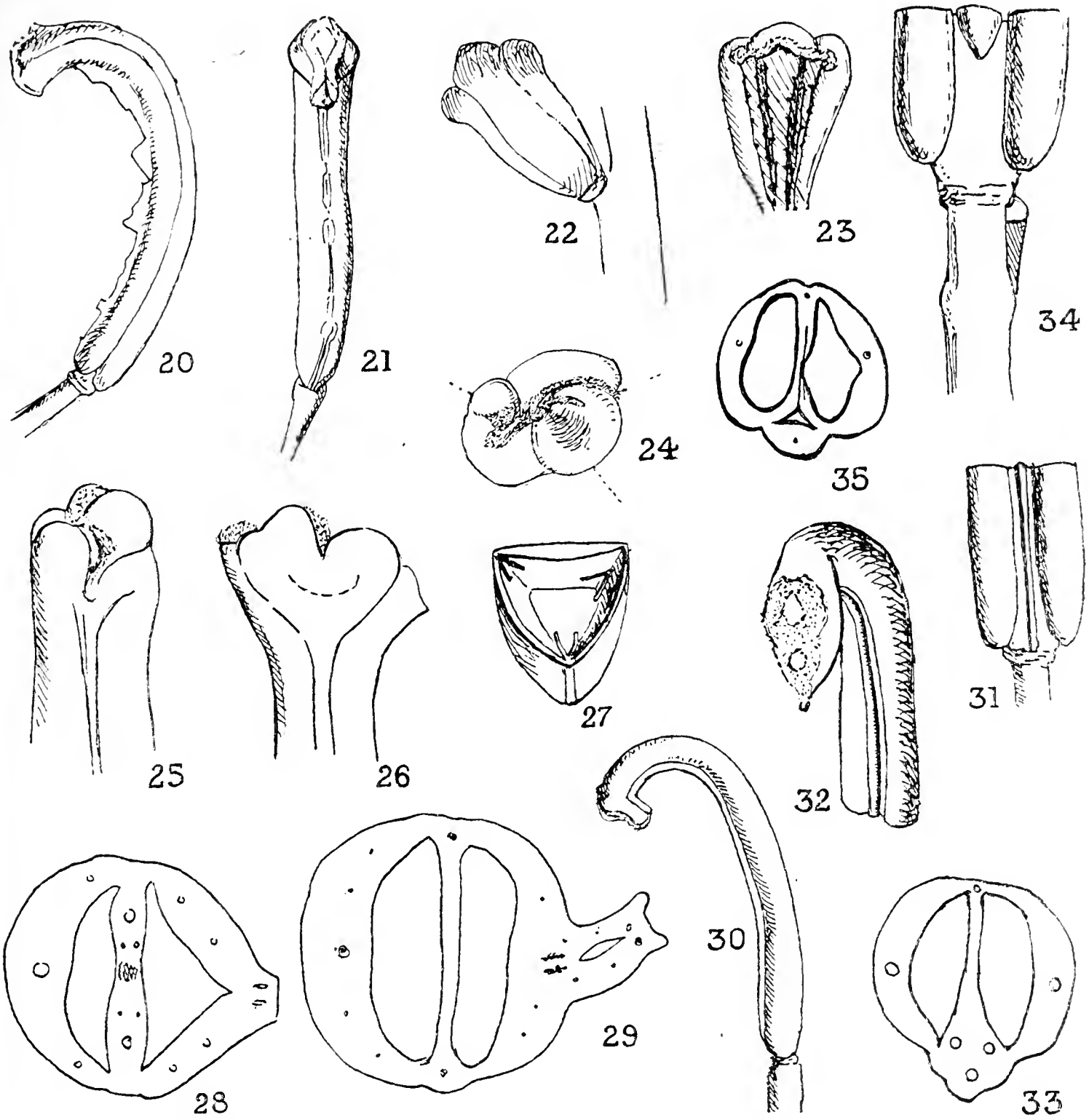
These exceptional fruits were almost invariably among the lowest on the axis.[1] For working purposes it could in fact be assumed that if they were not seen among the first half-dozen they would not occur.

5. *Conclusions to be drawn from the above facts.*

These examples of Stock fruits of various constructional types shed fresh light upon the original ground plan of the Cruciferous gynoecium, and enable us to conceive how the existing type has been derived from it through processes of reduction and consolidation. The above facts all go to show that the gynoecium in its fullest present-day development in the Stock consists of eight carpels, four of which assume the form of valves and are arranged in two pairs, the median pair appearing to arise at a somewhat higher level than the lateral pair.[2] The other four alternate with the valves as though the latter constituted a single whorl, in precisely the same manner as the four petals alternate with the two pairs of sepals. These latter carpels are solid, compressed into the small space between the valves, and appear outwardly as broad or narrow commissures. They are almost invariably fertile, whereas the hollow (valve) carpels are probably only so in

[1] See also Peyritsch, Pringsheim's Jahrb., viii, 1872, p. 117. It is in flowers in this position that other exceptional constructions seem most frequently to occur, as e.g. 'twinning' (see Journ. of Genetics, vol. xi, 1921, p. 69), and the formation of extra stamens (7–8).

[2] It is not, however, always the case that the level of swelling out into valve shape indicates the true level of origin (see Cases 2 and 5 above).



FIGS. 20–35. *Matthiola incana.* 20. A curved 3-valved siliqua with one lateral valve on the convex side and one of the pair of diagonal valves with ruptured edges on the concave side. 21. A similar 3-valved fruit seen from the side, showing partial rupture of the edges of the two diagonal valves. 22, 23. Very young 3-valved siliqua with the two diagonal valves completely disjoined. 22. Seen from the side of the lateral valve. 23. Seen from the opposite side, showing the disjunction of the diagonal valves. 24–6. Apex of a 3-valved siliqua with valves disjoined at the apex. 24. Seen from above, the dotted lines indicate the positions of the three commissures. 25, 26. Different views of the same seen from the side. 27. Transverse section of a symmetrical fruit with three valves and three commissures. 28. Transverse section of a siliqua with one lateral and two diagonal valves which have fused at their mid-ribs and formed a wing (cut away). 29. Transverse section of a similar siliqua; the two diagonal valves after fusing at their mid-ribs have separated again for a short distance and then reunite to form a two-winged edge. 30. A 2-valved siliqua, seen from the side, with a projecting rib extending the whole length of one commissure and terminating just below the sutural knob in a short process. 31. Lower end of the same siliqua seen from the front, showing the rib in the centre of the commissure. 32. Upper end of the same, showing the stigmatic area; a second small cavity (stylar canal) has been formed over the projecting rib. 33. Transverse section of the same siliqua, showing a triarch outline to the commissure carrying the projecting rib and three vascular cords. 34. Lower end of a siliqua, showing the development of a median valve 3 mm. above the lateral valves. 35. Transverse section of the same, showing the development of a small third loculus corresponding to the median valve. (The small vascular bundle in each of the front commissures is not shown.)

exceptional circumstances, as e. g. when the solid carpels are lacking (if this condition occurs, see above, p. 458) or when they are so small that the two edges of each lateral valve become conjoined at the point of contact, each thus forming a fertile closed cell to the exclusion of the solid median pair, as in *Menonvillea*, *Hexaptera*, *Biscutella* (Fig. 36). It is over the valves, however, in the Stock that the stigmatic papillae are centred,[1] though in the young siliqua before the sutural knobs enlarge they appear as an almost complete ring. In fruits of glabrous plants the sutural lines, as stated above (p. 452), can sometimes be seen to be continued over the shoulders of the knob, which in such case is derived in part from the edges of the two neighbouring valves (Fig. 37). We have, then, now to regard the typical siliqua as composed of four carpels, of which the lateral pair alone retain the valve form, the median pair having become solid. As a rule, solid and valve carpels regularly alternate, but we have already seen that in fruits in which both valve and solid carpels occur, but in which reduction has proceeded asymmetrically, juxtaposition of solid carpels and sometimes even of valve carpels may occasionally be seen. When two valves are contiguous their edges join where they meet in such a way as to show no sign externally of the soldering, the line of junction forming merely a blunt angle. Less rarely fruits were found exhibiting juxtaposition of solid carpels. In two instances (Cases 2 and 5) the interpolation of a valve part way up the siliqua showed that in reality the two well-developed solid carpels must have been separated by a very reduced third member, which assumed valve form as it became successful in the competition for space.[2] In another, however (Case 4), the triple vascular supply and triarch contour line of the cross-section of one commissure proved that a small solid carpel had been formed in each diagonal plane on either side of and in contact with one solid median carpel. Although the central member of the trio got as far as forming a projecting rib of tissue, it did not attain to valve form. We find in fact that the symmetrical alternate arrangement, though characteristic, is not invariable. In the ancestral form all the carpels must undoubtedly have been similar and valve-like, and the processes of reduction and consolidation here assumed to have taken place may well have proceeded by symmetrical stages. But a partial return to the primitive state is very likely to occur through some favourable condition so localized as to be one-sided in its effect.

[1] This arrangement, though very exceptional, is not confined to *Matthiola*. It occurs in the nearly allied genus *Moricandia* (*M. divaricata*, Coss.), where also the stigmas are sessile, and probably elsewhere (see below, p. 466).

[2] Occasionally in a wide commissure with two main fibro-vascular bundles the two bundles may diverge so much that a considerable breadth of non-vascular tissue is developed between them, simulating an interpolated valve. That such tissue does not repre-ent a true valve carpel is shown by the entire absence of vascular tissue and by the outline, which is V-shaped at the base instead of having the rounded contour of the true valve.

6. *The conception of the dimorphic carpel removes the anomaly of the 'false' partition and the 'commissural' stigma, and brings into harmony and renders intelligible many facts hitherto unexplained or without significance.*

Owing to its smooth polished surface, in addition to the considerable width of the commissures and the massive apex of the siliqua, the glabrous Stock offered exceptionally favourable material for the present investigation, but the conclusions here set forth are applicable to the Cruciferae in general. Moreover, they render intelligible certain facts already well known and others less familiar, the precise significance of which has hitherto not been apparent. In the first place, the nature of the replum is now clear. It is characterized by the same histological features as the valves. But in the rearrangement of the tissues of the median carpels necessitated by the consolidation process, the vascular bundles come to lie more or less in a ring, and the cells, which in the valve form extend beneath the lining epidermis along the whole extent of the loculus and are converted later into mechanical tissue, here become massed on the inner side of the fibro-vascular cord and form at the centre the characteristic fibrous strand. Sometimes, however, the median pair of carpels are arrested in their development before they come into contact, or even before any ingrowth at all has taken place, in which case the ovary remains unilocular.[1] When there are more than four carpels, the number of septa and the planes (orthogonal or diagonal) in which they will lie will depend upon the number and arrangement of the solid carpels and their relative vigour. In the 5-carpelled siliqua, with one lateral and two diagonal valves and two solid median carpels, the adjustment required generally results in considerable asymmetry. There may be no true septum, but the fusion of the inner bulging surfaces of the two diagonal valves may give rise to one closed loculus and to a second cavity not completely closed in by their free or disrupted edges. In 6-carpelled fruits with alternate arrangement of hollow and solid carpels a Y-shaped partition may be formed by the solid carpels, as was found in one Stock siliqua, and as is represented in one of de Candolle's[2] 3-valved *Lepidium* fruits. The presence in another of his figures[3] of *Lepidium*, and also in one by Godron[4] of a 3-valved *Cheiranthus* ovary, of a central triangular cavity is presumably due to splitting at the centre in the course of growth. When eight carpels are present, any one of the following conditions may no doubt occur: (1) One pair of the solid diagonal carpels only gives rise to an

[1] Constantly, e. g. in *Pringlea* (2-valved, dehiscent, many-seeded), *Enarthrocarpus* (jointed, indehiscent, many-seeded), *Peltaria* (indehiscent, one-seeded), *Braya Eschscholziana*, Benth. and Hook., and *Clypeola* (2-valved, each one-seeded), and in the fertile section of the fruit of *Cakile*, *Crambe*, and *Myagrum*. Exceptionally in many other genera, either partially or completely.

[2] Loc. cit., Tab. V, Fig. 16.

[3] Loc. cit., Tab. V, Fig. 17.

[4] Loc. cit., Pl. XVIII, Fig. 8.

antero-posterior septum. (2) Each pair forms such a septum. (3) All four converge to the centre, forming four complete or incomplete septa which lie in the two diagonal planes. The third form, as it happened, was not seen in the Stock, but a *Cheiranthus* fruit figured by de Candolle [1] was of this type.

This reversion to a multicarpellary condition, which has been observed in at least nineteen Cruciferous genera,[2] in a few fruits, in an individual here and there, may sometimes prevail through a large part of the individual. In plants of *Lepidium sativum*, L., raised by de Candolle from seed of multicarpellary Abyssinian parents, he found trilocular fruits almost as abundant as those of normal structure.[3] In the two forms originally named *Tetrapoma barbaraefolia*, Turcz., and *Holargidium Kusnetzowii*, Turcz., but now recognized merely as many-carpelled variants of *Nasturtium palustre*, DC., and *Draba* (? *hirta*, L.), respectively, the exceptional fruits are exhibited throughout the whole or the greater part of the inflorescence. Offspring from seed of *Tetrapoma* individuals have been found to exhibit the reversionary character. According to Hooker,[4] however, this is not invariably the case, and as a criterion of species, therefore, the character is useless. The reappearance of the character in the second generation of de Candolle's *Lepidium* plants grown in the spring, and its non-appearance in the third generation raised in the autumn, are of particular interest, as showing the dependence of the character upon conditions, as is indicated also in the Stock (see above, p. 457). The herbarium material of *Tetrapoma* which I was able to examine had mostly four valves, alternating with four solid carpels forming complete septa, but as many as five or even six of each occurred in individual fruits. In the specimen figured by Baillon, however, the septa are incomplete.[5] The short style, as in comparable cases in *Cheiranthus*, showed a corresponding increase in thickness. From the strictly radial type of symmetry exhibited by these fruits, it is clear that this multicarpellary condition does not arise through the morphological abnormality known as 'twinning'.[6] Rather it affords us a real glimpse of

[1] Loc. cit., Pl. XVIII, Fig. 9.

[2] *Alyssum incanum*, L., *A. libycum*, Coss, *Arabis alpina*, L., *Brassica Napus*, L., *Brassica oleracea*, L., *Capsella Bursa-pastoris*, L., *Cheiranthus Cheiri*, L., *Draba nemorosa*, L., *Diplotaxis muralis*, DC., *D. tenuifolia*, DC., *Erophila vulgaris*, DC., *Erysimum pannonicum*, Crantz, *E. repandum*, L., *Fortunia Garcini*, Shuttl., *Iberis sempervirens*, L., *Lunaria annua*, L., *L. rediviva*, L., *Matthiola incana*, R.Br., *Megacarpaea*, *Peltaria alliacea*, Jacq., *Raphanus sativus* (*caudatus*), L., *Ricotia Lunaria*, DC., *Sinapsis arvensis*, L., *Thlaspi arvense*, L. (For *Erysimum repandum* see Reichenbach, Icones, ii, Tab. LXXIV, Fig. 4406; for the rest see references in Penzig's Pflanzen-Teratologie.)

[3] Monstruosités végétales, p 13.

[4] Gen. Pl., i, 1862, pp. 83, 967.

[5] Nat. Hist., iii, p. 184, Fig. 213.

[6] The phenomenon of 'twinning' is not uncommon among the Cruciferae. I have elsewhere suggested (Journ. of Genetics, vol. xi, 1, 1921) that the occasional references in the early literature to the occurrence of semi-double flowers in the Stock are probably to be thus explained, as also I have no doubt is Darwin's statement that he had himself seen single and double flowers on the same Stock

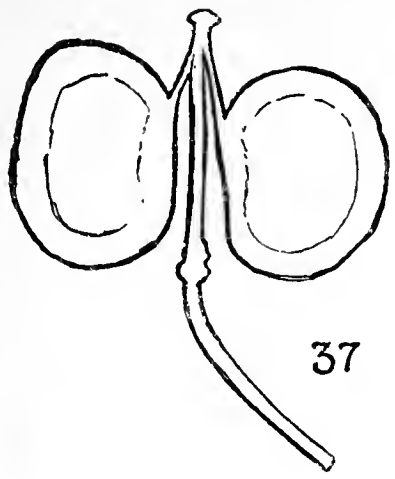
the past. For *there can be no doubt that in all these cases we are witnessing the reappearance of an ancestral character exhibited to a very slight degree and only sporadically in the several genera cited above* (see foot-note, p. 464), *but here manifested to a remarkable extent.* These occurrences, in fact, furnish concrete evidence that the progenitors of the present-day type of Crucifer were possessed of a many-carpelled ovary, and thus approached more nearly to the construction of some existing types among the Papaveraceae. Before leaving this part of the subject, it may be well to emphasize the point that the conception of two kinds of carpels is essential to the understanding of the relation of these multicarpellary fruits to the typical siliqua, and of the arrangement and position of the valves, especially in 3-valved fruits, in which the unpaired valve is generally lateral, though occasionally median. In the former case the suppression or consolidation of one lateral valve has been balanced by the production of hollow instead of solid carpels in the diagonal planes on that side. Similarly, in the latter case the development of one valve in the median plane has been compensated by the consolidation of the lateral carpels and the assumption of valve form by the two carpels in the diagonal planes. Several observers who have declared their adherence to the G 2 formula for the typical siliqua nevertheless cite these exceptional fruits as having a phylogenetic significance and as evidence of a fuller ground-plan composed of four orthogonal valve carpels. It is perhaps the contradiction involved in this position—for such a ground-plan does not satisfactorily account for the 3-valved siliqua—which has delayed recognition of the real character of these fruits now shown to be derived from a 4 + 4 ground-plan, and has caused them to be dismissed as 'sports' or monstrosities. On the other hand, Lindley, Kunth, and Klein, who recognized the commissures to be carpels, for whatever cause, do not appear to have extended their observations to these multicarpellary ovaries. How many cases of so-called 'sports' appearing under cultivation, or as the result of some other change in the external environment, are in reality the expression of some ancestral phase reappearing under a favourable combination of circumstances is a question which needs further investigation.

But the dimorphic nature of the carpel does more than rehabilitate the septum; it removes that most crucial difficulty, the commissural stigma. Observation shows that in the great majority of the Cruciferae the valve carpels have ceased to fulfil the receptive as well as the reproductive

plant (Animals and Plants, p. 381). Instances are also recorded by Godron (loc. cit., p. 303) for *Brassica oleracea*, L., by Eichler (Flora, 1872, p. 333) for *Brassica Napus*, by Wille (Bot. Centralbl., xxvi, 1886, p. 121) for *Capsella Bursa-pastoris*, L., and figured by Schnizlein (Iconographia, Fig. 181 *a*, Figs. 39, 40) for *Raphanus*. The difference in orientation of multicarpellary fruits arising from this cause which show isobilateral symmetry, from those due to reversion which are constructed on a radially symmetrical plan, is illustrated in Godron's figures of *Brassica oleracea*. (Compare Figs. 1 and 2 with Figs. 6 and 7, Pl. XVIII, loc. cit.)

function. The two solid carpels alone, in these cases, are prolonged upwards to form a style, consequently the lobes bearing the stigmatic surfaces are naturally centred, as is particularly well seen, e.g., in the Wallflower (*Cheiranthus Cheiri*, L.) (Fig. 40), over the two so-called commissures—now proved to be not true commissures, but consolidated carpels.[1] The few remaining genera fall under two heads: (*a*) those in which valve carpels as well as solid carpels are prolonged to form the style, and therefore presumably form part of the capitate stigma, as in *Biscutella* (valves separating but indehiscent) (Fig. 37) and *Guiraoa arvensis*, Coss. (indehiscent) (Fig. 41); (*b*) those with the style short or wanting, in which the valves, but little shorter than the solid carpels, bear the stigmatic papillae, as e.g. *Matthiola incana*, *Moricandia*, and possibly *Loucophora*. Hence, we need no longer subscribe to the time-honoured morphological fiction of the commissural stigma. In place of it, we have clearly shown to exist a morphological dimorphism sufficiently elastic to permit of a considerable degree of physiological interplay without change of ground-plan. It was the serious difficulty involved in the acceptance of a genuine commissural stigma which led Lindley nearly a hundred years ago to suggest that the Cruciferous placentae are in reality independent carpels (see above, p. 455). He was led to this view through a comparison of the typical siliqua, with an ovary, with four stigmas of *Eschscholzia californica*, Cham., which latter type of gynoecium he conceived might arise if the structures represented by the Cruciferous valves were contracted to mere threads producing partially aborted stigmas, and if the placentae, on the other hand, were widened to become the placentiferous valves of *Eschscholzia*, bearing well-developed stigmas (Figs. 38, 39). Though Lindley thus arrived at a correct interpretation of the siliqua, his evident conception that similarity of function indicated morphological identity caused him to miss the true homologies, and to propound his solution in a way which introduced a new difficulty almost as serious as the one it removed, for his scheme involves a shift of the orientation through 90°. Moreover, the replum is not double, as he supposed. Hampered by this idea of morphological equivalence, although he considers the possibility that his conjectured four carpels in *Eschscholzia* might produce four placentae and thus account for the multiple rows of ovules in this genus, he inclines against this view. It must further be noted that these theoretical considerations were unsupported at the time by any actual evidence, and that they still left unexplained other apparent contradictions. Thus, the unsoundness of the premises caused the correctness of Lindley's conclusion regarding the Cruciferae to remain unperceived, and botanical opinion has perforce acquiesced in the highly unsatisfactory alternative, the *commissural* stigma and the *spurious* partition. Only by

[1] It must be through some error that *Cheiranthus* is instanced by Eichler as a case where the stigma lobes alternate with the placentae (Blüthendiagramme, ii, p. 204).



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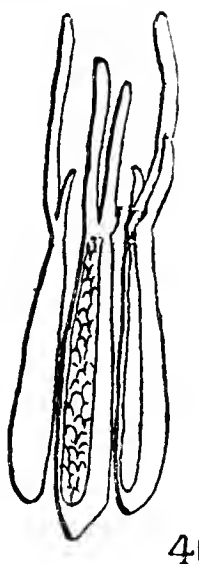

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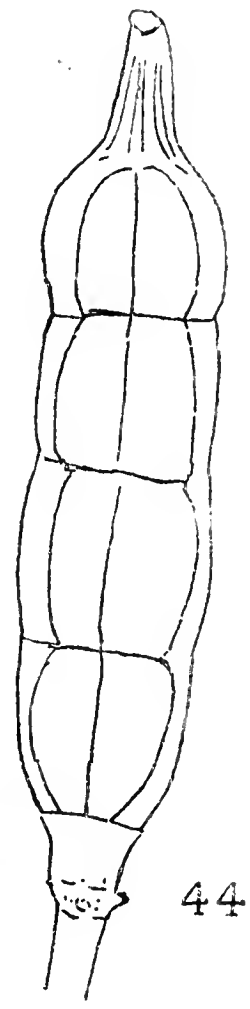

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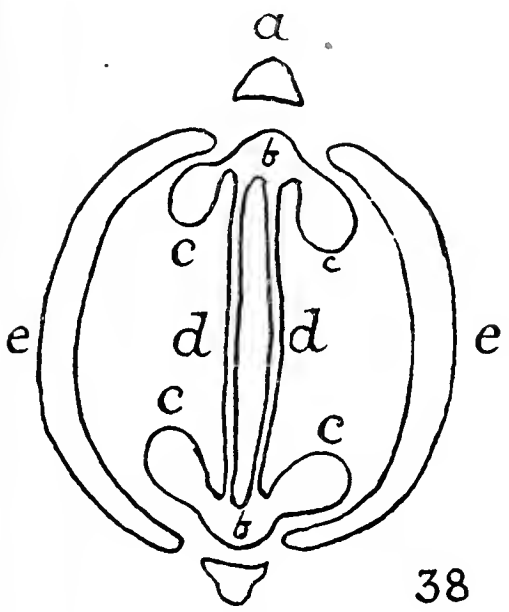

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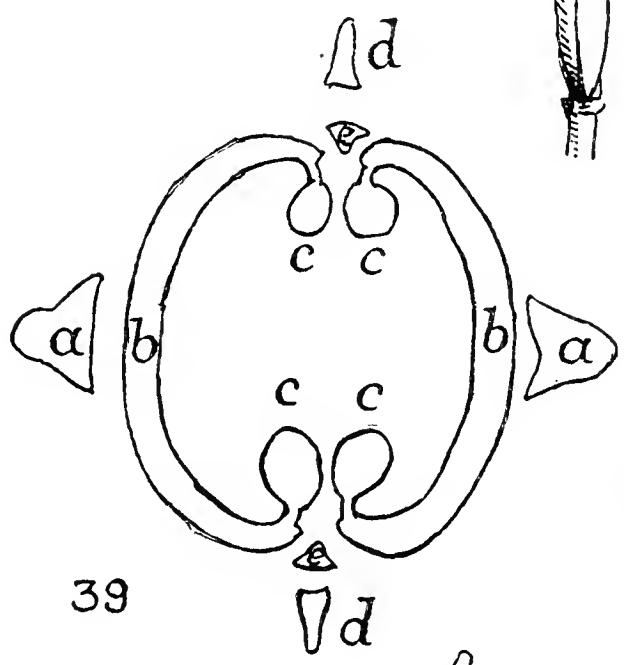

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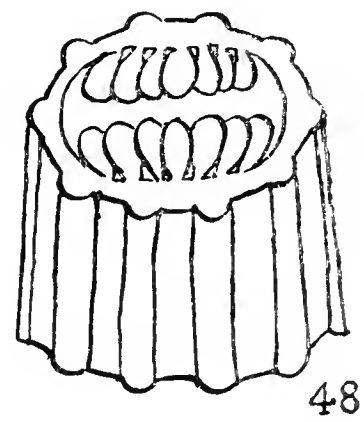

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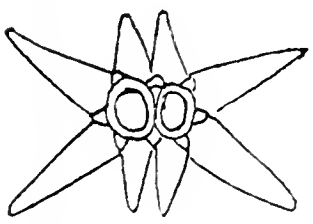

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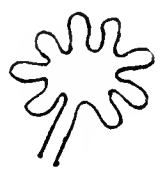

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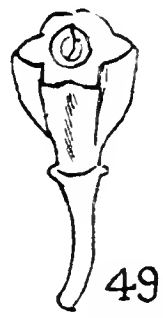

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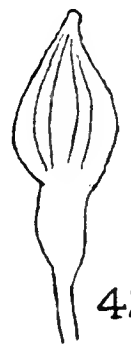

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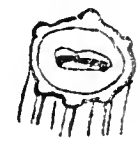

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FIGS. 36–7. *Biscutella frutescens.* 36. Transverse section of the fruit, showing the ovule-bearing valves. 37. Ripe fruit, showing that the valve carpels contribute to the formation of the short style. 38, 39. (Diagrammatic.) Showing Lindley's idea of the possible derivation of the *Eschscholzia* type from the Cruciferous ground-plan. 38. Cruciferous ground-plan (*a*, *a*, position of stigmas; *b*, *b*, placentae; *c*, *c*, ovules; *d*, *d*, double septum; *e*, *e*, valves). 39. Ground-plan of *Eschscholzia* (*a*, *a*, perfect stigmas; *b*, *b*, sides of pericarpium connecting the placentae; *c*, *c*, ovules; *d*, *d*, abortive stigmas; *e*, *e*, line of pericarp corresponding with the abortive stigmas). 40. Siliqua of *Cheiranthus Cheiri*, showing commissural outgrowths. 41. Transverse section of the fruit of *Guiraoa arvensis.* 42, 43, 49. *Rapistrum.* 42. Fruit of *R. perenne*, showing the smaller basal segment and the larger upper one. 43. Transverse section of the upper segment, showing the eight valves. 49. Transverse section of the lower segment of the fruit of *R. aegyptium.* 44. Many-valved lomentose fruit of *Enarthrocarpus clavatus.* 45. *Brassica cheiranthiflora*, siliqua cut across, showing the 3-ribbed valves. 46–7. *Eschscholzia crocea.* 46. Fruit with eight stigmas. 47. Ovary with two single filiform stigmas over the commissures and two 5-lobed plates over the valves. 48. *E. californica*, portion of fruit, showing ten ribs and numerous rows of ovules. 50–51. *Corydalis.* 50. Stigmatic disc of *C. bulbosa* with eight processes. 51. The same of *C. fabacea.* (Figs. 36, 37, 44 after Cosson; 38, 39 after Lindley; 41 after Willkomm; 42, 43, 45, 50, 51 after Reichenbach; 46, 49 after Baillon; 47 after Payer; 48 after Hooker; 40 original.)

realizing the historical position can one come to understand why evidence so plain to the eye in the overwhelming majority of the Cruciferae failed to reveal its meaning. In passing, it may be noted that a few years earlier the suggestion had been made by de Candolle[1] that we have to deal in the Cruciferae with a triple flower (i. e. three flowers in one), and that its component members when thus combined can conceivably assume a shape different from that exhibited by them when free. Thus presented, it is not perhaps surprising that the element of truth contained in this explanation should also escape recognition.

A common though less constant feature than the presence of the replum and the alternate position of valves and stigmas is the formation on the Cruciferous fruit of excrescences in the form of knobs, horns, wings, &c. A single such structure is frequently formed on the back (mid-rib) of the valves and enlarges considerably after fertilization. In any species in which it occurs it is constant, and may perhaps have originated as a secondary effect of sterilization of the valve carpel.

Less frequently similar outgrowths are developed by the solid carpels, or sometimes by both valve and solid members as in *Matthiola tricuspidata*. Possibly consolidation of the carpel may likewise act as a predisposing cause. The Wallflower is a puzzling exception, for the filiform processes or flanges which appear on the sutures occur only in a small proportion of fruits, generally among those in the lower part of the raceme. De Candolle[2] suggested that these structures, which can often be traced as a ridge to the base of the commissure, might represent aborted carpels, and the above-mentioned localization might well connote a case of such reappearance of a lost member, but the fact that several processes may arise one above another on the same suture seems definitely to preclude this explanation. Moreover, the fact that they contain no vascular tissue is also not in accord with such an interpretation. It seems that their true morphological nature has yet to be determined.

In other types the fruit may *constantly* exhibit a definite number of processes or wings, or it may show an unvarying number of conspicuous longitudinal parallel ribs. In illustration we may cite the following:—*Menonvillea* (fruit 4-winged), *Hexaptera* (6-winged), *Guiraoa arvensis*, Coss. (8-winged) (Fig. 41), *Decaptera* (10-winged); species of *Isatis* (6–10-valved) (Figs. 59–61); species of *Brassica*, *Sinapis*, *Sisymbrium* (valves 3-nerved) (Fig. 45); species (dehiscent) of *Carrichtera* and *Boleum* (with 3–6-ribbed valves); species (indehiscent) of *Rapistrum* (Figs. 42, 43, 49), *Enarthrocarpus*, and *Raphanus*, with from 6 to 24 ribs, ridges, or sutural lines on the fruit. These many-ribbed siliquas at once bring to mind the 10-ribbed siliquiform fruit of *Eschscholzia*.

[1] Théorie élémentaire de la botanique, 2nd ed., 1819, p. 144.
[2] Monstruosités végétales, p. 15.

It has already been recalled that it was from a comparison of the Cruciferae with *Eschscholzia* that Lindley was led to the view that the Cruciferous placentae are in reality independent carpels. It is, therefore, fitting that it should be from further comparison with this same genus that we are enabled to obtain new light on the construction of the many-ribbed siliqua. Lindley visualized the fertile carpel contracted to a placentiferous cord, but he failed to perceive the consolidation which produces *the multicarpellary valve*, and so missed the significance of the 10-ribbed character in both the Cruciferous and the Papaveraceous gynoecium—proof, as will appear, of the presence of a corresponding number of solid carpels in both families.

7. *Reduction and consolidation shown to have occurred in like manner in the Papaveraceae, Fumariaceae, Capparidaceae, and Resedaceae.*

Now a multicarpellary ovary in *Eschscholzia* would be a quite natural construction in view of the numerous carpels present in several other genera of the Papaveraceae. It is to this family, and the allied Fumariaceae, Capparidaceae, and Resedaceae, that we must now extend the inquiry, since the solid carpel explanation of the so-called commissural stigma must, we should suppose, apply equally to them. And this we find to be the case. For example, on the one hand may be cited *Platystemon californicus*, Benth., where the numerous carpels are all of the valve type *with single-line sutures*, and the stigmas, as we should then expect, superposed upon the midribs. On the other hand, the numerous genera with stigmas alternating with the valves, again in accordance with expectation, *show double-line sutures*; hence the distribution of the stigmas is not in reality exceptional, except in so far as they are borne by the solid carpels only. Among such may be mentioned *Bocconia*, *Chelidonium* with 2, *Roemeria* with 4, and *Meconopsis* and *Papaver* with 4–8–12 valves. The fact that in the band form of stigma characteristic of *Papaver* and its allies each stigmatic band can be seen to be double, was considered by Eichler as decisive against Lindley's view that the placentae in such cases represent whole carpels. But the solid carpel, although so contracted as to form merely a radial sheet of tissue, must yet be conceived to possess two edges like the expanded valve. Moreover, the ends of each pair of parallel stigmatic lines are continuous at the periphery of the stigmatic plate so that they form a very narrow V, a shape we might expect if compression were accompanied by a shearing action forcing inwards the tissues of the carpel lamina on either side of the midrib. It thus becomes unnecessary to make the entirely unsupported assumption required on Eichler's view that fusion occurs between every pair of neighbouring half-stigmas.

To return, however, to the case of *Eschscholzia*. The ovary in this

genus is always regarded as dimerous. Prantl and Kundig[1] describe it as composed of *two carpels with 2–4 stigmas, and the fruit as dehiscing either septicidally or by the two valves becoming detached from the placentae.* Figures are given of an ovary of four segments, with two pairs (one longer and one shorter) of filiform stigmas, and of a ripe, split, two-valved fruit with a row of seeds on one side only of each valve, both from *E. californicus* (Figs. 82 B and 83 C); also one (from Baillon[2]) (Fig. 46) of a fruit of *E. crocea* showing two valves with triple stigmas, the valves being detached from the two placentae, each of which carries a single stigma. Finally, in addition to two filiform stigmas, two others of 5-lobed plate-like form are shown by Payer[3] in a drawing of *E. crocea* (Fig. 47). On the accepted formula of G 2 there are several points in these statements and figures which require to be explained away, viz. the varying number of stigmas (2, 4, 8, 12); the two modes of dehiscence (the single split into two seed-bearing valves, and the double split into two seedless valves and two seed-bearing placentae, with the associated difference in length of the two pairs of stigmas); the numerous confused rows of ovules on the placentae. In order to arrive at a true interpretation of these somewhat contradictory relations, we must take into consideration the following facts:

1. All botanists to-day are agreed that *Eschscholzia* is closely related to the two genera *Dendromecon* and *Hunnemannia*, and that these three forms stand very near to a group in which the carpels are more than two, viz. *Platystigma* with three and *Romneya* and *Platystemon* with six to ten or more.

2. *Eschscholzia*[4] (Fig. 48), *Dendromecon*, and *Hunnemania* are all distinguished by having fruits with ten conspicuous ribs. In the intervening flat sections of the roughly 10-sided ovary there is an additional subsidiary vascular strand.

3. Dehiscence of the fruit in *Dendromecon* and *Hunnemannia* is by the two valves becoming detached from the two thread-like placentae, which form a frame (replum) as in certain of the Capparidaceae.

4. The two stigmas of *Dendromecon*, though stated to have three lappets ('Pflanzenfamilien', iii, 2, p. 138), are shown in the drawing (loc. cit. Fig. 87 C, also 'Bot. Mag.', t. 5134) to be in reality 5-lobed;[5] that of *Hunne-*

[1] Pflanzenfamilien, iii, 2, pp. 138, 139.
[2] Nat. Hist., iii, p. 118, Fig. 140.
[3] Organogenie, Tab. XLV, Fig. 38.
[4] See Bot. Mag., 56 (1829), t. 2887.
[5] On the ground that he himself had never found 5-lobed stigmas in the plants which he examined, Fedde asserts that these drawings must be incorrect (Pflanzenreich, iv, 104, p. 39). But *Dendromecon* may well show transition stages like *Eschscholzia*. Not only is there no ground for assuming these figures to be incorrectly drawn, but in the light of what has been made clear in the present account, such a 5-lobed outline to each valve stigma might well be expected from the outline of the ovary.

mannia is described as 4-lobed ('Pflanzenreich', iv, 104, p. 144; see also 'Bot. Mag.', 1831, t. 3061).

None of these facts are in good accord with a bicarpellary construction, and some are clearly incompatible with it. But in the light of what we have now learned from observation of certain Cruciferous genera, they can be satisfactorily harmonized and explained. There is no doubt that in *Eschscholzia*, as also in *Dendromecon* and *Hunnemannia*, we have not less than ten solid and ten other carpels present.[1] The processes of sterilization and consolidation, which have reached their height in the Chelidoneae among the Papaveraceae and in the Cruciferae generally, are here seen actually in progress. The ten solid carpels are indicated by the ten ribs of the ovary wall, and further, by the two 5-lobed stigmas seen in Payer's figure of *Eschscholzia* and again in *Dendomecon*. In the exceptional condition (hitherto unexplained) represented in Baillon's figure of a fruit of *E. crocea*, we have a stage in which division of labour among the carpels has reached a point at which six of the solid and eight, if not all, of the other carpels have already become infertile, though the former have retained their stigmas. The two solid members on either side of each median carpel, and, perhaps this same member as well, have remained ovuliferous, but show a decline of the stigmatic function, each median trio forming between them only a single stigma, which is less well developed than that of the middle member in each lateral group of three, but longer than that of its companion (outside) members. On dehiscence each triplet of sterile solid carpels, together with the contiguous valves, becomes detached *as a single lateral compound valve*. We see the next stage in the 4-sutured ovary with four stigmas, where the stigmas of the four outside solid carpels of the two triplets have now entirely disappeared. Finally, in the 2-stigma-bearing, 2-compound-valved fruit dehiscing by a single split, the ovuliferous carpels have also lost their stigmas, leaving only the two of the lateral solid carpels. The range thus illustrated within this one species is exhibited throughout the genus. Among more than 120 forms listed by Fedde ('Pflanzenreich', loc. cit.) the enormous majority have four stigmas generally of unequal length, apparently the most stable phase to-day. But of the rest one is cited with a doubtful six, two with eight, one with eight to ten, one with a doubtful twelve, while at the other end of the scale some three or four have reached the limit of reduction (2). In face of these facts, whether these numbers are invariable or only predominant for each type, G 2 becomes a meaningless symbol. Dehiscence usually occurs in the median plane. As it appears to be very usual for the split to arise so that one median carpel is left attached to each compound valve, the latter would show more rows of ovules on the one margin than on the other in a form where the

[1] The total of twenty is based on the supposition that each median flat face is composed of but one carpel. Only if this were not so would the number be higher.

adjacent solid carpels were also ovuliferous, as has been assumed to be the case in such plants as those figured by Hooker and Baillon, though ordinarily the median pair alone are fertile. In this way the massing of the ovules, hitherto a puzzling feature, now becomes intelligible. Owing to the thread-like character and close juxtaposition of the placentae, the impression is given that two placentae, superposed upon two ribs, are each crowned with some four or five rows of ovules, as depicted in the illustration referred to above (Fig. 48).[1] Presumably these placentae are placed on either side of the mid-line of each of these two ribs, as is characteristic in solid carpels.

Both the Fumariaceae and Capparidaceae include some genera which show two solid and two valve carpels, and dehiscence after the manner of the typical Crucifer, but here the ovary remains unilocular, the replum being merely a frame—a condition rare in the Cruciferae, but characteristic of certain Papaveraceae (see above, p. 470). These features are well seen in species of *Corydalis* (Fumariaceae) and in *Cleome* (Capparidaceae). In both families, however, forms are also to be found which point to this type of gynoecium having resulted from consolidation and reduction precisely as in Papaveraceae and Cruciferae. In *Capparis spinosa*, L., instead of G 4 (two hollow and two solid), we find G 16 (eight of each); and in *Morisonia*, another genus having a berry instead of a siliquiform fruit, G 8 (four of each). Also it seemed not impossible that these same processes might account for the great variation in number of the stigma lobes which is to be found in the Fumariaceae. In some forms there are two divaricate lobes placed over the placentae, in others (species of *Corydalis*) the style terminates in an erect plate-like structure with several small lappets, the number, though varying (4–6–8), being constant for any species (Figs. 50, 51). But though the vascular cord of the solid carpel sometimes appears to consist of three bundles (*Dicentra*), and the vascular strands of both valve and solid carpels pass up into the stigmatic plate, the increase in number of the vascular elements makes it difficult to determine any precise relation between the bundles and the lappets which are not themselves vascular.

The Resedaceae, though having an appearance of greater uniformity than the families already considered, nevertheless present an equally interesting and instructive series as regards the construction of the gynoecium. The number of carpels is stated to vary from two to six. They may be joined partially or completely, or altogether free. In *Reseda* the valves usually terminate in points which alternate with the placentae and bear the stigmas. In *R. odorata*, L., however, according to Buchenau,[2] the stigmas are placed midway between the valve points, i. e. they are over the placentae, whilst in the giant strain of this species commonly cultivated it is not unusual for stigmatic papillae to be developed in both positions.

[1] Bot. Mag., loc. cit.

[2] Bot. Zeit., 1853, Beiträge zur Morphologie von *Reseda*.

As Buchenau truly remarks, the stigmas do not appear to be tied to any particular morphological position, but he offers no explanation of this surprising fact, although in this genus they are so distant from one another that on question of fusion between neighbouring half-stigmas can arise. Another interesting fact from our present view-point is that in some species each placentiferous cord remains intact throughout its length, whilst in others it bifurcates above, a character of some importance in determining relationships. Both these features, viz. the variable position of the stigma and the behaviour of the placenta, hitherto unaccountable and apparently without import, now become significant and easy of explanation, for here too we have distinct evidence of consolidation resulting in dimorphism of the carpels. In such forms as *R. luteola*, L., *R. glauca*, L., *R. complicata*, Bory, *R. virgata*, Boiss. and Reut., the carpels are all of the valve type. Separation of the conjoined edges of the carpels above is accompanied by a preparatory separation of the two ovuliferous strands—the double placenta bifurcates. In such types the stigmas occupy a position over the midribs of the valves. In other species in which the double placenta remains whole, as e.g. in *R. lutea*, L., *R. alba*, L., *R. phyteuma*, L., the sutures present a *double* contour line—they are in fact not merely placentae but whole (solid) carpels and hence remain intact. In the light of this fact it is comprehensible why in those species which have a line of hairs or protuberances on the midrib of the valve, a line of these structures should also be present on the suture (the midrib of the solid carpel) as in *R. arabica*, Boiss. It is now also clear why even the young syncarpous gynoecium in *Reseda* is open at the top. The valve carpels are joined to the intervening placentiferous solid carpels, but the latter do not extend to the same height as the valves ; consequently where the solid carpels cease, the valves are disjoined and the ovarian cavity is not closed in. That the same condition obtains in the species with the few carpels present all hollow may be due to the form of the valve having become fixed before the disappearance of the solid members. We see in the *R. odorata*, L., type with its stigmas on the solid carpels, and the *R. luteola*, L., type with valve stigmas, an example of the same interchange of functional activity as has been shown to occur in the other families treated above. Finally in *Randonia*, where we have in reality not G 2 but G 4 (two solid and two hollow), we find a construction which is the counterpart of the typical siliqua, with this difference, that here it is the lateral carpels which become solid and the median which remain hollow. The general plan is precisely the same as in the Crucifer, but in *Randonia* there is great development of the flower in the median plane on the posterior side, and the plane in which consolidation takes effect is (? consequently) shifted to that in which the lateral carpels lie.

With the clue furnished by the dimorphic carpel yet another mystery is dispelled, for we are now able to interpret the extremely interesting and

hitherto unexplained phenomena exhibited by such forms as *Ceratocapnos* (*Corydalis*) *heterocarpa*, Ball. (Figs. 52, 53), among the Fumariaceae, and *Aethionema heterocarpum*, J. Gay, and *Diptychocarpus strictus*, Trautv., among the Cruciferae. These plants are peculiar in that they appear habitually to produce two kinds of fruit in the same inflorescence. Those first formed are short, thick, indehiscent, composed of four similar (hollow) carpels; those which succeed them are more elongated, slender, dehiscent, two of the carpels becoming detached as valves from the other two, which have undergone a partial transformation towards the solid type. The significance of this heterocarpism is now fully apparent. We have exhibited in these plants as a fairly constant feature a transition comparable with that observed occasionally in *Eschscholzia* (see above, p. 471). *The process of consolidation is taking place before our eyes!*

It is a striking fact that in these heterocarpic species among the Cruciferae and Fumariaceae it is the ancestral type of fruit composed of uniform hollow carpels which does not dehisce, whereas the later-evolved form in which some carpels have become solid is able to split. Can it be that differentiation into valve and solid carpels provided an easier and more reliable method of securing the opening of the fruit than rupture of the fruit wall at a spot which, though representing a junction of (valve) carpels, perhaps offered insufficient tissue differentiation to secure invariably the initiation of a split? Or has this modification of some of the carpels come about in the course of, and in association with, evolution from a primitive one—or few—seeded fruit in which dehiscence was unnecessary and perhaps did not occur, to a many-seeded condition in which it was essential? These questions we can scarcely hope to answer until a more exhaustive study of the structure of the gynoecium in these families has been carried out.

It has now been sufficiently made clear that in certain families of the Rhoeadales evolution has been accompanied by processes of reduction and consolidation of members of the gynoecium, leading to the production of two kinds of carpel, the hollow and the solid, and that in the dry dehiscent type of fruit consolidation has sometimes occurred without (apparently) a reduction in number, and has led in these cases to the formation of the *compound* (many-carpelled) valve. We can henceforth eliminate from the scheme of construction the *false* partition and the *commissural* stigma. Furthermore, we obtain a rational explanation of the hitherto unexplained occurrence of dimorphic fruits which are met with in many types, either as a rarity, or so constantly as to have been regarded at the outset as of generic value. In them we see the reappearance momentarily or for a longer space of some stage of the phylogenetic history.

8. *A cursory survey of the Ericales, certain Malvaceae, and some isolated genera which are held to have commissural stigmas, leads to the same conclusion. The commissural stigma also not a reality in the Orchidaceae, the gynoecium being composed of six carpels* (G 3 + 3).

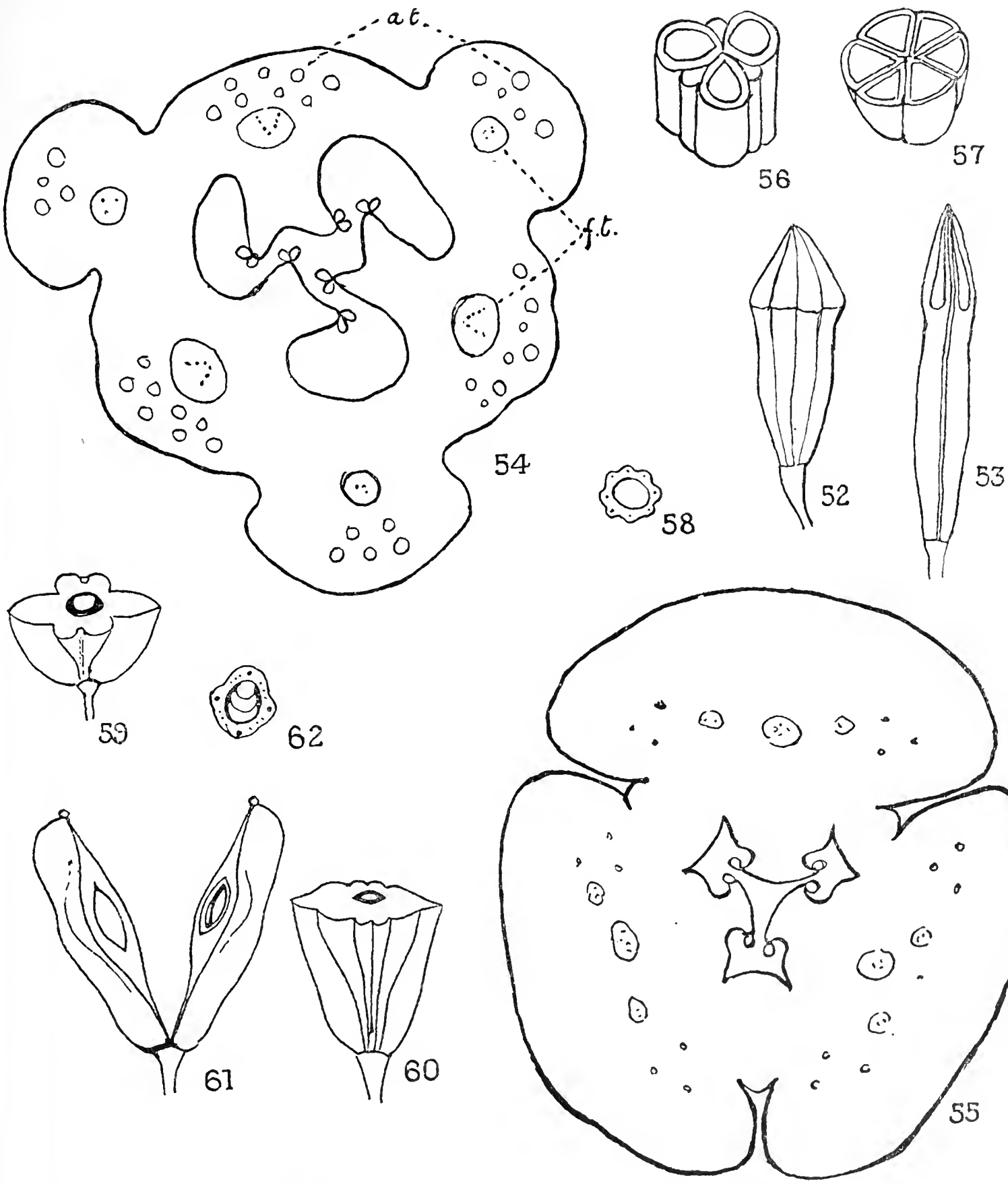
The elimination from the Rhoeadales of these anatomical anomalies, and the acceptance in their place of the differentiation of two kinds of carpels and of a certain interplay between them, necessitates at least a brief consideration of certain other unrelated families in which a similar disposition of the stigmas or mode of origin of the dissepiments has been held to prevail. Chief among these are the large group of families included in the series Ericales, the Orchidaceae and certain of the Malvaceae. To deal adequately with these cases within the limits of a single paper is impossible. It can, however, be stated at once that an examination of even a few types has already shown clearly that the same processes of reduction and consolidation have been in progress in these diverse groups as in the Rhoeadales. It is therefore proposed to conclude the present account with a brief reference to one Monocotyledon family, and to present the evidence in the case of the other families in a later communication. We will, therefore, very briefly consider the Orchidaceae from the present standpoint.

A perusal of Eichler's statement of the facts regarding the Orchid gynoecium ('Blüthendiagramme', ii, p. 182) and a consideration of the figure there given (Fig. 108) of a cross-section of the ovary of *Cypripedium Calceolus*, L., make it difficult to understand how any one not already committed to the commissural-stigma view and hence concerned to bring all facts into line with it, can fail to realize that the six structures composing the *Cypripedium* gynoecium, structures fundamentally equivalent in everything but size, represent six individual carpels, three of a pseudo-valve shape alternating with three which have become solid. Of the fact that in some Orchidaceous genera three of the six structures—'Zwischenstücke', as Eichler terms them—are sometimes wanting, he offers no explanation. In the light of our conclusions in regard to the Rhoeadales we have a clue to this varying conformation within the Orchid family. The full Orchidaceous ground plan is undoubtedly G 3 + 3, but in existing forms where all six are present one whorl of carpels has become solid and sterile, as can be seen from Figs. 54, 55, showing the appearance of the ovary of *Calanthe vestita*, L., and *Cattleya labiata*, L., in cross-section. A similar condition is depicted in the figures of the cross-section of the gynoecium of *Selenipedilum*, *Phragmopedilum*, *Paphiopedilum*, *Cypripedium*, and *Trichopilia fragrans*, Reichb., in 'Pflanzenreich', iv, 50, p. 22, Fig. 12 A, B, C; also 'Pflanzenfamilien', ii, 6, p. 82, Fig. 83 E, F, G, and p. 194, Fig. 208. The ripe fruit, generally a capsule, splits more or less completely into three broad strips (Eichler's 'Klappen') and three narrower ones (his 'Zwischenstücke') (as shown in Fig.

75, p. 73, Pflanzenfamilien, loc. cit.). A comparison of the above figures reveals the interesting fact that here, as in the Rhoeadales, a certain interplay takes place between the two kinds of carpels, that is to say, the association between form and function is not absolutely rigid. Thus in *Selenipedilum* three of the carpels are of typical valve form, the edges incurve in the normal manner, and re-entering, bear the ovules on the now free terminal surfaces; the other three carpels are much reduced, quite solid and sterile. In *Phragmopedilum* there are similarly three carpels of the latter type, but here the remaining three have become decidedly massive—the process of consolidation has already converted them into the pseudo or valve or semi-solid form. They are prolonged to the centre, where they meet, but although still *valve-like* in *outline* they exhibit the feature associated with the solid conformation, viz. *a shifting of the placenta position.* It seems possible that one whorl in this type having become sterile as well as solid, the form of the other has been evolved as a compromise: fertility is retained, but consolidation is arrested at a point which leaves enough of the primitive form to satisfy mechanical needs. In *Trichopilia* the trend of modification is in the same direction; the massiveness of the fertile carpels is even greater, but their later development is not carried so far: they do not meet centrally, hence the ovary remains unilocular. In *Cypripedium*, on the other hand, this shifting takes place without any marked departure from the valve form, so that the large ovarian cavity is retained with a deceptive appearance of typical parietal placentation. If, as follows from the statement accompanying one of the above figures (see legend to Fig. 12, 'Pflanzenreich', loc. cit.), the three valves in *Selenipedilum* are formed from the three which in the other genera cited become small, solid, and sterile, we have paralleled here among the carpels a reversal of their ordinary behaviour such as is seen in the Rhoeadales in the case of *Randonia* (see above, p. 473.) Finally, in such forms as *Angraecum* and *Pleurothallia* (see 'Pflanzenfamilien', loc. cit., p. 73) we find the counterpart of the 'compound valve' met with in the Papaveraceae and Cruciferae (see above, p. 474).

If further support for this interpretation of the Orchid gynoecium were needed, we find it in the appearance exhibited by certain of the Alismaceae. No more striking corroborative evidence could be adduced than that presented by the structure of the ovary of *Triglochin maritimum*, L., with its six fertile carpels, and *T. palustre*, L., with three normal-sized fertile valves alternating with three which, though still enclosing a cavity, are obviously much reduced and on the way to becoming solid (Figs. 56, 57).[1] Reduction carried a stage farther would produce the small sterile carpels of the Orchidaceae. Furthermore the parallelism between these reduced carpels and the commissures of the Cruciferous siliqua is shown by the mode of dehiscence. In *T. maritimum* the ripe fruit splits septicidally into the

[1] See illustrations in Flora Londinensis, v, Pls. XCVIII and XCIX.



FIGS. 52–3. *Ceratocapnos* (*Corydalis*) *heterocarpa.* 52. Earlier-formed indehiscent, urn-shaped fruit. 53. Later-formed dehiscent, siliquiform fruit. 54. *Calanthe vestita,* ovary in transverse section, showing three solid and three semi-solid carpels (pseudo-valves); *a.t.*, assimilating tissue; *f.t.*, fibro-vascular tissue. 55. *Cattleya labiata*, ovary in transverse section, structure as in *Calanthe*; the overlapping of the sunken solid carpels by the pseudo-valves gives a false appearance of single-line sutures. 56–7. *Triglochin.* 56. *T. palustre*, ovary in transverse section. 57. *T. maritimum*, the same. 58. *Hypecoum Geslini*, 8-ribbed ovary in cross-section. 59–62. *Isatis.* 59. *I. hebecarpa*, ovary 6-valved. 60. *I. littoralis*, ovary 10-valved. 61. The same splitting into two compound valves. 62. *I. iberica*, ovary in cross-section; the orthogonal carpels form four strong veins; the smaller veins probably represent an equivalent number of intervening carpels here so reduced that they do not form flutings on the surface. (Figs. 52, 53 after Le Maout and Decaisne; 56, 57 after Curtis; 58, 62 after Cosson; 59–61 after Delessert; 54, 55 original.)

six component valves, whereas in *T. palustre* the three valve carpels separate from below upwards, remaining attached by their apex to the tri-lamellate frame formed by the three reduced carpels.[1]

Having thus shown by reference to some half-dozen genera that we meet with precisely the same dimorphism and division of labour between the carpels in the Orchidaceae as in the Rhoeadales, and that the conception of the 'commissural' stigma and the 'false' partition is not more a reality in the one case than in the other, we must leave a more complete survey of this family for future investigation. It may be noted in passing, however, that the above interpretation is applicable equally to the neighbouring small family of the Burmanniaceae.

It remains to review briefly the principal conclusions to be drawn from the facts here investigated.

Summary of Conclusions.

General.

1. Evolution in the Rhoeadales has been accompanied by reduction and consolidation of the members of the gynoecium, leading to the production of two kinds of carpels, the 'hollow' or valve type and the 'solid' type.

2. When the two kinds of carpel coexist, they are disposed regularly and alternately, except in the rare cases where a member of the gynoecium is undeveloped.

3. The *solid* carpel is recognizable externally by its *double* contour line, as is well seen, e. g., in the typical Crucifer. Contiguous *valve* carpels on the other hand give rise to a *single-line* suture.

4. The morphological transformation from the valve to the solid type has been accompanied by redistribution of the carpellary functions.

5. When both types of carpel are present, the ovuliferous function is almost always taken over by the more recently evolved solid carpel, and the more primitive valve carpel becomes sterile.

6. The receptive (stigmatic) function is not rigidly associated with one particular type of carpel, but may be carried out by either the one or the other or by both. All three dispositions may be found within the same family, as is the case in the Cruciferae.

7. An alternate arrangement of solid and hollow carpels may act advantageously by facilitating a more complete dehiscence of the fruit than usually occurs where valve carpels only are present. In the latter case separation generally begins from above, and is only partial.

[1] The suggestion was made by Gaertner as long ago as 1791, that the three lamellae alternating with the three full-sized carpels might perhaps represent three other members which had been suffocated by their neighbours (De Fruct., vol. ii, p. 26). As Gaertner mentions that the stigmas are sometimes three and sometimes six, possibly we have here a parallel in this respect to the variability in stigma formation which occurs in *Eschscholzia californica* (see p. 471).

8. As a rule the hollow (valve) carpels separate individually from the contiguous solid carpels, but when the number of carpels remains large and many become sterile, a group of carpels may be detached in one piece as a 'compound' valve.

9. The conception of the solid carpel removes the difficulties inherent in the so-called 'commissural' stigma and the 'false' partition by establishing the normal position of the one and the genuine character of the other.

10. Furthermore, this conception affords an explanation of various hitherto unexplained phenomena, such as (*a*) the fairly constant occurrence in some species of two kinds of fruit on the same individual; (*b*) the occasional appearance of a few multicarpellary fruits in types normally having only the reduced number four; (*c*) certain more extreme cases, where this exceptional multicarpellary construction is found more or less throughout an individual plant, which on this account has in some instances, though on insufficient grounds, been accorded generic rank. From the present standpoint these exceptional fruits, hitherto considered for the most part as mere sports or monstrosities, are of cardinal importance on account of the light which they throw on the phylogenetic history.

11. Their occurrence points to the conclusion that in each family in the series the course of evolution has proceeded on the same lines: that those forms having numerous carpels all of the valve type and splitting septicidally are the older, those with a reduced number of carpels some of which are hollow and sterile, some solid and ovuliferous, the more recent.

Concerning the Papaveraceae and Fumariaceae.

12. Carpels generally numerous (twenty or more), but in some genera as few as three or four.

13. Many-carpelled fruits in which most of the carpels are sterile usually dehisce into two compound (several-carpelled) valves, as in *Eschscholzia* and its allies.

14. Evolution has probably proceeded from some form of the *Platystemon* type, with many carpels all of valve form. Thence in oned irection has arisen the group of the Papavereae, represented at first by forms with many carpels of the solid as well as the valve type, from which the few-carpelled forms were evolved later. In another direction the *Hypecoum* types (Fig. 58) with many-veined (-carpelled), generally lomentose or exceptionally dehiscent, compound-valved fruits leading on along one line to the 4-carpelled Fumariaceae types: along another perhaps to the Eschscholzieae, still many-carpelled with characteristic replum frame and compound valves.

15. The process of evolution of the solid carpel is to be seen as a regular feature in *Ceratocapnos* (*Corydalis*) *heterocarpa*, Ball. (Fumariaceae), where the earlier fruits are urn-shaped and the later ones siliquiform.

Concerning the Cruciferae.

16. The typical gynoecium is composed of four carpels, of which two form sterile valves, the other two which have become solid remaining fertile. In some few genera this relation is reversed. The solid members usually develop centripetally until they meet, and so form a complete partition (the replum), but in other cases the inward growth is arrested at an early stage, so that the partition is wanting or remains incomplete. Thus, the anomaly that from the first moment of their origin the placentae should be as large as, or even larger than, the carpels to which they belong disappears now that these placentae are shown to be independent carpels.

17. Outgrowths from the upper extremities of the carpels, such as are seen in *Matthiola bicornis*, DC., *M. tricuspidata*, R. Br., and *Parolinia ornata*, Webb, become intelligible when the tetramerous structure of the gynoecium and the division of labour among the two pairs of carpels is realized.

18. Ovaries of more than four carpels generally show a number of furrows, ridges, ribs, wings, or prominent parallel veins corresponding with the number of the solid or of the valve carpels present. Thus, e. g. in the genus *Isatis* the contour of the fruit in one species indicates the presence of six valves, in another of ten. From the character of the veining the number of carpels in species of *Brassica* and *Sinapis* appears to be sixteen ; from the number of wings, sixteen in *Guiraoa arvensis*, Coss.; from the ribbing, between forty and fifty in species of *Rapistrum*. It is necessary, however, to distinguish between formations associated with *parallel* veining and other outgrowths produced as the result of the presence of strong *lateral* veins, such as the horns in *Tetracme*, which are not significant of the carpel number.

19. The presence of this large number of carpels in various lomentose fruits accounts for the formation of the subsidiary loculi at the joints between the seed-containing sections.

20. The stigmatic function is usually restricted to the solid carpels, but it may be subserved by the valve carpels (*Matthiola incana*) or by both (probably the case in *Biscutella*).

21. The evolution of the solid carpel is to be seen in progress in certain types which produce characteristic dimorphic fruits, as e. g. *Aethionema heterocarpum*, J. Gay, and *Diptychocarpus strictus*, Trautv.

22. Many types produce a few reversionary multicarpellary fruits, generally, however, only at the beginning of the season and under favourable conditions (observed in some twenty genera).

23. Rarely this reversionary condition may be exhibited throughout the greater part of the plant, as in *Tetrapoma* (*Nasturtium*) *palustre*, *Holargidium* (*Draba*), and to a less extent in *Lepidium sativum*, L. In these cases the character is not inherited in the strict sense, but it may

nevertheless reappear fairly constantly in the offspring under unchanged conditions.

24. The widespread occurrence of reversionary fruit types indicates that the typical 4-carpelled siliqua and silicula have been derived by reduction and consolidation from an earlier ground-plan, in which G = 4 (hollow) +4 (solid); which in turn arose by simple reduction from one composed of a much larger number of both kinds of carpels, this construction being yet again the result of consolidation from an all-valve type with G numerous.

25. This tetramerous constitution of the gynoecium lends considerable support to the view put forward by several earlier observers, that the Cruciferous flower is composed of strictly alternating whorls, and that the ground-plan is K 4 C 4 A 4+4 G 4+4. The same causes which have led to the consolidation of the two carpels in the median plane have had their effect also on the members of the androecium which also lie in this plane, with the result that the posterior and anterior stamen have completely disappeared. To this same cause may also probably be attributed such inequality in the size of the two pairs of sepals as is not directly accounted for by the asymmetric distribution of the nectaries; while the forces tending to reduction, which in the first instance caused G ∝ to become G 4+4 (and perhaps A ∝ to become A 4+4), have led at a later stage to the complete suppression of the last members in the whole series—the second whorl of carpels.

Concerning the Capparidaceae.

26. The same processes of reduction and consolidation are exhibited here, as in the preceding families, the many-carpelled condition with both valve and solid carpels being exhibited by such forms as *Capparis spinosa*, L., the final 4-carpelled stage by the section Cleomidiae.

Concerning the Resedaceae.

27. Number of carpels three to eight. Where the carpels are separate, as in *Asterocarpus* (6), or with single-line sutures, as in *Reseda luteola*, L. (3), they are all of typical valve form with the stigma centred over the midrib. In *R. luteola* each edge is bordered by a placenta, hence the appearance of bifurcation as the two separate towards the top where the ovary is open. Where the valve junctions show a double outline the carpels are dimorphic, the extent of cohesion depending upon the height to which the solid carpels extend. As it is the latter which in this case bear the placentae, no bifurcation takes place. The stigmatic function is generally performed by the valve carpels, but in *R. odorata*, L., the solid carpels function as well or sometimes (according to Buchenau) alone. Reduction and consolidation combined reach their limit in *Randonia* with G 4.

The elimination from the ground-plan of the Rhoeadales of the *commissural* stigma and the *false* partition renders necessary a revision of a number of unrelated families in which the same constructions are held to exist, more especially the whole group included in the Ericales, some genera of the Malvaceae, and the Orchidaceae. Evidence in disproof of the commissural stigma in the Orchidaceae and in support of the formula G 3 + 3 is given in the present account. It is proposed to treat the other families here cited and certain isolated genera in a later communication.

In conclusion, I wish to express my very grateful thanks to Miss D. F. M. Pertz, who made the drawings of the Stock specimens and of the figures cited from other works; also to Miss M. G. A. Campin, who kindly prepared the microscopic sections of the Stock fruits, from which Figs. 19, 28, and 29 were drawn.

The necessary expenses incurred in connexion with the work have been defrayed by a grant from the Royal Society.

Further Observations on the Fungus present in Pellia epiphylla, (L.) Corda.

BY

W. F. F. RIDLER, M.Sc.

With three Figures in the Text.

THE distribution, isolation, identification, and physiological relationship to its host of the fungus present in *Pellia epiphylla* have been described

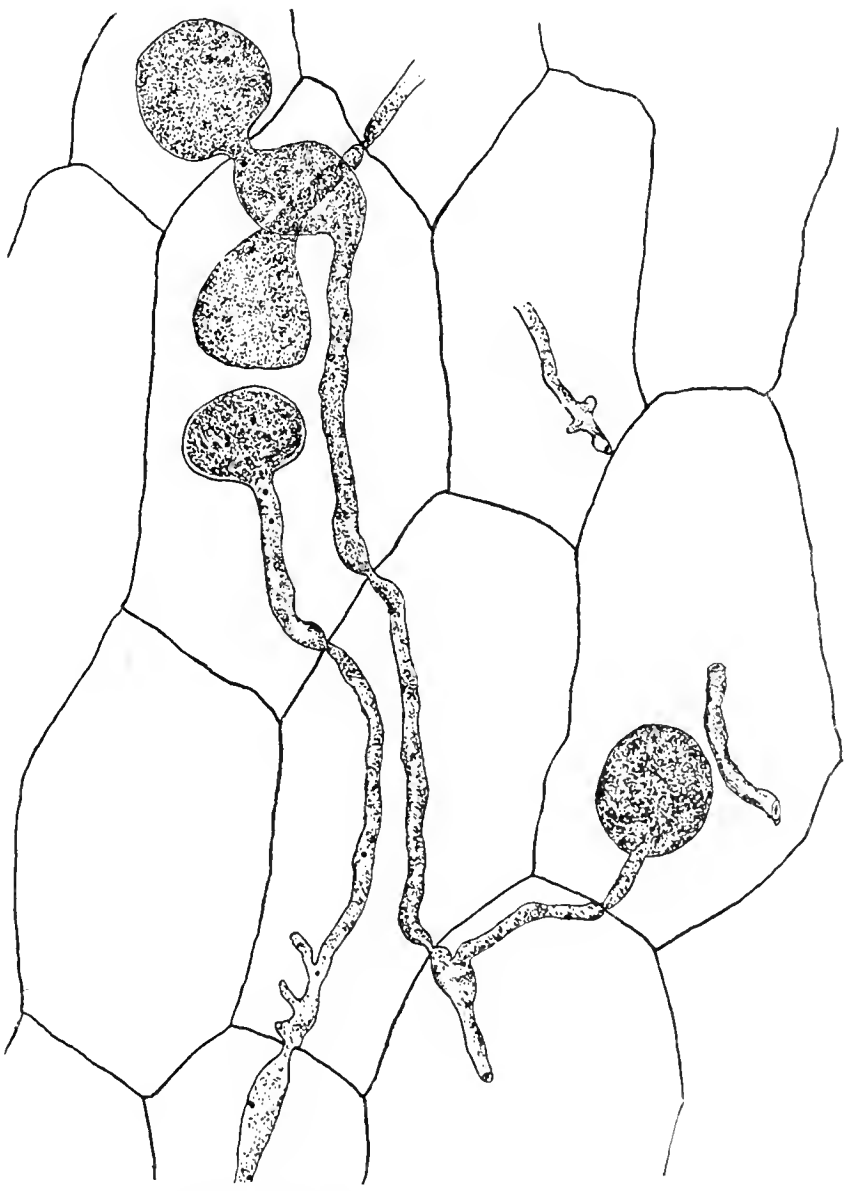


FIG. 1. Cells of *Pellia epiphylla* with hyphae and vesicles, indicating method of penetration of the cell-walls. × 290.

in a previous paper (Ridler, 1922). Some additional observations are recorded here.

Behaviour of the fungus in cells of the thallus. By reason of its light-brownish colour, the zone of cells occupied by the fungus in the thallus of

the liverwort can be recognized in sections of fresh material examined with a low power magnification (× 100), but individual hyphae cannot be so distinguished. Under a higher magnification (× 440) numerous relatively large hyphae are visible. These branch profusely, passing from cell to cell by piercing the cell-walls. Except at the points of penetration no injuries to the cell-walls, owing to the passage of the hyphae, have been detected, nor have any traces of the presence of an enzyme causing the destruction of the cell-wall been observed. Penetration of the cell-walls seems, therefore

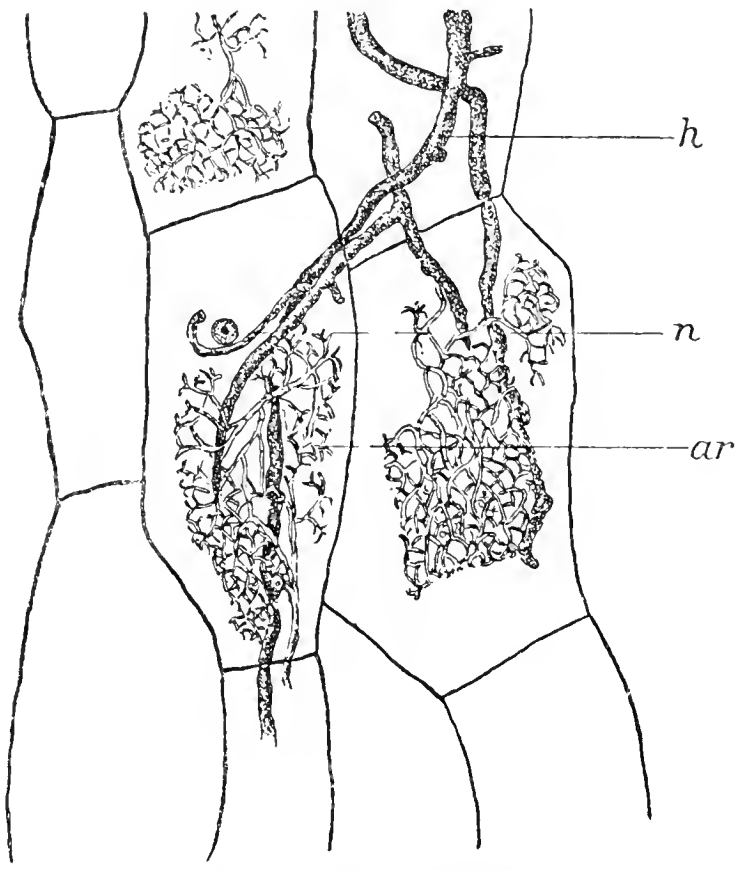


FIG. 2. Cells of *Pellia epiphylla* containing 'arbuscules'. *h.*, hyphae; *n.*, nucleus; *ar.*, arbuscules. × 290.

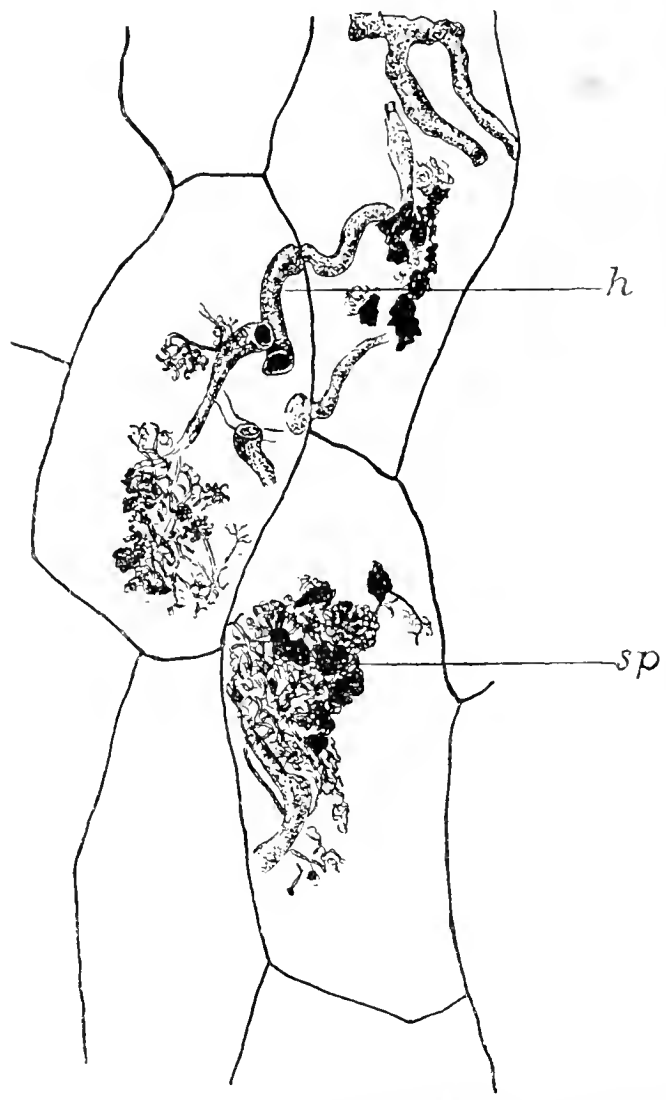


FIG. 3. Cells of *Pellia epiphylla* containing 'sporangioles'. *h.*, hyphae; *sp.*, sporangioles. × 290.

to be effected mechanically. The hyphae are distinctly swollen where their growth is arrested by cell-walls, and are constricted for their passage through them (Fig. 1).

Numerous very fine and profusely branching threads are produced from some of the hyphae, and form a network which completely fills some of the cells (Fig. 2). These threads, which can be made more conspicuous by staining with picro-nigrosine, probably correspond to the 'arbuscules' described by Noel Bernard (1911) in *Solanum Dulcamara*, and by J. Magrou (1921) in *Solanum tuberosum*, *Orobus tuberosus*, and *Mercurialis perennis*. These 'arbuscules' degenerate later into irregular masses termed by Bernard 'sporangioles' (Fig. 3). These masses are insoluble in water, alcohol, benzol, chloroform, and other similar solvents, also in the

common acids, whether in the cold or when heated. In the presence of iodine, hyphae, 'arbuscules', and 'sporangioles' turn yellow in colour, and with chlor-zinc-iodine they become a reddish brown. The mycosin test for chitin described by Wisselingh (1898) was tried, and after this treatment the 'sporangioles', the walls of the hyphae, and the 'arbuscules' all stained a reddish violet with chlor-zinc-iodine, while the cell-walls of the liverwort turned blue; a similar result was obtained with iodine and a trace of sulphuric acid. The main hyphae continue to ramify in the thallus, but the liverwort seems to possess some sort of control over the fungus, the nature of which is not exactly known, by which the latter is prevented from obtaining too great a hold on the plant and from becoming harmfully parasitic. The 'arbuscules' are not capable of passing from cell to cell, so that by their formation the growth of the fungus is restricted. Noel Bernard (1909) compared the condition of such a plant to that of a vaccinated animal, and suggested that the plant possessed *une immunité humorale*.

Although careful observations have been made, no differences have been noted in the size, shape, or structures of the nuclei in infected cells as compared with the nuclei of cells from which the fungus is absent. The only difference observed was in their position. In uninfected cells the nuclei are peripheral, whereas in infected cells they are more often in a central position in close proximity to the 'sporangioles' and 'arbuscules'.

In the case of young thalli infected at one point only, starch is found to be present in all the cells except those occupied by the fungus. In 'rejuvenation shoots' infected only at the thallus end, a considerable quantity of starch is present, except in the infected region, where there are only a few isolated granules. It would seem that the fungus is responsible for the absence of starch in the thallus of the fully developed gametophyte of *Pellia*. There is always a considerable amount of oil in the infected region, and especially in the swollen vesicles (Fig. 1). As a rule no reaction is obtained when these are tested with Millon's reagent, though in one instance a definite brick-red coloration resulted.

Culture of the liverwort. The constant occurrence of the fungus in the thallus of *Pellia epiphylla* might seem to indicate an obligate symbiosis between the two organisms. Attempts made in 1921 to grow *Pellia* plants from young gametophytes were unsuccessful; growth began, a few cell-divisions took place, and one or two rhizoids were produced, but growth did not proceed farther. Attempts were made again in the spring of 1922 when the capsules were mature. The medium employed was a modification of Knop's solution as used by Servettaz (1913) in his experiments on mosses. The cultures were made under sterile conditions in Petri dishes, at the bottom of which several rounds of filter-paper were placed. A capsule of *Pellia* was immersed in a one per cent. mercuric chloride solution

for a few seconds, washed in sterilized distilled water, burst with a sterilized needle, and removed by means of the latter to a Petri dish, where its contents were rubbed over the surface of the filter-paper, the young gametophytes being thus distributed. The dishes were supplied periodically with fresh medium, so that the filter-paper was kept damp. The cultures were placed in a north window, so that adequate light was obtained without direct sunlight. Growth began almost immediately and continued, but, owing to overcrowding, the thalli produced were somewhat attenuated, but otherwise quite healthy.

Isolation and inoculation of the fungus. Further attempts were made to isolate the fungus from the thallus, but, as before, these were unsuccessful. Bernard and Magrou found similar difficulty in isolating the endophytes described by them in species of *Solanum*, and suggested that the 'arbuscules' are more adapted to a parasitic life than the 'pelotons' formed by the endophytes of the majority of the orchids, and are incapable of developing in the autophytic state.

Attempts were also made to re-inoculate the fungus isolated from the sporophyte into uninfected *Pellia* plants obtained in the manner described above, but these were also unsuccessful. Confirmation that the fungus previously isolated is the true endophyte of *Pellia* is therefore still lacking.

Observations on the hydrogen-ion concentration. Soil on which *Pellia epiphylla* was growing was obtained from Leigh Woods, Somerset, and its hydrogen-ion concentration was measured colorimetrically. Two methods were used for obtaining the soil solution—the centrifuge method described by Gillespie and Hurst (1917), and the displacement method first described by Gola (1910) and quoted in a paper by Cavers (1914) on 'Gola's Osmotic Theory of Edaphism'. The figures obtained from these solutions were as follows:

Soil solution obtained by	*Indicator.*	*pH Value.*
Centrifuge method	Phenol red	6·8–7·0
Displacement method	Phenol red	6·8–7·0

Summary.

1. The growth and consequent distribution of the fungus in the thallus of *Pellia epiphylla* is restricted, as shown by the formation of 'arbuscules' and 'sporangioles'. Some sort of control is thus exerted by the liverwort, and the fungus is prevented from becoming harmfully parasitic.

2. The fungus undoubtedly obtains food material from the cells of the liverwort, in the form of starch which is replaced by oil after the entrance of the fungus. It is very doubtful whether the liverwort gains anything by the association. It is possible that nutrient substances may be absorbed by the fungus from the substratum, as Stahl (1900) suggests, but the con-

siderable quantity of starch present in the thallus before the action of the fungus does not support this view.

3. The pH value of soil upon which *Pellia* was growing was found to be slightly acid.

4. Notwithstanding the constant occurrence of the fungus in the liverwort, symbiosis is not obligate, as plants of the liverwort have been grown without the fungus.

My thanks are due to Mr. C. T. Gimingham for information regarding the displacement method of obtaining soil solutions; to Mr. C. Hunter for much valuable help and advice; and to Professor O. V. Darbishire for his interest in the work.

During the progress of this investigation I have been in the receipt of a maintenance grant from the Department of Scientific and Industrial Research.

Cryptogamic Research Laboratory,
University of Bristol,
November 1922.

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Observations on the Reaction of Protoplasm to some Reagents.

BY

WILLIAM SEIFRIZ.

With four Figures in the Text.

IN the following pages are given part of the results obtained in a series of experiments on the reaction of protoplasm to some common reagents. The effect of ethyl alcohol and of the three glucosides, saponin, smilacin, and senegin, on the living protoplast are here recorded.

Material and Reagents.

The leaves of *Elodea* served as material. All observations were made only on the superficial cells of the upper side of the leaf. The cells on the upper surface were found to differ somewhat from the upper cells in their sensitivity to certain of the reagents.

The reagents employed for treating the cells were ethyl alcohol, saponin, smilacin, and senegin. These reagents were all obtained from Merck (Darmstadt). The saponin (pur. albiss.) is extracted from the Levantine soaproot *Gypsophila struthium*. The smilacin comes from sarsaparilla roots, commercially known as radix sarsaparillae, and obtained from various species of *Smilax*. Senegin is extracted from the roots of *Polygala senega* .

The water used in making the solutions was spring water, the same in which the *Elodea* plants were kept growing in the laboratory ; it is in Geneva, very pure.

PART I.

THE REACTION OF PROTOPLASM TO ETHYL ALCOHOL.

Experimental Data.

Lethal Effect of Alcohol. Overton, whose pioneer work on osmotic properties of the cell gave us the first information we have on the effect of many reagents on protoplasm, states that methyl and ethyl alcohol of 3 per

cent. by weight are not harmful to the plant cell after a long time (18, p. 105). One would, therefore, expect no harm to result from a 3 per cent. solution of alcohol, and relatively little from a 10 per cent. solution, after no more than a few hours of treatment. Quite the contrary, however, is true. A 10 per cent. concentration of ethyl alcohol is sufficiently strong to kill, on an average, 60 per cent. of the cells in an *Elodea* leaf within half an hour, 80 per cent. of them within an hour, and 95 per cent. within two hours. A 3 per cent. concentration of alcohol will kill, on an average, more than half the number of cells in from four to six days. If the cells of the *Elodea* leaf are unusually sensitive to alcohol, a 2 per cent. concentration will kill 50 per cent. of them in less than two days.

An interesting fact in connexion with the toxicity of alcohol on protoplasm is the variability in sensitivity of cells both in the same leaf and in different leaves. It was a common instance to find a dead cell, killed by the reagents, next to an actively streaming one, and to find certain cells which would withstand the poisonous effect for twenty-four hours while others in the same leaf succumbed in half an hour.

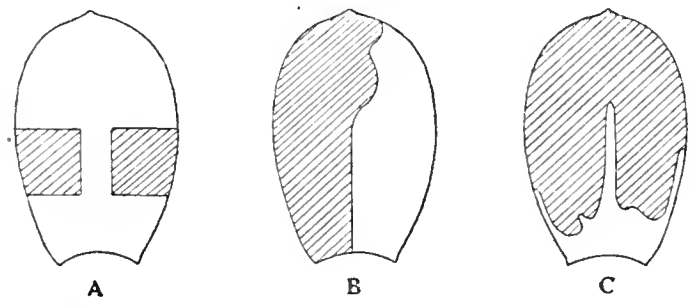

FIG. 1. Diagrammatic sketches indicating those regions of an *Elodea* leaf which first succumb to the toxic effect of ethyl alcohol. A. The cells killed may, as result of brief treatment in 10 per cent. alcohol, be situated in two blocks occupying corresponding positions on opposite sides of the midrib sharply delimited from the remaining leaf area of living cells. B. The dead cells, after longer treatment, may occupy the entire half of the leaf. C. The typical distribution of living and dead cells of a leaf treated for one hour in 10 per cent. ethyl alcohol. The very basal cells are the last to succumb.

The dead cells are usually grouped in patches, suggesting a common physiological state before death in these regions. The less resistant cells may be grouped into two blocks situated in similar positions on each side of the leaf (A, Fig. 1); or the cells in one entire half of a leaf may be killed while those in the other half remain alive and apparently normal (B, Fig. 1). In leaves treated in 10 per cent. alcohol, which kills quickly, there are definite regions which are in all cases more or less resistant to the alcohol. The few cells situated at the base of the leaf, extending a little way up each edge and still farther up the mid-rib, are the last to succumb to the toxic effect of 10 per cent. ethyl alcohol (C, Fig. 1). So pronounced is this difference in resistance that, while all the other cells, i.e. 95 per cent. of the total, may sometimes, in a more sensitive leaf, be killed in half an hour in 10 per cent. alcohol, it requires six hours or more of treatment to kill the two or three hundred cells situated in the base of the leaf. On the other hand, the alcohol-resistant basal cells are as sensitive as any in the leaf to some other reagents, e. g. to strontium chloride.

This variability in behaviour is not only true of different cells in a leaf,

but also of different leaves on the same shoot, and of different shoots in one lot of plants, and again, of different lots collected at different times and in different localities.

What all the controlling factors are, it is impossible to say. One or two of them were, however, ascertained. Young and luxuriantly growing *Elodea* plants are less resistant to alcohol than are older plants. Also, the longer the time plants remain in culture in the laboratory the greater does their resistance to alcohol become. (The osmotic pressure of the cell is likewise increased.)

The variability in reaction of the different cells of the same leaf can only be attributed to different physiological states of the cells, the causes of which are as yet unknown.

This variability in behaviour of the cells of *Elodea* leaves made it necessary to make many observations in order to obtain a general average of behaviour. Only such grand averages, unless otherwise stated, are given in this paper. In most instances these averages are based on the observation of more than a hundred leaves, representing many thousands of cells, for each time of treatment in every concentration of the several reagents used.

The average length of time necessary to kill an *Elodea* protoplast with 10 per cent. (1·7 M) ethyl alcohol is about (slightly less than) half an hour. In the most resistant leaf found every cell withstood the harmful effect of 10 per cent. alcohol for two hours, not a single dead cell being found. That the cells suffered to a considerable extent, which was not superficially noticeable, is inevitable. Such extreme resistance is, however, rare. In only one leaf in a hundred do all the cells survive the toxic effect of 10 per cent. alcohol for one hour. In contrast to these instances are those of two other leaves, in one of which 97 per cent. of the cells were killed in 10 per cent. alcohol in half an hour, and in the other, 99 per cent. were killed in one hour.[1]

The curve in Fig. 2 depicts the average rate at which a 10 per cent. solution of ethyl alcohol kills the cells of *Elodea*. The ordinates are minutes and hours of treatment in the alcohol. The abscissae are percentages of cells killed. It will be seen that in the average *Elodea* leaf no cells are

[1] The criterion of death used was inability to plasmolyse with a 10 per cent. solution of potassium nitrate. The average critical plasmolytic concentration, i. e. that concentration of plasmolysing salt which will, in a half-hour, plasmolyse about 50 per cent. of the total number of cells in a leaf, is 3 per cent. KNO_3; therefore it is reasonable to assume that a cell incapable of plasmolysis with 10 per cent. KNO_3 is dead. To prove the truth of this a 20 per cent. solution of potassium nitrate was applied after a 10 per cent. solution, and no additional cells were plasmolysed. To further convince myself that these alcohol-treated cells which could not be plasmolysed with 10 per cent. KNO_3, were dead, I observed whether or not cells treated in 10 per cent. alcohol for twenty minutes, half an hour, and one and a half hours would recover. There was no recovery after three days in water. Other indications of death are also usually present, such as an irregularly shrunken (coagulated) and discoloured protoplast.

killed in 10 per cent. ethyl alcohol within ten minutes, but in fifteen minutes several cells (0·1 per cent.) die, and in twenty minutes 1 per cent. are killed. The percentage of cells killed then increases with the length of time of treatment. After 95 per cent. are dead the curve rises very rapidly, due to the great resistance of the few basal cells. All cells, including the basal ones, succumb in 10 per cent. alcohol overnight.

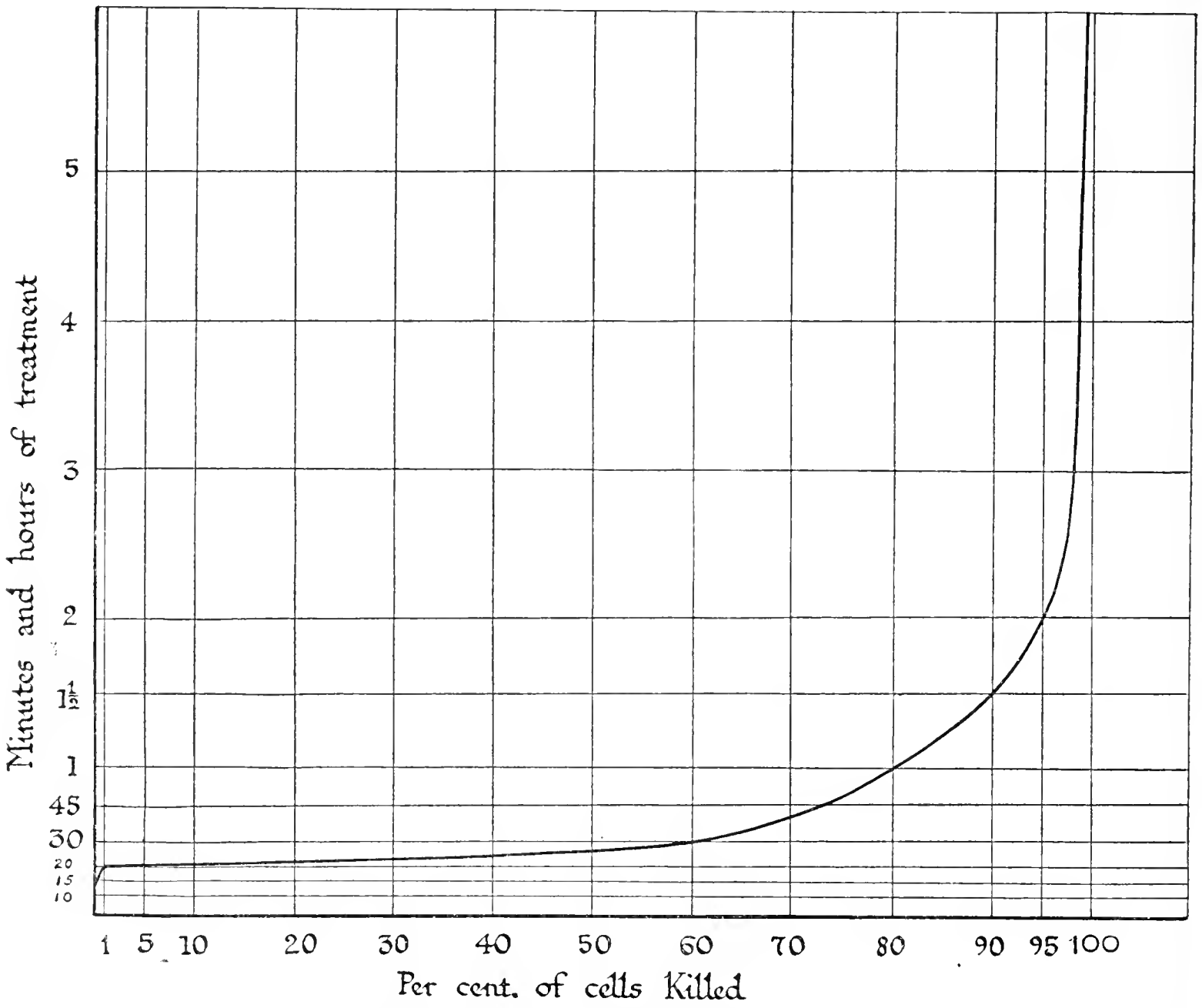

FIG. 2. The curve depicts the average rate at which *Elodea* leaf cells are killed in a solution of 10 per cent. ethyl alcohol. The ordinates are minutes and hours of treatment. The abscissae are percentages of cells killed. Thus, 0·1 per cent. of the cells of an *Elodea* leaf are, on the average, killed in 10 per cent. alcohol within fifteen minutes; 1 per cent. are killed in twenty minutes; 60 per cent. in half an hour; 95 per cent. in two hours. The sudden rise of the curve is due to the great resistance of the few basal cells of the leaf, which usually require from six to twelve hours to kill in 10 per cent. alcohol. The curve is an ideal one based on many averages, the variations in resistance of different cells in a leaf, and of different leaves, being very great.

The story of the toxic effect of alcohol on *Elodea* leaves can be told in another way. The curve in Fig. 3 represents the rate at which different strengths of solutions of ethyl alcohol kill the cells. The time in which a solution of alcohol would kill half the number of cells was chosen as a data upon which to plot the curve. By examining the curve one can ascertain how long an average cell may remain in an alcoholic solution of a certain strength and stand an even chance of surviving. Thus, in 5 per cent.

(0·9 M) ethyl alcohol half of the cells will be killed in forty hours, and half survive.

It must be remembered, however, that no cell probably escapes without considerable ill effect when the time of treatment is sufficient to kill 50 per cent. of them. For concentrations below 4 per cent. the curve can be regarded as only approximate, since in weak solutions the time required to

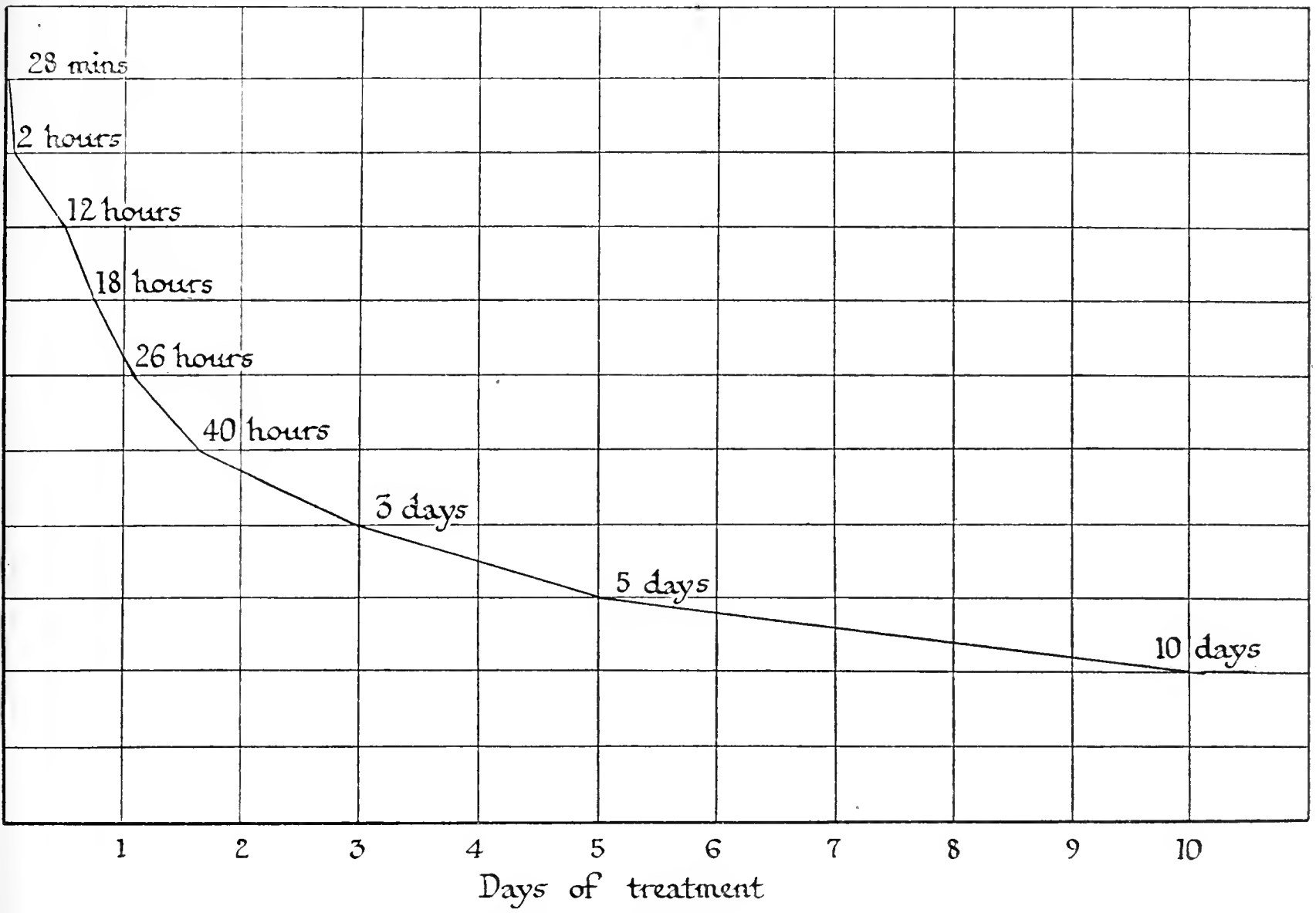

FIG. 3. The curve is based on the time required to kill half of the total number of cells on an *Elodea* leaf in solutions of ethyl alcohol of concentrations of 1 to 10 per cent. The ordinates are percentages of alcohol in solution. The abscissae are days of treatment. The curve shows, for example, that a cell will stand an even chance of surviving after being twenty-eight minutes in 10 per cent. alcohol, eighteen hours in 7 per cent. alcohol, &c.

kill varies greatly. Thus, in material subsequently worked upon in London[1] all cells in an *Elodea* leaf, including the more resistant basal ones, were killed in a 2 per cent. (0·3 M) alcohol solution in two days. Furthermore, the cells may become much deteriorated in a few days apparently without actually dying.

From the curve it will be seen that a concentration of about 9 per cent.

[1] Some of the experiments here recorded were repeated on *Elodea* collected near London with the purpose of ascertaining if different specimens growing in different localities reacted differently to the reagents. These duplicate experiments were carried out in the Huxley Laboratory of the Department of Botany of the Imperial College of Science and Technology, London. The writer wishes to express to Professor V. H. Blackman his appreciation of the courteous and helpful treatment which was accorded him while working in the Huxley Laboratory.

(1·6 M) is a critical point in the toxicity of ethyl alcohol on protoplasm. Above this concentration the protoplasm is killed in relatively few minutes. Below this concentration many hours are necessary to cause death. It is at about this concentration (1·5 M) that Stiles and Jørgensen (22, p. 60) obtained a marked increase in the exosmosis of electrolytes from potato in solutions of ethyl alcohol. They found that the rise in electrical conductivity of the potato tissue after twenty-four hours of treatment in water was 79; in 1·0 M (6 per cent.) ethyl alcohol it was only slightly higher, 110; while in 1·5 M (8½ per cent.) alcohol it rose to 509 in twenty-four hours.

Before death results in an alcohol-treated cell the protoplasm undergoes very pronounced changes in its physiological state, one of which is a change in permeability and a resulting change in osmotic pressure. This is determined by a reduction or an increase in critical concentration of the plasmolysing salt.

Changes in Permeability and Osmotic Value. In this work the critical concentration of plasmolysing salt is that concentration of potassium nitrate which will slightly plasmolyse 50 per cent. of the cells of a leaf within half an hour.

The critical plasmolytic concentration of untreated *Elodea* leaf cells varies considerably. It may be as low as 2 per cent. (0·3 M) or as high as 5 per cent. (0·9 M) KNO_3. It is, therefore, highly important that the critical plasmolytic concentration of control leaves be determined in every experiment, and that these control leaves should come from the same general region of the same shoot of *Elodea*. Whenever possible the treated and untreated leaves should come from the same, or at least from neighbouring whorls.

The concentration of potassium nitrate which is isosmotic with the contents of the average *Elodea* cell, i.e. the average critical plasmolytic concentration of untreated cells, is 3 per cent. (0·5 M), and unless otherwise stated this value will be employed for comparison.

Cells placed for one quarter of an hour in 10 per cent. (1·7 M) ethyl alcohol show little change in their osmotic values. After half an hour of treatment there is a noticeable reduction in the critical concentration of the plasmolysing salt—from 3 per cent. of the control leaves to 2·8 per cent. of the treated leaves. Prolonged treatment still further lowers the osmotic value of the alcohol-treated cells. The critical plasmolytic concentration of cells which have remained in 10 per cent. alcohol for three-quarters of an hour (and, of course, have remained alive) is 2·6 per cent. (the control still being 3 per cent.), and after one hour it is 2·5 per cent.

The reduction in osmotic value of cells treated in 10 per cent. alcohol cannot be so readily determined owing to the rapidity with which the cells are killed. With lower percentages of alcohol the gradual decrease in the

osmotic pressure of the cell, as determined by the critical concentration of the plasmolysing salt, is very marked and readily followed.

In 8 per cent. (1·4 M) ethyl alcohol the decrease in critical plasmolytic concentration is noticeable after one hour of treatment, although the amount of reduction is then slight, being 0·25 per cent. After four hours of treatment the decrease in critical concentration is 0·75 per cent., after seven hours 1 per cent. (control 3 per cent., treated 2 per cent.). There is no further reduction, although the critical concentration remains low for some hours.

With prolonged treatment in 8 per cent. alcohol there is a gradual *increase* in critical concentration of plasmolysing salt, until, after one or two days, the osmotic value of the treated and still living cell has risen from the low value reached during the first few hours of treatment to that of the normal untreated cell.

If we wish to follow more accurately the change in osmotic pressure of a cell due to the effect of alcohol it is best done with a solution of still lower percentage. This has been done for 3 per cent. (0·5 M) ethyl alcohol. The values are given in the form of a curve in Fig. 4. The abscissae are days of treatment. The ordinates are critical plasmolytic concentrations.

It will be noticed that there is no change in the critical concentration of salt (and therefore in the osmotic value of the cell) for eight hours. In eighteen hours the critical plasmolytic concentration has fallen to 2·2 per cent. (the normal being 3 per cent.). In twenty-four hours it has risen to 2·25 per cent. After two days of treatment the osmotic pressure of the treated leaves again equals that of the untreated. In two and a half days it

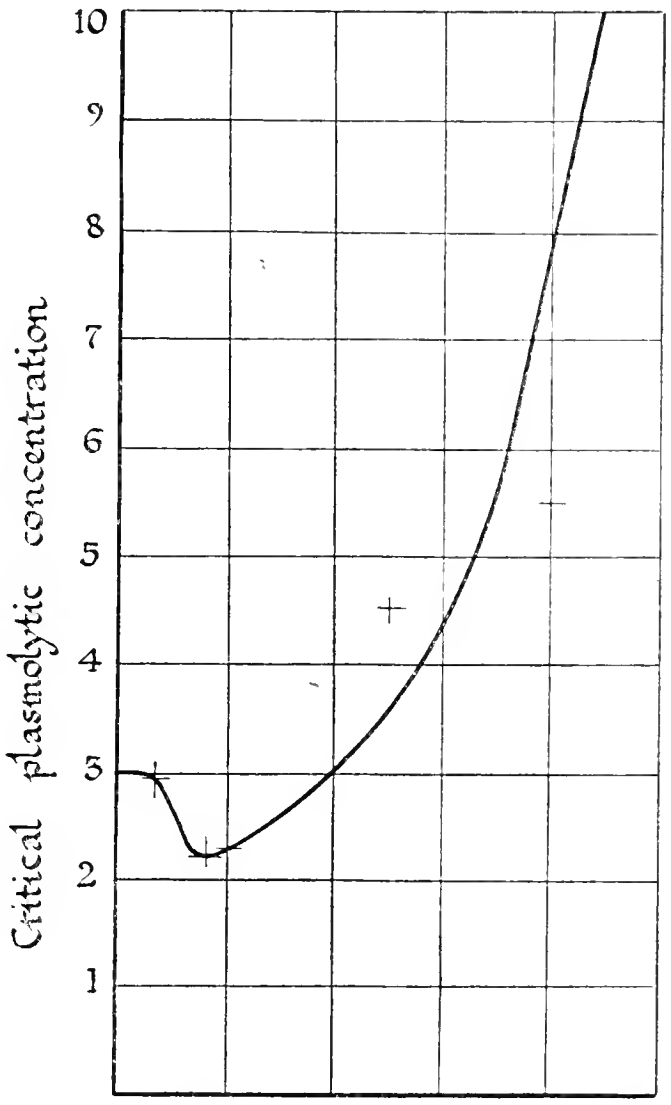

FIG. 4. The curve depicts the change in concentration of potassium nitrate necessary to plasmolyse cells treated in 3 per cent. ethyl alcohol. The ordinates are the critical plasmolytic concentrations, 3 per cent. being that of the untreated leaf. The abscissae are days of treatment. Eight hours' treatment in 3 per cent. ethyl alcohol are necessary before an appreciable reduction in critical concentration of potassium nitrate takes place. After eighteen hours' treatment the lowest critical plasmolytic concentration (2·2 per cent.) is reached. After two days' treatment the critical concentration has returned to the normal (3 per cent.). In two and a half days it has risen far above normal (to 4·5 per cent.), in four days to 5·5 per cent., and in five days to above 10 per cent. Since the osmotic pressure of the cell varies directly as the critical plasmolytic concentration, the curve indicates also the changes in osmotic value due to treatment in ethyl alcohol.

has risen 1·5 per cent. above the control (to 4·5 per cent. KNO_3), in four days to 2·5 per cent. above normal, and in five days the cells cannot be plasmolysed with a 10 per cent. KNO_3 solution. They are, therefore, in all probability dead (a very few cells remain alive).

While the reduction (and subsequent increase) in the osmotic pressure of alcohol-treated cells is quite clearly seen when the values of critical concentrations of salts are used to plot a curve, as in Fig. 4, the most convincing demonstration of the reduction in osmotic pressure of a cell resulting from treatment in alcohol is to be had by observing the immediate effect of a plasmolysing salt of a concentration slightly above the critical for untreated cells.

If *Elodea* leaves of average resistance to alcohol are left in 8 per cent. (1·4 M) ethyl alcohol for six or eight hours and are then plasmolysed, together with untreated leaves, by a 4 per cent. KNO_3 solution, practically *every protoplast in the treated leaves will be prominently plasmolysed into a sphere within two minutes, while of the control leaves only relatively few cells will be but slightly plasmolysed.*

Another of the physiological changes which an alcohol-treated cell undergoes is a pronounced stimulation of protoplasmic streaming.

Stimulation of Protoplasmic Streaming. Short treatment in 10 per cent. ethyl alcohol is too brief a time in which to arouse the cells to active streaming before death ensues, but in low grades of alcohol with longer treatment the increase in streaming—in rate, in number of cells exhibiting it, and in types of streaming—is very marked indeed. For example, in 3, 4, or 5 per cent. alcohol after two or three days of treatment nearly half of the total number of cells in a leaf exhibit active streaming.

While the amount and rate of streaming is very pronounced in alcohol-treated cells, the most interesting feature of this stimulation to protoplasmic activity is the great variety of abnormal types of streaming. In an alcohol-treated cell, the chloroplasts may be arranged in a belt encircling the protoplast at its centre. There may at times be two such belts, one at each end of the cell. Especially interesting is the fact that these two belts of chloroplasts in a single cell may be revolving in *opposite* directions. There may also be active streaming of a few chloroplasts around a mass of quiescent chloroplasts, the latter so fixed in their position that they are not dislodged and drawn into the stream even when the streaming chloroplasts rub against them.

Conclusion.

The initial effect of ethyl alcohol on the *Elodea* leaf cell is a pronounced increase in protoplasmic permeability permitting an exosmosis of electrolytes which results in a lower osmotic pressure within the cell, and, consequently, in a lower critical plasmolytic concentration. These

initial changes in the physical properties of the cell are followed by an increase in critical plasmolytic concentration, and therefore by an increase in osmotic value and at least a partial decrease in permeability, as a result of prolonged treatment in ethyl alcohol. Apparently, the first effect of alcohol on protoplasm is a dilution, a dispersal of the colloidal particles of the membrane. There at least takes place some kind of destruction, a breaking down of the membrane structure, which results in increased permeability, and consequent exosmosis of dissolved substances in the cells.

These results agree well with those of Stiles and Jørgenson (23, p. 427) who found that ethyl alcohol renders the plasma membrane of potato more permeable to the solutes dissolved in the cell sap, causing a pronounced reduction in osmotic value. These workers observed a slight initial rise in osmotic pressure of the cell, which apparently takes place before the alcohol has had time to alter the membrane. The brief initial increase in osmotic value is observed only in relatively dilute alcohol solutions. Stiles and Jørgenson did not, however, observe a *rise* in osmotic value after prolonged treatment in alcohol, as was so marked in these experiments on *Elodea*.

The subsequent rise in osmotic pressure of the cell after twenty-four hours of treatment in 3 per cent. alcohol (determined by an increase in critical plasmolytic concentration) is interpreted as meaning a decrease in permeability. That there has been at least some reduction in permeability from the porous condition of the membrane attained after eighteen hours of treatment is probable, otherwise the high osmotic value subsequently reached could not be maintained, but that there has been a further decrease in permeability beyond that of the normal, and comparable with the pronounced rise in osmotic pressure, is not conclusive. The pronounced increase in osmotic value following the initial decrease due to treatment in alcohol may not be the result of permeability change at all, but of death processes.

The ultimate effect of alcohol on the protoplasm of *Elodea* is coagulation. Death, which results from long treatment in alcohol, is undoubtedly due to coagulation, as is evident from the fact that the unplasmolysable protoplast is often contracted, i.e. it is slightly shrunken away from the cell wall; that the surface of this shrunken mass is rough; and that the dead protoplasm is coarsely granular in appearance, i.e. it is a coagulum. The reversal of the initial effect of alcohol is probably due to secondary reactions which take place within the cell as a consequent of the initial toxic influence of the reagent.

PART II.

THE REACTION OF PROTOPLASM TO THE GLUCOSIDES, SAPONIN, SMILACIN, AND SENEGIN.

Experimental Data.

Lethal Effect of the Glucosides. What has been said of alcohol could almost be repeated to describe the effect of saponin on protoplasm. The rate at which the cells are killed, the rapid decrease in osmotic value of the cell contents as a consequent of increased permeability, and the marked stimulation to streaming, are all equally pronounced in cells treated in saponin as in those treated in alcohol.

If leaves of *Elodea* are placed in a one per cent. solution of saponin, smilacin, or senegin, very few of the cells will be killed in two days by saponin, half will succumb from the effects of smilacin, and more than three-fourths will be killed by senegin. These values are averages. Some cells will resist the toxic effect of a one per cent. solution of saponin for six days (of a half per cent. solution for ten days), and of a one per cent. smilacin solution for three days. Senegin is much more toxic. A one per cent. solution of this glucoside will, in twenty-four hours, cause harmful effects which are observable to the unaided eye in the discoloration of the leaves.

Changes in Permeability. If the critical concentrations of a plasmolysing salt are periodically determined for cells which have remained in a one per cent. solution of saponin for from one-half to six days, the values will gradually fall below the normal for two or three days, the time depending on the resistance of the cells to saponin, and then rise to the normal and beyond it. The average reduction in critical concentration of salt is 0·5 to 0·75 per cent. The greatest reduction obtained was one per cent. after sixty-six hours of treatment (control 3·5 per cent. of KNO_3, treated 2·5 per cent.). After seventy-two hours' treatment the critical plasmolytic concentration of treated cells may be double that of the normal, namely 6 per cent. It is evident, therefore, that the curve in Fig. 4, based on data obtained from alcohol-treated cells, would, with slight modification, serve to demonstrate the change in critical plasmolytic concentration (and therefore of osmotic pressure) of saponin-treated cells.

As stated in the case of alcohol-treated cells, the most convincing demonstration of the reduction in critical plasmolytic concentration and osmotic value of the treated protoplast is to be had by observing the immediate effect on untreated and treated leaves of a plasmolysing salt of a concentration slightly above the critical value for normal cells. If this is done *in the control leaf some few cells will be slightly plasmolysed*

in a few minutes, while in saponin-treated material nearly every cell in the leaf will be prominently plasmolysed.

The change in critical concentration of salt just described for saponin material also takes place in smilacin and senegin treated cells, with the difference that the reduction in osmotic value of the cells is more rapid in senegin than in saponin.[1]

Throughout this work a *decrease* in the critical concentration of the plasmolysing salt is interpreted to mean that there has been a reduction in osmotic pressure of the cell contents due to an *increase* in permeability; that is, that during treatment there has been an increase in permeability of the plasma membrane which has permitted an exosmosis of dissolved substances from the cell resulting in a lower osmotic value; consequently, a salt solution which is isosmotic with the contents of the treated cell will be of lower concentration than the salt solution which is isosmotic with the untreated cell.

There are two criticisms which can be directed against the above point of view. First, a change in osmotic value of the cell contents is not in itself alone conclusive evidence of a change in permeability. Variations in the osmotic pressure of a cell might well occur as a result of chemical change within the cell without any alteration in the permeability of the plasma membrane. The synthesis of sugar from the organic acids, or of cane sugar from two molecules of glucose, or the formation of insoluble starch from cane sugar, would greatly lower the osmotic value of the cell without any change in permeability. The toxic substance itself (alcohol or saponin) might, and probably does, break down large indiffusible molecules into smaller diffusible ones, permitting exosmosis of them without any change in permeability. Second, a reduction in critical plasmolytic concentration may mean a *decrease* in permeability as a result of treatment. This opinion is held by some workers, the assumption being that a reduction in critical plasmolytic concentration implies that, because of decreased permeability, the plasmolysing salt can less readily enter the cell, and will, therefore, very slowly raise the concentration (osmotic value) of the cell interior up to that of the external salt: consequently, a lower concentration of salt will plasmolyse the treated cell than will plasmolyse the normal cell. Conversely, an *increase* in critical plasmolytic concentration may mean that there has been an *increase* in permeability, since then the external salt solution can more readily enter the cell and raise its osmotic value, therefore a higher concentration of salt is needed to plasmolyse the treated cell.

If the above interpretation is correct it would explain the apparent discrepancies between the results of Osterhout (17, p. 318) and those of the

[1] The critical concentration of smilacin-treated cells is determined with difficulty owing to the rapid collapse of the slightly plasmolysed cells. (This phenomenon will be considered later.)

writer (21) with $SrCl_2$. The writer has found that $SrCl_2$ causes an *increase* in permeability and not, as Osterhout maintains, a decrease. This conclusion is based on the assumption that a lower critical concentration of plasmolysing salt indicates increased permeability. It becomes important, therefore, to ascertain conclusively whether or not there has been an *increase*, as the writer believes, or a *decrease* in permeability of the protoplasmic membrane when the critical plasmolysing concentration is *lower* as a result of treatment. This was accomplished for smilacin-treated cells by the following experiment.

If, as a result of treatment, there has been *no* alteration in the permeability of the plasma membrane, then smilacin-treated cells should prove to be no more sensitive to alcohol than normal cells. If permeability has *decreased*, then the treated cells should actually be less susceptible to the toxic effect of alcohol. If, however, permeability has been *increased* by treatment in smilacin, as the writer believes a *decrease* in critical plasmolysing concentration indicates, then the treated cells should be *more* susceptible to alcohol than untreated ones, since the increased permeability of the treated cells will permit a more ready entrance of the alcohol.

Leaves which had been in a 0·5 per cent. solution of smilacin for eighteen hours were treated with 10 per cent. KNO_3 to ascertain the number of cells killed by the glucoside. The average number of dead cells was but 0·8 per cent. of the total. Other leaves which had undergone the same treatment in smilacin for eighteen hours were, together with some control leaves, put in 10 per cent. alcohol for ten minutes. By referring to the curve in Fig. 2 it will be seen that ten minutes in 10 per cent. alcohol is not sufficient time to kill any cell in a normal leaf. This was true of the control leaves above mentioned. *The cells of the smilacin-treated leaves, however, succumbed to the extent of* 92 *per cent.* as the result of a ten-minute treatment in alcohol. A similar experiment was performed, keeping the saponin-treated and the untreated leaves in 10 per cent. alcohol for twenty minutes. Seventeen per cent. of the cells were killed by alcohol in the control leaves. *In the smilacin-treated leaves* 95 *per cent. of the cells were killed* by the alcohol. The evidence seems conclusive that the plasma membrane is a more open one (since it is more permeable to alcohol) in smilacin-treated leaves than in normal leaves.

Further proof of a more permeable membrane as the direct effect of these glucosides on the plant cell is to be had from another observation. It was frequently observed that the chloroplasts of smilacin-treated cells suddenly scattered, just as if a miniature explosion had taken place within the cell, after the addition of potassium nitrate and before plasmolysis occurred. This is due undoubtedly to the effect of the salt which rapidly enters the smilacin-treated cell through its more permeable membrane.

Another phenomenon which adds further proof in support of the fact

that a greatly increased permeability of the plasma membrane results from treatment in the saponins, is the sudden collapse of smilacin-treated cells after slight plasmolysis has taken place following the application of the plasmolysing salt.

In attempting to obtain the critical plasmolytic concentration of cells treated in 0·5 per cent. smilacin, the immediate plasmolysis of many cells is often recorded, but on observing the leaves at the expiration of the half-hour period no plasmolysed cells are to be seen. Their collapse, i. e. a sudden return of the plasmolysed protoplast to its original size, was subsequently observed. The resulting protoplast was a much disorganized one. This collapse was less often observed in saponin and senegin treated cells, but was the rule in smilacin-treated cells.

Of fifteen smilacin-treated cells observed (after twenty-one hours in a 0·5 per cent. solution) all plasmolysed to a moderate degree after four minutes in 4 per cent. KNO_3. Then one plasmolysed protoplast suddenly collapsed. In five minutes five out of the fifteen protoplasts had collapsed. In nine minutes all had collapsed. This can only be interpreted to mean that the salt had entered at an abnormally rapid rate through a very porous membrane, and caused complete disorganization.

A fact worthy of note in connexion with this sudden collapse of smilacin-treated cells is that the collapse was only obtained with a low percentage of salt, slightly above the critical concentration, and never with a high percentage of salt. One might expect a more abundant entrance of ions through the open membrane from a 10 per cent. KNO_3 solution than from a 4 per cent. solution, yet this was not true. Smilacin-treated cells plasmolysed with 10 per cent. KNO_3 never collapsed. The explanation is clear. The 4 per cent. salt entered freely without causing any change in the very permeable membrane. The 10 per cent. salt, on the other hand, coagulated the dilute membrane, thus instantly decreasing its permeability and preventing the subsequent entrance of the salt.

The great readiness with which alcohol and potassium nitrate enter the protoplast of smilacin-treated cells is evidence of a much more permeable membrane in these cells, thus substantiating the interpretation that a *decrease* in critical plasmolytic concentration means an *increase* in permeability.

That saponin markedly increases the permeability of a protoplast is also the belief of Boas (3), who arrives at this conclusion from experiments of an entirely different nature than those of the writer. Boas subjected oats to a 5 per cent. solution of saponin for twenty minutes, then placed them in 25 per cent. cane sugar and measured the amount of carbon dioxide formed. In two hours the saponin-treated material had produced three times the amount of carbon dioxide that the control material had, and in six hours' time twice as much. The increase in fermentation of the sugar is a result of increased permeability of the cells.

The term plasma membrane is here employed to mean that part of the cell which is concerned in the phenomena of permeability without necessarily implying that all permeability phenomena are traceable to the activities of a morphologically definite membrane on the surface of protoplasm. That the surface layer of the protoplast is not the only region which is concerned in permeability changes is evident from the following observed phenomenon.

The layer of protoplasm lining the wall of a normal *Elodea* cell is relatively thin. In saponin-treated cells this protoplasmic layer may become *increased to five times its normal thickness* through excessive imbibition of water due to highly increased permeability of the protoplast *as a whole.*[1]

Protoplasmic Streaming. Streaming of protoplasm is greatly stimulated by the saponins. The number of cells exhibiting streaming, the rate, and the abnormal types are exceedingly great.[2]

Discussion.

The similarity in the reaction of protoplasm to two such widely differing substances as alcohol and saponin is most striking. It will be of interest to consider how far existing theories of permeability go towards explaining the effect of alcohol and saponin on protoplasm.

Overton (19), in his classical work on the osmotic properties of the cell, found that those alcohols which are most toxic to the living cell are also the alcohols which most readily attack lipoids. He therefore concluded that the chief constituents of the plasma membrane were lipoids. This lipoid theory of Overton was widely accepted at the time (1901) and prevailed until Traube (24) twelve years later advanced the hypothesis that a substance entered a cell in proportion to its capacity to lower surface tension; and the more rapidly it entered, the more toxic was its influence. Consequently, 'isocapillary' solutions of different narcotics produce the same end result because the same quantity enters in the same length of time.

Following Traube's surface-tension conceptions came the theory of Czapek (6). According to Czapek, water-soluble and surface-tension active substances begin to effect exosmosis of the contents of plant cells when of a concentration with the tension value of 0·685, which Czapek believed to

[1] One may here refer briefly to the bearing of this excessive imbibition of the protoplast as a whole on the structure of protoplasm. Some workers are in the habit of emphasizing the 'liquid nature' of protoplasm, and of regarding the living substance as miscible in water, apparently, therefore, assuming that the living colloid is an emulsion of protoplasm in water rather than one of water in protoplasm. Such excessive imbibition as above described could have been manifested only by a colloidal *jelly*. It is further of interest to note that *this highly swollen protoplasmic jelly continues active streaming.*

[2] A more detailed description of the effect of the saponins and of other toxic substances on protoplasmic streaming is given in an article recently published (20), to which the reader is referred.

be the surface-tension value of protoplasm based on a value of 1 for water against air. Czapek came to this conclusion because many of the substances with which he treated cells first began to noticeably affect the osmotic properties of the membrane when they were of such a concentration as to have a surface-tension value of about 0·685 (against air). He concluded, therefore, that the tension value of protoplasm must be 0·685, and that to enter the plasma membrane a reagent must be of a lower surface tension.

All of these theories have been discarded by Warburg (26), who has shown that the degree of toxicity of the methyl, ethyl, propyl, butyl, amyl alcohol series is much more closely proportional to the adsorptive powers of these alcohols than either to their lipoid solubility or their surface-tension values (capillary constants). Warburg arrived at this deduction in the following manner:

If red blood corpuscles (of bird) are frozen the thin cell membrane is burst. On thawing, a liquid mass is obtained in which the 'solid'[1] cell constituents float freely. If this liquid is centrifugalized two layers are obtained: an upper, clear, granule-free one, and a lower, cloudy layer containing the 'solid' cell particles. If one measures the respiration of the two layers separately one finds that only the lower layer respires. From this Warburg concludes that *respiration depends upon the solid constituents of the cell* (26, p. 135).

Warburg next studied the oxidation of inanimate substances, noticing the effect which narcotics have on their 'respiration'.

Freundlich (8, p. 163) has shown that if an aqueous solution of oxalic acid is shaken with blood carbon, there takes place a rapid decrease in concentration of the oxalic acid due to adsorption of the acid by the carbon. Warburg sought for a possible chemical reaction, and found that oxidation of the oxalic acid into carbon dioxide and water takes place. *This oxidation process can be retarded by narcotics just as can cell respiration.*

In a similar manner, if an aqueous solution of cystin—the sulphur containing amino-acid of egg albumin—is added to carbon and aerated, the amino-acid is oxidized into CO_2, NH_3, and H_2SO_4. The same end products are produced as in the case of the oxidation of albumin in living cells. This oxidation can also be retarded by narcotics.

The retardation of these inanimate oxidation processes by narcotics rises with the adsorption constants of the narcotics used (methyl-, ethyl-, propyl-, and phenylurethane). For example, methylurethane, which is at the bottom of the scale with a low adsorption constant, must be of a concentration of 0·5 mol. per litre to produce a 34 per cent. retardation of oxidation of the oxalic acid, while phenylurethane with a high adsorption con-

[1] The 'solid' cell constituents to which Warburg refers are the stromata of the blood corpuscles, not solid particles of colloidal size.

stant requires a concentration of but 0·005 mol. per litre to cause the same (34 per cent.) retardation in oxidation. This same relationship holds in the retardation of oxidation in living cells; thus, a 10 mols. per litre solution of methylurethane is necessary to cause a 60 per cent. retardation in respiration in red blood corpuscles, while but a 0·05 mol. per litre solution of phenylurethane is necessary to lower respiration the same amount.

The retardation of oxidation is looked upon as a consequent of a diminution of the free adsorptive surface of the carbon (or of the living solid particles) due to the adsorption of the narcotic. That is, since oxidation is augmented by an increase in adsorptive surface, just as are many other chemical reactions (e g. gas reactions are accelerated by the presence of quartz, porcelain, carbon, &c.), then a decrease in this surface by the adsorption of another substance, the narcotic, would cause a decrease in oxidation.

From the above experimental observations by Warburg, one can conclude that narcosis is an adsorption phenomenon; that the toxic effect of a narcotic is proportionate to its adsorption index; and that the chemical nature of the *adsorptive* substance (e. g. whether lipoid or protein) is not a factor in narcosis.

To consider the newest of these several theories first, it would seem clear that while Warburg's theory of narcosis is a most convincing one, and stands as the best explanation which we have of narcosis—in so far as narcosis consists in a suppression of respiration—it cannot be accepted as a theory which covers all permeability phenomena. It stands as a simple and attractive explanation of a very complex vital process, but whether the whole story is told is another matter. It is a debatable question whether the striking increase in permeability produced by such different substances as alcohol and saponin (and strontium and copper[1]) is in each case the outcome of the same single simple physical phenomenon.

The Overton lipoid theory of protoplasmic permeability is well supported by the reaction of protoplasm to the saponins, and possibly also by the toxic effect of alcohol. How far lipoids play a part in the toxicity of alcohol on protoplasm it is difficult to say. Lecithin is easily soluble in concentrated alcohol (12, pp. 153–4), but cholesterin is practically insoluble in cold aqueous solutions of alcohol (2, p. 1071). One would, therefore, not expect so low a concentration of alcohol as 10 per cent., which is highly toxic, and still less would one expect a 2 per cent. concentration, which we have seen is sufficiently concentrated to produce pronounced changes in permeability, to strongly attack the lipoid constituents of the plasma membrane. However, it is conceivable that higher concentrations of the alcohol could be arrived at as a result of adsorption on the membrane surface, a process which probably takes place. The concentration of alcohol

[1] For the results on strontium and copper see (21).

on the protoplasmic surface would then in time, even in a 2 per cent. solution, be sufficiently high to react with the lipoids. But, after all, there is no sound reason for believing lipoids to be the sole constituents of the plasma membrane. If we accept Nathansohn's (14) mosaic conception of membrane constitution—and it seems to me that we must—then we are forced to grant the likelihood of alcohol attacking proteins and other possible constituents of the cell membrane.

Saponins of low concentration attack (emulsify) lipoids. That lipoids are a part of the chemical make-up of the plasma membrane is generally accepted. MacDougal (11), without emphasizing the presence of any one single substance in the plasmatic membrane, regards lecithin as an important constituent. Other workers, Boas (4), Kahho (10), *et al.*, have been led by their experimental results to the belief that lipoids are one of the chief constituents of the plasma membrane. Bayliss (1, p. 130) regards certain permeability and other phenomena as attributable to the presence of a lipoid peripheral layer.

It is unwise, in the present limited state of our knowledge of the chemical constitution of protoplasm, to give undue emphasis to any one single substance in the membrane, yet that lipoids are one of the possible components of the protoplasmic surface layer seems likely. Whether they occur as free fat or, more likely, in chemical union with proteins (15), we do not fully know. Whatever the actual chemical make-up of the membrane is, the experimental data here given stands in opposition to the assumption of Grafe (9, p. 29), that a knowledge of 'the purely chemical constitution of the cell constituents has not given any explanation of the entrance and exit of different substances'.

The Overton lipoid theory must still stand as one of the possible factors concerned in permeability phenomena.

It is reasonably clear that both alcohol and saponin increase permeability in the *Elodea* cell. Such an increase in permeability means a more dispersed membrane, and therefore a lower surface tension. Conversely, a reduction in the surface tension of protoplasm will mean increased permeability. There is, consequently, good reason to regard surface tension forces as playing a part in changes in permeability. It seems likely, therefore, that the surface-tension ideas of Traube and Czapek may not be as fallacious as some have implied. That a reduction in surface tension actually does take place in the treated cells is confirmed by the following observations:

Untreated *Elodea* leaf cells mostly plasmolyse with *concave* surfaces, which are often very deeply concave, indicating a high surface-tension value of the plasma membrane of the normal cell. The treated cell (half an hour in 10 per cent. alcohol), on the other hand, plasmolyses, nine times out of ten, with a *convex* surface, even when the plasmolysis is ever so slight,

indicating lower surface tension, since the membrane readily loses its hold on the cell wall.

A still more beautiful demonstration of a reduction in surface tension is to be had from the absence in alcohol-treated cells of those delicate protoplasmic strands which radiate from a plasmolysed protoplast to the cell wall in an untreated *Elodea* cell (5, p. 18). These long fine protoplasmic threads could only be formed by a protoplasmic surface of high tension value.

Czapek maintained that a substance must have a tension value less than 0·685—which he thought to be the surface-tension value of protoplasm (water being 1)—in order to affect the diosmotic properties of protoplasm. Czapek based this theory on the idea that, since a lower tension value means greater surface activity, then an entering substance must possess greater surface activity, consequently a lower surface tension, than the constituents of the plasma membrane in order to pass through the membrane. It will be interesting to see how the surface-tension values of the saponins and the alcohols used compare with the critical value given by Czapek.

The surface-tension value of 1 per cent. ethyl alcohol is 0·933, of 8 per cent. alcohol it is 0·720, of 9 per cent. 0·700, and of 10 per cent. 0·682.[1] The surface-tension value of 1 per cent. saponin is 0·885, of 1 per cent. senegin 0·819, and of 1 per cent. smilacin 0·681.[2] It will be seen that while none of the surface-tension values of the three glucosides, with one exception, that of smilacin, are near the critical toxic value of 0·685 set by Czapek, yet the tension values of the glucosides and of all the more prominently toxic concentrations of alcohol are considerably less than 1.

It is of interest to note that 9 per cent. ethyl alcohol, which we found to be a critical concentration in the toxicity of this alcohol for protoplasm—since above this percentage cells are killed in relatively few minutes, while below it many hours are necessary to kill—has a tension value of very nearly the critical value set by Czapek.

We have seen, however, that a much lower percentage of ethyl alcohol than 9 is sufficient to effect osmosis of the cell contents. As low as a 3 per cent., even a 1 per cent., solution will, in several days, materially affect the diosmotic properties of the cell. We can only explain the effect of the low percentage of alcohol, on the basis of Czapek's theory, by assuming that there is a concentration of the alcohol at the surface of the protoplast. In time the alcohol thus concentrated on the plasma membrane will be of sufficient strength to have a surface-tension value low enough to cause a reduction in the tension of the protoplasmic surface, and consequently increase the permeability of the membrane. While such an assumption is

[1] These values are taken from Duclaux (6, pp. 22–3).

[2] These values were obtained by comparing the number of drops of water with the number of drops of reagent, of the same volume and temperature, from a Traube stalagmometer. The value of water is taken as unity.

possible, yet, without experimental data to support it, it seems preferable to regard the critical toxic tension value set by Czapek as one not proven to exist in the sense which Czapek intended it.

The theory of Czapek was early criticized by Vernon (25), and later received drastic criticism at the hands of Stiles and Jørgensen (22). These workers repeated the experiments of Czapek, employing the electrical conductivity method of Kohlrausch for determining the exosmosis of electrolytes. As a result of their experiments they conclude that, 'not only is the theory founded by Czapek upon his experimental results untenable, but . . . the facts on which he bases his theory are merely illustrations of experimentation due partly to the crudity of the method employed, and partly to a selection and arbitrary arrangement of experiments' (22, p. 50).

That Czapek's theory is not universally applicable is true. One need merely consider the 'oligodynamic' (9) action of copper water (the term is Nägeli's, and refers to the extremely high toxicity of water in which there is a slight trace of copper). The action of copper water (water in which several copper coins had remained overnight) on *Elodea* leaves was found by the writer (21), in its effect on permeability and protoplasmic streaming, to be similar to and as pronounced as that of saponin. The same is true of a weak (1 per cent.) solution of strontium chloride. Yet both of these highly toxic solutions possess a surface-tension value which differs exceedingly little from that of pure water.

Czapek himself found that some reagents do not fit in with his theory, and he assumed in these cases that a 'special toxic action' takes place. Such an assumption is destructive to his theory in so far as its universal applicability is concerned. However, in reply to Vernon, Czapek made one statement which stands in substantial support of his hypothesis. He (7, p. 111) stated that his theory must collapse *only* when there has been found *one* substance which in spite of a surface-tension value below 0·685 is *not* deadly.

Czapek's theory fails utterly as a complete explanation of all permeability changes due to the poisonous action of toxic substances. But the entire sweeping aside of the theory is not, in my opinion, justifiable; that is, we cannot completely ignore the possibility of alterations in surface tension being a factor, and possibly a very prominent factor, in permeability changes.

It is most remarkable that substances which differ chemically so widely as do ethyl alcohol and saponin (and strontium chloride and copper oxide) should all cause the same pronounced change in the physiological condition of protoplasm. One hesitates to believe that any one theory will explain the reaction of protoplasm to all these reagents even though the end result, an increase in permeability and a stimulation to streaming, is the same in each case.

It seems that there are two criticisms which can be directed against all theories of protoplasmic permeability so far advanced: first, their authors have attempted to explain a complex vital phenomenon on the basis of a single physical process; and second, results obtained on one type of tissue are allowed to form the foundation of a theory intended to cover all living systems.

Summary.

1. Ethyl alcohol of a concentration of 10 per cent. will kill the cells in the average leaf of *Elodea* at the following rate: 0·1 per cent. of the total number are killed in 15 minutes, 1 per cent. in 20 minutes, 60 per cent. in 30 minutes, 80 per cent. in 1 hour, 95 per cent. in 2 hours.

2. The average cell of the leaf of *Elodea* is killed in the following concentrations of ethyl alcohol in the following respective lengths of time: 10 per cent. alcohol in 28 minutes, 9 per cent. in 2 hours, 8 per cent. in 12 hours, 7 per cent. in 18 hours, 6 per cent. in 26 hours, 5 per cent. in 40 hours, 4 per cent. in 3 days, 3 per cent. in 5 days, 2 per cent. in 10 days.

3. Short treatment of the *Elodea* leaf cell in ethyl alcohol results in a reduction of osmotic pressure within the cell due, apparently, to an increase in permeability and consequent exosmosis of the cell contents. With longer treatment the osmotic value of the cell rises until it far surpasses the value of the untreated cell.

4. Treatment in ethyl alcohol causes a pronounced stimulation to streaming in the *Elodea* leaf cell.

5. The permeability of the *Elodea* leaf cell is increased and the osmotic value, therefore, decreased by brief treatment in a 1 per cent. solution of the glucosides, saponin, smilacin, and senegin. Longer treatment results in an increase of osmotic pressure.

6. A change in the critical plasmolytic concentration of the salt employed for determining the osmotic pressure of a cell may mean either an increase or a decrease in permeability depending upon the interpretation. It was possible to show by several different observations that a *lower* critical plasmolytic concentration means *increased* permeability in the case of saponin-treated cells.

7. The saponins greatly stimulate the streaming of protoplasm.

8. The theories of Overton, Traube, Czapek, and Warburg, bearing on permeability and narcosis, are considered, and it is shown that each theory is supported by experimental evidence, but none of them are alone sufficient as a complete explanation of all permeability phenomena.

The experimental work on which this article is based was carried out in the laboratories of the Botanical Institute of the University of Geneva, Switzerland, where I enjoyed the privileges of a guest through the courtesy

of Professor R. Chodat. I wish to express to Professor Chodat my appreciation of his kindness in placing the facilities of the Institute at my disposal and of his helpful assistance during the progress of the work. To Professor W. Stiles, of Reading, England, I am also indebted for valued criticism of the manuscript of this article.

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Diurnal Variations in the Total Nitrogen Content of Foliage Leaves.

BY

ALBERT CHARLES CHIBNALL.

(*From the Biochemical Department, Imperial College of Science and Technology.*)

DURING the past two or three years the writer has had occasion to refer to the literature dealing with the nitrogen content of leaves. It is a subject that has been treated by chemist, botanist, and agriculturist, consequently papers concerning it are scattered in a number of journals of diverse interests, often difficult of access. Czapek (3) has made a praiseworthy effort to collect them, but the limitations imposed on the writer of a handbook such as his have naturally prevented him giving anything but the briefest discussion on the subject-matter contained therein.

In the present paper the author proposes to discuss only one aspect of the subject, that concerning the diurnal variations in the total nitrogen content of leaves. He is prompted to do this because some workers have failed to grasp one significant fact when estimating these variations, namely, that the total nitrogen makes up but a small fraction of the total weight of a leaf, and that the large normal variation in other constituents of the leaf, such as carbohydrates or water, may quite prevent the change in total nitrogen appearing in its true value unless proper allowances are made.

A diurnal change in the nitrogen content of a leaf at night, if it can be definitely established, is a point of physiological interest, in that it may be used as a stepping stone to the elucidation of a complex and important problem that is still more or less a mystery, that of the nitrogenous metabolism in the leaf.

At any moment the total nitrogen content of a leaf depends on the two following variables:

1. The rate of translocation through the petiole *into the leaf* of simple nitrogenous substances, such as nitrates, ammonium salts, &c., from the root system.

2. The rate of translocation of nitrogenous substances *out of the leaf* through the petiole to other parts of the plant. If (1) rises relative to (2) nitrogen will accumulate; whilst if (1) falls relative to (2) nitrogen

will be lost. Now the inward and outward flow of nitrogen through the petiole cannot be tapped, consequently it is impossible, by direct chemical analysis, to determine the nature of the nitrogenous substances that pass out of the leaf. But if it can be definitely established that the total nitrogen in a leaf falls at night, then, by chemical analysis, it may be possible to determine from what substances (such as protein) the outgoing nitrogen has originated. This would be an important advance, for though it is known that the protein is synthesized from nitrates, &c., translocated from the root system, very little is known as to its function in the leaf cell and the composition and ultimate fate of its degradation products. Such analyses have already been made (Kosutany (6), Suzuki (12), Schulze and Schütz (11), Chibnall (2)), but it is not proposed to discuss them here. Their interpretations depend primarily on the method by means of which the comparison of the day and night leaves have been made. In the following pages these methods are discussed in some detail, and the conclusion is drawn that one of them, that which estimates the nitrogen as a percentage of the dry weight of the leaf, gives an inaccurate and misleading result.

In comparing the day and night samples of leaves three methods have been used to estimate the total nitrogen present, namely, those in which it is expressed as

A. Weight in terms of a certain number of leaves,
B. A percentage of the dry leaf-weight,
C. A percentage of the fresh leaf-weight.

It is proposed to examine each of these in detail to ascertain if the diurnal change given by them is reliable.

Method A. If every leaf on a plant was directly comparable with every other, this method would give the absolute diurnal change; but since this is not so the question of sampling errors at once arises. The two workers whose results obtained by this method are discussed later have not determined these. An idea as to their magnitude, in the case of annuals, may be gathered from some experiments made by the present author with the leaves of *Vicia Faba maj.* Twelve plants, about six weeks after they had appeared above ground, were used. All the leaves from each plant were picked and weighed. Probable errors, calculated by Peter's formula, are given in Table I.

TABLE I.

Showing Probable Errors of Leaves from Vicia Faba maj.

	Number of leaves per plant.	*Weight of leaves per plant in grm.*	*Average weight of a leaf in grm.*
Mean of 12 plants	35	19·75	0·574
Probable error of the mean of 12 plants	±1·17	±0·99	±0·025
Percentage error of the mean	3·34	5·0	4·42

The total number of leaves from the twelve plants was 419, of total weight 237 grm. For a sample of this size and weight the probable errors in the total nitrogen are given in Table II, the nitrogen being determined by the Kjeldahl-Gunning method modified for the presence of nitrates.

Table II.

Showing Sampling Errors in the Total Nitrogen of the Leaves of Vicia Faba maj.

	Weight of nitrogen per 100 leaves. (*Method A.*)	*Percentage of nitrogen per total dry leaf-weight.* (*Method B.*)	*Percentage of nitrogen per total fresh leaf-weight.* (*Method C.*)
Mean	0·432	5·71	0·753
Probable error	±0·0195	±0·036	±0·0068
Percentage error	4·50	0·63	0·91

The percentage error in estimating the nitrogen by Method A was 4·50, a figure five times as great as Method C and seven times as great as Method B. Undoubtedly, if circumstances are such that leaves of similar age and size can be used in the diurnal samples (such as the opposite leaflets of the pinnate leaves of the weeping ash) the probable errors by Method A would be lower, but in the absence of any definite data as to the size of these errors, Method A must be considered inferior to Methods B and C.

Method B. Variation in the total dry weight of a leaf will depend on the assimilation of carbon dioxide from the air, on respiration, and translocation of substances into and away from the leaf through the petiole. If the diurnal variation is estimated over a period of darkness, the assimilation of carbon dioxide will be nil. But of the substances leaving the leaf through the petiole only part, probably a small part, will be nitrogen. Clearly then, when the diurnal change in the nitrogen content of a leaf is being estimated by this method, it is not the absolute amounts of nitrogen in the leaves that are being compared, but only the concentrations of nitrogen in terms of total solids. For example: suppose the percentage loss due to translocation of total solids and total nitrogen away from leaf to be the same, then the percentage of nitrogen per dry weight remains unchanged. Similarly, if the percentage fall in the total nitrogen is greater than that of total solids the percentage of nitrogen per dry weight will fall, whilst if the percentage fall in the total nitrogen is less than that of the total solids, the percentage of nitrogen per dry weight will rise. Obviously this method is quite inapplicable for expressing the true diurnal change in the nitrogen content of leaves, since a loss, should it be proportionately less than that of the total solids, will appear as by a rise.

It is true that Brown and Morris (1), Parkin (9), and Davis, Daish, and

Sawyer (4) in their estimations of the diurnal change in sugars and starch have all used the percentage dry weight basis. But in these cases it must be remembered that the major part of the loss in total dry weight of the leaf at night is to be ascribed to the translocation of sugars either pre-existing as such in the leaf, or formed during the night by the hydrolysis of starch. Consequently, as starch and sugars make up only 20–30 per cent. of the total dry weight, it follows that the percentage loss in these substances will always be much greater than that of total solids. Their results then, though they show a lesser fall than that which actually occurred, are of the utmost value.

Method C. When using this method it is necessary to consider not only changes in the total solids, as has been done above, but in the water-content of the leaf as well. The diurnal change in the water-content of leaves has been the subject of several researches, but only two are worth considering here. Livingston and Brown (7) obtained the water-content of the leaf by drying in an oven at 105°. Analysis of the tables given by them for several different types of plants shows that the chief fluctuation in the water-content took place around midday, when it was natural to expect that the rate of transpiration from the stomata would be a maximum. During the night the leaves remained more or less saturated, and the values that they give for the percentage of water over the hours 6–10 p.m. do not differ by more than 1 per cent. from those over the hours 3–6 a.m. Their work was carried out in the summer at the Desert Laboratory at Tucson, Arizona, U.S.A., and as they themselves point out, in cooler or more humid climates, such as are experienced in England or Germany, smaller fluctuations can reasonably be expected.

Knight (5), using the same procedure, has recently demonstrated that this is indeed so, and his figures for *Eupatorium adenophorum* indicate a change of about 0·1 per cent. between 5.30 p.m. and 8.30 a.m. It is by no means certain, however, that these authors are justified in assuming any change in the water-content of their leaves at all. All that they really show is that there is a change in the dry weight of the leaf expressed as a percentage of the fresh weight. This may well be brought about by translocation of solids away from the leaf, for Parkin's (9) work on the snowdrop and Davis, Daish, and Sawyer's (4) on the marigold both show a fall in total sugar at night of 3 per cent. of the dry weight, equivalent to about 0·5 per cent. of the fresh weight of the leaf. It would appear, then, that the only conclusion to be drawn from these results is that the variation in the water-content of the leaf at night must be very small. This view is confirmed by another observation of Knight's (5). He removed a series of leaves from a plant and allowed them to wilt for ten, twenty, and thirty minutes respectively before weighing for water determinations. In these cases no translocation of solids away from the leaves was possible, consequently his obser-

vation, that extreme flaccidity results in a decrease of approximately only 1 per cent. of the water-content of a leaf, shows that the diurnal variation must be extremely small. Now water makes up 80 per cent. to 90 per cent. of the total weight of most fresh leaves. It would appear then that the diurnal change in the total nitrogen, expressed by Method C, will approximate very closely to the actual diurnal change in the leaf. It has already been pointed out that Method A expresses this also, but that the sampling errors, as shown in Table II, are greater. Method C, then, seems the best to adopt as a working basis.

It is now proposed to examine some of the published results in detail.

Schulze and Schütz (11) These workers have made a very thorough investigation into the seasonal and diurnal changes in the leaves of *Acer negundo*. The leaves were picked from two trees fifteen to twenty years old, and those in each sample were chosen as far as possible of equal weight and size. The evening leaves were picked between 6 p.m. and 6.30 p.m., those on the following morning (except in case of 3–6 September) between 5 a.m. and 6.30 a.m. depending on the season. Their results, in so far as they concern the diurnal change in the total nitrogen, are given in Table III. The authors themselves used Methods A and B; it is possible from the data they give to calculate C.

TABLE III.

Showing the Fall in the Nitrogen Content of the Leaves of Acer negundo at Night, expressed as a Percentage of Day Value.

(Schulze and Schütz)

Date of Picking.	*Method A.*	*Method B.*	*Method C.*
7 May	10·16	4·50	9·07
6 June	9·99	2·08	6·88
5 July	7·27	1·76	1·48
2 Aug.	3·97	0·16	−1·76
3–6 Sept.	18·50	0·78	11·90
25 Sept.	−7·54	1·36	−7·18

These figures are probably the best available at the present. In the absence of sampling errors those under Method A must be accepted with caution. Method C shows that when the leaves are young there is a large withdrawal at night, the amount decreasing as the leaf ages until it reaches a value of only 1 per cent. The apparent large gain at night observed on Sept. 25 may be due to dehydration, as the leaves at that date had already begun to fall. It will be observed from Method B that the loss of nitrogen at night was proportionately greater than that of other solids in the leaf.

Suzuki (12) has examined the diurnal changes between 6 p.m. and 6 a.m. of the plants given in Table IV, the figures in round brackets after each name denoting the number of leaves per sample used.

TABLE IV.

Showing the Fall in the Nitrogen Content of Leaves of Various Plants at Night, expressed as a Percentage of the Day Value.

(Suzuki)

Name of Plant.	*Method A.*	*Method B.*
Wisteria brachybotrys (400)	13·6	−1·45
Phaseolus vulgaris (50)	4·3	0·0
,, *mungo* (200)	10·9	−2·24
Pueralia Thunbergiana (51)	4·8	1·39
Solanum tuberosum (220)	8·4	−28·2
Batatas edulis (100)	4·0	−1·33
Polygonum fagopyrum (450)	8·3	−3·82
Helianthus annuus (33)	9·7	−2·09

Sufficient details are not given for the fall by Method C to be calculated. Futhermore there is no mention that any regard has been paid to sampling errors. In view of the percentage error of 4·5 quoted above for *Vicia Faba* Suzuki's results by Method A must be considered inconclusive. It will be noted that Method B shows in most cases a gain at night, probably indicating nothing more than a large translocation of sugars.

Kosutany (6) has taken a series of diurnal readings for the nitrogen content of the leaves of *Vitis riparia*. The vine was so placed that it was in the shade after 2–3 p.m. At 3 p.m. one-half of a certain number of leaves was removed by cutting alongside the mid-rib, the other half being removed at 3 a.m. the following morning. He expressed his results by Methods B and C (summarized in Table V), but drew all his conclusions from the former.

TABLE V.

Showing the Fall in the Nitrogen Content of the Leaves of Vitis riparia at Night, expressed as a Percentage of the Day Value.

(Kosutany)

Date picked.	*Method B.*	*Method C.*
4–5 June	−1·15	0·0
19–20 June	−2·80	7·68
3–4 July	−1·62	−5·99
14–15 Aug.	−1·66	−0·71
28–29 Aug.	−1·42	7·84
25–26 Sept.	−2·03	4·86
9–10 Oct.	−2·74	4·20

He therefore stated that leaves became enriched with nitrogen during the night, and concluded, from further analyses, that this was due to the accumulation of protein. In light of the discussions given above it will be seen that this conclusion is not justified. By Method C there is in four cases a fall, in one no change, in one a very small rise, and in one only any evidence of a pronounced rise.

Otto and Kooper (8) have made a careful examination of the morning and evening nitrogen content of the leaves of *Aesculus hippocastanum*, *Philadelphus coronarius*, *Phlox Drummondi*, *Sambucus nigra*, and *Syringa vulgaris*. Leaves were picked at 6 p.m., and at 6 a.m. the following morning, at frequent periods throughout the season. Samples were picked with care from plants of the same age. They express their results by Method B, showing in all cases a very small fall in the nitrogen content at night. As it is impossible to believe that the leaves become richer in sugars, &c., through translocation into the leaf, their results undoubtedly do indicate a fall in the nitrogen at night. But it would appear that the authors did not completely realize that they were only comparing relative concentrations in terms of total solids, as mentioned earlier on page 513. Unfortunately Otto and Kooper do not give the fresh weight of the leaves used, so that an estimate of the actual fall in the nitrogen content at night cannot be calculated by Method C. Their results, then, though they undoubtedly indicate a fall in the nitrogen at night, do so only because in the samples examined the loss of nitrogen happens to have been greater than that of sugars, &c.

Pigorini's (10) results for the mulberry, with samples picked at 5 p.m. September 7 and 5 a.m. the following morning, show a fall at night by Methods B and C, whilst those of the present author (Chibnall (2)), for *Phaseolus vulgaris var. multiflorus*, for samples picked 8.30 p.m. July 3 and 2.15 a.m. July 4, each consisting of all the leaves from 12 plants, show a rise by Method B, and a fall by Method C.

Reviewed as a whole the results quoted above undoubtedly establish the fact that in general there is a withdrawal of nitrogen from the leaves at night. Schulze and Schütz show that for trees the amount decreases as the leaf ages, but, though one would expect the same for annuals, no evidence has yet been presented.

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A Study of the Growth in Culture of Verticillium albo-atrum, B. et Br.

BY

H. CHAUDHURI, PH.D.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

With twelve Figures in the Text.

THIS paper deals with the growth of *Verticillium albo-atrum*, B. et Br., with special reference to the effect of temperature and aeration. A comparison is also made of the method of estimating growth by surface spread of the fungus on a solid medium with a method of dry-weight determination.

The later portion of the paper deals with zone formation, and an attempt has been made to determine some of the conditions which bring about such formation.

The original culture of *V. albo-atrum* was received from Dr. Bewley of Lea Valley Experiment Station, where it is a cause of wilt in tomatoes.

In most of the experiments described, the fungus has been grown in Coon's (1) medium; so the effect of this medium in various dilutions and of its chief constituents on the growth of the fungus was first studied. The fungus also grows well in oat broth, oatmeal agar, wheat broth, prune juice agar, tomato-extract agar, potato-extract agar, and potato-mush agar. It grew in Dox's or Czapek's solution, and also in Richard's synthetic solution. With solid media, agar was added in a concentration of $1\frac{1}{2}$–2 per cent., according to the acidity of the solution; with prune juice 3 per cent. agar was found necessary to ensure setting of the medium.

Very luxuriant growth takes place in potato mush and potato extract, and also on cooked banana and oatmeal agar. In Dox's solution and in corn-meal agar, it grew rather indifferently.

The sizes of the conidia varied with different media and with the liquid or solid nature of the medium. Sometimes the conidia, on germination in the hanging drops of water, produced abundant small secondary conidia; the average size of these was $4{\cdot}5\,\mu \times 2{\cdot}5\,\mu$, while that of ordinary conidia was $6{\cdot}8\,\mu \times 2{\cdot}5$–$5\,\mu$.

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Coon's medium (1) consists of $MgSO_4$, KH_2PO_4, asparagin, and maltose. Coon advises the steaming of the solution on three successive days, but no difference in the nutritive value of the medium was to be observed between steaming and sterilizing at 120° C.

V. albo-atrum grew very well in Coon's normal solution. No significant difference in the dry weight of the growth occurred when, in a series of experiments, the asparagin was reduced by half; but whenever the maltose is reduced, the reduction in growth is very great. When maltose is present in full strength the reduction of asparagin does not make much difference; again, if maltose is decreased to half neither can the addition of asparagin in full strength enhance the growth nor the reduction of asparagin diminish it.

The following gives the actual dry weight of the growth of *Verticillium* in Coon's medium with varying amounts of maltose and asparagin. These figures have been arrived at by estimating the dry weight of the fungus after sixteen days' growth on various modifications of Coon's medium with agar. The fungus was incubated at room temperature (18°–20° C.). The method of determining the dry weight is given later.

Normal Coon.	*Coon's with full maltose and half asparagin.*	*Coon's with half maltose and full asparagin.*
0·0460 grm.	0·0380 grm.	0·0303 grm.
0·0363 ,,	0·0313 ,,	0·0241 ,,
0·0324 ,,	0·0267 ,,	0·0221 ,,
0·0262 ,,	0·0182 ,,	0·0185 ,,
0·0252 ,,	0·0173 ,,	0·0170 ,,
0·0242 ,,	0·0164 ,,	0·0158 ,,
Av. 0·0317 ± 0·002 grm.	Av. 0·0246 ± 0·0019 grm.	Av. 0·0213 ± 0·0012 grm.

Coon's with half maltose and half asparagin.	*Coon's with full maltose and no asparagin.*	*Coon's with no maltose and full asparagin.*
0·0302 grm.	0·0271 grm.	0·0166 grm.
0·0231 ,,	0·0239 ,,	0·0102 ,,
0·0205 ,,	0·0234 ,,	0·0084 ,,
0·0183 ,,	0·0230 ,,	0·0076 ,,
0·0167 ,,	0·0225 ,,	0·0074 ,,
0·0155 ,,	0·0203 ,,	0·0066 ,,
Av. 0·0207 ± 0·0013 grm.	Av. 0·0234 ± 0·0006 grm.	Av. 0·0094 ± 0·0008 grm.

Coon's with half maltose and no asparagin.	*Coon's with no maltose and half asparagin.*	*Coon's with neither maltose nor asparagin.*
0·0297 grm.	0·0124 grm.	0·0074 grm.
0·0277 ,,	0·0084 ,,	0·0040 ,,
0·0220 ,,	0·0074 ,,	0·0040 ,,
0·0149 ,,	0·0067 ,,	0·0038 ,,
0·0148 ,,	0·0066 ,,	0·0036 ,,
0·0132 ,,	0·0061 ,,	0·0036 ,,
Av. 0·0204 ± 0·0018 grm.	Av. 0·0079 ± 0·0008 grm.	Av. 0·0044 ± 0·0004 grm.

The above data are represented graphically in Fig. 1, and here the importance of maltose is very manifest.

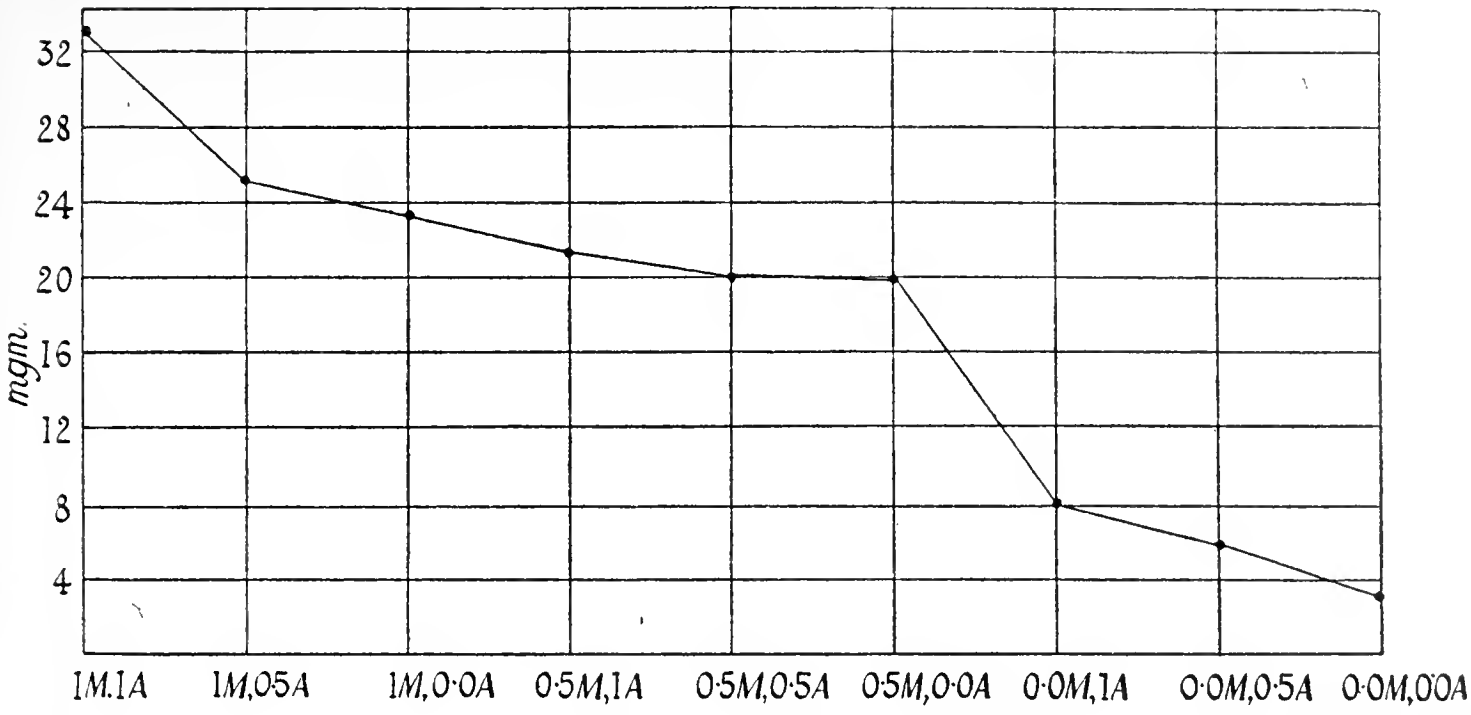

FIG. 1. Graph, showing the dry-weight production (in milligrams) of *V. albo-atrum* grown for sixteen days in Coon's medium with varying amounts of maltose and asparagin. 1 M = normal amount of maltose, 1 A = normal amount of asparagin, 0·5 M = half the normal amount of maltose, &c.

THE EFFECT OF AERATION AND OF TEMPERATURE ON THE GROWTH OF THE FUNGUS.

Method. The various temperature conditions were obtained by means of incubators for the higher temperatures, viz. between 14° and 30° C. For the lower temperatures, 5°–10° C., an automatically regulated refrigerator was used, and for 12° C. a cool storage room. Experiments on aeration were first carried on with flasks of different capacity containing the same quantity of the liquid medium. It was found more growth took place in the flasks with greater capacity. Next, flasks of same size were taken, and were aerated once every day for a few minutes. This gave no better growth than no aeration. Successful results were obtained by aerating very gently but continuously. This was done by means of a glass blower attached to the water tap. About fifty flasks were aerated equally and simultaneously. The main tube was connected with a flask having five branch tubes; these being connected to small jars with ten branch tubes, which were connected with the flasks to be aerated.

Throughout the course of experiment, data have been obtained both by measurement of the superficial growth on agar plates, and by determination of dry weight; and an attempt has been made to determine how far the spread of a fungus on an agar plate is a satisfactory measure of its rate of growth.

By measuring at regular intervals the spread of the fungus on an agar medium it has been found that *there is little or no difference in the rate of spread between Coon's normal, half-normal, and quarter-normal medium, though there is a marked difference in the amount of mycelium produced* (p. 534).

The following are the average measurements in mm. of the diameter of colonies in mm. at 25° C. from the fourth till the sixteenth day in Coon's medium of full strength, half strength, and quarter strength:

Days.	*N*/1	*N*/2	*N*/4
4	7·5	7·8	8·3
5	9·5	10·0	10·3
6	12·5	13·2	13·2
7	15·0	17·0	17·0
8	18·2	19·8	19·8
9	21·8	23·8	23·8
10	25·5	27·0	27·0
11	29·2	31·0	31·0
12	33·0	34·5	34·5
13	36·8	38·0	38·6
14	40·0	41·8	41·6
15	43·8	45·0	44·6
16	47·5	48·2	48·6

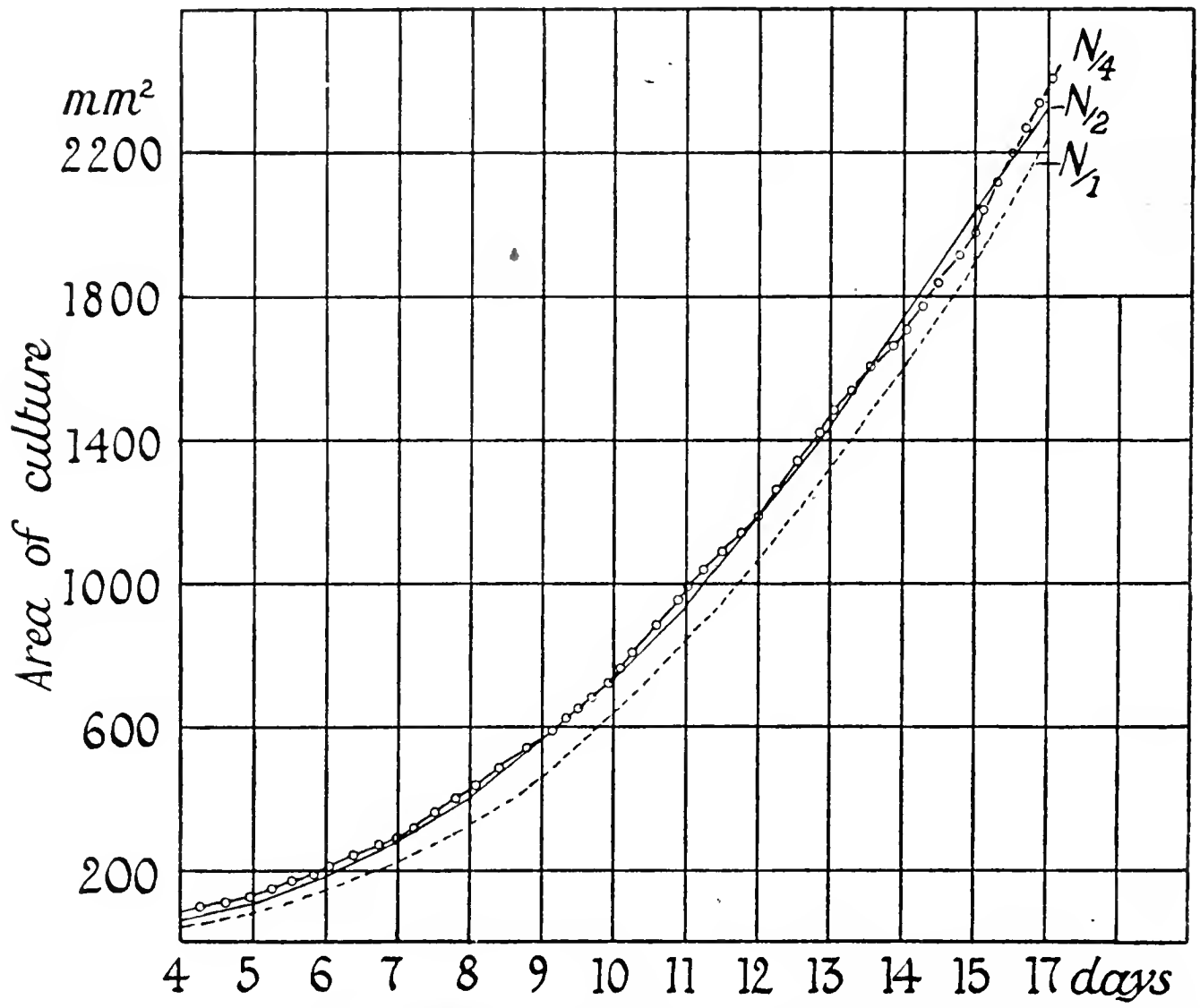

FIG. 2. Growth of *V. albo-atrum* on the different strengths of Coon's medium with agar. The broken line represents growth on full strength (N/1), the continuous line that on half strength (N/2), and the dotted line that on quarter strength (N/4). The growth is measured by the area of the plate covered by the mycelium; the area is estimated in terms of the square of the diameter measured in mm.

Fig. 2 shows the result graphically. It depicts surface growth of the fungus as determined by taking the squares of the diameter of the circular area covered by the fungus growing at 25° C. The rate of growth in area is seen to increase with the increasing diameter of the mycelium, thus following approximately the compound interest law.

Dry-weight determination. The procedure employed in determining dry weight is as follows: Weighing bottles with a filter-paper inside were placed in a drying oven at 65° C. for twenty-four hours, then cooled in a desiccator and weighed carefully to a tenth of a milligram. The liquid medium is passed through the filter-papers placed in a funnel, care being taken to scrape out any mycelium that might stick to the sides of the flask. The filtrate is then washed in distilled water, and placed in the weighing bottle and put in the drying oven at 65° C. for three days. The bottles are then placed in a desiccator and the weights determined. The difference is taken as the actual weight of mycelium produced.

In the case of an agar medium, the portion over which the fungus has spread out is carefully cut out and placed in a test-tube, with distilled water, which is boiled to melt the agar; the whole is then passed through the filter. Washing in this case is done with boiling distilled water three or four times. After that the material is dried and weighed as before.

The washing, especially with hot water, no doubt dissolves away a certain portion of the dry matter, but the amount so lost is slight, and is probably roughly proportional to the original mass of material.

Growth and Temperature.

Effect of temperature. The fungus grows very well both in darkness and light at room temperature in summer (18°–20° C.), whereas in winter little or no growth takes place at room temperature. Series of cultures in various media were grown at different temperatures, viz. 5°, 10°, 12°, 14°, 16°, 18°, 20°, 21°, 22·5°, 24°, 25°, 27°, and 33° C. It is found that the best growth takes place at 22·5° C. No growth took place in cultures incubated at 33° C. for a week, and when removed to 22·5° C. such cultures showed no growth; apparently the fungus had been killed or lost the power of germination. No growth took place in a week at 5° C., but when transferred to 22·5° C. the growth of such cultures was very vigorous. Even at 10° C. no apparent growth took place. Above that, growth begins, reaching its optimum at 22·5° C. At 25° C. the growth is less, and at 27° C. there is no growth.

Fig. 3 represents graphically the growth at different temperatures, determined first by measurement of the diameters of colonies after fifteen days, secondly by measuring the length of the germ tubes in hanging drops of distilled water after twenty-four hours. In measuring the length of the germ tube, all the tubes from a single spore were counted.

The two curves are very similar and have their optimum at 22·5° C. The difference lies in their maximum, for many spores produced short germ tubes at 27° C. in hanging drops, whereas when examining the agar medium of Coon's normal solution, no visible growth was found after fifteen days, though the spores remain living, for at a lower temperature a mycelium soon appears.

Measurement of spread of colonies at different temperatures after fifteen days:

	10° C.	12° C.	14° C.	16° C.	18° C.	21° C.	22·5° C.	25° C.	27° C.
Average diameter in mm.	0	10	14	35	45	55	60	45	0

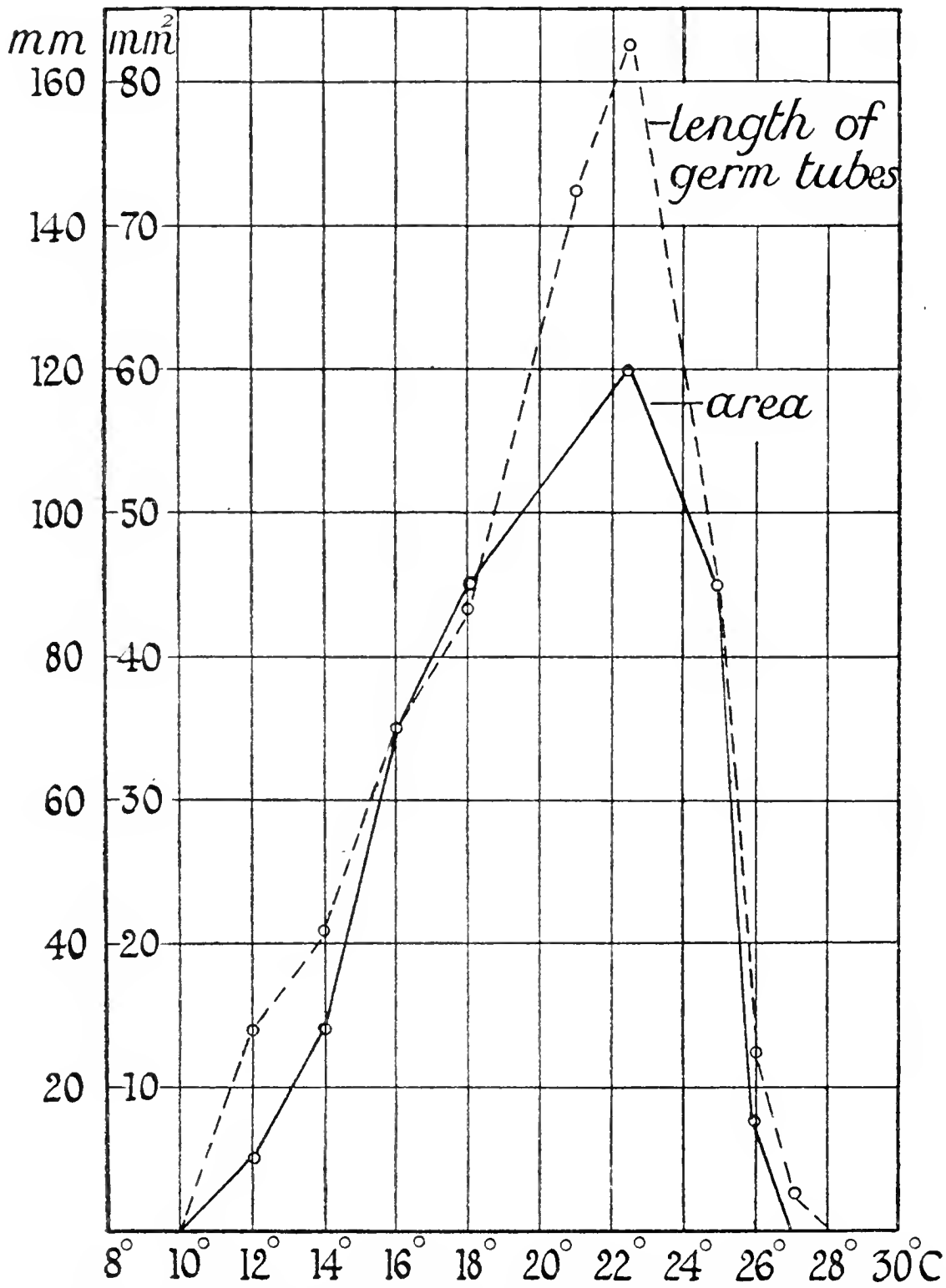

FIG. 3. The effect of different temperatures on (1) the spread after fifteen days of the mycelium on Coon's medium in terms of the diameter of the circular area covered, (2) the length of germ tubes developing from conidia after twenty-four hours in hanging drops. The right-hand vertical scale is that of the length of the germ tubes, the left-hand that of the diameter of the mycelial areas.

Measurement of germ tubes after twenty-four hours' growth, the unit being 0·5 μ:

12° C.—16, 16, 15, 18, 12, 12, 14, 10, 7, 8, 10, 15, 10, 8, 10, 16, 12, 8, 10, 15, 25, 25, 15, 20, 28, 18, 15, 18, 18, 15, 12, 15, 10, 10, 10, 8, 15, 18, 20, 12. Average, 14 = 28 μ.

14° C.—30, 32, 24, 15, 15, 22, 28, 25, 30, 18, 16, 14, 12, 20, 15, 25, 18, 30, 25, 10, 10, 12, 20, 22, 22, 18, 18, 20, 20, 18, 22, 30, 30, 22, 30, 12, 18, 12, 15, 30. Average, 21 = 42 μ.

16° C.—42, 45, 48, 25, 35, 28, 28, 55, 25, 40, 45, 50, 35, 40, 45, 55, 38, 22, 32, 40, 20, 25, 28, 24, 30, 35, 20, 60, 50, 55, 25, 20, 40, 35, 30, 25, 45, 50, 40, 35. Average, 36 = 72 μ.

18° C.—40, 25, 20, 25, 45, 40, 35, 30, 30, 45, 45, 35, 40, 45, 50, 55, 60, 60, 45, 40, 50, 60, 35, 40, 28, 35, 40, 45, 65, 65, 28, 45, 50, 35, 40, 38, 45, 55, 40, 55. Average, 43 = 86 μ.

20° C.—65, 60, 80, 65, 65, 70, 55, 85, 80, 75, 80, 60, 75, 80, 85, 45, 50, 90, 90, 85, 65, 75, 70, 85, 60, 60, 70, 80, 85, 95, 90, 75, 60, 65, 80, 75, 55, 40, 45, 62, 38. Average, 72 = 144 μ.

22·5° C.—64, 80, 95, 110, 120, 65, 70, 80, 90, 80, 85, 60, 80, 80, 60, 50, 125, 100, 75, 120, 80, 75, 80, 100, 65, 85, 90, 100, 55, 60, 80, 70, 75, 65, 95, 90, 130, 68, 70. Average, 82 = 164 μ.

25° C.—30, 35, 40, 45, 45, 30, 35, 32, 45, 45, 50, 40, 35, 40, 45, 30, 35, 40, 45, 60, 65, 45, 50, 35, 50, 50, 45, 45, 30, 35, 45, 50, 40, 45, 40, 50, 75, 60, 70, 45, 50. Average, 45 = 90 μ.

27° C.—6, 30, 18, 13, 6, 10, 8, 8, 6, 6, 10, 8, 13, 16, 18, 6, 6, 8, 10, 8, 8, 10, 6, 13, 18, 10, 13, 8, 16, 6, 6, 6, 6, 8, 8, 11, 16, 18, 10, 8. Average, 10 = 20 μ.

Edson and Shapovalov (2), in their experiments to determine the growth of various fungi at various temperatures, found for *V. albo-atrum* an optimum at 25° C. They incubated the fungus at 10°, 15°, 20°, 25°, and 30° C. If, however, they had experimented with smaller differences of temperature, as has been done in this paper, the optimum might have been found to be between 20° and 25° C. or between 25° and 30° C.; and the growth at the real optimum might have been considerably greater than that found at 25° C., which was arbitrarily assumed to be the optimum.

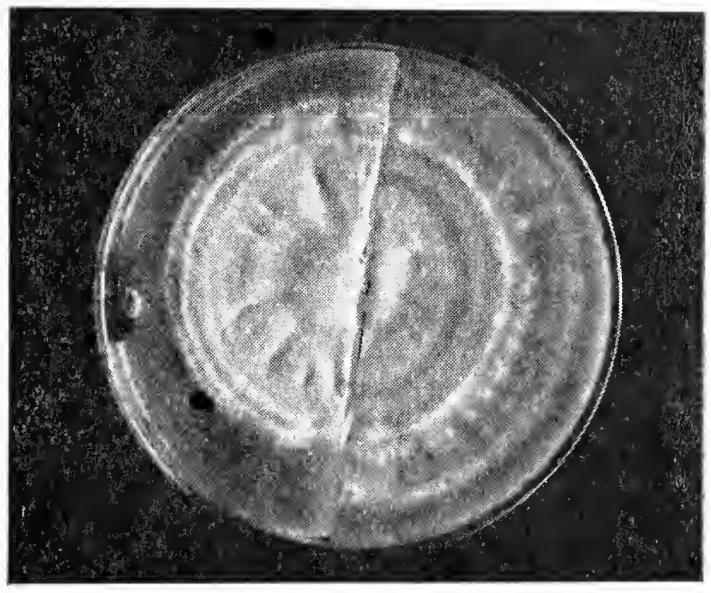

FIG. 4. Photograph showing the difference in the amount of mycelial 'spread' due to difference in thickness of the medium, that on the left hand being the thicker. It also shows the difference in zonation produced by the difference in thickness. Incubated at 25° C.

It may be mentioned that with the fungus here investigated the *maximum* varied with different food material, as will be described later on, but the *optimum* always remained the same.

Edson and Shapovalov determined the optimum by measuring the spread of the fungus on solid media. It should be pointed out, however, that with this method great care must be taken that the thickness of the medium is constant; for the spread of the fungus varies with thickness unless the medium is very thick, i.e. about 10 mm., in which case a little difference does not affect the growth. Fig. 4 shows a photograph of halves of two plates containing media, 4 and 6 mm. thick respectively. The difference in size of the fungal growth is well marked.

It was found that *V. albo-atrum* could under no circumstances withstand 33° C. for one week. An attempt was therefore made to discover how long it could withstand high temperatures. The period was gradually decreased and various food materials used. It was found that the lethal temperature varied with different media. Thus with potato-mush agar, pea-broth agar, Dox agar, corn-meal agar, and cooked banana exposed to 33° C.

for forty-eight hours, there was no survival, but on pea agar, corn-meal agar, and prune agar it survived the temperature for twenty-four hours, but not on potato mush or tomato mush. When, however, the period was reduced

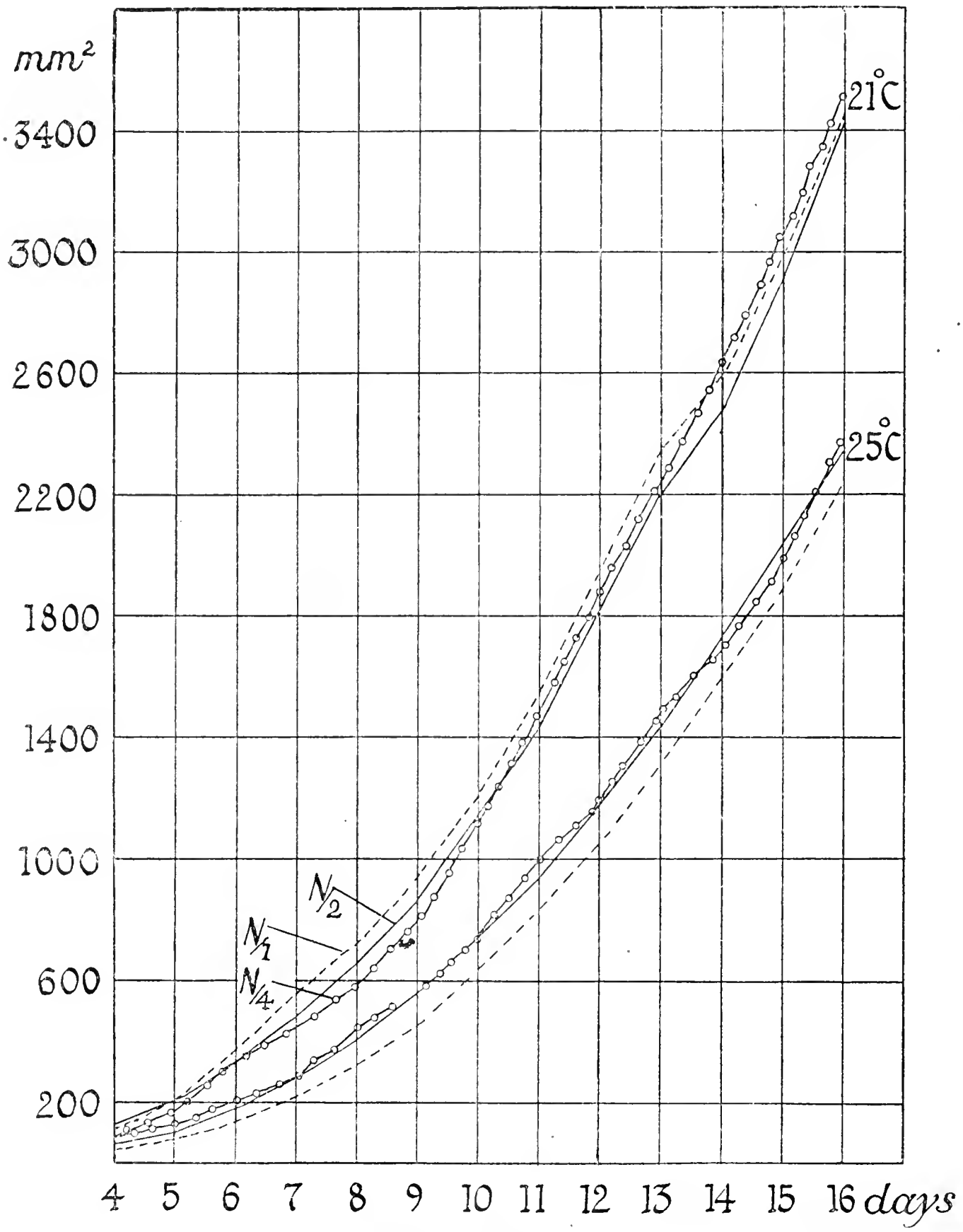

FIG 5. Curves showing the relative area (diameter in mm. squared) covered by the mycelium at 21° C. and 25° C. in agar cultures of Coon's medium, full strength (N/1), half strength (N/2), and quarter strength (N/4).

to eighteen hours it survived on all the media. The power of withstanding high temperatures agrees to a certain extent with that recorded for the two strains used by Edson and Shapovalov, though both had a higher maximum temperature. They write: 'The two strains do not survive exposure for several days to a temperature of 35° C. or higher;' and again: 'With

sufficient moisture present no growth took place in 30° or 35° C. after seven, fourteen, or twenty-one days.'

The usual bacterial method was followed to determine the thermal death-point of the fungus. A number of tubes of melted potato-mush agar were inoculated and kept at 40° C. for ten minutes and then poured into sterile Petri dishes and cooled rapidly. Next the temperature of the bath was raised to 43° C., and another set of tubes inoculated and kept there for ten minutes, and then poured into sterile Petri dishes and cooled rapidly. Similar experiments were made at 46°, 49°, 52°, and 55° C. These Petri dishes were incubated at 22·5° C., the optimum temperature for this fungus. Innumerable colonies appeared on the second and third day from those exposed to 40°, 43°, and 46° C. A smaller number of colonies appeared at 49° C. No colonies appeared at 52° C. Next a similar set of experiments were made at 48°, 50°, 52°, and 54° C. A number of colonies appeared at 48° and some at 50° C. One or two colonies appeared in some cases at 52° and none at all at 54° C. So the thermal death-point may be put between 52° and 54° C. for a ten minutes exposure.

Growth in Media of Different Concentration at One Temperature.

It has been found that if certain amounts of food material are present the increase in diameter of the mycelium is fairly constant for a particular temperature, provided that the thickness of the medium is the same in all cultures. Thus a colony will spread equally in Coon's normal, half normal, and quarter normal, if incubated at the same temperature. The following are the actual measurements (squares of the diameter in mm.) for Coon's N/1, N/2, and N/4 at temperatures 21° and 25° C. It will be seen from Fig. 5 that spread is equal for different concentrations at the same temperature.

21° C.				25° C.			
Days.	*N*/1	*N*/2	*N*/4	*Days.*	*N*/1	*N*/2	*N*/4
4	81	100	77	4	56	60	68
5	182	188	150	5	90	100	106
6	361	324	324	6	150	175	182
7	529	494	461	7	225	289	270
8	702	665	575	8	330	319	410
9	930	900	870	9	475	560	560
10	1225	1135	1135	10	650	729	729
11	1560	1444	1482	11	860	961	990
12	1936	1831	1849	12	1089	1190	1190
13	2304	2190	2227	13	1354	1444	1480
14	2601	2570	2650	14	1600	1740	1720
15	3025	3100	3080	15	1916	2025	1980
16	3481	3457	3600	16	2240	2325	2350

In one case, however, the effect of a difference of temperature was not manifest. This was with a six-months-old solution (Coon's normal) in which growth had previously stopped; the pH was 8·2. This medium was filtered,

agar added, and then plated. A very thin mycelium was produced, only just visible to the naked eye, but this grew very rapidly, and there was no difference in the diameters of the colonies incubated at the two temperatures. The following are the measurements in mm. from the fourth till the seventeenth day:

	21° C.				25° C.			
Days.	*Pl. I.*	*Pl. II.*	*Pl. III.*	*Pl. IV.*	*Pl. I.*	*Pl. II.*	*Pl. III.*	*Pl. IV.*
4	10·0	9·0	9·0	8·0	10·0	9·0	10·0	10·0
5	14·0	13·0	12·0	12·0	14·5	12·5	13·0	14·0
6	18·0	16·0	17·0	17·0	16·5	16·0	16·0	17·0
7	22·0	21·0	21·0	21·0	22·0	21·0	20·0	21·0
8	26·0	26·0	26·0	25·0	26·0	26·0	24·0	25·0
9	30·5	29·0	29·0	28·0	31·0	29·0	27·0	30·0
10	34·0	33·0	33·0	33·0	36·0	32·0	32·0	34·0
11	38·5	37·0	37·5	37·5	39·0	37·0	37·0	38·0
12	41·5	40·0	40·0	40·5	42·5	40·0	40·0	41·5
13	45·5	45·0	45·0	45·5	46·0	45·0	43·0	45·0
14	50·0	51·0	50·0	49·5	49·5	48·5	47·0	49·0
15	54·5	54·0	54·5	53·5	53·0	62·0	52·0	53·0
16	58·5	57·0	57·5	57·0	57·0	56·0	56·0	56·0
17	61·5	62·0	61·5	60·0	60·0	60·0	59·5	60·0

Relation between Rate of Surface Spread of the Fungus and its Increase in Weight.

The amount of spread may be taken as a satisfactory measure of growth when determining the effect of temperature with a definite medium of constant thickness. But when growth-data at a given temperature for different media are required it gives fallacious results. Almost the same rate of spread is found with normal N/2, N/4, N/8, and N/16 Coon's medium, though the determinations of dry weight show the increase in fungal material to be very different. The difference is especially marked for growth in liquid media.

Fig. 6 shows graphically the relation between area and dry weight in Coon's medium with agar at a temperature of 22° C. The diameters were measured on the 1st, 4th, 8th, 11th, 14th, and 17th days, when also eight plates at a time were taken for dry-weight determination. The following gives an average figure for the area (diameter in mm. squared) and dry weight:

1st Day.
Area . . . . . 9 mm.²
Weight . . . . . Nil.

4th Day.
Area . . . . . 100 mm.²
Weight . . . . . 0·0015 ± 0·0003 grm.

8th Day.
Area . . . . . 900 mm.²
Weight . . . . 0·0148 ± 0·0012 grm.

11th Day.
Area . . . . . 1,764 mm.²
Weight . . . . . 0·0254 ± 0·0014 grm.

14th Day.
Area . . . . . 3,025 mm.²
Weight . . . . . 0·0380 ± 0·0021 grm.

17th Day.
Area . . . . . 4,900 mm.²
Weight . . . . . 0·0557 ± 0·0032 grm.

It will be seen from the graph that the area curve more or less follows the compound interest law, at least for the period when the fungus is growing vigorously, but the dry-weight curve runs almost in a straight line.

The graph in Fig. 7 shows the increase in mycelial 'spread' obtained by

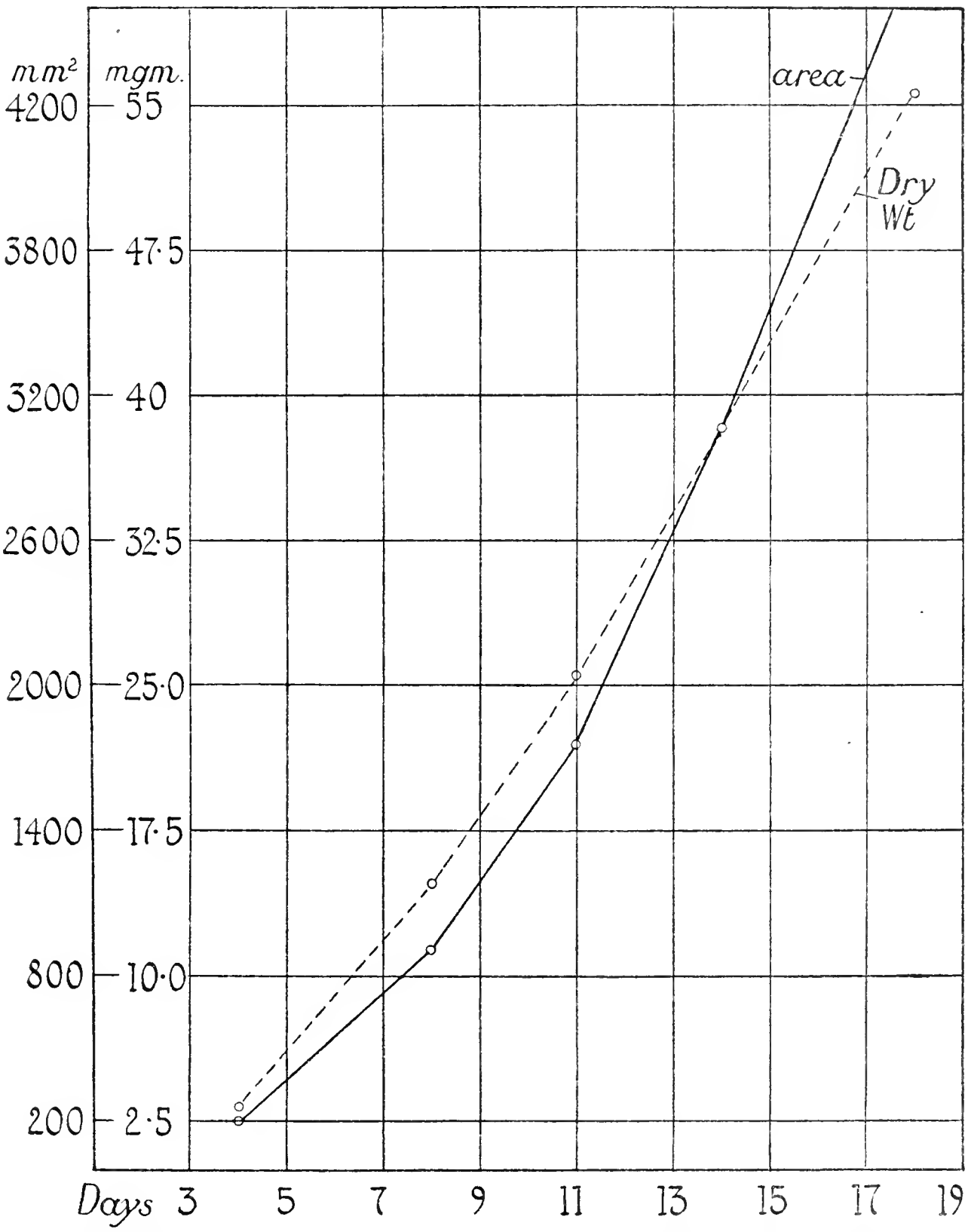

FIG. 6. Curves showing the relation between the daily increase in dry weight of *V. albo-atrum* in Coon's liquid medium, and the daily increase in 'spread' of the mycelium on the same medium with the addition of agar. The scale represents area covered by the mycelium as given in terms of the square of the diameter measured in mm. The vertical scale on the left represents mm.², that on the right milligrams.

measuring diameters daily up to ten days at different temperatures. At the end of that period the dry weight for the total growth was determined. The area curve and dry-weight curves are very much alike, and it is only in this case where we are estimating the effect of *different* temperatures that the spread is a satisfactory measure. By following the daily growth curves,

we find that the growth at the optimum temperature 22·5° C. is always the best, only it is not so marked during the first twenty-four hours, when almost equal amount of growth has taken place at 20° C. But when the spore germination curve is studied in Fig. 3 we find that the difference between 20° C. and 22·5° C. is quite significant even for the first twenty-four hours.

The following are the average figures for different temperatures of the

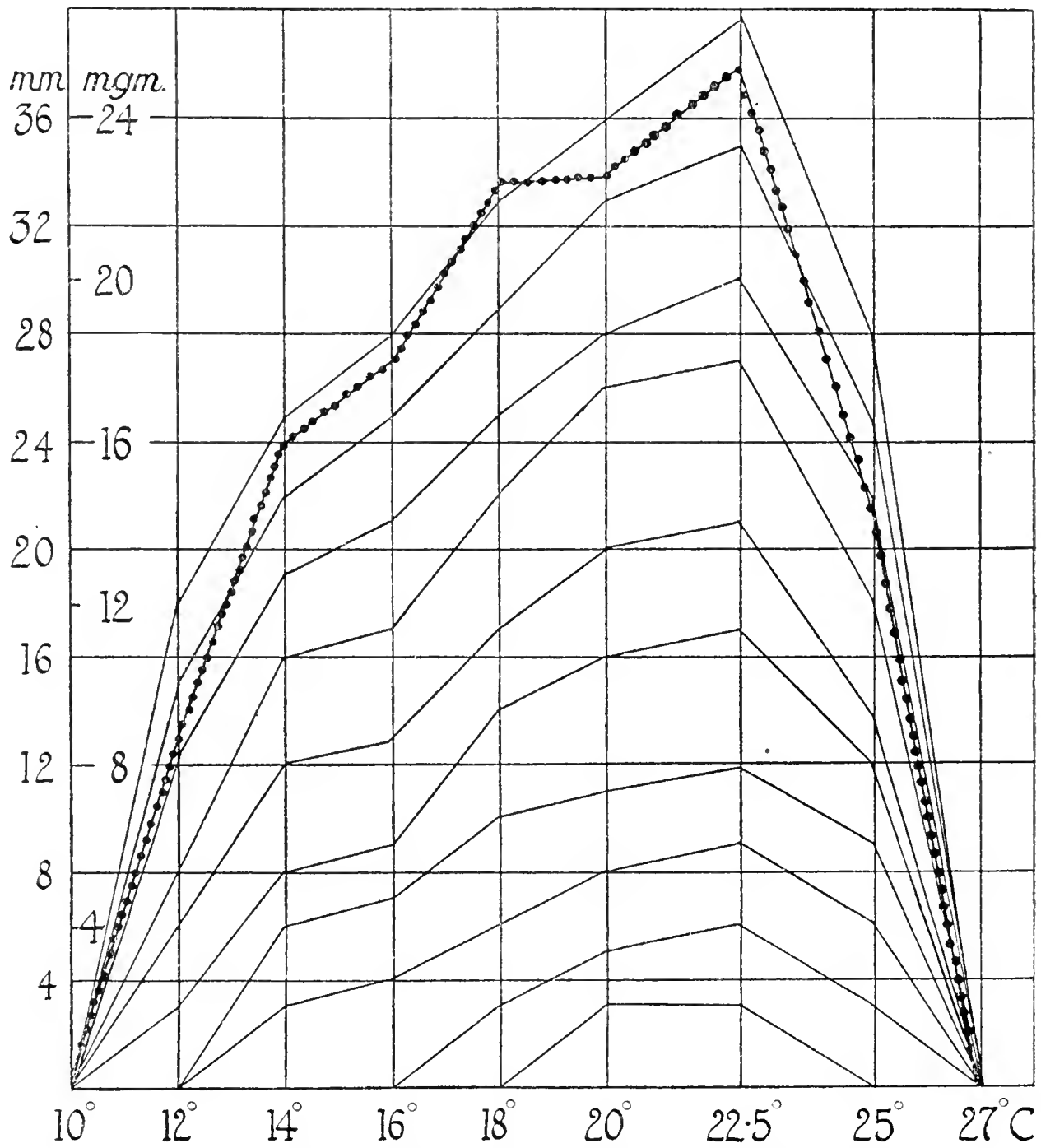

FIG. 7. The continuous lines represent the daily growth in *diameter* of mycelium on Coon's medium (plus agar) at the temperatures indicated. The thick dotted line represents the dry weight of mycelium produced in ten days at the different temperatures. The two vertical scales represent on the left mm., on the right mgm.

diameters of the mycelia taken daily. Fig. 8 shows a photograph of the colonies grown at different temperatures. It will be seen that no growth has taken place at 27° C. nor at 10° C.

The following data show the dry weight at different temperatures after a period of ten days; the average is that of three samples:

12° C.	14° C.	16° C.	18° C.	20° C.	22·5° C.	25° C.
0·0087 grm.	0·0161 grm.	0·0181 grm.	0·0224 grm.	0·0225 grm.	0·0253 grm.	0·0144 grm.

Aeration.

The effect of aeration was studied by aerating a series of culture flasks continuously, and determining at regular intervals the dry weight of mycelium produced. These experiments were all carried on at 21° C. in a large incubator, and the flasks were aerated with the help of a blast pump connected with the water supply. Control non-aerated flasks were placed in the same incubator. In the first set of experiments, the flasks were

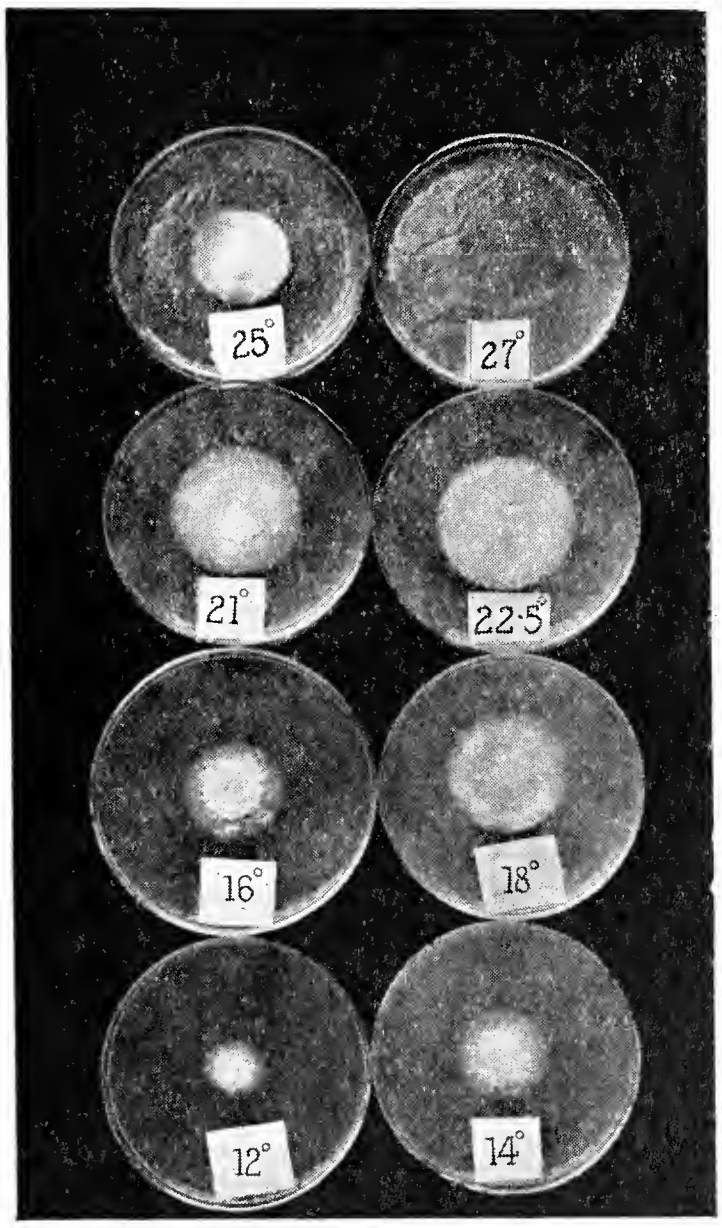

Fig. 8. Photographs of cultures of *V. albo-atrum* after fifteen days at 12°, 14°, 16°, 18°, 21°, 22·5°, 25°, and 27° C.

aerated discontinuously, once for three minutes daily, and at the same time a series of flasks were shaken. The aerated ones were found to show less growth than the non-aerated ones, and about the same as those shaken. For five successive weeks a number of flasks were taken, and dry weights determined, and each time the aerated ones gave less growth, as did the shaken flasks. Obviously, the fungal hyphae were torn by the direct shaking or by that caused by rapid bubbling and this retarded growth. It was also found in this connexion that the best growth had taken place by the

end of the second week, and that in the following two weeks there was very little growth.

A second set of experiments was then started in which air was gently bubbled continuously through the liquid in the flasks, and a set of eight flasks from the aerated ones and another set of eight from the non-aerated ones were taken away every five days for dry-weight determination for a period of fifteen days. The result was very striking; after the first five days the dry weight was nearly double that of the non-aerated ones. After ten days the difference was still very great; after fifteen days, how-

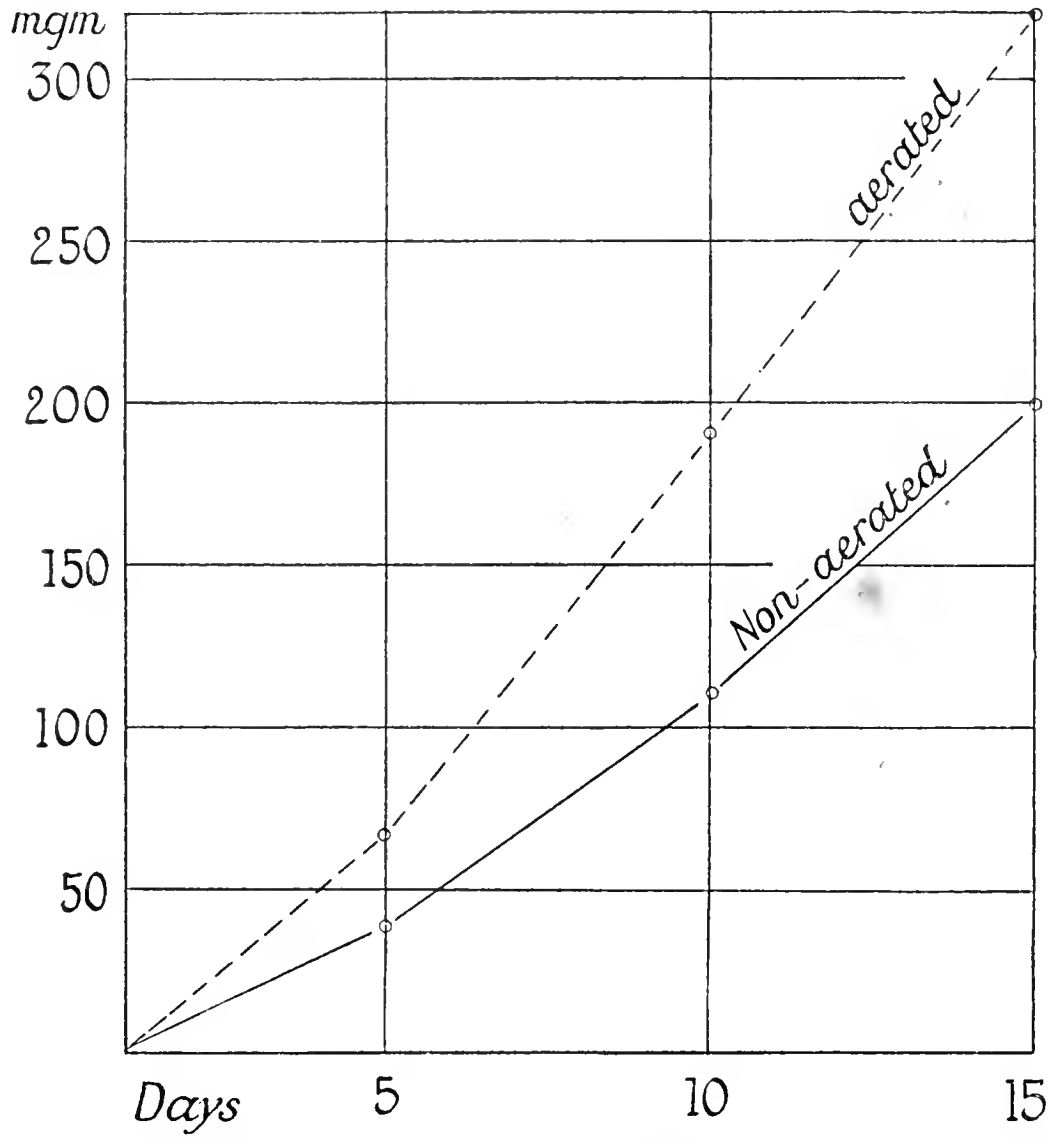

FIG. 9. Dry-weight production of *V. albo-atrum* in aerated and non-aerated cultures in Coon's medium.

ever, the ratio was falling. These results are represented graphically in Fig. 9. At the end of five days the aerated cultures weighed on an average $0{\cdot}0659 \pm 0{\cdot}0027$ grm., whereas the non-aerated ones gave only $0{\cdot}0381 \pm 0{\cdot}0028$ grm. After ten days the aerated gave $0{\cdot}1942 \pm 0{\cdot}006$ grm. and the non-aerated $0{\cdot}1107 \pm 0{\cdot}0056$ grm.; and after fifteen days $0{\cdot}3239 \pm 0{\cdot}0114$ grm., and $0{\cdot}2099 \pm 0{\cdot}0058$ grm. respectively. These figures, which are in all cases the mean of eight samples, show that the differences are very significant.

In this experiment all the media, viz. Coon's, pea broth, tomato juice, prune juice, potato extract, Richard's synthetic, &c., in which the fungus grew were rendered alkaline. The highest degree of alkalinity reached in Coon's medium was pH 8·2 after six months, the normal pH of the

medium being 4·3. No particular relation between growth and change of pH could be established. It was however observed that pH remains low when the media is aerated, though more growth takes place.

Experiments were next carried on to find out how long the weight continued to increase in the aerated and in the non-aerated cultures, and whether, when the growth had stopped in both, the total weight was the same. This experiment was carried on in two sections. In the first aerated and non-aerated flasks were inoculated and incubated at 21° C., and dry weight

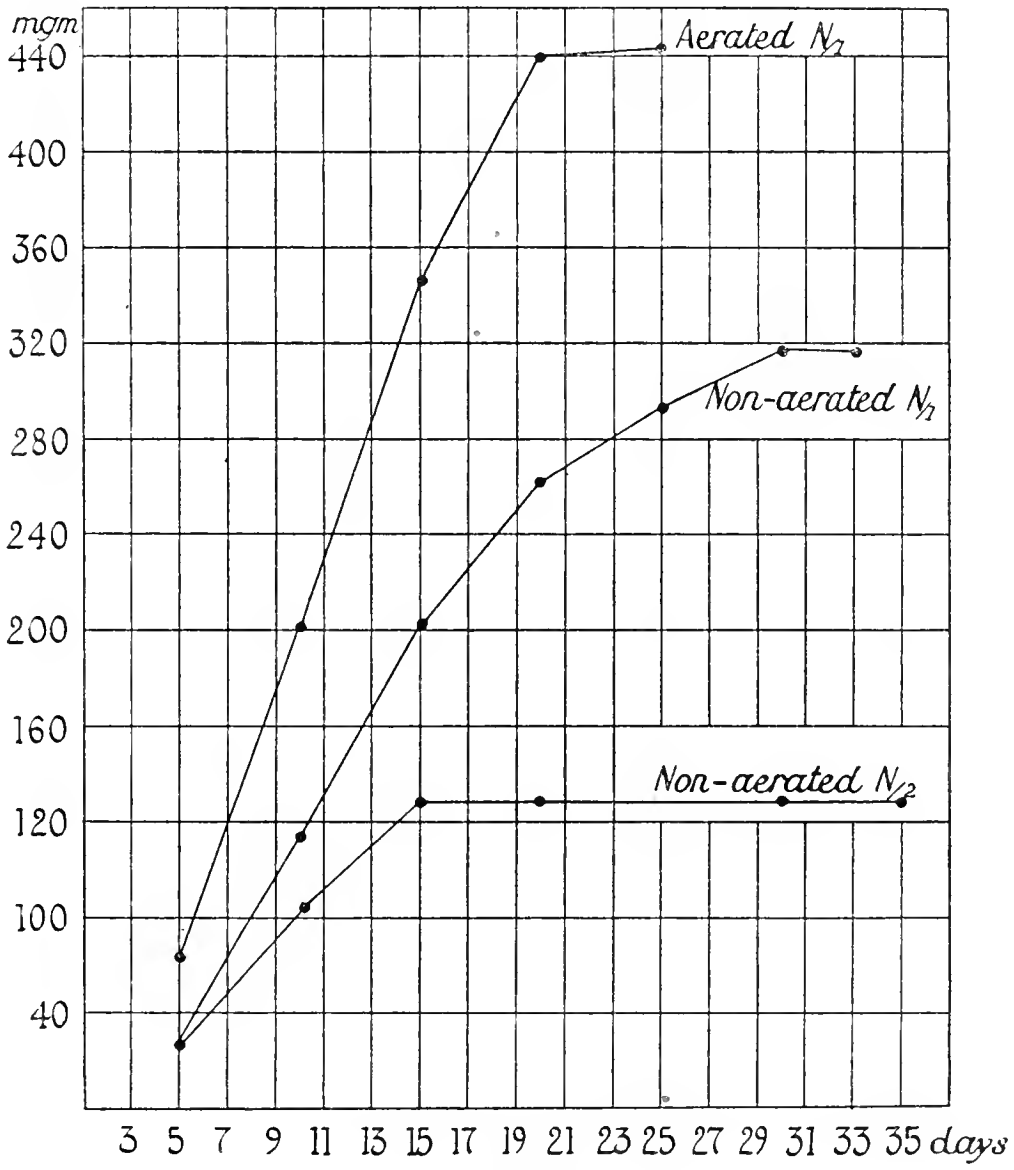

FIG. 10. Dry-weight production of *V. albo-atrum* in aerated and non-aerated Coon's medium of normal strength (N/1), and in non-aerated Coon's medium of half strength (N/2).

determined every five days for twenty-five days. Inoculations were made every time with equally old cultures, generally three weeks old ones on potato mush in tubes. Eight flasks were taken every five days for determination of the dry weight. In the third interval nine aerated flasks were lost accidentally, so for the remaining three intervals only five flasks could be examined at a time. This would cause a greater probable error, but fortunately the results were very good, the error being quite low. As the eighty flasks required for the two sets, aerated and non-aerated, could not be started at once owing to the limited space available, the experiment was begun first with aerated ones only (forty flasks), and as, at each interval of five days, eight were taken out, fresh non-aerated ones were put in their place,

so that when the last set of aerated ones were taken out and the last non-aerated ones put in, only another five days were required to complete the whole experiment. Every care was taken that the parent cultures were of the same generation and age.

We find by studying Fig. 10 that by the end of twenty days maximum growth had taken place in the aerated ones, for at the end of twenty-five days there was no increase of growth over that of twenty days.

In the non-aerated ones, however, maximum growth had not been reached by the end of twenty days, so they were carried on to thirty-five days. At the end of thirty days the dry weight still showed a certain gain, but by the end of the thirty-five days there was no further gain. So the maximum growth had taken place, and the total weight was much less than the total weight of the aerated ones. This shows the significant effect of aeration.

Another set of experiments was carried out at the same time to determine the actual difference in dry-weight production in a normal and half-normal medium; it had been found previously, on the bases of the rate of spread of the mycelium, that there was no difference. These flasks were not aerated, and were examined on the fifth, tenth, twenty-fifth, thirtieth and thirty-fifth days. We find very little difference in growth between the tenth and twenty-fifth days, and after that there is no further increase in growth. The total weight is only about a third of the normal. When this result is compared with the result obtained by the method of increase in diameter it is clear that the increase in surface area of a culture on a solid medium does not give a satisfactory measure of the growth of this fungus in media of different concentrations.

The following are the determinations of dry weight on Coon's normal liquid medium at 21° C. The figures are in all cases the means of a number of cultures; this number is given in the fourth column.

Coon's Medium (Full Strength).

Dry Weight (grm.).

Days.	*Aerated.*	*Non-aerated.*	*No. of samples.*
5	0·064 ± 0·003	0·037 ± 0·001	8
10	0·203 ± 0·004	0·115 ± 0·004	8
15	0·336 (5 samples)	0·205 ± 0·006	8
20	0·440 (5 samples)	0·262 ± 0·010	8
25	0·444	0·294	5
30	—	0·318 ± 0·003	8
35	—	0·318 ± 0·003	8

Coon's Medium (Half Strength).

5	—	0·037	5
10	—	0·085	5
25	—	0·129	5
30	—	0·130	5
35	—	0·128	5

In Fig. 10 the curves for the growth of the aerated and non-aerated mycelia during the early period when there was plenty of food material show that the growth does not follow the compound interest law, as does

the increase in surface area. Instead, there is approximately the same increment of weight for each period. This may be attributed either to the old mycelium ceasing to produce new growing hyphae or to the young mycelium, though increasing in amount, growing less and less vigorously.

An experiment was carried out to throw some light on the action of aeration. Does it act by oxidizing the staling products or by reducing their production? An experiment was started with a medium staled for thirty-five days which had been used for the non-aerated cultures and in which growth had stopped. The medium was filtered and put in flasks under aseptic conditions, and then inoculated and aerated for fifteen days. A similar set was left non-aerated. Very little growth took place in either, and the growth in the aerated ($0{\cdot}0034 \pm 0{\cdot}0004$ grm.) was not greater than the growth in the non-aerated ones ($0{\cdot}0036 \pm 0{\cdot}0005$ grm.). This indicates that aeration favours growth, not by oxidizing waste products, but by keeping down the production of waste products.

Zone Formation by *Verticillium albo-atrum*.

In connexion with the experiments in temperature, it was found that the fungus formed zones regularly at a certain temperature, viz. 25° C. in all media. Fig. 11 shows zonation in Coon's medium with agar, and Fig. 12 zonation in corn-meal agar.

An attempt has been made to discover some of the conditions affecting ring formation. ' It has been studied in three different relations, viz. the medium, heat, and light.

It has been mentioned before that this fungus always causes the medium to become alkaline. Coon's normal, normal with 0·3 per cent. HCl, and normal with 0·3 per cent. NaOH, as well as Coon's N/2, N/4, and a fourteen days stale liquid, were plated with agar. These were inoculated and incubated at 14°, 16°, 18°, 21°, 22·5°, 25°, and 26° C., but zones appeared at 25° C. only, and the zoning in half-normal, quarter-normal, stale medium, and alkaline medium was more marked than in the others. Though the amount of growth at 18° C. was the same as that of 25° C., no rings appeared at the lower temperature. A study of a graph showing the daily spread in an agar colony at the two temperatures exhibits the lines crossing and re-crossing many times, showing that the growths at these temperatures are the same. The total growths are also the same. If equal amounts of growth produce equal amounts of waste products, and staling products are the cause of zone formation, then we should expect rings equally at 18° C., as at 25° C. But no rings appeared at 18° C.

The rings at 25° C. do not appear successively as growth proceeds, as in *Penicillium*. The colony grows uniformly for a certain time, and then suddenly zones appear in the mycelium already formed, which had previously

shown uniform growth. Zones generally appear by the end of the second week, when an area of 45–50 mm. in diameter has appeared. Zones appeared at a lower temperature than 25° C. only when the Petri dishes were continuously lighted. Thus ordinary Petri dishes were kept at 21°–23° C. under continuous illumination, with some plates covered with black paint to prevent the entry of light. Zoning appeared only in the illuminated dishes,

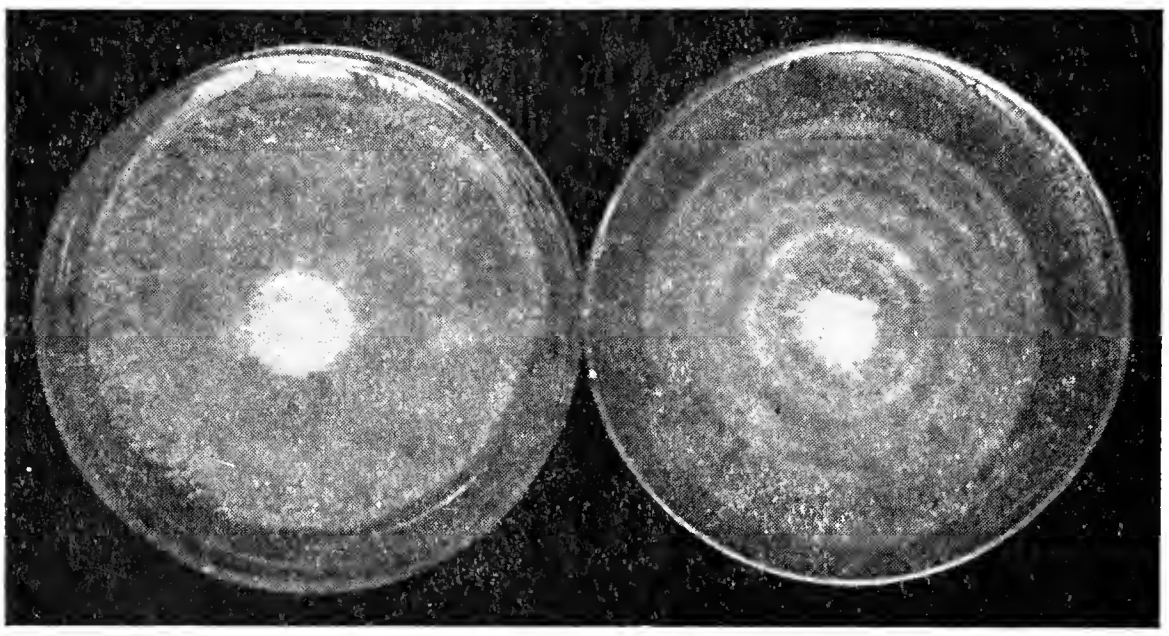

FIG. 11. Two cultures of *V. albo-atrum* on Coon's medium plus agar. That on the left was incubated at 22·5° C. and shows no zonation, that on the right at 25° C. and shows well-marked zonation.

FIG. 12. A culture of *V. albo-atrum* on corn-meal agar at 25° C.; zonation is clearly visible.

which definitely shows that it was the effect of light that brought about zoning at this lower temperature. Zones failed to appear in the dark even at 25° C. when the media was acidified to a pH of about 2·8, but the same medium produced zones when exposed to light. Normal Coon (pH 4·3) produces zones at 25° C., but these are not very prominent. Very marked zone formation takes place at 25° C. when a fourteen days stale medium (pH 5–5·2) is used. It is the same for 21°–23° C. in the light. At 25° C. in

darkness and 21°–23° C. lighted, ring formation is very marked when about 3 per cent. NaOH is added (pH 7·0). In connexion with zone formation at a lower temperature in light, it is noteworthy that the rings do not appear suddenly as at 25° C. in darkness, but gradually one after another from the very beginning. The results now may be tabulated as follows:

Medium.	22·5° C. *Darkness.*	21°–23° C. *Light.*	21°–23° C. *Darkness.*	25° C. *Darkness.*
Coon's normal	–	+	–	+
Coon's ½ normal	–	+	–	+
Coon's ¼ normal	–	+	–	+
Coon's 14 days stale	–	+	–	+
Coon's normal with 0·3 % HCl	–	+	–	–
Coon's normal with 0·3 % NaOH	–	+	–	+

At 20° C. and below this temperature, light produced no zonation in any medium.

We may now conclude that an alkaline medium favours ring formation (up to pH 7·0, or just over it), but if the medium becomes very alkaline (pH 8·0), as in a very stale six months old medium, the fungus grows, but no rings are formed. Also that *in the dark* zone formation is practically confined to 25° C., which, however, can be lowered slightly by exposure of the culture to light.

Zone formation has been held to be due to a periodic external factor, e.g. alternation of light and darkness; but in these experiments rings were formed not only in constant darkness but also under continuous illumination.

It has been claimed that the production of staling substances is the cause of zone formation. *Verticillium*, however, grows best at 22·5° C., so should then form most waste products, yet no zones are formed in darkness at that temperature, although they appear under similar conditions at 25° C. The way in which the zones appear suddenly at 25° C. does not support the alkali diffusion theory of Munk (3). Also Moreau's (4) view of alternation of light and darkness cannot be applicable as already stated. Hedgecock (5), in his experiments on zonation with *Cephalothecium*, found light to be the determining factor, and showed that variation of temperature did not cause zonation; he showed also that no zonation occurred in darkness, and that light of different wave length had different actions on zone formation. Similar experiments were carried out by Knischewsky (6) with *Penicillium luteum*. Munk (7) had also found that on exposure to light zonation develops, but not at constant temperature in darkness. He had found that increase in the amount of nutrient makes the zones narrower and tends to diminish coremium formation.

Plates were grown at 25° C. with the medium 6 mm. and 12 mm. thick,

Coon's normal medium agar being employed. In twenty days' time the total growths were as follows:

In medium 6 mm. thick . . Diameter of mycelial area 80 mm.
In „ 12 „ „ . . „ „ „ „ 60 mm.
The rings were more closely packed in the thicker medium (Fig. 4).

Summary.

Verticillium albo-atrum, B. et R., grows on a large variety of media, and always renders the medium alkaline.

The asparagin of Coon's medium can be markedly reduced without much affecting the growth of the fungus; on the other hand, reduction in the concentration of the maltose in the medium markedly reduces the growth.

The optimum temperature for growth is 22·5° C., the maximum 30° C., and the minimum 10° C.

Aeration in liquid media markedly increases not only the rate of growth but also the total amount of growth in a given volume of medium.

Aeration appears to increase growth by reducing the production of waste products, rather than by removing (oxidizing?) waste products already formed.

The rate of surface 'spread' of a culture on a solid medium was compared with the rate of increase of material as measured by dry-weight production. The rate of 'spread' as a measure of actual production of fungal material was found to be extremely untrustworthy. It gives, however, a satisfactory measure of the effect of different temperatures on the rate of growth in a medium of *constant composition and constant thickness.* When, however, different media are concerned the same rate of surface spread may be associated with extremely different rates of mycelium production.

In surface 'spread' the fungus follows approximately the compound interest law, but the dry-weight production there is more nearly a linear relation of time.

For cultures on solid media in the dark, zonation is confined to a temperature of about 25° C.; there is no zonation at 24° C. or 26° C. In the light, however, such cultures will show zonation at about 23° C.

The zones are more closely packed in a culture on a thick layer of medium than in that on a thin layer.

There is no evidence that accumulation of waste products in any way favours zone formation.

In conclusion the writer wishes to thank for his valuable help Professor V. H. Blackman, at whose suggestion this work was undertaken.

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NOTE.

THE LAWS OF PROBABILITY AND THEIR MEANING.—The point has more than once been raised, in conversation and in correspondence, that the constancy of form shown by the frequency distributions of sizes of genera—the charts showing numbers of genera of 1, 2, 3, . . . species—has no bearing on vital phenomena, but is in some way or other a necessary outcome of 'the laws of probability'.

But the laws or rules of probability are only rules for the combination of chances and can give us nothing by themselves, any more than the rules of multiplication and division. If the chance of a given event occurring is p_1 and the chance of an independent event occurring is p_2, the chance of both events occurring is $p_1 p_2$: if the two events are not independent but alternative, the chance of *either* the first *or* the second occurring is $p_1 + p_2$—such rules alone can only lead us to a form of frequency distribution by applying them to given assumptions concerning the facts. A few illustrations may make the matter clearer.

If we have n dice, and we assume (a) that the chance of throwing sixes is the same for every die, (b) that the same dice are used throughout the experiment, so that the chance of throwing sixes is the same at every throw, (c) that the result of the throw of every die is independent of that of every other, then the laws or rules of probability show that the frequencies of 0, 1, 2, 3, . . . sixes in N throws of the n dice are given by the successive terms of $N(q+p)^n$. If we find the results of a long series of throws differ significantly from this theoretical expression, we may conclude that our assumptions are in error: the dice are not all the same, or two different sets of dice with different values of p have been used, or it may be that we have merely taken an incorrect value of p.

Again, if we assume (a) that an error of observation is compounded of a number of elementary errors, (b) that the number of these elementary errors is large, (c) that positive and negative elementary errors are equally frequent, (d) that elementary errors are independent, the form of frequency distribution deduced is the normal curve of errors. If we make different assumptions as to the way in which an error of observation is built up, we will—or may—arrive at a different form of distribution, and conversely, if the curve observed is not the normal curve of errors, we are justified in concluding that our assumptions as to the genesis of an error of observation are wrong.

The following case, taken from some recent work,[1] is particularly illuminating as regards the light which the form of the frequency distribution may throw on the facts. Data are given as to the number of girls in a munition works who have met with 0, 1, 2, 3, . . . accidents (trivial accidents, sufficient to send the girl to the Welfare Room) during a certain period. The actual form of frequency distribution is not unlike that

[1] Greenwood and Yule, Journ. Roy. Stat. Soc., March, 1920.

observed for sizes of genera, girls with no accidents being most frequent, but there is not so long a 'tail' towards high numbers of accidents.

A. It is assumed that every girl is equally likely to meet with an accident during any short interval of time. The form of frequency distribution deduced does not fit the facts.

B. It is assumed that the girls may be regarded as forming two groups, careful and careless, a girl of the second class having a higher chance of meeting with an accident during any short interval of time than a girl of the first class. The form of frequency distribution deduced still cannot be made to give a good fit to the facts.

C. It is assumed that the girls are of all degrees of 'carelessness', i. e. of chance of accident during a short interval of time, and that the form of frequency distribution of carelessness is given by one of Pearson's curves with origin at zero, so that there are or may be girls of zero carelessness. The form of frequency distribution now deduced for numbers of girls with 0, 1, 2, 3, . . . accidents is found to give an excellent fit to the facts, and from the constants of the fitted distribution we can deduce the distribution of carelessness amongst the girls. The interpretation thus arrived at is confirmed by finding that the number of accidents met with by a girl during one interval is correlated with the number she shows during a subsequent interval.

We may conclude that assumptions A and B are in error, whereas C *may* be right, and since it is reasonable and probable and otherwise confirmed it probably *is* the right interpretation.

Similarly, from the view of evolution now discussed, the form of the frequency distribution for sizes of genera can be deduced, and it appears to give a very good fit to the facts. This is, in so far, confirmatory evidence of the truth of the view.

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AND

R. THAXTER, M.A., PH.D.
PROFESSOR OF CRYPTOGAMIC BOTANY IN HARVARD UNIVERSITY, CAMBRIDGE, MASS., U.S.A.

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The Trisomic Mutations of Oenothera.

BY

R. RUGGLES GATES, PH.D., F.L.S.,

Professor of Botany, University of London (King's College).

With Plate XI.

THE purpose of the present paper is to record the occurrence and cytological behaviour of a new mutation having 15 chromosomes, and to discuss various questions relating to trisomic mutations in *Oenothera*. The breeding experiments involved will be described elsewhere, but it is necessary to refer to them briefly in this connexion.

Reciprocal crosses were made between a pure homozygous race of *Oenothera rubricalyx* and *Oe. Hewettii*, Ckll., in 1915 at the Missouri Botanical Garden and the following year at the University of California. It should be pointed out that *Oe. Hewettii* has not bred entirely true in my cultures. There is a green-stemmed as well as a red-stemmed form, and a second generation culture of 73 red-stemmed plants includes one small dwarf. *Oe. Hewettii* differs throughout from *Oe. rubricalyx*, the most conspicuous differences being the narrower leaves, which are practically smooth (crinkled in *rubricalyx*) with midribs red on the upper surface and green below. The petals of *Oe. Hewettii* are also much narrower, so that gaps are left between them at the base where they fail to overlap. The buds are green and covered with soft pubescence, while the stems are free from the characteristic red papillae of the *Lamarckiana* forms. Four generations of hybrids have been grown from *Oe. Hewettii* × *rubricalyx*, and three generations of the reciprocal. With the exception of one culture these have been grown at the Royal Botanic Gardens, Regent's Park. The F_1 hybrids are intermediate between the parents, but in both cases more like the seed parent. They both have the red bud colour pattern of *rubricalyx*, but it is considerably diluted. In later generations these differences between the reciprocal hybrids are maintained. As regards bud colour in F_2 and later generations, there is a continuous range from full red to completely green buds,

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and it was found impossible (contrary to the usual experience with this character in other hybrids) to classify the individuals with any certainty in the F_2, and the usual ratios are not obtained. Nevertheless, there is segregation of this character in later generations, although the green-budded segregates usually retain a tinge of red on the hypanthium. Whether segregation actually occurs in the other characters need not be discussed in the present connexion, although the first impression is that of a blend which essentially retains its blended condition.

In the present strain of *Oe. Hewettii*, a native of New Mexico which was kindly sent me by its discoverer Dr. T. D. A. Cockerell (1913) in 1914, as well as in these hybrids, occasional aberrant forms appear, as already mentioned. Reference will here be made only to a type which appeared in 1920 in the F_1 of the cross *Oe. rubricalyx* × *Hewettii*. This culture (No. 54) numbered 53 plants, including two which were strikingly aberrant and precisely alike. The rosette leaves were very much broader and more crinkled than the typical F_1 hybrids. In this feature these mutants resembled *Oe.* mut. *lata*. The stem leaves were also more crinkled, but were pointed at the tip and a resemblance to the mutant *lata* was not at once suggested, although the plants were shorter than the type and the stem was slightly zigzag. These plants probably most resembled *Oe.* mut. *semilata*, Gates. For a discussion of the various occurrences of this mutation see Gates (1915, p. 111). Examination of the pollen in these plants showed that the grains were all three-angled. A flower of one of them was selfed and produced 33 seeds (as against the usual 100–300 in typical members from these crosses), but the seeds failed to germinate.

Descriptions and photographs of this type will be published elsewhere. Cytological material was collected from one of them and shows that this mutant had 15 chromosomes. I am indebted to Miss E. M. Rees, B.Sc., for making the microscopic preparations for this study, and to Mrs. N. Ferguson, B.Sc., for preparing the drawings. The remainder of this paper will be devoted to pointing out the main cytological peculiarities of this new 15-chromosome type. A large number of chromosome counts both in pollen mother-cells and in somatic tissues established beyond doubt that the chromosome number is 15, but in two somatic cells 16 chromosomes were clearly present. The explanation of such departures from the somatic number has been discussed elsewhere (Gates and Thomas, 1914).

In the first place, it may be pointed out that the occurrence of mutations with an extra chromosome in a pair of individuals in a culture is not uncommon, and it furnishes clear evidence as to how they have arisen. For it means that the irregular 8–6 distribution of chromosomes took place on the heterotypic spindle in a pollen mother-cell and resulted in the production of two pollen grains with eight chromosomes, both of which functioned in fertilization to produce mutants. If the irregular division had occurred in

a megaspore mother-cell, only one functional megaspore with eight chromosomes would have resulted. The occurrence of any aberrant form in a pair of individuals therefore suggests at once that they have probably arisen from an irregular chromatin distribution in the heterotypic mitosis of a pollen mother-cell of the previous generation. In this case the non-disjunction of a pair of chromosomes must then have occurred in a pollen mother-cell of *Oe. Hewettii.*

We may now consider briefly the main cytological features of this aberrant type, confining ourselves to diakinesis and the heterotypic mitosis. Figs. 1 and 2, Pl. XI, represent late stages in diakinesis. In the former 15 chromosomes can clearly be counted. It will be seen that nearly all of the chromosomes, but not all, are arranged in pairs, these pairs in many cases forming complete rings. It has long been known that in Oenotheras of the *Lamarckiana* and *biennis* group the formation of ring chromosomes seldom occurs (Gates, 1908 ; Davis, 1910), while Davis (1909) has described the constant formation of ring chromosomes during diakinesis in *Oe. grandiflora*, and Cleland (1922) has recently figured the regular occurrence in *Oe. franciscana* of five pairs of chromosomes in rings, the other four forming a single large ring. In Figs. 1 and 2, referred to above, there are five pairs of ring chromosomes in each case. In no case have more than five ring-pairs been found, although there are sometimes less, and usually not more than one ring persists on the heterotypic spindle. Unfortunately no material of the parent species *Oe. Hewettii* at this stage has been examined, so it is unknown whether this species is like *Oe. grandiflora* in having only ring chromosomes, but it appears more probable that it may be like *Oe. franciscana*, having five ring-pairs and four other chromosomes more loosely united into a single ring.

As previously pointed out by the author, the absence of a clear pairing of all the chromosomes during diakinesis and the heterotypic mitosis is an indication of a weak attraction between the chromosomes constituting a pair. This is the characteristic condition in *Oe. Lamarckiana* and its derivatives. Davis and Cleland have argued that the occurrence of ring chromosomes in *Oe. grandiflora* and *Oe. franciscana*[1] is a sign of a 'pure species'. In the present instance, however, we have as many as five ring-pairs of chromosomes in a 15-chromosome mutant occurring in an F_1 hybrid between forms belonging to quite distinct species. One of these rings might of course be descended from the pair which failed to separate in the *Hewettii* pollen mother-cell, but there is no evidence that such a pair will persist in this

[1] It should be pointed out that the writer made a study of the variability of *Oe. franciscana*, which Cleland stresses as a pure species. While in California in 1916 a study was made of large wild colonies growing near San Francisco, and also of the variations of this species in cultures. The wild variations were particularly conspicuous in size of flower, pigmentation, and the number of red papillae on the sepals. It was evidently a population of interbreeding forms and must have been heterozygous for a number of characters.

way. It appears probable that in *Oe. lata* (Gates and Thomas, 1914) the three chromosomes belonging to a particular pair ($A\ A_1\ A_2$) may all undergo synapsis with each other equally according to chance. Bridges (1916) has shown that in XYY males of *Drosophila* the synapses of these three chromosomes are probably according to chance, while in XXY females homosynapsis occurs much oftener than heterosynapsis.

In this mutant from *Oe. rubricalyx* × *Hewettii* the unpaired chromosome is sometimes clearly indicated (see Fig. 5). Here we must suppose that four at least of the ring-pairs were made up of one chromosome from *rubricalyx* paired with the corresponding one from *Hewettii*. So far as can be judged from analogy, another pair might equally be made up from the descendants of the original non-disjoined *Hewettii* pair or from one of these and the corresponding *rubricalyx* chromosome. The odd chromosome is then likely to be a different one in different pollen mother-cell nuclei of this mutant according to how synapsis has taken place. But in any case it is clear that all these ring-pairs but one must be constituted from a union between a *rubricalyx* chromosome and a *Hewettii* chromosome, unless we make the very unlikely assumption that in this hybrid the pairing of homologous chromosomes does not take place. It is therefore impossible to regard the mere presence of ring chromosomes as evidence for a homozygous condition of the species. It is rather a useful indication of the strength of the attraction which produces ring-pairs, but it appears that that attraction may on occasion be quite as great between the chromosomes of forms belonging to distinct species as between the chromosomes of a homozygous species.

The chromosome rings may persist on the multipolar heterotypic spindle, although, as shown in Figs. 3 and 4, the number of rings is usually fewer at this time. Occasionally a ring is found persisting even in the heterotypic metaphase (Fig. 6), but this is unusual. That one or two ring-pairs of chromosomes are occasionally formed in diakinesis and may persist on the heterotypic spindle in *Oe. Lamarckiana* and *Oe. lata* is shown in an early paper (Gates, 1907, Figs. 17–20, 33, 34), although they were differently interpreted at the time. There appears to be no fixity in the number of such rings except in forms in which the attraction is so strong that all the pairs form rings.

One striking feature of this 15-chromosome mutant is the fact that the pollen is not conspicuously sterile, although the fact that only 33 seeds were produced from the self-pollination of a flower shows that a much higher percentage than usual of the pollen was non-functional. The irregularities in meiosis are much less numerous than in the various *lata* forms (cf. Gates and Thomas, 1914). They apparently begin in the heterotypic anaphase through chromosomes lagging behind, as in Fig. 7, where seven chromosomes are arriving at one pole, five at the other, and three are lagging behind. Fig. 8 is a heterotypic telophase in which apparently eight

chromosomes have reached one daughter nucleus, six the other, while the fifteenth is left out in the cytoplasm. As is usual, a number of these chromosomes already show the split for the homotypic mitosis.

Discussion.

It has long been recognized that if the chromosomes of *Oenothera* are differentiated from each other in their hereditary qualities, as they clearly are in some organisms, then seven different types with 15 chromosomes might be expected. For a number of years *Oe. lata*, or the *lata*-like assemblage of forms, was the only mutation in which the chromosome count of 15 was clearly authenticated. The possibility remained either that *any* chromosome might constitute the extra one and give rise to a *lata*-like condition, or that the disturbance of duplicating one of the other chromosomes was so great that the result was non-viable. It seems highly probable that non-disjunction of any of the seven pairs of chromosomes may take place, but it was possible that only one of the seven simple trisomic types (to use Blakeslee's term) was viable. This possibility was perhaps strengthened by the discovery (Blakeslee, Belling, and Farnham, 1920) that *Datura*, which has twelve pairs of chromosomes, produces twelve trisomic mutations (i. e. forms with an extra chromosome), most of which have, like *Oe. lata*, high pollen sterility and transmit their characters almost entirely through the female sex. For since in *Datura* only one chromosome in twelve is duplicated, the germinal disturbance will be less than in *Oenothera*, where a chromosome constitutes one-seventh of the whole chromosome series.

In recent years, however, a long array of 15-chromosome mutants in *Oenothera* has been demonstrated. Indeed the number now far exceeds the seven types originally anticipated, and this raises a number of interesting questions regarding the relationship and manner of origin of these types. Blakeslee has also found two additional *Datura* mutants with 25 chromosomes, which he interprets as due to the presence of another genetic factor in certain trisomic forms. It may be pointed out here that Blackburn and Harrison (1921), in their studies of chromosomes in the roses, have found one plant of *Rosa pimpinellifolia* with sterile anthers and 15 instead of 14 chromosomes. But they apparently observed no other differences between this plant and the type. We may now enumerate the various *Oenothera* forms in which 15 chromosomes have been found. They are summarized in the following table. A (?) indicates either that the number of chromosomes is inferred from the genetic behaviour, or that it has not been determined with certainty.[1]

[1] It may be pointed out that Lehmann (1922, pp. 366–99) has treated all these forms with an extra chromosome in a somewhat different way in his 'Oenotheraforschungen', and Tischler (1922, pp. 604 ff.) has also treated the subject from the cytological point of view.

Table I.

Mutants with 15 chromosomes.

Mutation.	*Parent form.*	*Author.*[1]	*Remarks.*
lata, de Vries	*Oe. Lamarckiana*	Lutz, 1912 Gates, 1912 Gates and Thomas, 1914	
semilata, Gates	*Oe. lata* × *Lamarckiana*	Gates and Thomas, 1914	
albida, de Vries	*Oe. Lamarckiana*	Lutz, 1908, 1917	
incurvata, Gates	*Oe. Lamarckiana*, Swedish race	Gates, 1915	
lata	*Oe. biennis* race	Gates and Thomas, 1914	The conception of parallel mutations was based on this form.
simplex lata	*Oe. Lamarckiana*, mut. *simplex*	de Vries, 1919 Boedijn, 1920	*Oe.* mut. *simplex* differs from *Lamarckiana* in having lost a lethal factor. It has no empty seeds, no *velutina* gametes, and hence does not give twin types in F_1 crosses. It produces *lata*, *oblonga*, *scintillans*, &c., as mutations.
secunda lata	*Oe. Lamarckiana*, mut. *secunda*	Boedijn, 1920	
albinervis, van Overeem	*Oe. biennis semigigas* × *biennis*	van Overeem, 1920	
lata rubricalyx	*Oe. rubricalyx* × *grandiflora* F_2	Gates and Thomas, 1914	
de Vriesii	*Oe. Lamarckiana semigigas*	van Overeem, 1920, 1922	
bipartita, Lutz	*Oe. Lamarckiana*	Lutz, 1917	
type 5509 (modified *oblonga*)	,,	,,	
subovata, de Vries	*Oe. Lamarckiana* and *Oe. lata* × *Lamarckiana*	,,	
type 2806	*Oe. Lamarckiana*	,,	'Having many points in common with type 5509.'
type 4499	*Oe. lata* × *Lamarckiana* and *Oe. lata* selfed	,,	
type 5365	*Oe. lata* selfed	,,	
exilis, Lutz	*Oe. lata* selfed	,,	
exundans, Lutz	*Oe. lata* selfed	,,	
superflua, de Vries?	*Oe. lata* selfed	de Vries, 1916	'A mutant from *lata*,' probably from *Oe. lata* × *Lamarckiana*.
cana, de Vries	*Oe. Lamarckiana*, &c.	van Overeem, 1922	
pallescens, de Vries	*Oe. Lamarckiana*	van Overeem, 1920	
Lactuca, ,,	,,	,,	
liquida, ,,	,,	,,	
lata, de Vries	*Oe. suaveolens*, Desf.	de Vries, 1918	Chromosomes counted by van Overeem.
jaculatrix, de Vries?	,,	van Overeem, 1920	See below.
scintillans, de Vries	*Oe. Lamarckiana*	Hance, 1918	
oblonga, de Vries	,,	Lutz, 1917 van Overeem, 1920, 1922	There are certain contradictory features in the record of '*oblonga*', but van Overeem (1922) has counted 15 chromosomes in 10 plants.
unnamed	*Oe. erythrina*	van Overeem, 1920	

[1] This refers to the author who determined the chromosome number.

Mutation.	*Parent form.*	*Author.*	*Remarks.*
sublinearis, de Vries?	*Oe. Lamarckiana*	de Vries, 1909	This is based only on genetic behaviour.
elliptica, de Vries?	,,	,,	,,
nanella lata	*Oe. nanella*	Lutz, 1917	
dwarf type 2256	,,	,,	
aberrans, Lutz	*Oe. lata* × *Lamarckiana*	Lutz, 1916	14^{+1} chromosomes.
rubrinervis, de Vries	*Oe. Lamarckiana*, &c.	,,	14^{+1} chromosomes. See remarks below.
lasiopetala, Bart.?	*Oe. stenomeres*, Bart.	Bartlett, 1915	Chromosomes not counted.
saligna, de Vries	*Oe. biennis*, Chicago, de V.	de Vries, 1916	,,
lata	*Oe. grandiflora* × *biennis*	Davis, 1913	
unnamed	*Oe. rubricalyx* × *Hewettii*	Here recorded	

For purposes of interpretation we may consider (1) the parallel mutations (*lata*) which have appeared in *Oe. Lamarckiana*, *biennis*, *suaveolens*, also as mutations from 14-chromosome mutants, and in various hybrids; (2) the various 15-chromosome types which have arisen directly from *Lamarckiana*. The now widely adopted conception of parallel mutations was based upon the fact (Gates, 1912 *a*) that *Oe. Lamarckiana* mut. *lata* and *Oe. biennis* mut. *lata* were corresponding types resulting from the same kind of germinal change in different species. A partial list of parallel mutations in *Oenothera* has been published elsewhere (Gates, 1921). It has long been recognized that *lata* is in many respects intermediate between *Lamarckiana* and *gigas* although it has but one extra chromosome. Van Overeem (1922) shows that the papillary cells of its stigma are conspicuously larger than in *Lamarckiana*, and concludes that in *lata* there is a lack of balance between cell size and turgor, the lack of turgor accounting for the habit of the plant.

The question of the relationships between the various mutants with 15 chromosomes is a very difficult one. In the first place, we may eliminate from present consideration all such forms arising from different species than *Lamarckiana* or from hybrids between forms belonging to two distinct species. Such cases of course all arise through a chance irregular meiotic division during the distribution of the hybrid differences in the germ cells, as was originally explained in the case of *lata rubricalyx* (Gates, 1914). This leaves us to consider the various 15-chromosome forms arising from *Lamarckiana* and its mutants. In various combination mutations, such as *nanella lata* and *simplex lata*, the explanation is evidently similar to that given above. If we examine the 15-chromosome forms arising directly from *Lamarckiana*, we find in the first place the following from the experiments of de Vries: *lata*, *albida*, *scintillans*, *oblonga*, which are all widely different and have long been known, and *pallescens*, *Lactuca*, *liquida*, and *cana*, which are closely related, are more like *Lamarckiana* and have been discovered more recently (de Vries, 1916). The chromosome number in *cana* has apparently not been actually counted, but its genetic behaviour is exactly like that of the other forms with 15 chromosomes, leaving no doubt

that it must have the same unbalanced chromosome condition.[1] The genetic behaviour of all these forms will be referred to later. The mutant *incurvata* (see Table I) should probably not be included in this series, for, while it is evidently a distinct type, it appeared in the Swedish race of *Oe. Lamarckiana* and may owe its peculiarities to certain genetic differences which exist between it and the *Lamarckiana* of de Vries's cultures.

We now come to the various 15-chromosome forms described by Miss Lutz (1917). In the first place is *subovata*, de Vries, a sterile form which de Vries (1909) obtained not only from *Lamarckiana* and *lata* × *Lamarckiana*, but also from *sublinearis* and from *scintillans*. The latter has 15 chromosomes (Hance, 1918), and the genetic behaviour of *sublinearis* indicates that it also probably had 15. *Elliptica*, de Vries, also, from its genetic behaviour, is probably in the same condition. Miss Lutz describes a 15-chromosome form which she calls *bipartita* and which is much more like *Lamarckiana* than most of these types. It differs in that flowers with more than four stigma-lobes are much commoner than in *Lamarckiana*, and the petals frequently develop diagonal clefts owing to their not overlapping in regular order. Cleft petals, owing to conditions which are apparently somewhat different, have been observed in various other forms (van Overeem, 1920; Gates, 1917, 1923). The flowers are smaller and the leaves more finely crinkled than in *Lamarckiana*. Much of the pollen is obviously bad and it was very difficult to get seeds from self-pollination. There is apparently no genetic evidence to indicate the relation of *bipartita* to other 15-chromosome mutants, except that in one instance *Lamarckiana* × *lata* gave two *bipartita* (?) in addition to 63 *Lamarckiana*, one *lata*, and certain other forms. Miss Lutz also refers to two other mutations from *Lamarckiana* with 15 chromosomes, but they are not described. They are 'type 5509', which is said to be a modified *oblonga*, and 'type 2806', which is said to have 'many points in common with type 5509'. Both then appear to be nearest *oblonga*. In addition to the above 15-chromosome mutations from *Lamarckiana*, others have so far been obtained only from *lata* selfed or from *lata* × *Lamarckiana*. These will be referred to later.

For the purpose of further analysis it appears best to confine ourselves largely to the eight and more forms with an extra chromosome which have been identified by de Vries. The genetic relationships of these forms to one another renders the whole problem a very difficult one. The next evidence to be considered concerns the genetic behaviour of these forms when selfed or in crosses. In general they are more or less completely male sterile, and when pollinated from *Lamarckiana* give the two parental types with a greater or lesser preponderance of *Lamarckiana* offspring. Eight forms arising directly from the *Lamarckiana* of de Vries's cultures and

[1] Van Overeem (1922) has recently shown that the somatic cells of *cana* have 15 chromosomes.

showing this type of behaviour are known to have 15 chromosomes. Miss Lutz has shown that *subovata* also has 15 chromosomes, and the same may reasonably be inferred of *sublinearis* and *elliptica* from their genetic behaviour. In addition, we have *bipartita*, making a total of twelve forms. 'Type 5509' and 'type 2806' of Miss Lutz may be looked upon as modified oblongas, the modification being due, perhaps, to the presence of independent genetic factors, although in *Oenothera* so few factors of this kind have been demonstrated that great weight cannot be attached to this suggestion.

We may next consider the relationships of the trisomic forms as indicated by the offspring they produce when selfed or pollinated from *Oe. Lamarckiana*. As formerly pointed out (Gates and Thomas, 1914), *lata* and *semilata*, Gates, are peculiarly related, since the latter is essentially intermediate between *lata* and *Lamarckiana* and is only known to occur in the offspring of *lata* × *Lamarckiana*. Similarly mut. *superflua*, de Vries (1916), occurred as a mutant from *lata* (presumably pollinated by *Lamarckiana*) in 1914, and when selfed gave the usual dimorphic progeny characteristic of trisomic forms. It probably had 15 chromosomes. Then *cana*, *pallescens*, *Lactuca*, and *liquida* are found by de Vries (1916) to arise apparently with equal facility from *Lamarckiana* or from *lata* × *Lamarckiana*. Indeed, with far fewer seeds developed, owing to the sterility, they appear to arise with a much higher frequency from the latter source. Mut. *subovata* has also arisen both from *Lamarckiana* and from *lata* × *Lamarckiana*. Furthermore, the 'type 4499' of Miss Lutz has arisen from *lata* × *Lamarckiana* and from *lata* self-pollinated, while her *exilis*, *exundans*, and 'type 5365' are only known from the offspring of *Oe. lata* selfed. Hence there appears to be a peculiar relationship between *lata* and some at least of the other trisomic forms.

Oe. cana mutants have been obtained (de Vries, 1916) from *Lamarckiana*, *lata*, *pallescens*, *scintillans*, and *laevifolia*, but its frequency as a mutant from *Lamarckiana* is only 0·03 per cent., while from *lata* it may be as high as 9 per cent. When selfed, *cana* gives, in addition to *cana* and *Lamarckiana*, a significant frequency of *nanella*, as well as occasional stray mutations, including *albida*. *Pallescens*, which is said by de Vries to differ the least from *Lamarckiana*, was not discovered until 1911. It has since been obtained from some of the derivatives of *Lamarckiana*. When selfed it gives, in addition to the usual dimorphic progeny, *cana*, *liquida*, *scintillans*, *lata*, *albida*, and *rubrinervis* mutants, the mutants reaching as high as 4 per cent.[1] Mut. *Lactuca*, first observed in the offspring of *lata* × *Lamarckiana* in 1913, gave when selfed 39 *Lamarckiana*, four *Lactuca*, and one *nanella*. Mut. *liquida* most resembles *scintillans*. It was first observed in

[1] The question of *rubrinervis* will be discussed below.

1912, and has arisen once from *Lamarckiana*, four times from *lata* × *Lamarckiana*, and once from *pallescens*. When selfed it yields *liquida* and *Lamarckiana* in nearly equal numbers, and also the mutants *scintillans*, *pallescens*, *cana*, and *oblonga*. It thus appears that while these four simple trisomic forms arise more frequently from *lata* × *Lamarckiana* than from *Lamarckiana* itself, and frequently produce other trisomic forms as mutants in their offspring, yet they never give rise to *lata* itself. This fact appears to be significant as indicating that they are perhaps in some sense modified from *lata*, which appears as the basal form in the series. Thus the records show that *cana* can give rise to *pallescens* and *pallescens* to *cana*, also that the same interchangeable condition exists between *pallescens* and *liquida*. But while any of these eight trisomic forms can apparently arise from *lata* × *Lamarckiana*, none of them except *pallescens* apparently can give rise in turn to *lata*.

That *scintillans* can also give rise to *lata*, however, is shown by the following record (de Vries, 1913, p. 248). Eight *lata* mutants were derived from *scintillans* in a strain of *Oe. Lamarckiana* obtained from Messrs. Vilmorin, Andrieux & Cie, of Paris. *Lata* also appears (p. 257) in the offspring of *scintillans* in de Vries's strain of *Lamarckiana*. In addition, *scintillans* is said to have appeared 14 times as a mutant up to 1900, and to arise equally from *Lamarckiana* or *lata*. Hence this reciprocal relation exists between these two trisomic forms. Further, *lata* × *scintillans* (l. c., p. 257) produces only *lata* and *Lamarckiana* (736 plants) and *oblonga* × *scintillans* gave 61 *oblonga*, 13 *Lamarckiana*, and one *lata*. In another record (p. 265) *oblonga* × *scintillans* gave 81 per cent. *oblonga*, while *scintillans* × *oblonga* gave 18 per cent. *oblonga*. It has long been known (de Vries, 1909, i. 390) that a special relation exists between *oblonga* and *scintillans*, the latter producing a progeny which fluctuates widely but usually includes a high percentage (2–15 per cent.) of *oblonga* in addition to *scintillans*, *Lamarckiana*, and about one per cent. of *lata* and *nanella*. On the other hand, mut. *oblonga* when selfed is found to breed essentially true except that it gives occasional mutants, including *albida*, *elliptica*, and *rubrinervis*. It does not apparently produce *scintillans*, but it has arisen as a mutant both from *lata* and from *scintillans* × *nanella*.

By selfing certain *lata* plants, Miss Lutz (1917) has succeeded in getting 360 seeds from three individuals. Of these seeds 129 germinated and 126 developed. Three of the *lata* offspring were again selfed and yielded 259 seeds, of which 99 germinated. Of the 226 plants so produced 109 were *lata*, 8 *lata* (?), 57 *Lamarckiana*, 4 *Lamarckiana* (?), and 23 are said to have belonged to seven different 15-chromosome types (names unfortunately not given), 4 (belonging to three types) were 14-chromosome mutants, and one each had respectively 16, 21, and 22 chromosomes. The remaining 18 plants were apparently not classified. The offspring may be said to

have included approximately 50 per cent. of *lata* and 25 per cent. of *Lamarckiana*, the remainder being aberrant forms. Of the latter those known to have 15 chromosomes belonged to seven types. Only four mutants were known to have 14 chromosomes, and it is believed there were no others in this group. It appears from the rather involved account that the 18 unclassified forms were believed to have probably had more than 14 chromosomes. From *lata* × *Lamarckiana* Miss Lutz obtained 27 *lata*, 15 *Lamarckiana*, 1 *albida*, 1 *aberrans*, and one doubtful form. From *Lamarckiana* × *lata* she succeeded in getting 1 *lata*, 63 *Lamarckiana*, 2 *bipartita* (?), 1 *nanella*, and 9 of the *rubrinervis* type with 14 chromosomes which is probably *subrobusta* (see below).

From all these and many other rather jumbled facts, several significant points seem to emerge as regards these trisomic forms: (1) *scintillans* gives a large percentage of *oblonga* offspring and evidently stands in a peculiar relation to that type; (2) there is a reciprocal relationship between *lata* and *scintillans* in that each can give rise to the other; (3) *lata* can produce directly *albida*, *oblonga*, and *scintillans*, while *oblonga* can produce *albida* but apparently not *lata* or *scintillans*, and *scintillans* produces *oblonga* and *lata* with relatively high frequency but apparently not *albida*; (4) that *semilata*, Gates, stands in a peculiar relation to *lata* has already been pointed out.

We may next refer to the fact that *albida* and *oblonga* apparently breed true notwithstanding their unbalanced chromosome number. In the case of *albida* (de Vries, 1909) this involves only 122 offspring grown in 1897–8. At any rate no *Lamarckiana* appeared among them. For *oblonga* the evidence is better. Some 2,919 offspring were grown during 13 years, and were all *oblonga* except three *albida*, one *elliptica*, and seven *rubrinervis*. Miss Lutz (1917) suggests that such trisomic forms would breed true if all the gametes of one sex contain seven chromosomes and all those of the other sex eight chromosomes. In *lata* and other 15-chromosome forms it is clear that very few 8-chromosome pollen grains ever function in fertilization. In some cases, as I have previously suggested, the extra chromosome may be lost during the mitoses in pollen grain or pollen tube. It would therefore be necessary to assume that in *albida* and *oblonga* all the functional eggs contained eight chromosomes and all the functional pollen seven chromosomes. On the other hand, Bartlett (1915) probably overstates the case in assuming that *all* 8-chromosome pollen grains are eliminated, for various results indicate that trisomic forms do occasionally produce functional 8-chromosome pollen grains. As already pointed out in this paper, the frequent occurrence of trisomic mutants in pairs (de Vries, 1916, also notes such cases) shows that these have probably arisen from non-disjunction in a pollen mother-cell of the previous generation.

An effort has been made to systematize the many records of trisomic mutants in *Oenothera* as regards their origin, their offspring, and their

consequent relationships with each other, with the intention, if possible, of bringing order out of what appears to be chaos. But it seems that, with some exceptions, any trisomic form is capable of giving rise to any other. The experiments of de Vries with *cana* and other forms lead him to the conclusion that none of these trisomic mutants transmit their characters through the pollen. This is in accord with the cytological studies of *lata* (Gates and Thomas, 1914), in which it was shown that very few pollen grains can be formed having eight chromosomes. But in trisomic mutants such as the one described in this paper, where a larger percentage of functional pollen is produced and the pollen nuclei not infrequently receive eight chromosomes, it is probable that the extra chromosome may often be lost in the later nuclear divisions of the male gametophyte. When used as the female parent in crosses, these trisomic forms frequently appear in numbers approaching 50 per cent. of the offspring. From this fact it seems clear that in megaspore formation the chances are equal that the extra chromosome will pass to either pole, the defect in numbers of the trisomic form being the result of a lesser viability of gametes with the extra chromosome, or of the extra chromosome sometimes being left behind on the spindle. There is, on the contrary, some evidence of greater viability on the part of zygotes with an extra chromosome (at least in the case of *lata*).

We must now refer to certain other interesting forms in Table I (p. 548) which have not yet been mentioned. These are *aberrans*, Lutz, and *rubrinervis*, de Vries. Miss Lutz (1916) describes *aberrans* as a mutant from *lata* × *Lamarckiana*, two individuals occurring from this cross in 1908 and 1909. The type differs from *Lamarckiana* by no very marked characters except the almost complete pollen sterility. A moderate abundance of pollen was produced, but containing few good grains, and it was found almost impossible to obtain seed by self-pollination. The plants had more slender, tapering buds than *Lamarckiana*, but produced an abundance of flowers with slightly lighter yellow petals. Cytological examination of root tips showed the constant presence[1] in both plants of a small chromosome fragment in addition to the usual 14 (hence 14^{+1}). As Miss Lutz points out, this type no doubt arose through a fragment of a disintegrating chromosome becoming included in one of the daughter nuclei, a condition which was shown actually to occur (Gates and Thomas, 1914) in the meiotic pollen divisions of *Oe. lata* forms. The inclusion of such a fragment has been sufficient to alter appreciably the characters of the plant; the fragment has regularly divided in mitosis, but its presence during the meiotic division was a sufficient disturbance to cause almost complete pollen sterility.

The other fact recorded by Miss Lutz (1916) which is germane to the present discussion is that in a *rubrinervis* mutant arising from *lata* × *Lamarck-*

[1] Occasionally the small chromosome could not be found in cells in which it apparently could not have been concealed.

iana in the garden of de Vries and identified by him as typical *rubrinervis*, cytological examination of the root tips showed a small extra chromosome, somewhat larger than the small one in *aberrans*. It is significant that this mutant also arose from *lata* × *Lamarckiana*. While the origin of this chromosome fragment is therefore clear, its significance is less certain. Mut. *rubrinervis*, de Vries, is recorded as a form which breeds true and has no marked pollen sterility (i. e. the usual 50 per cent). It is therefore not clear whether this fragment will prove to be a constant feature of the type which de Vries calls *rubrinervis*. It has long been evident that the form identified in American cultures as *rubrinervis* differs in certain features from the type of de Vries. It is a taller, stronger plant without a zigzag stem but with the same red stripes on the sepals, and appears to be the same as the derivative from *rubrinervis* crosses which de Vries (1913, p. 192) has since called *subrobusta*. The latter may in turn give rise to *rubrinervis* (according to de Vries) when self-pollinated. The taller, stronger plant is the one which has been called *rubrinervis* in all of my experiments. It certainly has 14 chromosomes without any extra fragment (Gates, 1908). There would appear to be still some uncertainty regarding the unity of the forms which de Vries has called *rubrinervis*.

Finally, brief reference may be made to mut. *lasiopetala*, which originated from *Oe. stenomeres* in the cultures of Bartlett (1915). It has hairy petals and is very late in flowering, forming terminal rosettes on its branches. Less than half the pollen appears viable, and as its offspring are dimorphic, like those of other trisomic mutants, it probably also has 15 chromosomes. The hairy petals are a new character in the genus and it is totally different from any other trisomic form. The tetraploid mutant *gigas* from *Oe. stenomeres* also gave rise to a secondary mutation *lanosa* with hairy petals like *lasiopetala* and also the 'filaments of the stamens alternate with the petals were densely lanose'. It is therefore parallel with *lasiopetala* but more extreme. Possibly this form had 29 chromosomes.

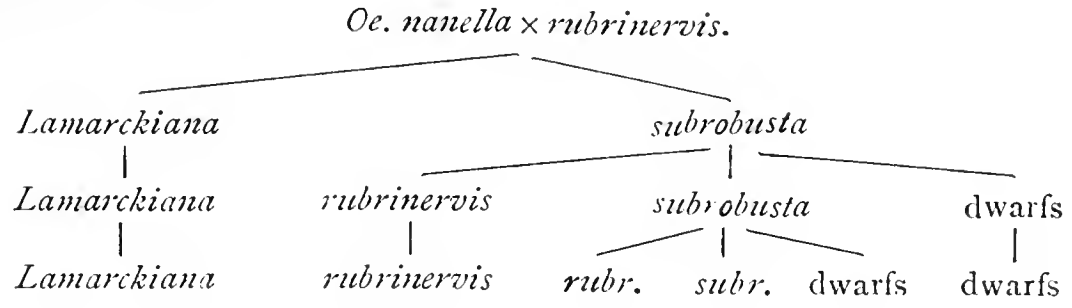
We may next consider the occurrence of plants with 16 chromosomes, either as mutations or in the offspring of simple trisomic forms. Their appearance is rare, and this confirms the fact that 8-chromosome pollen grains rarely function or rarely retain their full chromosome complex in the male nuclei. Miss Lutz (1917) obtained two *lata*-like mutants from separate cultures of *Lamarckiana* in 1908 and 1910. Both had 16 chromosomes, but they were 'in no sense identical forms'. Like *lata*, they had crinkled leaves, one broad, the other narrower; both had yellow-green foliage, irregularly shaped buds, and were male sterile. A third form with 16 chromosomes was a dwarf arising from *lata* selfed and having broad leaves in the rosette stage. In the experiments of van Overeem (1920) several forms with 16 chromosomes (double trisomic) have appeared. In the offspring of *Oe. Lamarckiana semigigas* the chromosome number ranged from 14 to 28 and

included three plants with 16 chromosomes, one of which resembled *cana*. Also from *semigigas* and its crosses with *Lamarckiana* and *gigas* the following 15-chromosome forms were obtained: *lata, cana, pallescens,* and *liquida*. Similarly from *Oe. biennis semigigas* × *biennis* was obtained *albinervis* with 15 chromosomes and three rosettes with small blue-green leaves, one of which was shown to have 16 chromosomes.

Such forms may of course arise from 8 + 8 chromosomes, or particularly in cases where the mother plant is triploid, from 9 ♀ + 7 ♂. That the condition is still an unbalanced one is shown by the male sterility of these forms.

We may now consider briefly the significance of these complicated facts, and it is well to point out in the first place a fact which has as yet been recognized by very few geneticists, namely, that the great majority of the known mutations in the Oenotheras involve altered chromosome numbers, chiefly simple trisomic. Indeed almost the only exceptions are (1) *brevistylis*, *rubricalyx*, and *gigas nanella*, which involve simple Mendelian differences, and (2) *rubrinervis*, *nanella*, perhaps *laevifolia*, and a few others which may possibly have arisen through crossing-over. Irregular chromosome distributions are then concerned in the great majority of *Oenothera* mutations.

It was first pointed out many years ago (Gates, 1908) that owing to the usually weak attraction between the chromosomes in *Oenothera* during meiosis, irregular chromosome distributions (now spoken of as non-disjunctions) may and do occur, giving rise to pollen grains (and probably also megaspores) with eight chromosomes. It was also pointed out that simultaneous non-disjunction of two pairs of chromosomes in opposite directions would lead to the formation of pollen grains all having seven chromosomes, but two of them lacking both members of one pair and two both members of another pair. Since then an attempt has often been made, by giving letters to the chromosomes, to work out the behaviour of the various mutations on a chromosome basis. Since no consistent scheme could be arrived at, the results were not published. Miss Lutz (1917) has made a similar attempt, but confesses failure. It is perhaps worth while to view the matter afresh in the light of our present knowledge. Let us apply this view first to mutations like *rubrinervis* and *nanella*. The striking facts in their genetic behaviour are (1) that they arise sporadically from *Lamarckiana*, (2) that they breed true except for occasional aberrant forms, (3) that in crosses with *Lamarckiana* they give the two parent types in various proportions, (4) that when crossed together they give (de Vries, 1913, p. 214) in F_1 *Lamarckiana* and *subrobusta* (which we have seen is closely related to *rubrinervis*). The *Lamarckiana* breeds true, while *subrobusta* in F_2 splits out dwarfs (*rubrinervis, nanella*) as a Mendelian recessive, and *rubrinervis*, both of which breed true. *Subrobusta* continues to split in the same manner in later generations. This is shown in diagram form on p. 557.



It may be worth while determining how many of these complicated facts of genetic behaviour can be explained by the simple hypothesis that in the origin of *nanella* and of *rubrinervis* there has been what we may call double non-disjunction in one or both parental germ cells, so that we have for *nanella* the formula $\frac{AACDEFG}{AACDEFG}$ (the B chromosome being absent), while for *rubrinervis* we have $\frac{BBCDEFG}{ABCDEFG}$. *Oe. nanella* × *rubrinervis* will then give an F_1 $\frac{AACDEFG}{BBCDEFG} + \frac{AACDEFG}{ABCDEFG}$. The former combination would be

Lamarckiana *subrobusta.*

expected to produce *Lamarckiana*, and in its pollen formation synapsis might be expected to take place between the A and B chromosomes instead of between the two A's and the two B's. It should therefore breed true, as it actually does. The other F_1 combination (*subrobusta*) might be said to be heterozygous for an AB pair of chromosomes. When selfed it would therefore be expected to give the following:

$$\frac{AACDEFG}{AACDEFG} + \frac{AACDEFG}{ABCDEFG} + \frac{ABCDEFG}{ABCDEFG}$$

nanella 2 *subrobusta* *Lamarckiana.*

But according to de Vries, we get in fact *rubrinervis* where we should expect to get *Lamarckiana*. In view of the fact that there is some confusion over the *rubrinervis* forms and that *subrobusta* was only recognized as a separate type in 1913, we might suppose that there was something incomplete in the analysis. But the hypothesis of double non-disjunction meets with other difficulties. It will explain the result of *Oe. rubrinervis* × *Lamarckiana* thus:

$$\frac{BBCDEFG}{ABCDEFG} \times \frac{ABCDEFG}{ABCDEFG} = \frac{BBCDEFG}{ABCDEFG} + \frac{ABCDEFG}{ABCDEFG}$$

rubrinervis *Lamarckiana* *rubrinervis* *Lamarckiana.*

But, as Miss Lutz pointed out, *nanella* × *Lamarckiana* introduces a fatal difficulty, for we have $\frac{AACDEFG}{AACDEFG} \times \frac{ABCDEFG}{ABCDEFG} = \frac{AACDEFG}{ABCDEFG}$, which is *subrobusta*, whereas we are supposed to obtain the two parent forms in the F_1 of this cross. It therefore appears to be impossible to explain the

behaviour of such 14-chromosome mutants as *nanella* and *rubrinervis* through double non-disjunction, although their origin and the fact that they breed true may be accounted for in this way. There are a few other forms whose origin could be explained by crossing-over, but this hypothesis equally fails to explain their later hereditary behaviour. Perhaps a critical repetition of some of the breeding experiments might throw light on these difficulties.

Let us now consider the situation as regards the trisomic forms. We have already seen that *lata* can apparently give rise to all the other trisomic forms, and that they usually arise with higher frequency from *lata* × *Lamarckiana* than from *Lamarckiana* itself. This must mean that when they arise from *lata* × *Lamarckiana* it is, usually at any rate, by the union of an 8-egg with a 7-male cell. The mutation giving a different trisomic form might then arise from a male gamete in which double non-disjunction has taken place. Thus if we suppose that *lata* is $\frac{AABCDEFG}{ABCDEFG}$, its female gametes will be *AABCDEFG* and *ABCDEFG* in equal numbers unless some irregularity occurs. Double non-disjunction in a pollen mother-cell of *Lamarckiana* might produce a male gamete *ACCDEFG*. Hence a trisomic individual could arise which was $\frac{AABCDEFG}{ACCDEFG}$. Let us suppose this is the formula for *scintillans*. Then the germ cells of this mutant could form various recombinations of chromosomes, such as (considering only the unbalanced pairs) $\frac{AABC}{ACC}$, $\frac{AACC}{ABC}$, $\frac{ABCC}{AAC}$, &c. In fertilization such recombinations could occur as $\frac{AABC}{ABC}$ (*lata*), $\frac{AABC}{ACC}$ (*scintillans*), $\frac{AACC}{ACC}$ (which might represent a different trisomic form), &c. Hence we can understand how *lata* could give rise to *scintillans* and vice versa, and also how *lata* might be the starting-point for a whole series of other trisomic forms arising through further irregular distributions of the chromosomes. Whether such a form as, e.g., $\frac{AACC}{ACC}$ is viable would depend on how far the chromosomes are differentiated from each other.

It has recently been found that a haploid mutant can arise in *Datura* (Blakeslee, Belling, Farnham, and Bergner, 1922). This number of chromosomes is then sufficient for the production of a complete sporophyte. If duplication and redistribution of chromosomes could go on indefinitely it might be possible to produce an organism with all *A* or all *C* chromosomes. Weismann's theory of the germ-plasm contemplated the view that each id contained all of the representative germinal material, each chromosome being made up of a number of ids. Speculation has since swung to the opposite extreme, and it has been rather tacitly assumed that the chromo-

somes, at least in the haploid series, all differed from each other throughout. This assumption is quite possibly too extreme, at least for plants. It appears probable that in animals, which are more highly integrated organisms than plants, the various chromosomes are differentiated from each other throughout their length. But even here there may be a common substratum for each chromosome which has remained undifferentiated in an evolutionary sense. It is reasonable to suppose that the amount of difference between chromosomes in one organism may be much greater than in another. We know this to be the case as regards sizes and shapes of chromosomes, since in some organisms the chromosomes are all visibly alike, while in others they show many constant differences. It is probable that there are equally great differences as regards chemical differentiation, which is presumably at the basis of mutations of the *Drosophila* type. On the other hand, it seems necessary to assume *some* difference between the chromosomes of every organism, for if there were no such difference it is difficult to see how they would maintain their identity, as they appear to do, while passing through the resting stage of the nucleus. But it does not necessarily follow that this difference is sufficiently great so that replacement of one or two chromosomes by duplicates of others would produce a non-viable result, or one which produced a new genus or family. It may be, for example, in *Oenothera* that its generic and family characteristics are equally represented in all the chromosomes, while minor differences may have arisen more recently through mutations of the type represented by *rubricalyx* and *brevistylis*.

An analogy may make this general position clearer. It seems evident that the differentiation of the sexes is much greater in some organisms than in others. Thus in *Hemiptera* the relation of sex differentiation to dimorphism of the *X* and *Y* chromosomes seems clear. On the other hand, Schaffer (1918) has given good reasons for the conclusion that in hermaphroditic flowering plants sex differentiation has 'absolutely nothing to do with segregation or association of chromosomes or allosomes'. In a similar way I believe that there is evidence in the Oenotheras that the chromosomes are much less differentiated than in animals and some plants, i. e. the undifferentiated substratum occupies a greater portion of the whole chromosome. And the occurrence of such a large number of trisomic mutants in *Oenothera*, a number of which have interchangeable relationships with each other, seems to find its explanation in further assortments of non-homologous chromosomes. It seems unlikely that this process could go further than the production of forms with four members of one chromosome and none of another.

The almost complete sterility of trisomic forms in *Oenothera* renders the solution of these problems almost impossible, but it is to be hoped that in *Datura*, where there is greater viability and where there are also more

Mendelian characters whose inheritance can be used as a check, a further analysis of the situation will be possible. There is apparently, however, no evidence at present that any of the trisomic mutants in *Datura* are interchangeable, so the conditions in the two genera differ in some respects. The further study of the offspring of triploid *Oenothera* mutants and hybrids will also throw further light on these questions.

One question which remains to be discussed is the possible visible differentiation of the seven pairs of chromosomes in *Oenothera*. Hance (1918) claims that the chromosome pairs of *Oenothera scintillans* form a graded series in length, but it must be said that his treatment of the subject is not entirely convincing. The difficulties of obtaining all the chromosomes of a large number of equatorial plates exactly in the plane of section are very great, and the frequent bendings of the chromosomes render accurate measurements still more precarious where small differences in length are involved. Van Overeem (1922), in an important paper, has, however, reached a similar conclusion. He identifies the extra chromosome in *lata* (which we have called *A*) as belonging to a trio which are long and strongly bent. He calls this chromosome No. 1 (see his Pl. II, Figs. 9–12). In *cana* he identifies the extra chromosome as belonging to No. 5 pair, a pair which he believes is probably of medium length and is bent near the end and often somewhat constricted (Pl. II, Figs. 1–8). If these differences can be proved to be constant this will help to substantiate the views expressed in the present paper. But more critical and extensive measurements of these chromosomes must be made before one can regard it as proved that the chromosomes of *Oenothera* form a series which are constantly differentiated in size and shape. The facts, as far as they go, are entirely in accord with the views here expressed, and it is to be hoped that with further evidence it may be possible to identify the chromosome composition of each form. The possibility should be kept in view that some forms with an extra chromosome may at the same time be lacking both members of a different pair.

It may be pointed out here that van Overeem (1922) concludes from the genetic data that the extra chromosome in *lata* belongs to the *gaudens* complex (since in crosses it gives, like *Lamarckiana*, the twin types *laeta* and *velutina*) and that the same is true of *scintillans* and *oblonga*, although the extra chromosome is a different one in all three. In a paper now in the press I have shown reasons for believing that the difference between *gaudens* and *velans* resides in a single pair of *Lamarckiana* chromosomes. How these two views are to be harmonized can only be determined by further breeding experiments with various trisomic forms. There seems no reason for assuming that in any of these mutants the *gaudens-velans* pair of chromosomes is the extra one. There should however, on my view, be one trisomic mutant in which this was the case, and this should distort the

ratios of *laeta* and *velutina* obtained by pollination from, e.g., *Oe. Hookeri* or *Oe. strigosa*, according to whether the extra chromosome in the mutant was carrying *laeta* or *velutina*.

Summary.

This paper records the occurrence in the F_1 of *Oenothera rubricalyx* × *Oe. Hewettii* of two mutants having 15 chromosomes, but of a type different from *Oe. lata* and having viable pollen. It probably most nearly resembles some of the *semilata* mutations, which also have 15 chromosomes. The occurrence of a pair in such cases is significant, indicating that non-disjunction of a pair of chromosomes in the heterotypic division of a pollen mother-cell of the male parent (in this case *Oe. Hewettii*) led to the formation of two pollen grains having eight chromosomes, both of which afterwards functioned in fertilizing eggs.

In diakinesis in the pollen mother-cells of this mutant as many as five ring-pairs of chromosomes are found. One of these ring-pairs may perhaps be descended from the original pair of chromosomes which underwent non-disjunction in a pollen mother-cell of *Oe. Hewettii*. The others must be formed by the union of homologous chromosomes from *rubricalyx* and *Hewettii*. The presence of chromosome rings, while an indication of strong attraction between synaptic mates, cannot therefore be regarded as evidence that the species is homozygous.

Some of the chromosome-rings persist on the heterotypic spindle even until metaphase. The later irregularities in the chromosome behaviour of this mutant are not so great as in *Oe. lata*, but chromosomes are frequently left behind in the cytoplasm.

This is followed by a discussion of all the trisomic forms of *Oenothera* (having 15 or 16 chromosomes). The origin of mutations with 14 chromosomes such as *nanella* could be accounted for by double non-disjunction as well as through crossing-over, but neither theory explains the later genetic behaviour of these forms.

The evidence now indicates that forms with aberrant chromosome numbers make up the great majority of *Oenothera* mutations. There is an older group of 15-chromosome mutants from *Oenothera Lamarckiana* which includes *lata*, *scintillans*, *albida*, *oblonga*, *subovata*, and probably several others. More recently a series of others more like *Oe. Lamarckiana* has been recognized, including *cana*, *pallescens*, *Lactuca*, and *liquida*. Apparently *lata* × *Lamarckiana* can give rise to any of these.

Trisomic mutants appear much more frequently in the offspring of *lata* than in the offspring of *Lamarckiana*. Again, several at least of the simple trisomic mutants are interchangeable. Thus *lata* gives rise to *scintillans* and *scintillans* to *lata*. The view is developed that this results from secondary irregular chromosome distributions.

This leads to the question how far the chromosomes of *Oenothera* may be differentiated from each other. The conclusion is reached that the degree of differentiation probably varies in different organisms, being generally greater in animals than in plants. Such differentiation may be increased by the occurrence of Mendelian mutations, but in *Oenothera* mutations of this type are relatively infrequent.

In conclusion, I wish to record my thanks to the Royal Society and the British Association for grants in connexion with this work, and also to the Royal Botanic Gardens, Regent's Park, for the facilities afforded.

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EXPLANATION OF PLATE XI.

Illustrating Professor Gates's paper on the Trisomic Mutations of *Oenothera*.

All figures were drawn with a $\frac{1}{12}$-inch Koristka immersion lens N.A. 1.30 and compensating ocular 12, and reduced one-fourth in reproduction, giving a magnification of 2,250 diameters.

Fig. 1. Pollen mother-cell in diakinesis, showing five ring-pairs of chromosomes and five single chromosomes.

Fig. 2. Another cell at the same stage, showing five rings.

Fig. 3. Multipolar spindle stage, showing 15 chromosomes, including a looped ring.

Fig. 4. Another cell at the same stage, showing a looped ring.

Fig. 5. Heterotypic spindle in early anaphase, showing the odd chromosome ϵ.

Fig. 6. Heterotypic bipolar spindle, showing a persistent ring chromosome.

Fig. 7. Heterotypic anaphase, showing 15 chromosomes, seven of them approaching one pole, five the other, and three lagging behind.

Fig. 8. Heterotypic telophase with probably eight chromosomes in one daughter nucleus, six in the other, and one left behind in the cytoplasm. Most of the chromosomes show their split character at this time.

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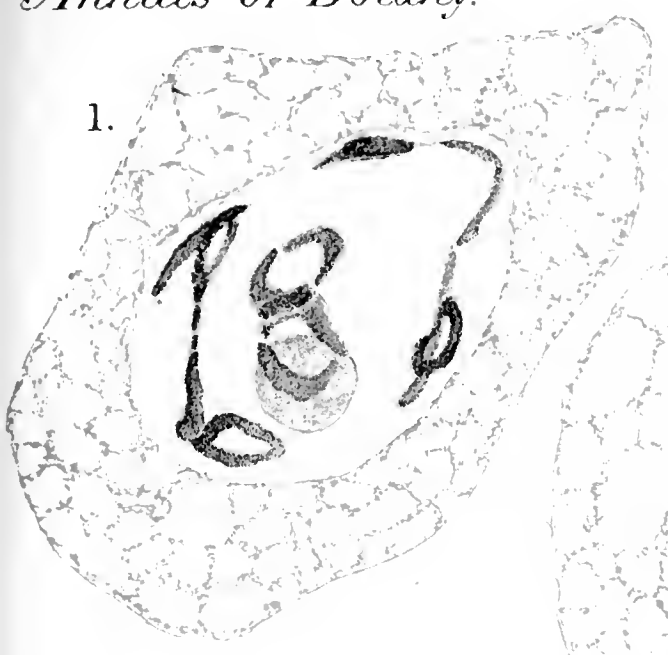

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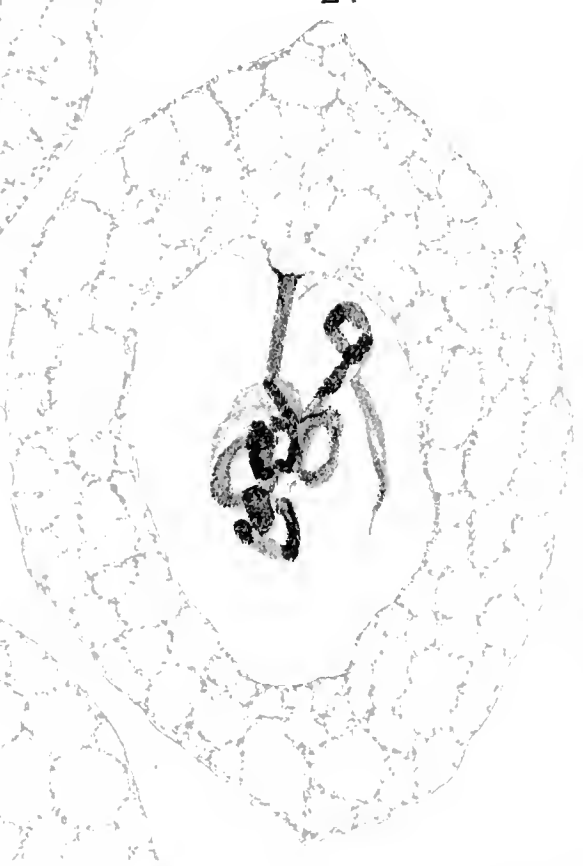

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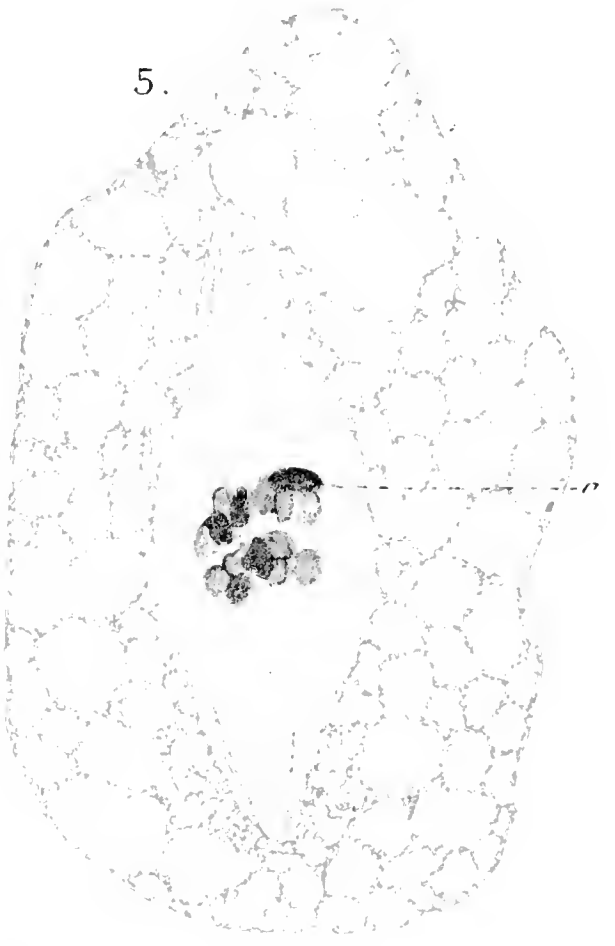

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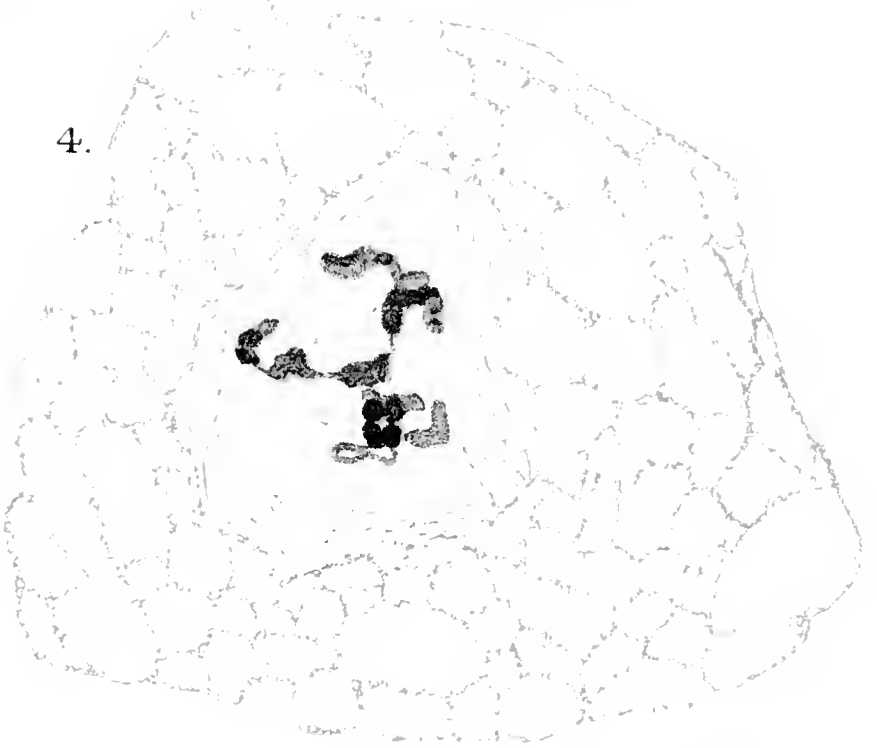

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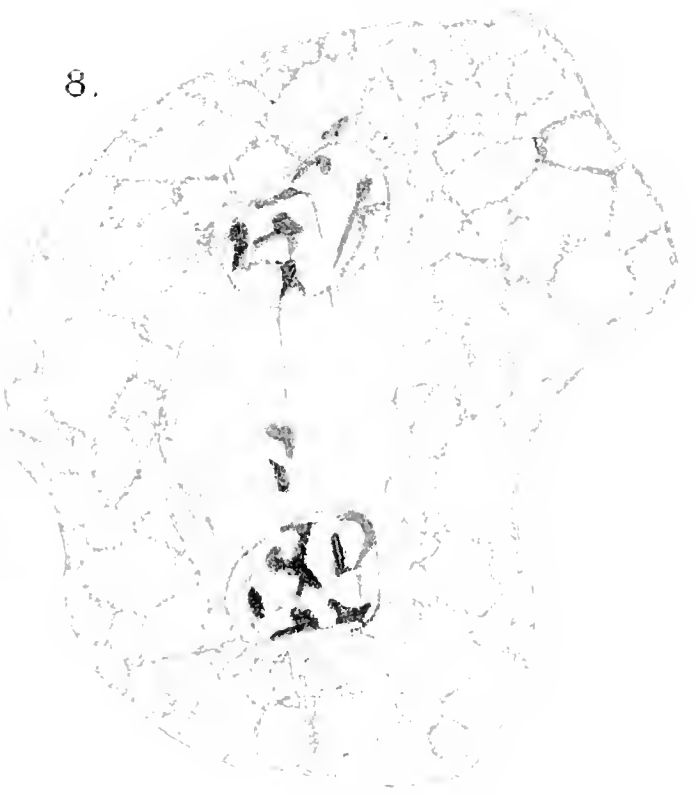

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GATES — OENOTHERA.

The Chromosomes of a Triploid Oenothera Hybrid.

BY

R. RUGGLES GATES, PH.D., F.L.S.,

Professor of Botany, University of London (King's College).

With Plate XII.

IN 1912 crosses were made at the John Innes Horticultural Institution between *Oenothera rubricalyx* and *Oe. gigas*. The *Oe. rubricalyx* parent, having 14 chromosomes, was descended in the third generation from the original mutant which occurred in 1907. The *Oe. gigas* parent belonged to the second generation from seeds obtained from the Palermo Botanical Garden under the name of *Oe. cognata*. This race was identical in character, chromosome number (28), and variability with the *gigas* mutant of de Vries's cultures, and it doubtless represented an independent origin of the tetraploid condition. A further account of this race was published elsewhere (Gates, 1913).

From the cross *Oe. rubricalyx* × *gigas* 199 seeds were obtained in one capsule. They were sown in 1913 at Rothamsted, but only one plant developed. The rosette leaves were large, broad, and crinkled like those of *gigas*, and the midribs were green below, not red as in *rubricalyx*. The plant grew very large, stout and taller than the parent forms, as is usual in triploid hybrids. The leaves stood out from the stem and the flowers were 'very large', the buds showing the red sepals and hypanthium of *rubricalyx*, but conspicuously pale in colour. The leaves were less crinkled than in *gigas*. Thus in this hybrid with 2 x chromosomes from the male parent *gigas* and x from the female parent *rubricalyx*, the usually dominant red pigmentation character was so diluted as to be invisible on the rosette leaves and pale on the buds. The foliage was intermediate, but more like that of *gigas*. Examination of the pollen showed that 'bad' grains and 'good' grains were in the ratio of about 4 : 1. The apparently functional grains were four-lobed (as in *gigas*) or three-lobed in the ratio of about 2 : 1. The lobing of *Oenothera* pollen grains has been discussed elsewhere (Gates,

1915, p. 212), and it was shown that grains with less than the full diploid number of chromosomes may still be four-lobed. Probably in this plant the four-lobed grains, which numbered about 12 per cent. of all the pollen grains, had not less than 10 chromosomes. Further reference will be made to this point later.

At the end of the season this plant was potted and placed in a greenhouse. Its main stem died and was cut down. Then a number of short side shoots developed from the base bearing rosette leaves. These rosettes were mostly like *gigas* with broad-pointed crinkled leaves. One shoot, however, was quite different, nearer *Lamarckiana*, with leaves pointed and nearly smooth. Another shoot was intermediate in character between these. Unfortunately no photograph was obtained, and although cuttings were attempted the plant finally died and was lost. The variation in the character of the leaves on different shoots was no doubt due to the loss of certain chromosomes from their growing points. This undoubtedly occurs, as in the meiotic divisions, through the dropping out of certain chromosomes in the cytoplasm, a process which may be expected to take place when a plant with an unbalanced chromosome number is placed in unusual conditions.

Cytological material was collected in 1913 for a study of the meiotic divisions in this plant, but the embedded paraffin material was laid aside and was only sectioned last year. I am indebted to Miss E. M. Rees, B.Sc., for making the preparations (stained in iron-haematoxylin), and to Mrs. N. Ferguson, B.Sc., for the careful drawings. Fig. 1, Pl. XII, is a side view of the heterotypic spindle in the pollen mother-cell. It shows 21 chromosomes somewhat scattered along the spindle, as is usual in many Oenotheras. The other illustrations have been selected to show how the 21 chromosomes are distributed in the reduction divisions and how the number gradually drops down, through the omission of chromosomes from the daughter nuclei in both divisions, until many of the pollen nuclei receive only 7.

Fig. 2 is a homotypic metaphase showing 10 chromosomes in the right-hand group and probably 11 in the other. Usually this is the segregation which takes place on the heterotypic spindle, 10 chromosomes entering one daughter nucleus and 11 the other. Chromosomes may however be left behind on the heterotypic spindle. Thus in Fig. 3 we have a homotypic anaphase with 18 chromosomes on the left-hand spindle and 20 on the right. Under the microscope these are clearly in two separate groups of 9 and 10 respectively, although the groups necessarily overlap somewhat in the drawing, since the spindles were obliquely placed. Hence 9 split chromosomes have separated normally on the left-hand spindle and 10 on the right. This means that a total of 19 chromosomes only is now present, and hence the other two must have been left behind on the heterotypic spindle and disintegrated. Similar conditions have been observed in other pollen mother-cells.

Fig. 4 shows another homotypic anaphase in which more chromosomes have been lost, for there are only 8 pairs of separating chromosomes on the left-hand spindle and 7 pairs on the right. Hence the remaining 6 whole chromosomes must have been left out of the daughter nuclei on the heterotypic spindle.

In Fig. 5 a homotypic spindle is in telophase, with 7 chromosomes at either pole and an eighth divided chromosome remaining behind. Only 8 chromosomes reached the daughter nucleus, which is here dividing. As the number of chromosomes on the other homotypic spindle is unknown, it is impossible to say how many of the other 13 chromosomes reached the opposite pole of the heterotypic spindle. Fig. 6 shows a homotypic spindle in which 8 chromosomes have reached either pole and a ninth split chromosome is disintegrating on the spindle. Probably in this mother-cell at least one chromosome was lost from this end of the heterotypic spindle. Fig. 7 represents a not infrequent condition in which only 7 chromosomes have arrived at the poles of a homotypic spindle, the eighth disintegrating.

These figures show that a varying number of chromosomes may be lost during either of the reduction divisions in this triploid hybrid. Occasionally all the chromosomes may reach the daughter nuclei in the heterotypic mitosis, but usually one, two, or more unpaired chromosomes are left behind to disintegrate. All the chromosomes which reach the daughter nuclei in the heterotypic division apparently undergo a split. When the homotypic spindles are formed and these split chromosomes separate, a certain number of them may again be left behind, so that the number of chromosomes ultimately reaching the four daughter nuclei is usually only 7 or 8.

In an early paper (Gates, 1909) on *Oenothera lata* × *gigas* I showed how the 21 chromosomes usually separate 10–11 on the heterotypic spindle, but sometimes 9–12.[1] Apparently in that material all the chromosomes usually reached the two daughter nuclei. The chromosome numbers in interkinesis therefore ranged only from 9 to 12, and this entirely owing to the sometimes irregular chromosome distributions. In the present hybrid *Oenothera rubricalyx* × *gigas*, however, there is evidence that from one to six chromosomes are frequently lost on the heterotypic spindle, since the numbers on the homotypic spindles are frequently only 8, 9, or 10.

That similar conditions occur in the pollen formation of other triploid *Oenotheras* is indicated by recent results of van Overeem (1920), to which brief reference may be made. From *Oe. Lamarckiana* mut. *semigigas* (21 chromosomes) crossed with *Lamarckiana* he obtained plants as follows: 1 × 14, 9 × 15, 4 × 16, 2 × 17, 2 × 18, 1 × 20, and 1 × 21 chromosomes; from *semigigas* × *gigas* 1 × 21, 2 × 22, 12 × 23, 21 × 24, 25 × 25, 19 × 26, 6 × 27, and 1 × 28. *Oe. biennis semigigas* × *Lamarckiana gigas* gave corresponding

[1] These results have recently been confirmed by van Overeem (1922) in an important paper dealing with various triploid and other forms.

results. The most common chromosome number was therefore 24, 25, or 26, when *gigas* was the pollen parent. The reciprocal, *Lamarckiana gigas* × *semigigas*, gave 3×21, 1×22, 1×27, 10×28, and 3×29. Hence 28 chromosomes was the commonest number when *gigas* was seed parent. The exact manner of origin of the large number of 28's and 29's in *gigas* × *semigigas* is perhaps uncertain. These results confirm the writer's early observations that in triploid forms the segregation of chromosomes is usually 10–11, but occasionally 9–12. They indicate further that the segregation may be occasionally 8–13 and rarely even 7–14.

From *Oe. biennis semigigas* × *biennis* van Overeem obtained a somewhat narrower range of variation. There were 41 plants with 14 chromosomes, 26 (*albinervis*) with 15, and 4 with 16. In *biennis semigigas* × *gigas*, on the other hand, the chromosome numbers were 21, 22, 23, 24, 25, 26, 27, 28, and one plant with 36, while *Lamarckiana gigas* × *biennis semigigas* gave 33 plants with 28 chromosomes, 2 with 29, and 6 undetermined. Van Overeem suggests with probability that the plant with 36 chromosomes arose from an egg cell of *biennis semigigas* having 8 chromosomes uniting with a pollen grain of *gigas* having 28. That such a pollen nucleus may arise in *gigas* has already been shown (Gates, 1915, Fig. 73 f.).

In a plant from *Oe. gigas* × *lata rubricalyx* which had 22 chromosomes (Gates, 1915, p. 189) I showed that the chromosome segregation in the pollen heterotype was usually 10–12, but other daughter nuclei were observed having 11, 13, and 9 chromosomes, as well as $6\frac{1}{2}$ and $9\frac{1}{2}$, which points to the production of a range of new numbers in the next generation.

The present study of the reduction divisions in the pollen of *Oe. rubricalyx* × *gigas* helps to make clear the chromosome behaviour of triploid forms, and explains the origin of the wide range of chromosome numbers found in their offspring.

Summary.

This paper deals with the distribution of the chromosomes in the microspore meiosis of a triploid hybrid, *Oe. rubricalyx* × *gigas*. It confirms earlier results with similar triploid hybrids and further explains how the variety of chromosome numbers found in the second generation of such hybrids arises.

In the heterotypic mitosis the chromosome distribution is usually 10–11, but from 1 to 6 chromosomes may be left out of the daughter nuclei. This is shown by the chromosome numbers on the homotypic spindles, which may be 11, 10, 9, 8, or 7, and usually differ in the two homotypic spindles of a cell. Cases occur where these two spindles have 10 + 11, 9 + 10, or 8 + 7. All the chromosomes regularly split and separate on the homotypic spindles, but certain split chromosomes frequently lag behind and are left out of the daughter nuclei, as on the heterotypic spindle.

Thus pollen grains will be formed having every chromosome number between 11 or 12 and 7. As pairs of tetrad nuclei usually have the same chromosome number, aberrant forms should be looked for in pairs in the next generation.

These experiments were aided by grants from the Royal Society and the British Association.

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EXPLANATION OF PLATE XII.

Illustrating Professor Gates's paper on the Chromosomes of a Triploid *Oenothera* Hybrid.

All figures were drawn with a $\frac{1}{12}$ in. Koristka immersion lens N A. 1·30 and compensating ocular 12, tube length 152 mm., and reduced one-fourth in reproduction, giving a magnification of 2,250 diameters.

Fig. 1. Heterotypic anaphase in a pollen mother-cell of *Oe. rubricalyx* × *gigas*, showing twenty-one chromosomes somewhat scattered.

Fig. 2. Homotypic metaphase, showing ten chromosomes on right-hand spindle and probably eleven on left-hand.

Fig. 3. Homotypic anaphase, showing ten split chromosomes separating on right-hand spindle and nine on left-hand. The other two chromosomes were evidently lost on the heterotypic spindle.

Fig. 4. Homotypic anaphase, showing the two daughter groups of eight chromosomes on the left-hand spindle and two groups of seven on the right-hand. The remaining six chromosomes must have been lost in the heterotypic mitosis.

Fig. 5. Homotypic telophase, showing seven chromosomes entering each daughter nucleus, and an eighth divided but left behind.

Fig. 6. Homotypic telophase, showing eight chromosomes at either end of spindle and a ninth split chromosome disintegrating on the spindle.

Fig. 7. Homotypic telophase, showing seven chromosomes at each pole of the spindle, the eighth divided but left behind.

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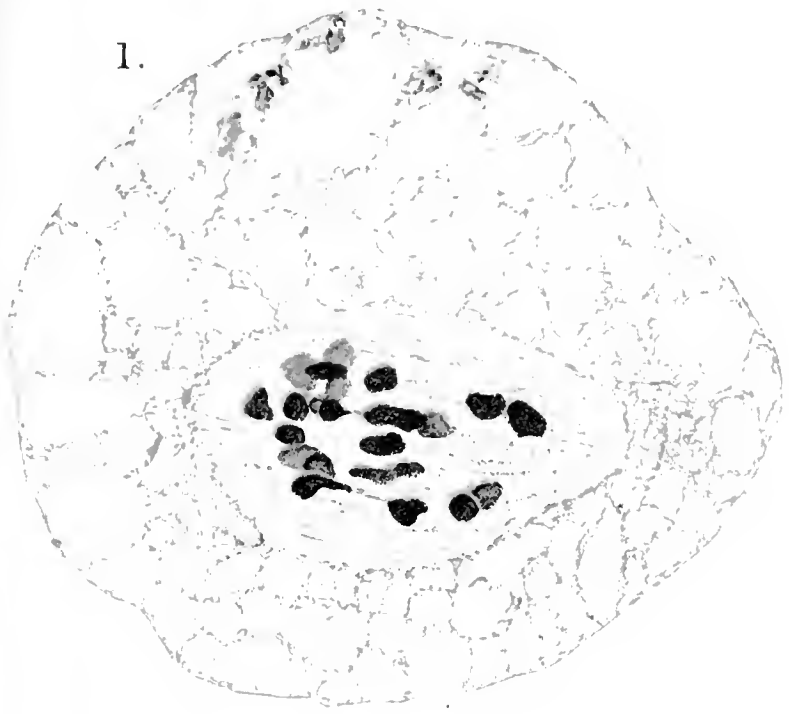

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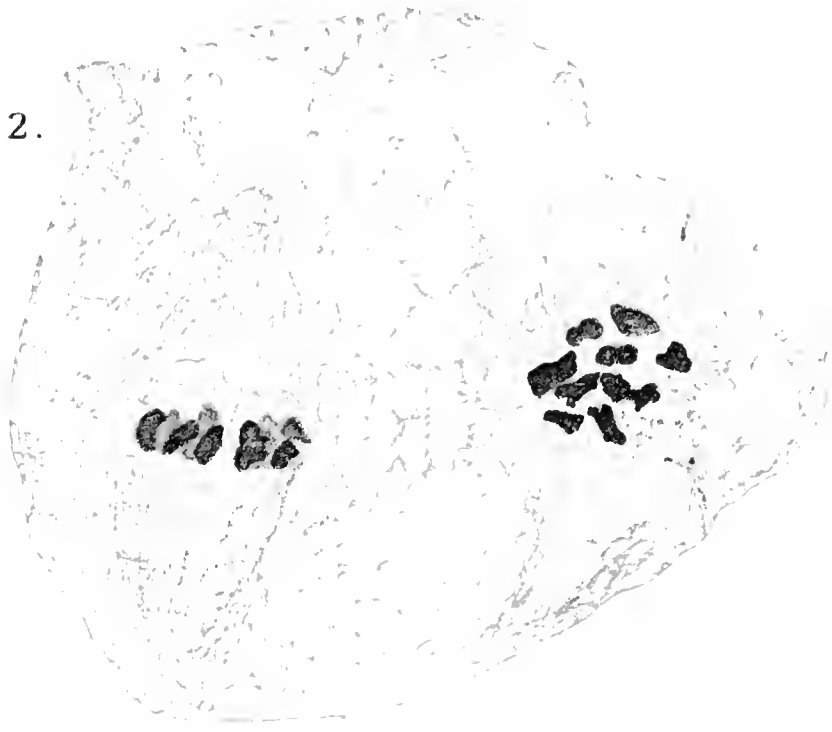

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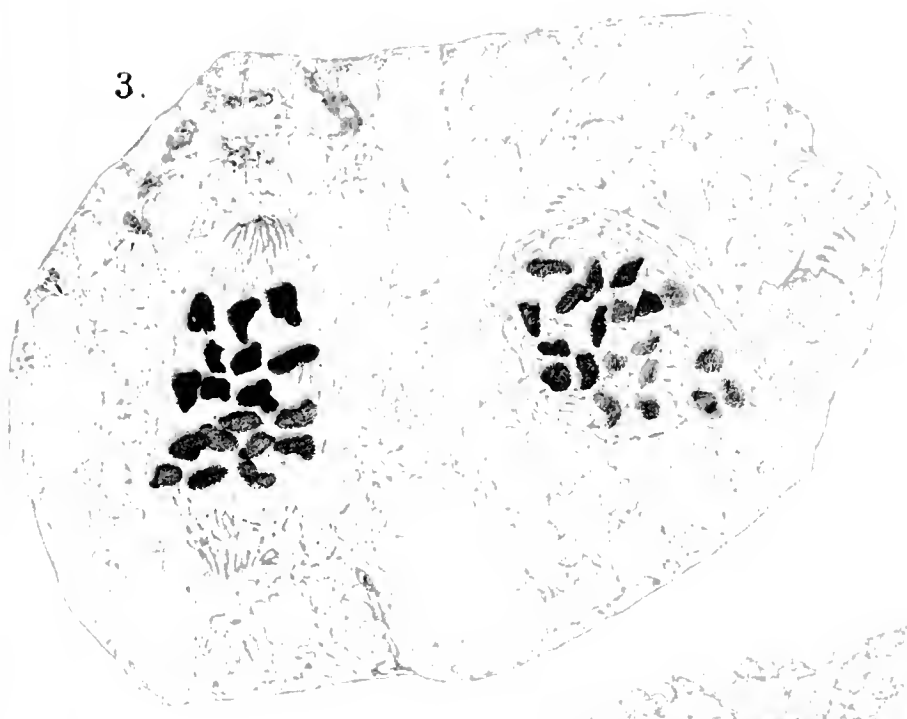

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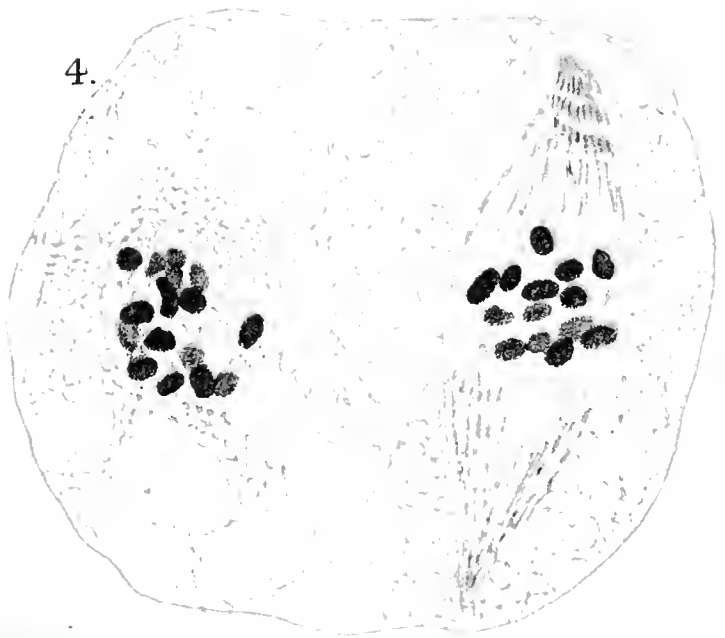

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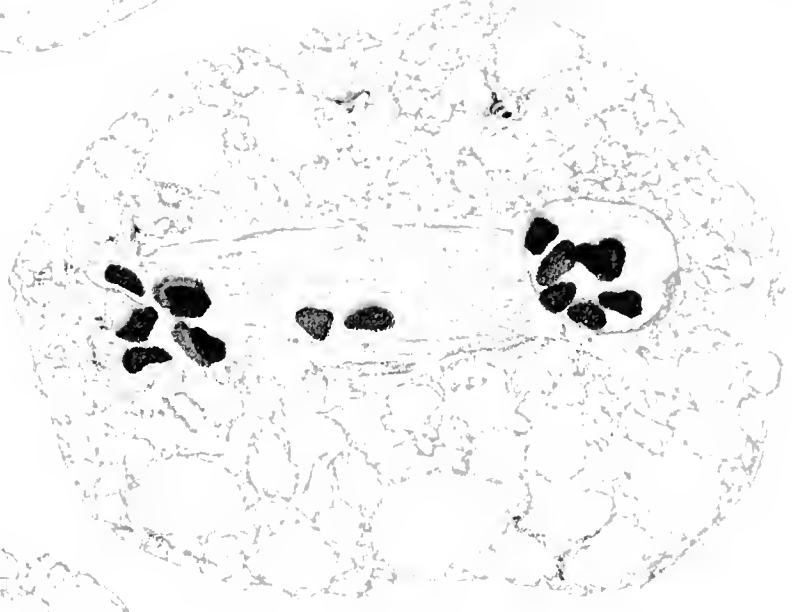

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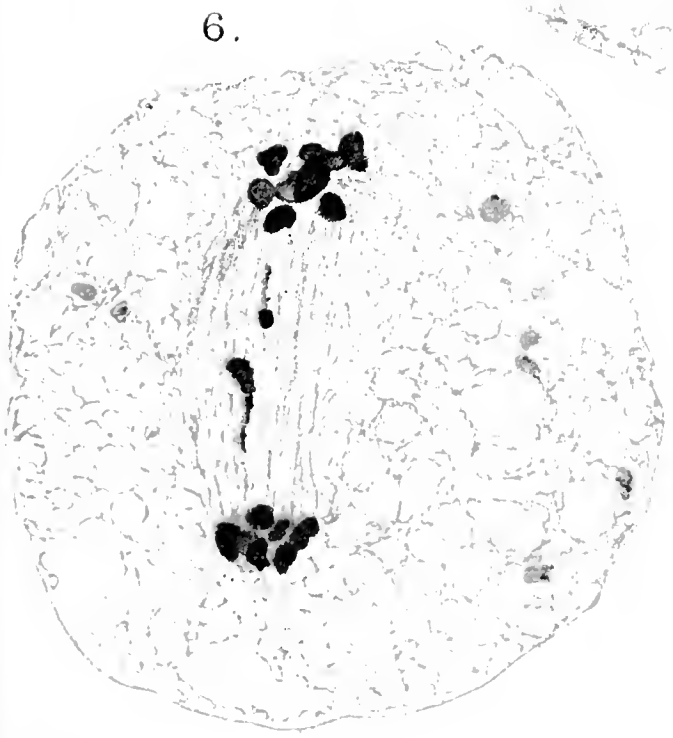

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GATES — CHROMOSOMES OF OENOTHERA.

On the Seedling Structure of Acer Pseudoplatanus.

BY

H. S. HOLDEN, D.Sc., F.L.S.,

AND

DOROTHY BEXON, M.Sc.,

University College, Nottingham.

With seventy-four Figures in the Text.

Introduction.

THE morphology of the later stages in the development of the embryo of the sycamore and the germination of the seed were worked out by Lubbock (11) and described in his classic memoir 'On Seedlings' in 1892, and since then it has frequently figured in elementary text-books as an example of epigeal germination. A further description, again morphological, of the polycotylous seedlings so frequently occurring in this plant is given by Thiselton-Dyer (15), whilst Tansley and Thomas (14) make a brief reference to its anatomy in a short abstract dealing with their work on the vascular structure of seedlings. A more detailed knowledge of the early anatomy of the normal seedling was a necessary preliminary to projected work on polycotylous and syncotylous specimens, and the present paper is primarily the outcome of that need.

The Food Reserves in the Embryo and Young Seedling.

The embryonic food reserves comprise at least four substances. The first of these, *starch*, is distributed through the whole of the general parenchyma of the embryo and rapidly disappears as growth proceeds, until, apart from that located in the starch sheath of the hypocotyl, it is absent by the time that the cotyledons are expanded. It has been recently shown by Briggs (2) that the sycamore seedling belongs to a type in which assimilates are produced immediately on exposure to light, so that these must be

[Annals of Botany, Vol. XXXVII. No. CXLVIII. October, 1923.]

utilized as rapidly as they are produced in addition to the stored starch of the embryo during the early phases of rapid growth. Following on the expansion of the cotyledons, which apparently coincides with the advent of full assimilatory activity, the formation of storage starch commences. This appears to be closely associated with the developing vascular structures, the major portion being situated in the outer part of the pith and the inner part of the cortex. As the secondary xylem of the hypocotyl develops, abundant starch also occurs in the xylem parenchyma and in the medullary rays.

In addition to the starch reserves there appear to be at least two *proteins*. During the early stages of the investigation free-hand sections were cut from embryos which had been preserved in methylated spirit, and it was noticed

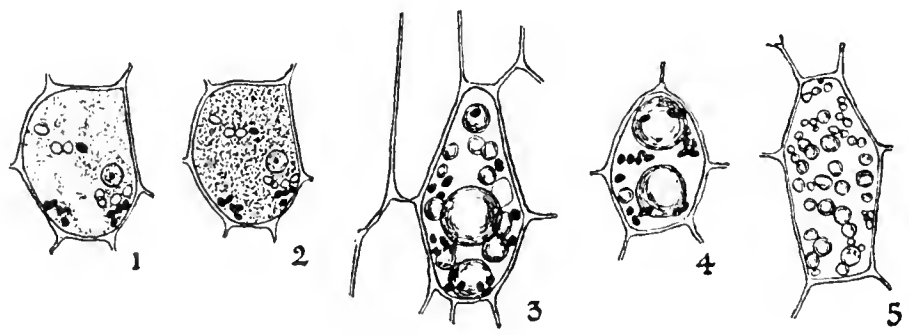


FIG. 1. Cell from the cotyledonary parenchyma mounted 'dry'.
FIG. 2. The same cell irrigated with tap-water, showing the emulsoid phase of precipitation.
FIGS. 3–5. Cells showing variations in the final phase of precipitation. The starch grains are indicated as black granules throughout.

that the cell-contents were obscured by numbers of globules which stained brown with iodine (Fig. 3). Subsequent investigation showed that the globules were also present in sections of material fixed in formalin or chromacetic fixatives, and of *fresh* material mounted in tap-water. It was not until the precaution was taken of mounting sections of fresh material in tap-water and examining at once that the clue to the formation of these globules was obtained. It was then discovered that they were the result of precipitation, presumably from colloidal solution. The first stage in precipitation is the development of a fine haziness in the cell-contents, succeeded by the formation of minute granules showing active Brownian movement (Fig. 2). The coalescence of these granules produces the globules (Figs. 3-5). Sections of fresh material cut and mounted in distilled water developed a haziness of the cell-contents similar to that of the preliminary stages of precipitation produced by tap-water, but no further precipitation was observed.

Sections were also mounted in dilute glycerol, and here precipitation in the form of globules was immediate, there being no emulsoid phase. After some time the cell-contents became clear owing to the solution of the globules, and this suggested the probability that, with higher concentrations of glycerol, no precipitation would occur. Owing to the difficulty of obtaining anhydrous glycerol the 'pure glycerine' of the British Pharma-

copoeia was taken as a standard and the effects of varying dilutions of this on the precipitation phenomena were studied, with the results indicated in the following table:

Parts 'Pure Glycerine', B.P.	*Parts Aq. Dest.*	*Result.*
100	—	No globules formed.
75	25	No globules formed as a general rule: a few transient globules occurred in one section.
50	50	Immediate precipitation of globules, followed by rapid disappearance within twenty minutes.
25	75	Precipitation of globules almost immediate. Solution of globules complete in about two hours.
10	90	Precipitation of globules within a few minutes, followed by slow solution. Several hours elapse before solution is complete.

It would seem probable that the proteins are immediately soluble in glycerol which contains relatively small amounts of water, and that the explanation of the slow disappearance of the globules in the higher dilutions lies in the gradual concentration of the glycerol owing to the evaporation of the water. Although the usual chemical tests for proteins did not give clearly defined results owing to the obscuring effects of the general protoplasm, a number of digestion experiments were tried with sterile pepsin and trypsin extracts. Whilst the majority of the globules were dissolved by the pepsin some were resistant: all, however, were digested by the trypsin. This difference in behaviour indicates the presence of at least two proteins, and is borne out by the fact that whilst, when mounted in 10 per cent. KOH, all the globules disappear within 24 hours, the rate of disappearance is differential, some going relatively quickly, others very slowly, and also by the staining reactions, some staining much more deeply than others with iodine solution, whilst some take aqueous gentian violet well and others remain clear and refringent. Like the starch grains, the reserve proteins are generally distributed through the parenchyma of the embryo and young seedling: they are absent from older seedlings.

The third type of reserve substance occurring in the sycamore is of a *fatty nature*,[1] and was chiefly studied in sections which had been treated with trypsin solution to remove the proteins, and in some cases with diastase solution also to remove the starch. The fatty substance, which, like the proteins, occurs as globules, is also generally distributed, but appears to be specially abundant in and near the outer cell layers of the cotyledons. After preliminary treatment to remove the proteins, the action of various solvents was tested in two ways, namely, by immersing thin sections in each of the solvents for 24 hours and then staining with a specific stain, and also by staining with a specific stain and mounting in each of the various solvents

[1] The authors are indebted to Professor J. H. Priestley, of Leeds, for several helpful suggestions in this section.

under direct microscopic observation. Judged by these tests this substance is insoluble in alcohol, xylol, or benzene, but readily soluble in acetone, ether, and chloroform. It stains well with both Scharlach R and Sudan III, and gives an intense black with 1 per cent. osmic acid solution.

Although its general staining and solubility characters are sufficiently indicative of its fatty nature it does not agree entirely with either the true fats or the lipoids described by Cramer (7), and it may be that more detailed investigations will show that it is a mixture of substances.

The Vascular System.

(i) *The Embryo and Young Seedling.*

The earliest stage studied in connexion with the development of the

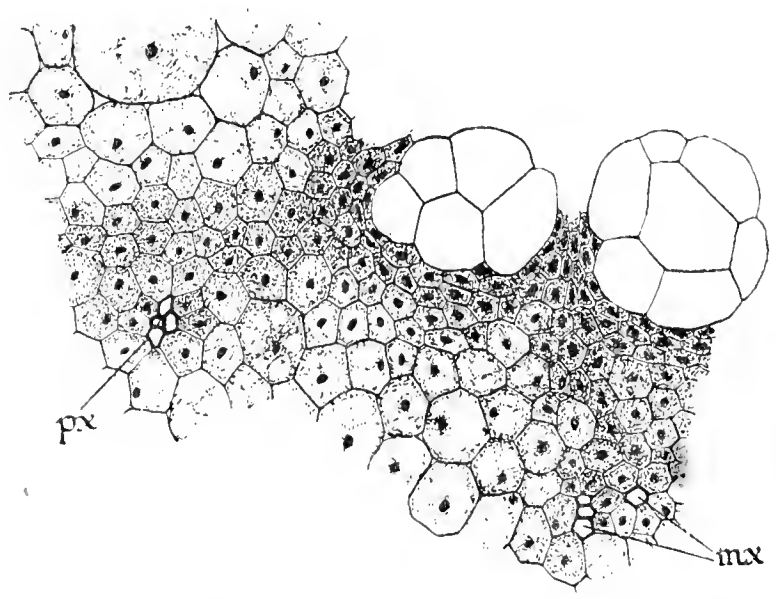
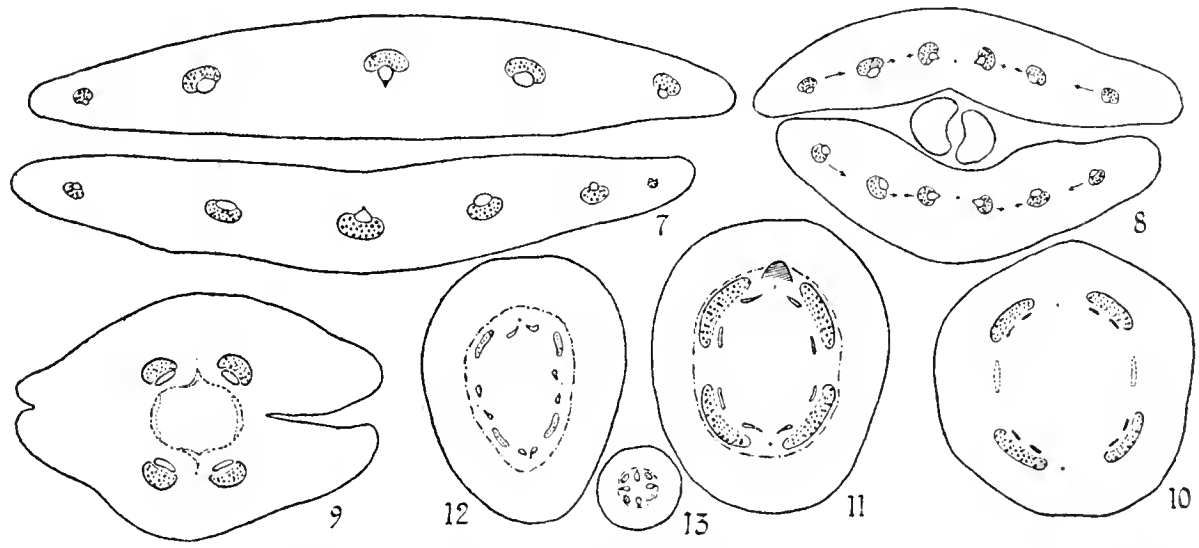


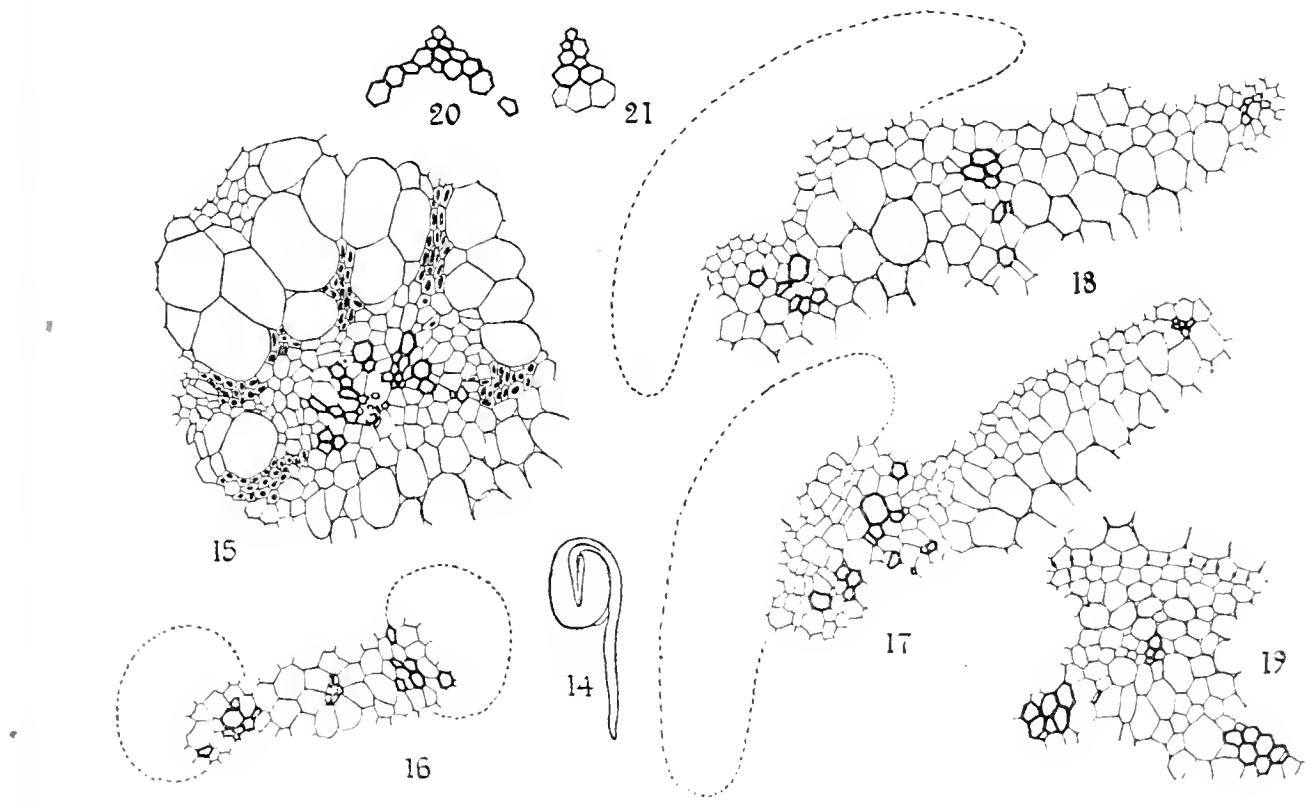
Fig. 6. Transverse section of a portion of the upper end of the hypocotyl of a very young seedling before the radicle has penetrated the testa. Note the isolated protoxylem (*px.*) and the first-formed metaxylem elements (*mx.*). × 750.

vascular system was that of the embryo at the stage when the fruit is shed from the tree in autumn. There is no differentiation of the xylem at this stage, but the position of the vascular strands is clearly indicated owing to the presence of large secretory sacs in the phloem areas. Embryos examined in the spring show the earliest stage of xylem differentiation, the protoxylem being well marked and the first metaxylem, though very immature, also being present (Fig. 6). Each seed-leaf is traversed by five main vascular strands, namely, the midrib, two laterals, and two marginals (Fig. 7). Towards the base of the leaf the midrib divides into three parts, these consisting of a central isolated protoxylem with a group of metaxylem and phloem forming a collateral bundle on either side (Fig. 8). These collateral bundles *move outwards* and unite with the lateral and marginal bundle so that at the cotyledonary node there are two isolated median protoxylem strands with four collateral bundles in the diagonal planes (Fig. 9). At some dis-

tance below the cotyledonary node the xylem of each of the four diagonally placed bundles divides into two, so that at this level there are eight groups of metaxylem associated in pairs, each pair lying within one large group of phloem (Fig. 10). This arrangement of the vascular tissues is maintained



FIGS. 7–13. Series of semi-diagrammatic figures, showing the behaviour of the vascular strands during the transition from the cotyledons to the younger part of the root. Figs. 11 and 12 are from sections below the collet, and illustrate the leisurely manner in which root structure is attained.



FIGS. 14–21. Fig. 14. Young seedling from which the sections illustrated in Figs. 15–21 were drawn. Fig. 15. Cotyledonary midrib. Fig. 16. Bifurcation of midrib. Fig. 17. Isolated protoxylem and one diagonal bundle at the top of the hypocotyl. Fig. 18. Portion of section from the middle of the hypocotyl: the xylem of the diagonal bundle has divided into two. Figs. 19–21. Successive stages in the formation of the cotyledonary root pole. Fig. 21 is near the lower limit of differentiation. (Figs. 15–21 × 600.)

in the very young seedling to the lower limits of differentiation, thus showing that the bulk of the initial growth is intercalary in character. The epi-cotyledonary leaves, two in number, are very minute at this stage and show very little differentiation of tissues. At the cotyledonary node six desmogen strands are present in the intercotyledonary plane, these being in two groups

of three, one associated with each of the two leaves. In older seedlings (root $\frac{1}{2}''$–$\frac{3}{4}''$) (Fig. 14) the structure of the hypocotyl is essentially the same, but it is noteworthy that secondary thickening is very evident in the cotyledonary midrib (Fig. 15) and is beginning at the upper end of the hypocotyl (Fig. 17) whilst the protoxylem is already disorganizing. The transition to root structure takes place *below the collet* and proceeds very slowly with the ultimate establishment of tetrarchy (Figs. 11–13). During the assumption of root structure the metaxylem groups flanking the cotyledonary plane gradually approach the protoxylems in that plane and fuse with them, thus constituting two root poles (Figs. 19–21). This method is constant in all seedlings. The behaviour of the strands concerned in the formation of the root poles in the intercotyledonary plane is, however, somewhat variable. In some seedlings a central protoxylem group appears in the intercotyledonary plane, the adjacent metaxylems fusing with this to form a root pole. In other cases there is no such differentiation of a central isolated protoxylem, but the two metaxylems simply approach one another and after fusion become organized into a normal root pole. The approximation of the metaxylem groups may be accomplished by the tangential development of elements in continuity with the main body of elements, or in many cases elements may appear irregularly on the inner side of each metaxylem group, first in isolation, then linked up with the main group.

The tetrarch root in all cases possesses a large pith throughout its whole length.

It is interesting to note that from the time that the cotyledons are unrolled the bulk of the cotyledonary xylem (Fig. 22) and that associated with it in the hypocotyl are secondary and the primary elements occupy a subordinate position in the conducting system. The six plumular strands connected with the two epicotyledonary leaves show little differentiation at this stage. The median strand consists of a fairly well developed phloem group, whilst the development of the xylem is just beginning, a single element appearing within the phloem. The two lateral strands, which are small and undifferentiated, move outwards to join the adjacent diagonal phloem groups. The behaviour of the median strand is very variable. In some cases it bifurcates, each half moving across as a small collateral strand to join the adjacent lateral strand, whilst in others the phloem alone divides and the xylem remains central to the lowest level of differentiation. In other cases, again, the bundle does not divide at all and moves as a unit to the right or left. Even in the same seedling the behaviour is inconstant, one midrib behaving in one way and one in another. The plumular xylem does not appear to have any influence whatever on the formation of the intercotyledonary root poles, since many seedlings occur in which the plumular midrib forks at the cotyledonary node or moves laterally, whilst a new intercotyledonary root protoxylem is organized below the collet.

(ii) *The Anatomy of the Older Seedling.*

The period following the full expansion of the cotyledons is one of rapid anatomical change which is to be correlated with the growth and expansion of the plumular structures. The change, as far as it affects the hypocotyl, consists essentially of the development of a purely cauline system of secondary xylem, the upper portion of which connects directly with the epicotyledonary leaf-traces. The cotyledonary vascular supply and its connexions have attained practically their full dimensions at the beginning of this period and only add slightly to their bulk subsequently. The further vascular history of the seedling is one of steady change in the relative importance of cotyledonary and epicotyledonary constituents, the former

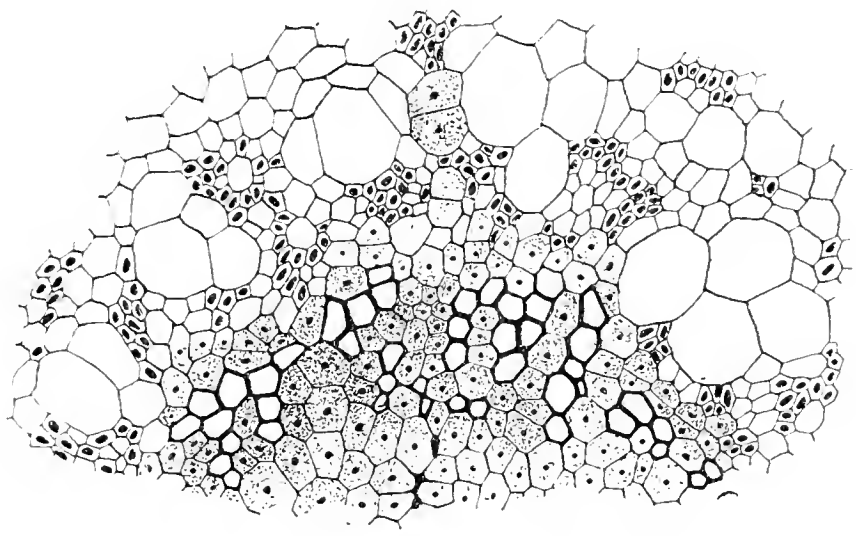
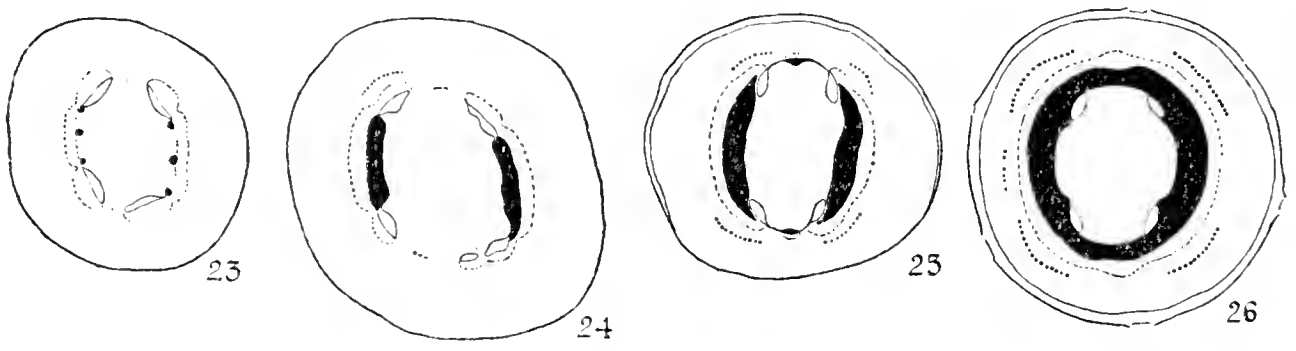


FIG. 22. Transverse section of the midrib of the cotyledon when completely unrolled. Note the disorganized and partly resorbed protoxylem and the fact that the bulk of the functional xylem is secondary. The large cells are secretory sacs. × 750.

assuming a subsidiary position as the latter increase in size. Although it is impossible to correlate precisely the various stages in vascular development with the morphological changes, since these vary slightly from seedling to seedling, the broad features are as follows: At the time that the cotyledons are first expanded the isolated cotyledonary protoxylems are disorganized and in some cases partly resorbed (cf. Fig. 15), but are still recognizable. This disorganization and resorption is shared to some extent by the older elements of the primary metaxylem which lie on the inner faces of the four large diagonally situated bundles. The epicotyl, which has not yet begun to elongate, contributes six well-differentiated strands to the hypocotyledonary vascular system, namely, the midrib and laterals of the first pair of epicotyledonary leaves. Near the apex of the hypocotyl the lateral bundles are reinforced by desmogen strands representing the vascular supply of the axillary buds of the cotyledons. The three epicotyledonary bundles of each side are united by a continuous band of phloem which, at a slightly lower level, connects with that of the cotyledonary traces (Fig. 23). The xylem of the lateral bundles also soon unites with that of these traces, but,

as was noted in the case of younger seedlings, the behaviour of the midrib is somewhat variable. In the majority of cases it bifurcates and the half-bundles move apart and fuse with the cotyledonary strands ; but occasionally the phloem alone bifurcates, the xylem moving as a single unit to one side or the other. In none of the seedlings of this age examined does the midrib retain its original median position in the intercotyledonary plane. Below the level of fusion of the epicotyledonary bundles with those from the cotyledons the general vascular structure of the hypocotyl shows no significant change from that of the earlier stages and its elements are wholly associated with the cotyledonary traces.

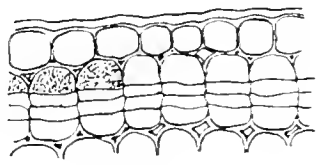
In seedlings in which the first pair of leaves projects about a quarter of



FIGS. 23–6. Camera lucida outlines of hypocotyls from progressively older seedlings (× 20). Fig. 23 is from a seedling in which the cotyledons are just fully expanded ; Fig. 24, from one in which epicotyledonary growth has commenced ; Fig. 25, from one in which the first epicotyledonary leaves are expanded ; Fig. 26, from one in which the secondary pair of epicotyledonary leaves are expanded. The cotyledonary xylem is outlined ; the epicotyledonary xylem and its connexions are solid black ; the phloem is indicated by fine dots, and the pericyclic fibres by coarse dots.

an inch above the median fissure between the cotyledons considerable changes have taken place. The upper part of the elongating epicotyl shows six well-differentiated strands derived from the first pair of leaves and, alternating with these, six desmogen strands representing the vascular supply of the second pair of leaves. At a lower level fascicular cambium is present in the larger strands, whilst the smaller strands each possess a group of three or four xylem elements. The bundle fusions characterizing the epicotyledonary nodes, which will be described in detail below, occur in this instance at or near the apex of the hypocotyl, so that there are six large strands in two equal groups at this level. These are united at a slightly lower level by a band of secondary xylem, and as the diagonally placed cotyledonary bundles move inwards they come to lie at the extremities of the arcs of xylem so produced (Fig. 24). At or near this level the site of the now completely disintegrated protoxylem is frequently occupied by a small group of cambiform cells, whilst still farther down the hypocotyl the eight strands which result from the bifurcation of each of the cotyledonary bundles are linked by a thin but continuous zone of secondary xylem often only one cell in width. In some seedlings of this age the continuity of the secondary xylem is broken in the lower half of the hypocotyl, but in others it remains constant down to root level.

In older seedlings a steady increase in the thickness of the secondary xylem occurs, the cambium adding elements external to the cotyledonary system and linking up with a small collateral strand which fills the position formerly occupied by the isolated cotyledonary protoxylem in each half of the hypocotyl (Fig. 25). By the time the second pair of epicotyledonary leaves have expanded there is a continuous ring of secondary xylem five or six elements in width throughout the hypocotyl and the lower half of the first epicotyledonary internode (Fig. 26).



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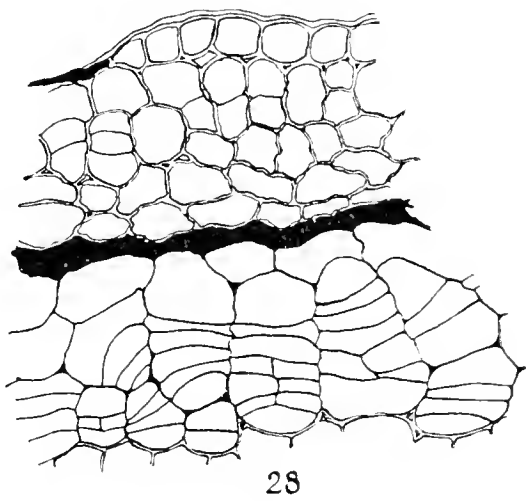

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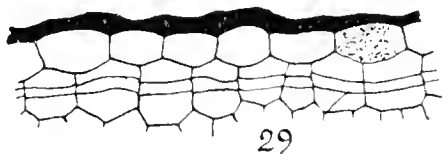

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FIG. 27. Sub-epidermal formation of cork in the hypocotyl : the granular contents of three outer cells only shown.

FIG. 28. Pericyclic cork formation in the region of the collet involving more than one layer of cells. Immediately outside these is a layer of crushed and disorganized cells (indicated in solid black), and beyond these the dead outer cortical tissues.

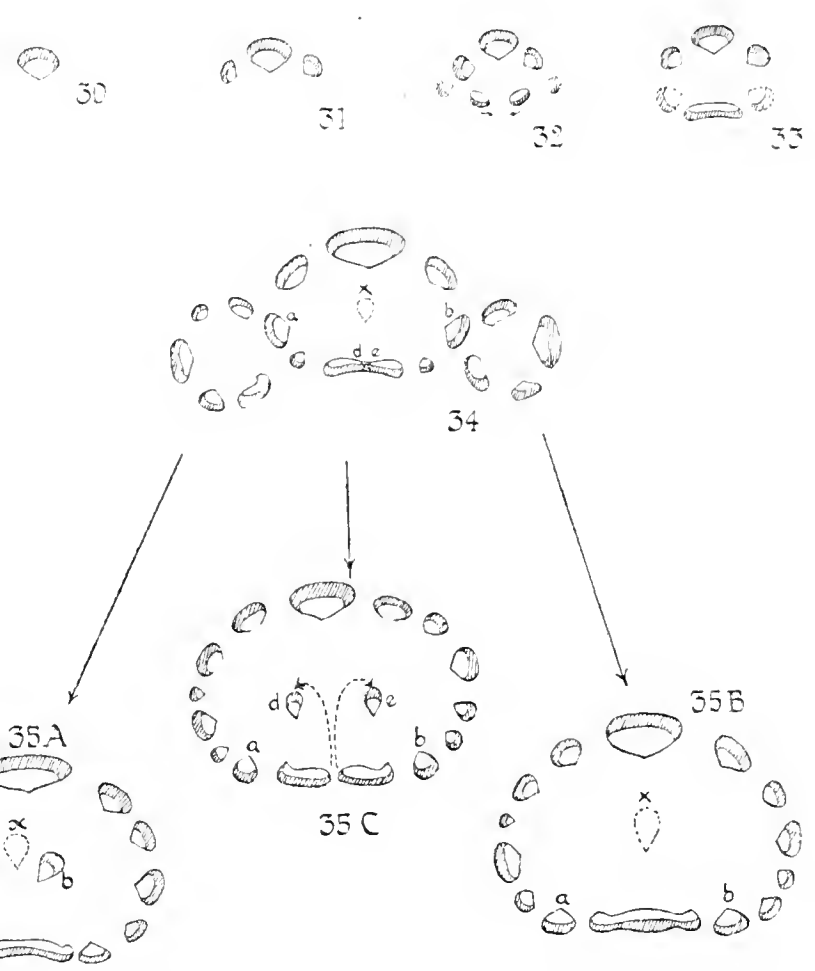
FIG. 29. Pericyclic cork formation in the root. The layer of solid black indicates the same region as in Fig. 28.

Accompanying these changes there occurs a development of pericyclic fibres. These first appear as clearly differentiated arcs in the hypocotyl lying outside the four diagonal cotyledonary bundles about the time the first epicotyledonary leaves are fully expanded. They are succeeded in older seedlings by others which develop both in the epicotyl and hypocotyl opposite the strands derived from the midribs of the first pair of leaves (Figs. 25, 26). It is interesting to note that traumatic stimulus may induce their formation in younger seedlings even though the wound is relatively slight and local in character. Somewhat deeper but still superficial wounds frequently result in an excessive development of secondary xylem adjacent to the injured area. The cork cambium is sub-epidermal in origin (Fig. 27). The period of its inception is variable, since many seedlings gathered in 1921, with the first pair of epicotyledonary leaves alone expanded, had a well-developed cork cambium, whilst in the wetter season of 1922 it was only just commencing to show in seedlings in which the second pair of epicotyledonary leaves were expanded. It first appears at the top of the hypocotyl and often involves the bases of the cotyledons, the cells of which divide to a depth of several layers. From this region it spreads downwards and in some cases is remarkably irregular, frequently leaving one or two vertical strips of green tissue bounded by the epidermis when all the rest of the hypocotyl shows the characteristic grey-brown resulting from cork formation. The cork cambium in the root is pericyclic in origin, as is normally the case (Fig. 29), but it is interesting to note that in the collet, where the pericycle is becoming several

cells deep, more than one layer of cells is involved in the subdivision to form cambiform elements (Fig. 28). We were unable to detect any indication of junction between the pericyclic cork cambium of the root and that arising sub-epidermally in the stem.

(iii) *The Vascular System of the Leaf and Petiole.*

The midrib, at the tip of the leaf, is represented by a small collateral strand which, as it is added to by lateral veins, gradually forms a shallow



FIGS. 30–5. Diagrammatic figures illustrating the building up of the petiolar vascular system. Figs. 35 A, B, C show the common variants derived from Fig. 34. The bundles *a* and *b* are lateral bundles which may undergo displacement near the base of the lamina, and *d* and *e* are portions of the adaxial bar which may undergo similar alterations in position: the bundle *x* is one which is occasionally displaced at a higher level.

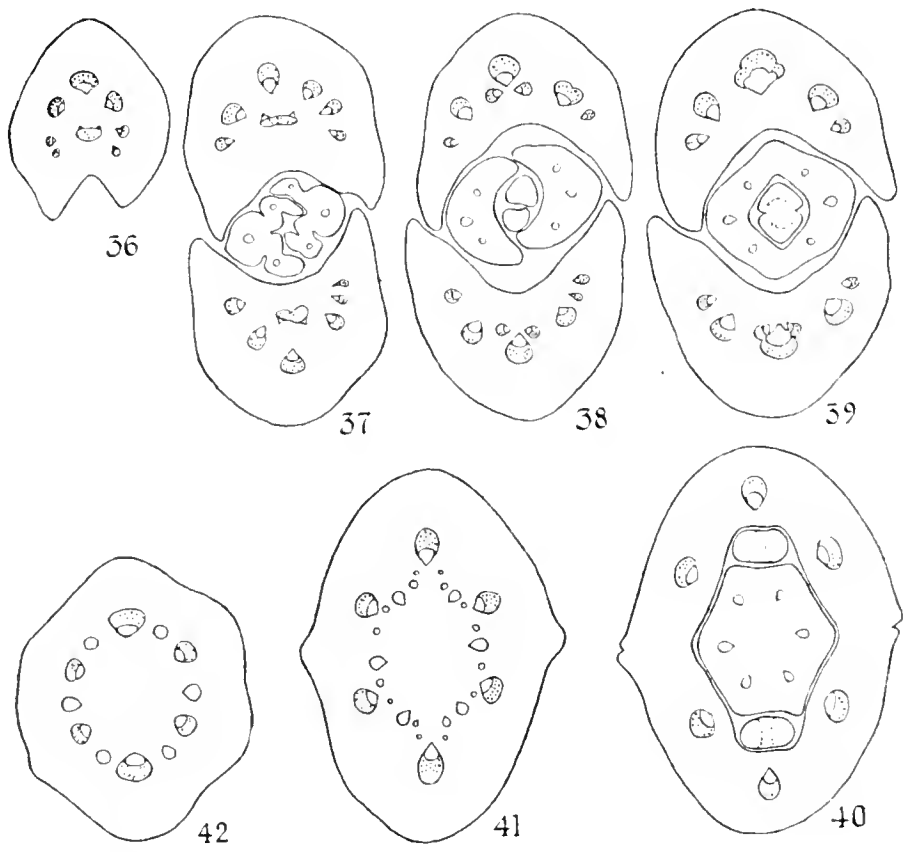
gutter-shaped system of collateral bundles the concavity of which is adaxial (Figs. 30, 31). At a lower level, as further contributions from the lateral veins occur, it becomes 'C'-shaped, as seen in transverse section (Fig. 32). The incurving of the free margins appears to be due to the displacement towards the adaxial side of some of the components from a higher level and, nearer the basal portion of the lamina where the largest lateral veins join the midrib, results in the formation of a closed ring of bundles (Figs. 33, 35 B). The adaxial portion of this closed ring is flattened and undergoes considerable secondary thickening, so that it is sometimes difficult to determine the limits of the original components. Whilst the formation of the closed ring is characteristic of all the leaves examined, a number show from one to three small internal strands in addition. These may occasionally consist only of phloem (Fig. 51 *a*), but are usually collateral and show an inverted orienta-

tion compared with the adaxial portion of the outer ring. Their formation is due to further bundle displacements, and is apparently the result of the compression of the adaxial portion of the ring or the lateral bundles on either side of it (Figs. 35 A, 35 C). This compression causes an infolding or displacement of vascular tissue from the part affected to the inside of the ring, the xylem and phloem rotating during their passage inward, so that they come to have an inverse orientation to the system from which they are derived. More complex internal bundle systems of the same type often occur in the vascular systems of the leaves of mature plants, and earlier observers appear to regard the more elaborate types as an expression of greater size and vigour. Thus Col (4) says, 'Le *pétiole* des plus larges feuilles montre dans sa moelle un arc, surmonté d'un plus petit, ayant tous deux leur bois tourné en avant. Dans d'autres pétioles, il n'y a qu'un arc interne. D'autres encore ont montré un très petit nombre de fascicules médullaires. Ainsi, sur quatre feuilles d'une pousse d'automne, toutes offrent au milieu du pétiole, un ou trois faisceaux libéroligneux médullaires très petits.' The size of the leaf, in the early stages at any rate, does not seem to us to have any direct bearing on the presence or absence of internal bundles, since we have noted their absence in the larger of one of the first pair of leaves and their occurrence in the smaller, and also their occurrence in seedling leaves distinctly below the average size.

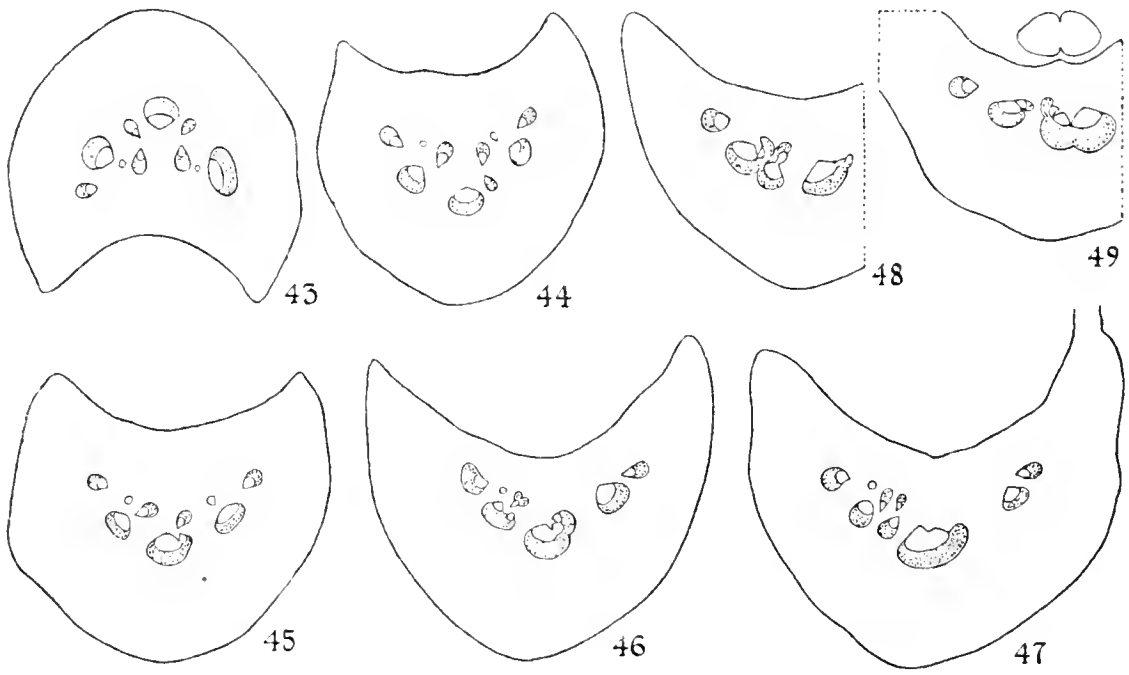
The vascular grouping prevailing at the base of the lamina is maintained throughout the greater part of the petiole, but the adaxial portion often forms a continuous bar, or two contiguous bars, as a result of secondary thickening. The first indication of rearrangement is shown by the internal bundles where these are present. Although too small a number of petioles showing this feature were examined to enable us to make a general statement, it appears to be the rule that, as the base of the petiole is approached, the internal bundles undergo a reversal of the series of changes which resulted in their formation. Thus those derived from the central portion of the adaxial system rotate and reunite with this (Fig. 54), a gap being developed to admit of their insertion, whilst those derived from the lateral portions of the ring behave similarly.

As the base of the petiole is approached there is a considerable increase in its transverse diameter, and this is accompanied by a series of bundle fusions which result in the passage into the stem of three bundles from each leaf (Fig. 40). These bundle fusions are somewhat complex, but they always involve two processes. These are (i) the concentration and fusion of the lateral bundles, and (ii) the moving outwards of the bundles constituting the adaxial system and their union with one or another of the abaxial series. The regroupings may be arranged in a series showing progressively greater complexity, although there is no evidence that this constitutes an evolutionary sequence.

The simplest type, (*a*), which is relatively rare, is that shown in Figs. 36–9, in which the adaxial bar divides into two bundles, each of which rotates and unites directly with the midrib. An advance, (*b*), on this type



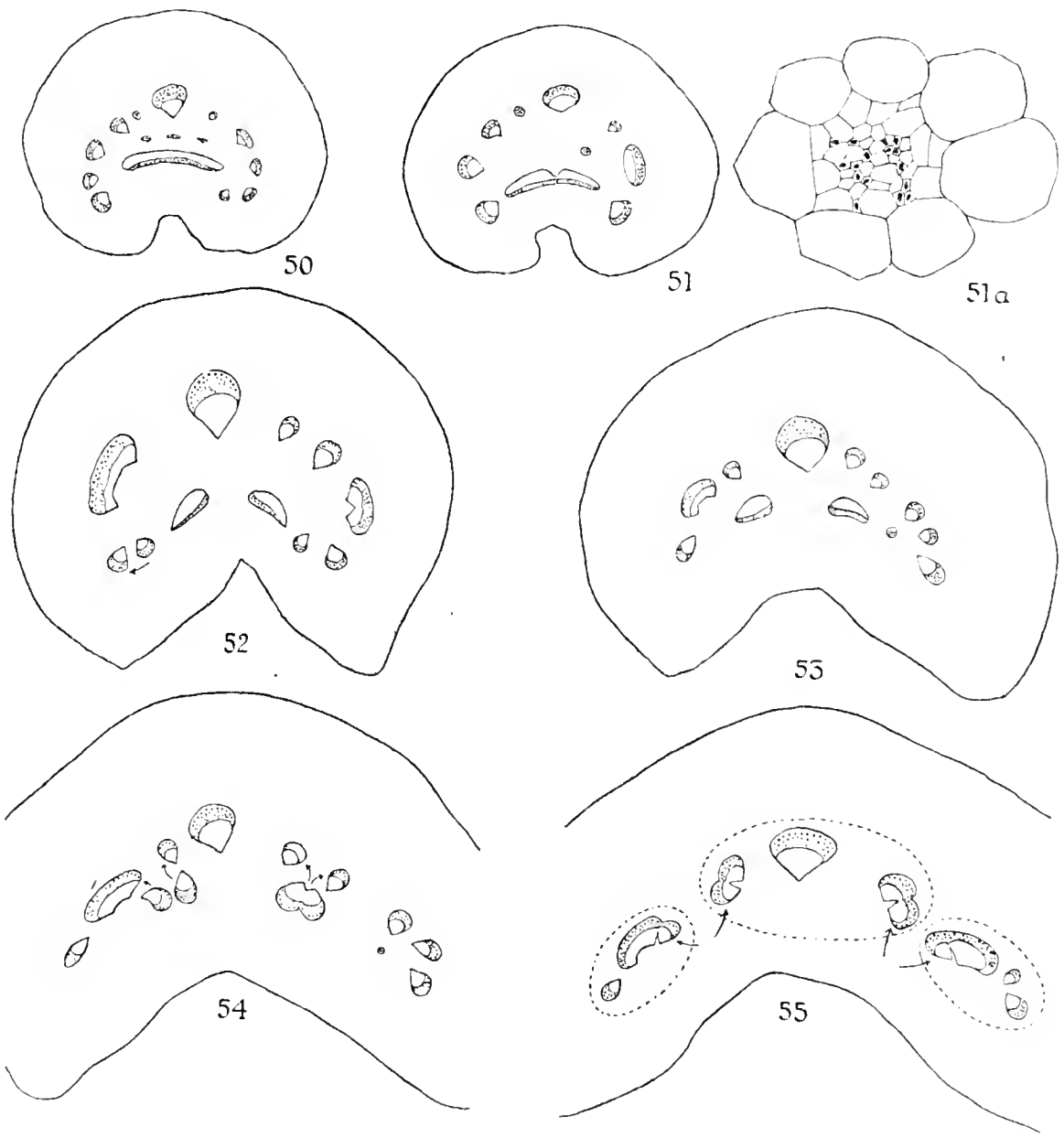
FIGS. 36–42. Camera lucida outlines of successively lower sections through the young plumular bud, showing the simplest type of bundle fusions occurring at the base of the petiole (Figs. 36–40) and the passage into the epicotyl of the three bundles from each leaf. × 20.



FIGS. 43–9. Transverse sections through a petiole in which a slightly more complex type of fusion of the adaxial bundles with the abaxial constituents occurs. Figs. 44–9 are from the petiole which is fellow to that shown in Fig. 43, the adaxial bundle on the right behaving similarly, whilst that on the left shows a still further complexity. (Camera lucida outlines × 20.)

is shown by those petioles in which each of the two bundles derived from the adaxial bar rotates and unites with a small lateral strand adjacent to the midrib, the compound strand thus formed then uniting with the midrib (Fig. 43). A still further advance, (*c*), is illustrated by the division of each of the two original bundles derived from the adaxial bar (Figs. 54, 55). Of

the four bundles so produced the two more centrally placed ones rotate and unite with small lateral strands which in turn join the midrib as in the previous type. The two distally situated bundles also rotate, but unite with strands farther from the midrib which contribute to the formation of the large laterals passing into the stem. An additional complication is introduced by the fact that irregularities occur, not only in any two petioles

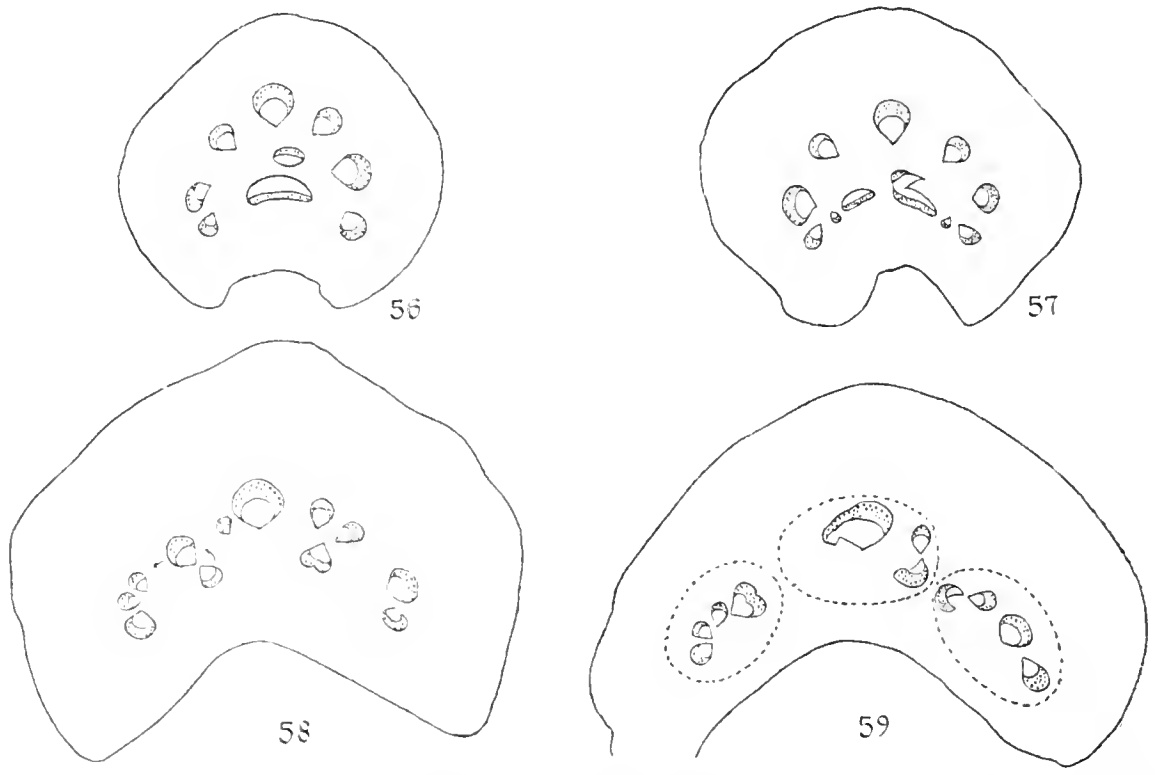


FIGS. 50–5. Transverse sections of a petiole in the upper part of which three feebly developed medullary bundles occurred locally, but died out at a lower level without fusion. The one persisting longest (Fig. 51*a*) was represented by phloem only for the greater part of its course. Figs. 54 and 55 show the bundle fusions near the base of the petiole. Fig. 51*a* × 750, the remainder × 20.

constituting a pair, but even on opposite sides of the same petiole. Such a case is shown in Figs. 44–9, in which the adaxial bundle on the right behaves in the manner described under (*b*) above, whilst that on the left bifurcates and its halves behave in the manner described under (*c*), but with the further peculiarity that the two bundles to which they unite are in their turn produced by the bifurcation of a parent bundle (Figs. 46–9).

A further irregularity is occasionally produced by the union of the whole of one of the adaxial bundles with a strand which forms part of the main basal lateral instead of its uniting with one supplementing the midrib (Fig. 58). Whatever the type of bundle fusion, the ultimate result is always

the production of three bundles at the base of the petiole, so that six bundles enter the epicotyl at each node (Fig. 40), these alternating with the six bundles entering the stem from the node above (Fig. 42).



FIGS. 56–9. Transverse sections of a petiole, showing a single asymmetrically situated medullary bundle (Fig. 56) which at a lower level unites with half the adaxial bar (Fig. 57). Figs. 58 and 59 show differing methods of bundle fusion on opposite sides of the same petiole. (Camera lucida outlines × 20.)

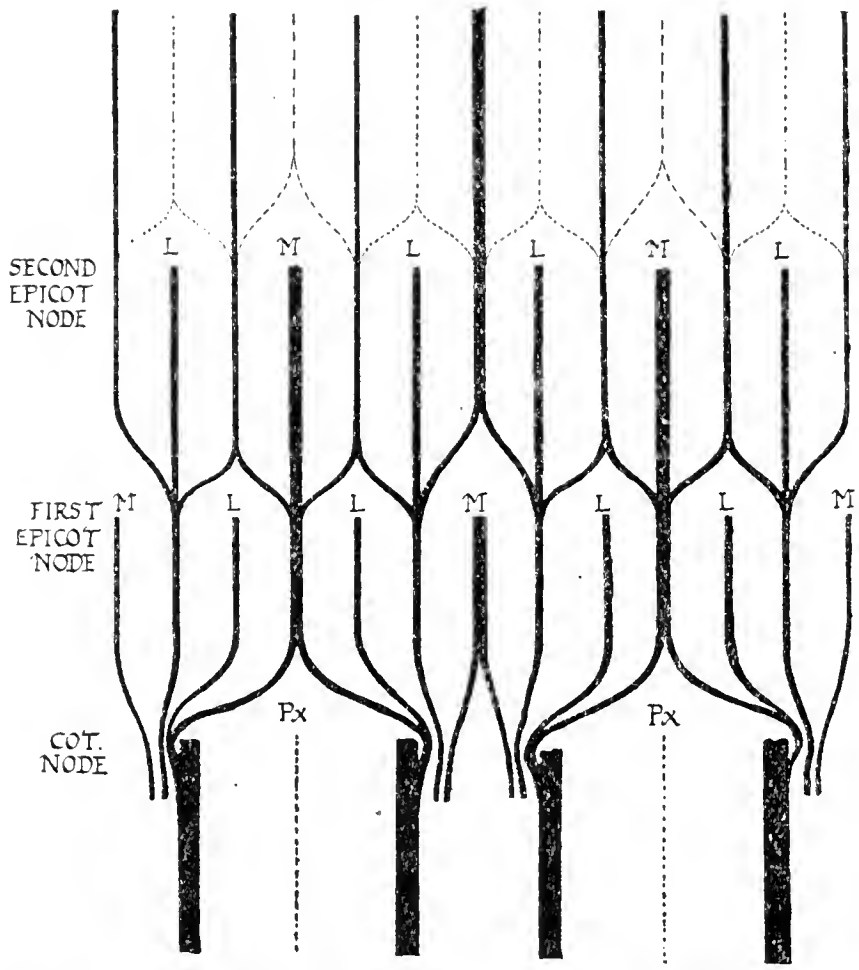


FIG. 60. Diagrammatic representation of the vascular system of the young seedling shown as if cut through in the intercotyledonary plane and spread out flat. M., midribs; L., laterals.

The bundles derived from each pair of leaves, with the exception of those at the first epicotyledonary node, travel down through two internodes. At the base of the second internode each bundle bifurcates to admit of the

insertion of the six bundles from the leaves arising at that node (Fig. 41). The half-bundles so produced move apart and unite with the bundles entering the stem at the previous node. The bifurcation of the midrib bundles is always somewhat precocious compared with that of the laterals (Figs. 41, 60), this admitting of the insertion, on the adjacent faces of the two half-bundles, of the strands representing the vascular supply of the axillary buds. The six bundles entering the epicotyl at the first epicotyledonary node only traverse a single internode before entering the hypocotyl. The laterals unite with the diagonally placed cotyledonary metaxylems *without bifurcation*, whilst the midrib, as has been stated previously, generally bifurcates, its halves behaving similarly. The six strands from the second epicotyledonary node are also involved in the bundle fusions occurring at the apex of the hypocotyl. Of these the midribs bifurcate in the usual way, but become joined to the laterals from the node below a little earlier than is generally the case. No division of the laterals has been detected, but they appear to move outwards with the half-bundles of the midribs of the first epicotyledonary node and so link with the cotyledonary strands. It is, however, somewhat difficult to determine their exact fate, since by the time that they are sufficiently differentiated in this region the limits of the individual bundles are obliterated by secondary thickening.

Abnormalities.

Of the fifty-three seedlings examined, nine show some degree of departure from the normal in the behaviour of their vascular strands during transition and are best dealt with individually. With two exceptions they may be arranged in two groups. In the first of these the abormality is generally the result of the persistence of plumular contributions, chiefly phloem, beyond the upper part of the hypocotyl and in some cases down into the root. It is not always possible, however, to refer the abnormality to plumular strands (e. g. in seedlings *E* and *H* below).

In the second group the abnormality takes the form of the development of accessory groups of metaxylem in the root, which may or may not give rise to additional root poles.

Group 1.

Seedling A. This is a very young seedling in which the plumular elements are not well differentiated. A single phloem group appears in the intercotyledonary plane of one side and after persisting for some time dies out.

Seedling B. This is also very young and is characterized by two groups of phloem in the intercotyledonary plane on opposite sides of the stele, one of these developing at a lower level than the other. Both persist to the

limit of differentiation, but this seedling is too young to determine the effect of the persistence of the phloem groups on the root structure.

Seedling C. This is an older seedling of the same type as seedling *A*, the phloem of one of the plumular midribs persisting throughout the hypocotyl and below the collet, though subsequently dying out. Preparation for the formation of the intercotyledonary root poles is evident below the collet, small xylem elements appearing associated with the adjacent margins of the lateral metaxylem groups. These are separated at first by the intrusive plumular phloem, but approach each other after this has disappeared.

Seedling D. This is similar in type to seedling *C*, except that the phloems of both plumular midribs are persistent to the limits of differentiation.

Seedling E also resembles seedling *C*, but in this case the persistent plumular strand consists of both xylem and phloem, a small collateral vascular strand appearing therefore in the intercotyledonary plane throughout the hypocotyl. As in the seedlings previously described, small xylem elements appear on either side of the persistent bundle in preparation for the formation of the intercotyledonary root pole, the xylem of the bundle bifurcating, and the halves moving towards these small elements. Meanwhile, a group of phloem has appeared on the other side of the axis and small xylem elements are differentiated on either side of this second ploem also. At the limit of differentiation these arrangements still prevail, but the phloem group which was last to appear is becoming much reduced in size and seems about to disappear.

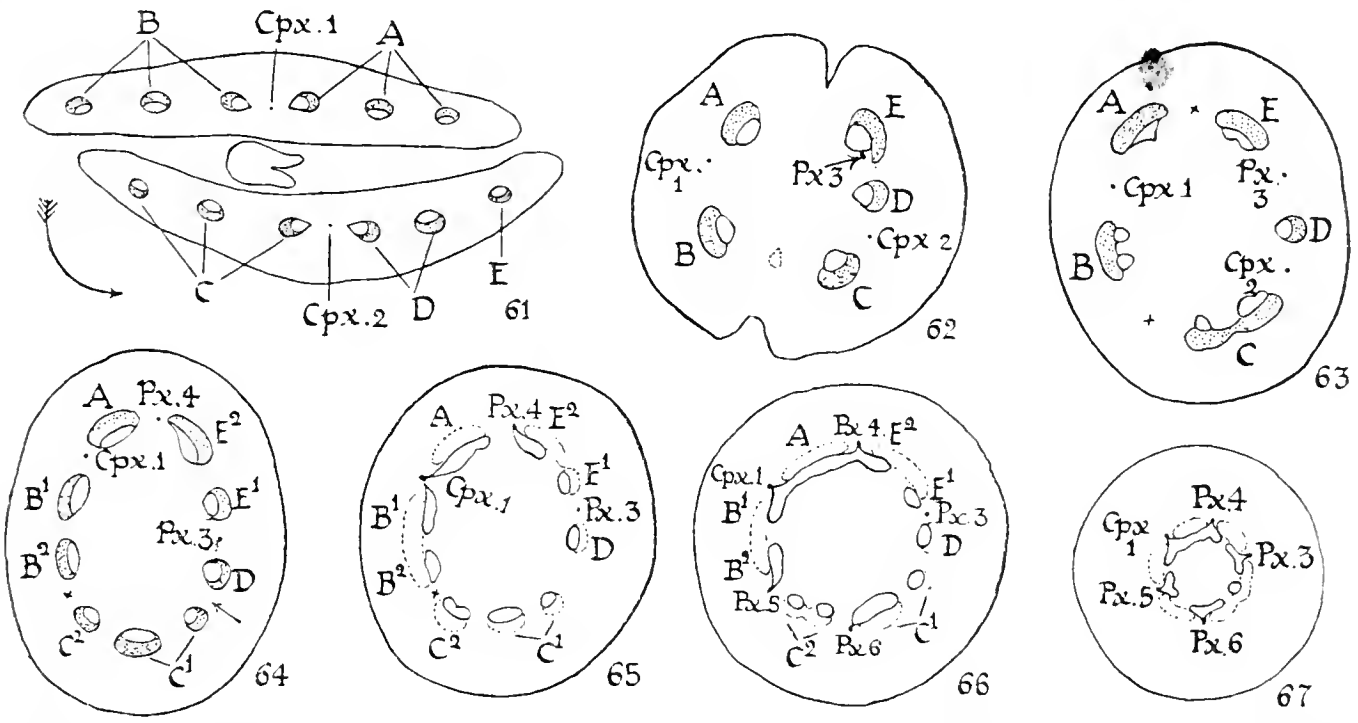
Group 2.

Seedling F. In this seedling normal structure obtains throughout the hypocotyl and the upper part of the root. When the organization of typical root structure is almost completed, however, a group of metaxylem appears lying inside the phloem between a cotyledonary and an intercotyledonary pole. This does not cause any disturbance of root symmetry, and in the lowest sections is approaching the adjacent cotyledonary pole.

Seedling G is obviously of the same type as seedling *F*, and like it shows normal structure in the hypocotyl and upper part of the root. At this stage, however, a fifth xylem group appears within one of the four phloem groups. The phloem bifurcates and a fifth root pole is established, this being followed at a slightly lower level by a sixth pole developed as the result of the intrusion of a new xylem group directly opposite the fifth. Hexarchy prevails throughout the remaining part of the root.

Seedling H, though showing distinct affinities with the two seedlings of Group 2, has additional peculiarities of considerable interest. The behaviour of the epicotyledonary strands is normal, as is that of the cotyledonary strands, except that the central isolated protoxylems die out. One appears again sporadically as one or two small elements throughout the hypocotyl

and only becomes well defined in the region of the collet. The site of the other is occupied, a little way below the apex of the hypocotyl, by a small but well-defined collateral bundle which persists for a little over a centimetre but gradually dies out. As it disappears a similar bundle appears in the intercotyledonary plane at one side and persists for about a centimetre and a half before it too dies out. Meanwhile, the four diagonally situated xylem groups, with one exception, have each divided into two in the normal way. The one showing delay is in close proximity to a wound involving the greater part of the cortex in that particular part of the hypocotyl, and



FIGS. 61–7. Series of semi-diagrammatic outlines, showing the transition features of seedling *J*. The small crosses in Figs. 63 and 64 mark the positions of the single protoxylem-like elements which appear sporadically in the hypocotyl; the arrow in Fig. 64 marks the position which would be occupied by *Cpx.* 2 if it had persisted.

the traumatic stimulus has led to a precocious development of secondary xylem which obscures the behaviour of the affected strand. Transition to root structure, however, apart from this disturbing factor, is typical. Subsequently there appear, at slightly different levels, three xylem groups lying inside three of the four root phloems and, apart from the repetition, resembling the condition found in seedling *F*. Of the three groups of xylem one persists for about half a centimetre and then dies out, whilst the other two are still well developed in the lowest sections obtained.

Seedling J is one which stands quite apart from the remainder and is peculiarly complex. Only one epicotyledonary leaf is developed, the fusion of the cotyledons being slightly earlier on the side opposite to this. In one cotyledon the vascular strands behave in the normal manner, so that a protoxylem strand (*Cpx.* 1) and two widely separated collateral groups (*A* and *B*) enter the hypocotyl from it. In the second cotyledon the vascular strands of one side fuse in the usual manner to form one large bundle (*C*), whilst on the other side the half-midrib bundle fuses with the

lateral bundle only, producing a compound strand (*D*) the marginal (*E*) remaining independent. From this cotyledon, therefore, a median protoxylem strand (*Cpx.* 2) enters the hypocotyl with a single collateral (*C*) on one side and two such strands (*D* and *E*) on the other. A few small xylem elements become detached from the inner side of the marginal strand (*E*) and move away from the parent group until they occupy a position midway between strands *D* and *E*. Although at first they lie within the phloem of the main bundle they ultimately resemble a protoxylem group closely (Fig. 63, *Px.* 3) and during the development of root structure behave as such. This condition is maintained for some distance down the hypocotyl, the only change being the division of the metaxylem groups *B*, *C*, and *E* into B^1, B^2, C^1, C^2, E^1, E^2 ; the metaxylem group *A* does not divide until considerably later and then becomes resolved into two very unequal portions. During the passage of these half-bundles down the hypocotyl a solitary, small protoxylem-like element appears and disappears between *A* and *E* and a similar one, at a slightly lower level, between *B* and *C*. Towards the base of the hypocotyl the protoxylem from the cotyledon showing abnormal transition (*Cpx.* 2) dies out and the metaxylem group C^1 divides into two.

From this complex five root poles are organized as follows :

1. A normal cotyledonary pole is formed from B^1 and the bulk of *A*.
2. The remnant of *A* forms a second pole with E^2.
3. Strands E^1 and *D* concentrate on *Px.* 3 to form a third pole.
4. Strand C^2 divides into two, half forming a fourth pole with B^2 and half forming a fifth pole with C^1 : the remaining part of C^1, though persistent, takes no part in root-pole formation.

It is worthy of note that the poles formed by the remnant of *A* with E^2, and that of B^2 with C^2, occupy the positions of the isolated protoxylem-like elements which appeared and disappeared higher up in the hypocotyl.

Discussion.

In considering the possible theoretical significance of the seedling anatomy of the sycamore it will perhaps be well to outline the ontogenetic succession which Chauveaud (3) has shown to be characteristic of a large number of angiosperm seedlings. This investigator has established the fact that the vascular elements pass through a series of phases which are remarkably uniform and which he regards as constituting a phylogenetic sequence. He considers that each xylem group has its phylogenetic origin in a radial file of vessels developing centripetally, the phloem at this stage being represented by a series of tangentially distributed elements occupying alternate radii to the xylem. This condition is termed the ‘ disposition alterne ’. The xylem and phloem elements developed subsequently to those exhibiting

the 'alterne' grouping show a characteristic alteration in relative position. In this phase, showing what Chauveaud terms the 'disposition intermédiaire', the xylem vessels arise in a tangentially extended series to the right and left respectively of the plane occupied by those of the 'alterne' phase, whilst the new phloem elements develop in a plane roughly parallel with the earlier ones, but are situated nearer the xylem. In continuity with the latest formed xylem elements of the second phase ('disposition intermédiaire') arise other elements developed in a centrifugal series, whilst the new phloem elements lie still nearer the xylem and thus lie on the same radii as the centrifugal vessels. As a result of this, two collateral bundles are formed, one on either side of the original plane of xylem differentiation. This third phase is termed the 'disposition superposée'.

The xylem of all three phases may be represented at one time, or the development of each of the later phases may be accompanied by the disappearance of the preceding one.

In the absence of the first phase the elements of the second appear as two separate groups of vessels inclined to one another like the arms of the letter V, whilst the third phase, in the absence of the two earlier ones, takes the form of two quite separate collateral bundles. Chauveaud has shown that in the ontogeny of the seedling vascular system not only the root, but also the hypocotyl and even the basal portion of the cotyledon, may exhibit the 'alterne' grouping of the xylem and phloem. In the root this vascular grouping is persistent, and though further conducting elements possessing the 'intermédiaire' arrangement may augment the original units (e.g. *Phaseolus vulgaris*), they do not obscure its character. In the hypocotyl and cotyledon, however, it is far otherwise, since the development of each new phase is followed by the partial, or total, resorption of the vascular elements of the phase preceding it. The result is that in these organs the early stages in vascular development are only to be observed during the initial stages of germination and those which immediately follow it. Chauveaud considers that seedlings exhibit in their vascular development an acceleration from below upwards, so that whilst the primitive exarchy is retained in the root it is transient in the aerial parts of the seedling, this transient character being shared to a less degree by the intermediate elements. Where this acceleration is accentuated it leads to the total elimination of the earlier phases from the ontogeny in the upper portion of the hypocotyl and in the whole of the cotyledon and to their early resorption at lower levels. It will thus be seen that, according to Chauveaud, the differences in orientation exhibited during the transition from the radial distribution of the xylem and phloem in the root to their collateral distribution in the stem are simply the result of the persistence of only the phyletically more recent stages at successively higher levels of the hypocotyl and cotyledons.

Whilst Chauveaud's theory supplies a consistent explanation of the

march of events in the vascular development of many seedlings, there are a number which present difficulties requiring further elucidation and explanation. The chief of these are two in number, and reference has already been made to them by previous investigators. The first is the difficulty presented by the behaviour both of the protoxylem and metaxylem which constitute the intercotyledonary poles in tetrarch roots, and here, as Dr. E. N. Thomas (16) points out, Chauveaud's theory breaks down to some extent, the metaxylem of these poles being 'continuous with the "elements superposés" of the lateral strands of the cotyledon'. Although the collateral segments may be almost wholly concerned in augmenting the cotyledonary vascular supply, they frequently combine this function, as Compton (6) has shown, with that of ensuring a closer contact with the epicotyledonary strands. Occasionally, as in *Juglans nigra* (8) and *Caesalpinia separia* (6) for example, this function may be the only one, and the intercotyledonary root poles are then directly continuous with the epicotyledonary traces and contribute no elements to the cotyledons. This extreme condition is one which is frequently associated with hypogeal germination, and the fact of its occurrence raises the question as to what extent the intercotyledonary root protoxylems generally, in tetrarch types, are referable to epicotyledonary sources. Miss Davey, in a paper dealing with the seedling anatomy of the *Amentiferae* (8), suggests that this may be the case in certain species (e. g. *Myrica Gale*, *Alnus*, *Carpinus*) even though direct connexion between the epicotyledonary protoxylem on the one hand, and that of the root on the other, cannot be established. This lack of continuity is held to be due to the delay in plumular development. As a result of this delay 'differentiation is not complete at the upper end of the seedling, so that the protoxylem centre "dies out" in ascending the hypocotyl, and actual connexion cannot be established'. The same author (8, p. 598) cites Compton as describing a similar distribution of the intercotyledonary vascular units between cotyledons and plumular leaf in the Leguminosae 'as a phenomenon of replacement in which the cotyledons are being supplanted by plumular leaves'. It would appear, however, that Miss Davey had misinterpreted Compton's views, since this investigator (6, pp. 102–4), after stating the following alternatives: '(i) The plumular traces may tend to replace the intercotyledonary root poles, or vice versa their relation to one another being *complementary* and one of supplantation. (ii) The intercotyledonary xylem may combine with the leaf-trace to form a joint conducting channel, their relation to one another being *supplementary*,' and after discussing the evidence for each, pronounces in favour of the latter.

The evidence in the case of the sycamore seems definitely against the view that the intercotyledonary protoxylems are plumular in origin. In the majority of cases the midrib bundles fork at a relatively high level in the

hypocotyl, though they may move laterally as a whole to join the adjacent cotyledonary strands, and subsequent to such displacement it is difficult to see how the intercotyledonary root protoxylems which appear below the external collet can be referred to them. Further, when, as in some of the abnormal types described in Group 1, the epicotyledonary midribs persist below their normal level, they appear to delay the formation of the intercotyledonary poles rather than assist in their development. It should also be noted in this connexion, although we do not wish to stress the point, that in the abnormal seedling *J*, although only *one* epicotyledonary leaf is present, *two* root poles are formed in the positions which would normally be occupied by the intercotyledonary poles.

The second criticism of Chauveaud's theory was originally put forward by Compton (5), who pointed out that the sequence of alternate, intermediate, and superposed phases is not realized in types such as *Lupinus hirsutus*, in which, at the base of the cotyledon, an isolated median protoxylem is present, flanked on either side by a widely separated collateral bundle, from which it may be separated by as many as fifteen parenchymatous cells. It is equally evident that this sequence is not realized in the sycamore. If the upper part of the hypocotyl is examined in the very young seedling (Fig. 6) it is found that the formation of the central protoxylem is followed by the development of metaxylem elements in the diagonal plane, and that a series, often exceeding twenty, of parenchymatous cells intervenes between the protoxylem and the metaxylem. It requires a good deal of faith to believe that this parenchymatous gap represents a region occupied ancestrally by intermediate xylem elements, and the facts seem to be met better by a different explanation.

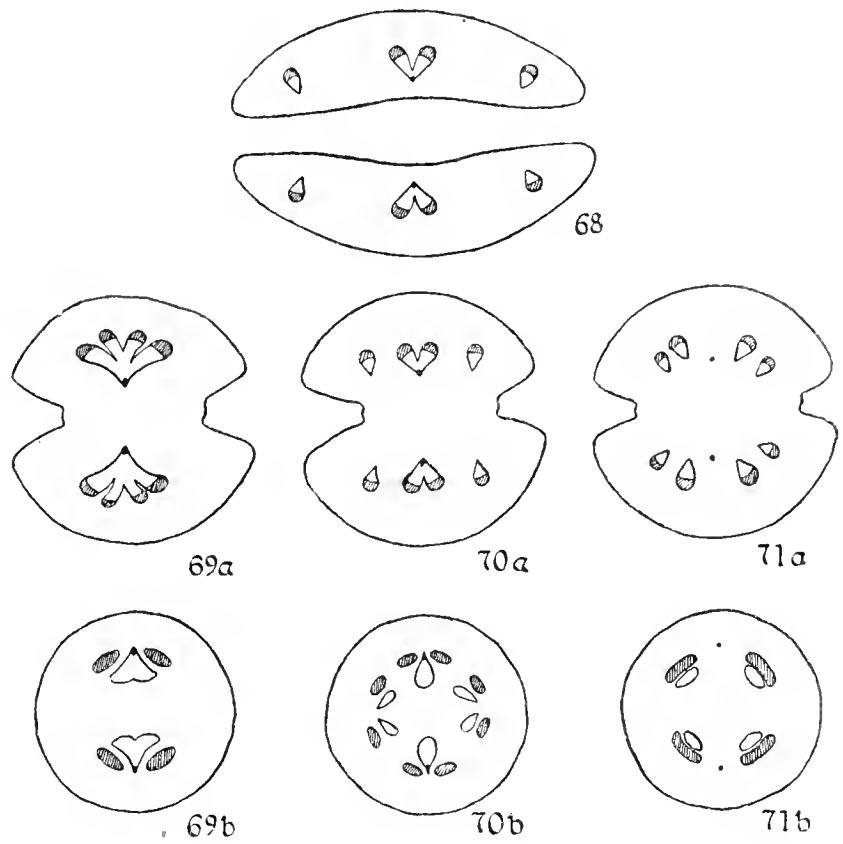
We may say, generally speaking, that in each seed-leaf of a dicotyledonous seedling the vascular components consist of a midrib and a lateral system (Fig. 68). These may react on one another in various ways. Thus there may be a *median concentration* of vascular components leading to their aggregation in the cotyledonary plane so that *two compound strands* enter the hypocotyl (Fig. 69 *a*, *b*); alternatively the midrib and laterals may remain independent so that *six simple strands* enter the hypocotyl, two in the cotyledonary plane and four in the diagonal planes (Fig. 70 *a*, *b*). Finally, there may be a *lateral concentration* of the vascular components so that the metaxylem and phloem of the midrib are detached from the central protoxylem, each half moving outwards and frequently uniting with its adjacent lateral. In this event *four compound strands* will enter the hypocotyl in the diagonal planes, whilst the cotyledonary plane will show two isolated protoxylems (Fig. 71 *a*, *b*). Any of these three types of grouping may produce either a diarch or a tetrarch condition in the root, so that there does not appear to be any close connexion between the number of root poles and the type of vascular arrangement found in the hypocotyl.

Of the three types we believe the last to be the most recently evolved, and to be the one which gives the clue to the origin of the majority of those forms showing diagonal tetrarchy in the root (e. g. *Calycanthus*).

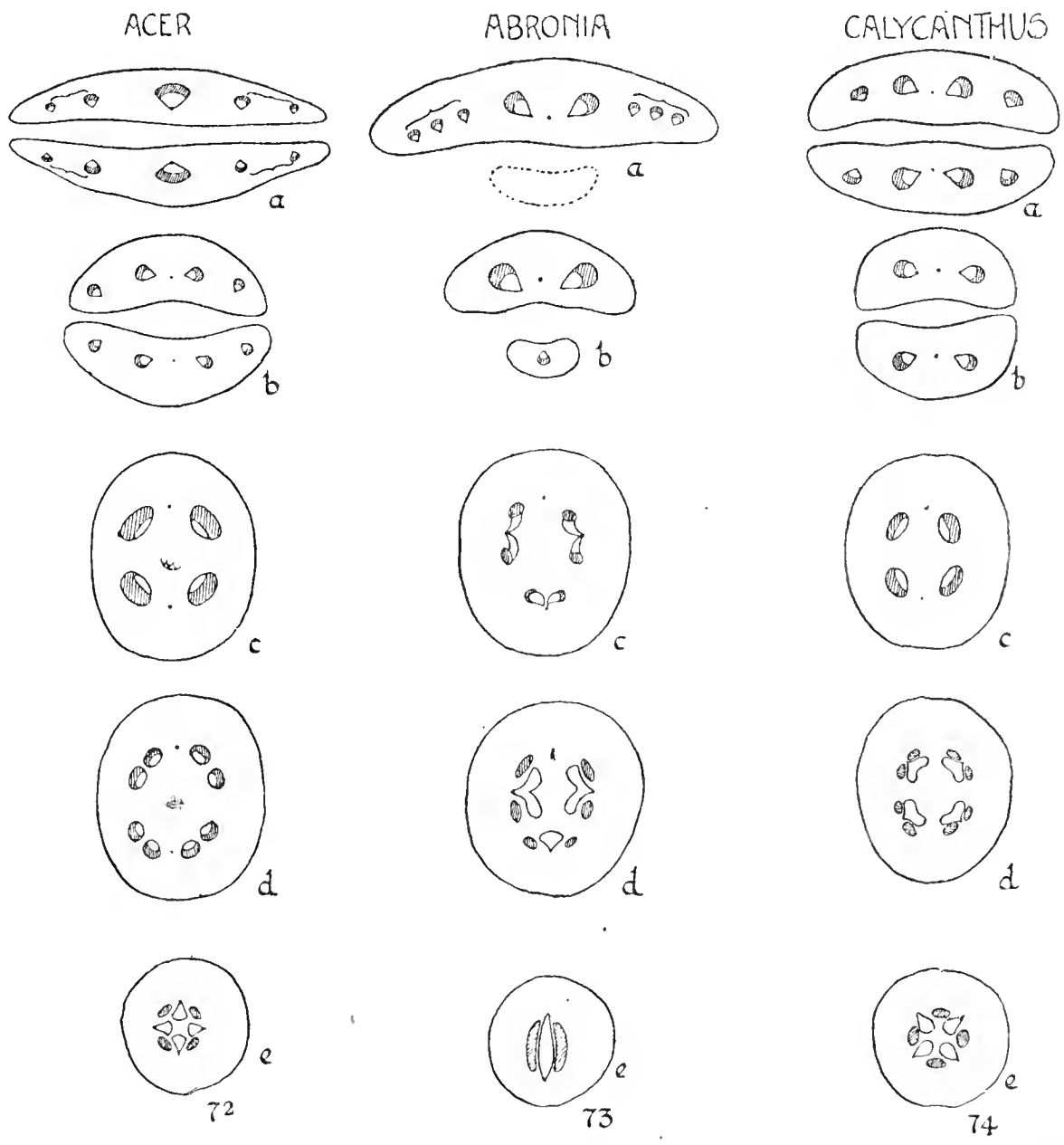
We consider that the sycamore exhibits this lateral concentration and that there has been *a definite moving outwards* of the major portion of the midrib constituents. It represents a relatively early stage, however, of this line of evolution, and the vascular components forming the compound diagonal bundle are soon dissociated, probably in part at least owing to the importance of the laterals which form the intercotyledonary poles. We may trace the later stages in a number of seedlings. In *Lupinus hirsutus*,[1] *L. albus*, and *L. mutabilis*, for example, the diagonal arrangement extends throughout the hypocotyl, and a still later stage is exhibited by the seedlings of *Abronia* spp. (9). Here the diagonal grouping is not only continued through the whole of the hypocotyl, but involves the upper part of the root. The result is that well-developed root poles are developed in the diagonal position, whilst only a rudimentary pole, represented by the isolated protoxylem, is present in the cotyledonary plane. This condition may be persistent, but more usually there is a gradual return to the older symmetry and the diagonally situated poles dwindle whilst the cotyledonary poles become progressively more important, so that the tap-root is ultimately diarch. The situation is complicated somewhat in *Abronia* owing to the fact that the lateral concentration only affects one cotyledon, the other, which is much reduced in size, showing median concentration (Fig. 73 *a*). The final term in this series is shown by forms like *Calycanthus*. Here the diagonal position is permanent throughout the seedling, so that four diagonal root poles occur without normally any reversion to an ancestral diarchy. It is interesting to note, however, that Thomas (16) records a reversion to the diarch state as an occasional abnormality in *Calycanthus*. A further point of similarity in the series is also worthy of mention. There exist in *Calycanthus* a number of small secondary collateral bundles at the base of the cotyledons between the widely separated diagonal strands, these appearing quite soon after germination has commenced. A single strand of a similar character is frequent in *Lupinus mutabilis*, though here it appears later, and it is also present as a rare abnormality in the sycamore. There is thus good reason for regarding the series *Acer–Lupinus–Abronia–Calycanthus* as illustrating a well-marked tendency in seedling anatomy.

It is not proposed to discuss in this paper the possible significance of the vascular structure of the leaves and epicotyl, as it is felt that more work of a comparative character is necessary before its real bearing can be adequately determined.

[1] Thomas (16) records a number of species showing a condition similar to that found in *Lupinus hirsutus*, e. g. *Decaïsnea Fargesii*, *Laurus Sassafras*, *Liquidambar styraciflua*.



FIGS. 68–71. Diagrams illustrating the three types of transition from the cotyledons to the hypocotyl described in the text.



FIGS. 72–4. Comparative diagrams illustrating the transition features in *Acer*, *Abronia* and *Calycanthus*.

Summary.

1. The development of the seedling vascular system of the sycamore, *Acer Pseudoplatanus*, is described from an early stage in its differentiation up to a period in which the epicotyl is beginning to assume a dominant rôle.

2. It is held to illustrate an early stage in the evolution of diagonal root symmetry of which the stable tetrarch stage is shown by *Calycanthus*.

3. An account is also given of the food reserves found in the embryo and young seedling. These include starch, a fatty substance, and at least two proteins, all of which are digested and utilized in the early stages of seedling growth.

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Anomalous Traces in the Cone of Equisetum maximum, Lam.

BY

ISABEL M. P. BROWNE.

IN an account of the anatomy of the cone of *Equisetum maximum*, Lam., published in 1915, a brief comment was made on the abnormal behaviour of certain traces of a cone of this species (Browne, 1915, pp. 247–8). Since 1915 numerous facts concerning anomalies in the course and structure of the traces of the sporangiophores of *E. maximum* have come under my notice, and I propose to give a short account of the phenomena observed.

The anomalies about to be described are of three kinds.

In the first or A anomaly the phloem of the trace enters into connexion with that of the axis, and the tracheides of the trace enter the bundle but, failing to join on to the axial protoxylem, die out among the metaxylem elements.

A second anomalous type of trace is that in which the phloem joins on to the phloem of the axial bundle, though the tracheides of the trace do not penetrate the bundle, but die out in the cortex. This kind of anomaly I propose to call the B anomaly.

The third anomaly is the most striking of those about to be considered and is that mentioned in my paper of 1915. A trace, passing in from a sporangiophore, dies out in the cortex without any of its elements joining on to the corresponding elements of the axial bundle. Such a trace may conveniently be called a free trace, or a trace showing the C anomaly.

These three kinds of anomaly are not confined to the traces of the lowest whorl of the cone of *E. maximum*, but they are much more frequent in that position. Consequently, in order to obtain reliable data as to these anomalous traces, I have made a detailed study of the traces of the lowest whorl in four cones of *E. maximum*. Two of these cones are those already described in my paper of 1915 as Cones A and B; the two others have never been described and I propose to refer to them as Cones F and G.

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The following table gives the particulars as to the number of vascular strands, normal and anomalous, entering the axis from the sporangiophores in the lowest whorls of the four cones studied.

Lowest whorl of cone.	*Strands showing the A anomaly.*	*Strands showing the B anomaly.*	*Strands showing the C anomaly.*	*Normal strands.*
A	2	0	4	18
B	1	0	0	41
F	7	4	14	5
G	6	1	0	20

As already pointed out these anomalous traces also occur, though more rarely, in other positions and in the cones of other species. For instance, in the upper part of Cone A of *E. maximum* one of the strands belonging to a sporangiophore of the twelfth whorl died out in the cortex. So did one of the strands entering the cortex from a bifascicular sporangiophore of the second whorl of a cone of *E. sylvaticum* (Browne, 1921, p. 438). The B anomaly seems to be rather rare. Besides the cases recorded in the above table I have observed a case in which a vascular strand entering the cortex from a bifascicular sporangiophore of the sixth whorl of Cone F of *E. maximum* showed this peculiarity. The sporangiophore was, to judge from its size and form, single in nature, but peculiar in that its two vascular strands were vertically superposed, the upper one showing the B anomaly and the other, which originated about 200 μ lower down, the C anomaly. The B anomaly has also been observed in a cone of *E. limosum* (Browne, 1915, p. 248), and once in a cone of *E. sylvaticum* (Browne, 1921, p. 438).

It might be supposed that the A anomaly would be commoner than the B and C anomalies, since it shows a less great departure from the normal than these. I have, however, only observed it in *E. maximum*. In the case of Cone F there were, besides the seven cases in the lowest whorl included in the above table, seven other examples of the A anomaly. All were situated in the lower half of the cone, since they did not occur above the level of the seventh whorl, at which level there were three of them. In the five lowest whorls of Cone G—the portion of this cone studied for the present purpose—there were six strands of this type belonging to sporangiophores of the lowest whorl and one to a sporangiophore of the fourth whorl. It should be borne in mind that the A anomaly is much less obvious than either of the other two anomalies and requires careful and prolonged examination to distinguish it from the normal type. Discontinuity between the axial protoxylem and that entering the stele from a sporangiophore can only be established when the series of sections is complete and the preservation of the tissues good. In the case of the A anomalies described in this paper I was, however, able to satisfy myself of the real discontinuity between the axial protoxylem and that entering the stele from the sporangiophores. In view of the difficulties and lengthiness of the process of distinguishing those traces showing the A anomaly from normal traces, and in view also of the

essentially similar results obtained from a study of the lowest whorls of Cones A, B, F, and G of *E. maximum*, I did not attempt to make a detailed study of the attachment of the xylem of the traces to the protoxylem of the stele throughout the whole of Cones A, B, and G, although this was done for Cone F.

Before further considering these anomalous traces it may be well briefly to recall certain points as to the normal traces of the sporangiophores of *E. maximum*. As is usually the case in the genus the xylem of the traces of the sporangiophores is given off from the axial protoxylem. The traces of the lowest whorl, and to a less degree those of several of the succeeding whorls, are constantly deflected downwards as they pass outwards through the cortex. This deflexion is especially marked at maturity. In Cone B, which was mature, the average downward divergence of the traces of the lowest whorl was 997 μ, and in one case the extent of the downward deflexion reached 1,500 μ. A similar downward divergence, often varying considerably in extent, is characteristic of anomalous as well as of normal traces in the lower region of the cone.

As a result of the unequal downward deflexion of the traces in the cortex the difference of level between the traces at their insertion on the stele is often greater than the difference in level between the sporangiophores that they supply, and in some cases is sufficient to make it doubtful in examining reconstructions of the stele of the cone to which whorl certain traces belong. It is interesting to note that a similarly variable course is characteristic also of the protoxylem destined to the sporangiophore during its passage through the axial bundle. This feature is not shown in either of my reconstructions of the steles of Cones A and B of *E. maximum*, since they show the distribution of the xylem generally without indicating the course of the protoxylem (Browne, 1915, Pl. XII and Pl. XIII). The protoxylem destined to pass out as the xylem of a trace may become detached from a (relatively) main axial protoxylem strand at the height at which it passes out of the bundle or considerably above or below this level. For example, taking the group of protoxylem strands that pass out as portions of the traces 7–13 of the lowest whorl of Cone A (see Browne, 1915, Pl. XII), we find that the protoxylem supplying the seventh and eighth traces of the diagram is given off slightly above the point at which it makes its exit from the bundle. In other words, the tracheides of the trace are slightly deflected within the bundle. Those composing the protoxylem of the ninth and twelfth traces are more markedly deflected within the bundle, while those of the tenth and thirteenth traces pass outwards through the bundle in an upward direction, the former becoming free 750 μ and the latter 280 μ higher up. The course of the protoxylem of the eleventh trace is highly suggestive. This trace shows the A anomaly, for the protoxylem of the incoming trace does not unite with that of the axis. Its elements run

steeply upwards and inwards within the bundle until they are separated from the axial protoxylem by but a single parenchymatous cell. Their number then rapidly decreases and they soon die out, so that the connexion is not effected. One of the traces of the sixth whorl of Cone F of *E. maximum* was interesting because it appeared to be intermediate between the trace just described and normal traces. In it all the tracheides of the incoming trace died out in the metaxylem of the bundle, except one which joined on to the axial protoxylem. It should be borne in mind that whether the protoxylem of the traces of the lowest whorls of cones of *E. maximum* passes out more or less horizontally or upwards or downwards through the bundle, it is always deflected downwards in its passage through the cortex.

The extent to which the protoxylem of a trace may be deflected within the bundle is variable. In Cone B, in which the axis of the cone had elongated fully, the deflexion was sometimes as much as 420 μ. This was, of course, exclusive of the usually much greater deflexion of the trace in the cortex. Though on the whole greatest in the lowest whorls the deflexion within the bundle of the protoxylem that is about to depart is not confined to these whorls. Cases of a downward deflexion within the bundle of over 150 μ were observed to occur at the level of the fourth and fifth whorls of Cone G.

The cases in which the protoxylem destined to the trace passes upwards and outwards through the bundle seem to be examples of an early preparation for the departure of the trace. They are by no means confined to the region of the lowest or lower whorls. I have observed cases in which a small canal, left by the destruction of the protoxylem elements, separated from a relatively main protoxylem canal of the axis about 2 mm. below its departure from the bundle. So great a distance between the separation of the protoxylem of the trace and its departure from the bundle is very exceptional. Quite often, however, the distance is between 0·5 and 0·75 mm.

As the metaxylem was still undifferentiated at the time at which the tracheides of the trace were undergoing lignification, traces showing the A anomaly afford examples of a disjunction between the vascular system of the axis and that of some of the appendages. This isolation of appendicular protoxylem is to a large extent remedied later by the development of axial metaxylem round the incoming protoxylem. It should, however, be borne in mind that in this species the metaxylem remains separated from the more deeply seated protoxylem (Barratt, pp. 223-4; Browne, 1921, p. 447).

We may, I think, see in this separation of protoxylem and metaxylem in the cone of *E. maximum* a feature correlated with the presence in this species of the A anomaly. In other words, it is suggested that the inconvenient depth of the axial protoxylem in the lower part of the cone may have increased the number of cases in which the xylem of the sporangiophore fails to enter into connexion with the protoxylem of the axis. It is

true that separation of protoxylem and metaxylem, though less constant than in the cone of *E. maximum*, is characteristic also of the cone of *E. arvense*, in which the A anomaly has not been observed. But, apart from the inconspicuousness of the anomaly, which may have led to its being overlooked, the actual distance between metaxylem and protoxylem is markedly greater in *E. maximum* than in *E. arvense*, and is especially considerable in the lower part of the cone of the former species, in which region the A anomaly seems chiefly to occur.

At the same time, though the depth of the axial protoxylem may have led to an increase in the number of cases in which the protoxylem of the sporangiophore fails to enter into connexion with that of the axis, it is clear that the xylem of the incoming trace shows, in *E. maximum*, a tendency towards reduction in the inner part of its course. For in the second or B anomaly the tracheides of the trace do not even enter the bundle. This very obviously represents a further stage in the reduction of the protoxylem of the trace within the axis of the cone. In this type of trace the phloem has not suffered any reduction.

In the third or C type of anomaly—in the free trace—the reduction in the course of the trace in the axis is carried still farther. The phloem as well as the xylem ceases to be formed over a part of the course normally traversed by traces. In some cases the free strands of the sporangiophores approach very close to the stele ; in other cases they die out at the base of the sporangiophore without penetrating into the cortex.

I have examined twenty examples of vascular strands belonging to sporangiophores which failed to reach the axial stele, five in Cone A and fourteen in Cone F of *E. maximum*, and one belonging to a cone of *E. sylvaticum*. In two cases only, both in *E. maximum*, did the free bundle die out without entering the cortex. Of the eighteen other free strands some only died out when they had approached very close to the axial stele, and others died out almost as soon as they entered the cortex. A series could have been constructed showing traces dying out at every depth in the cortex. In *E. maximum* it is quite common for the individual tracheides of the free traces to become markedly wider (often about twice as wide) before they die out. Barratt has shown that in the cone of *E. palustre* the first tracheide of the trace to be differentiated abuts on the axial protoxylem and that the further differentiation of the tracheides proceeds outwards into the stalk of the sporangiophore (Barratt, p. 223 and Text-figure 19, p. 222). The enlargement of the tracheides before the dying out of the free trace suggests the possibility that, at least in the lowest whorl of the cone of *E. maximum*, the differentiation of the tracheides of the trace may begin in the distal part of the latter's course and proceed inwards. On the other hand, any marked increase in the width of the tracheides as we pass inwards is not a feature of the normal traces. In this connexion it may be pointed out that should

such a difference as that suggested above be found to exist it might, perhaps, be brought into relation with the fact that in the cones of *E. palustre* the downward deflexion of the traces of the sporangiophores in the cortex is insignificant even in mature cones and at the level of the lowest whorl; while in the cone of *E. maximum* a downward deflexion of the traces in the cortex is characteristic of the cone, at the level of the lowest whorl, even of very young specimens, and possibly exists even at the moment of lignification of the tracheides of these traces (Browne, 1915, p. 247).

Cone B of *E. maximum* possessed no free traces. Some, however, of the traces of the lowest whorl, e.g. Nos. 5, 36, and 40 of this whorl in my diagrammatic reconstruction of the stele (Browne, Pl. XIII, 1915), appeared, as they entered the axis from the sporangiophore, to be approximately twice as large as the average traces. These traces rapidly diminished in size by the dying out of numerous tracheides in the cortex, and before reaching the axial stele they are indistinguishable in size from the other traces. They might be held to show preparation within the cortex for an early division of the trace in the sporangiophore. Two facts, however, suggest that these traces show a certain tendency towards a reduction in the inner part of their course through the cortex, though the tendency is not strong enough to lead to the development of a free trace. In the first place, these traces do not divide unusually early within the sporangiophore; secondly, the elements that die out as the trace passes inwards through the cortex (or make their appearance as it passes outwards) are aggregated chiefly on one side of the strand.

The presence of free strands and of other anomalous traces is frequently associated with the existence of bifascicular sporangiophores, apparently single in nature, and still more often with the prevalence of complexes consisting of two or several more or less concrescent sporangiophores. In the only case in which a free strand was observed in *E. sylvaticum* it was one of two strands entering the axis from a sporangiophore clearly single in nature. In Cone A of *E. maximum* four out of five free strands formed part of the vascular supply either of a sporangiophore single in nature but possessing two distinct bundles, or of a double sporangiophore, i. e. a sporangiophore formed by the concrescence of two sporangiophores.[1] Only the last free trace in the diagram on the reader's right (cf. Browne, 1915, Pl. XII) represents the whole vascular supply of an ordinary monofascicular sporangiophore. In my first note on free traces, published in 1915, three out of four free strands found at the level of the lowest whorl of Cone A of *E. maximum* were regarded as belonging to sporangiophores single in nature. On a careful re-examination of the sporangiophores of this whorl, however, I have

[1] One of the free strands at the level of the lowest whorl is not shown in my reconstruction of the stele of Cone A of *E. maximum* (Browne, 1915, Pl. II), because it died out within the sporangiophore. This strand was situated between the fifth and sixth traces of the diagram.

come to the conclusion that two of these sporangiophores are double in nature and represent two concrescent members, while one is a single, unusually large, bifascicular sporangiophore.

The lowest whorl of Cone F of *E. maximum*, in which the free traces are more numerous than in any other whorl examined (fourteen out of thirty), shows an extraordinary degree of concresence among the sporangiophores. Only one of the free traces supplied a single, monofascicular sporangiophore. All the sporangiophores had very short stalks, so that their heads tended to be close together, a condition obviously favourable to concrescence. The stalks of the sporangiophores are often dilated at their insertion on the axis. This, coupled with the unusual shortness of their stalks, gives on superficial examination a fallacious impression that the sporangiophores are all slightly coherent basally. I have in the following paragraphs attempted to analyse the constitution of this whorl. The term complex is used throughout to denote a series of sporangiophores showing a considerable degree of concrescence. The presence in a complex of a given number of mutually independent vascular strands does not necessarily indicate that the complex is composed of a number of members equal to the strands. Bifascicular sporangiophores, single in nature, i. e. sporangiophores whose traces divide while still in the cortex, are common in *E. maximum*, and as such sporangiophores tend to be rather large they are inclined to be more closely approximated to their neighbours and more frequently fused with them. Thus bifascicular members are relatively common in complexes. On the other hand, there seems to be at least one possible case in which a more or less reduced or arrested member of a complex was devoid of a vascular strand (cf. under 10 of analysis). These two considerations introduce an element of difficulty, even of uncertainty, in analysing complexes. In the following section, however, I have indicated the only two cases in which I felt any doubt as to the number of constituents in a complex.

Analysis of the Lowest Whorl of Cone F of *E. maximum*.

1. A complex of four sporangiophores. This is supplied by a normal trace, two free traces, and another normal trace. This complex is locally concrescent (over the middle region of the stalk and part of the head) with:

2. A single, monofascicular sporangiophore. This sporangiophore is followed by:

3. A complex of two sporangiophores. The first and largest member contains two closely approximated vascular strands, one a free strand and the other showing the B anomaly. The second sporangiophore of the complex contains a free strand. This complex is followed by:

4. A complex of three sporangiophores. The first member possesses

a free trace, the middle and largest member two closely approximated strands, one free and the other showing the A anomaly. The third member also has a trace showing the A anomaly. This complex is followed by :

5. A still larger complex consisting possibly of three, more probably of four, almost completely fused sporangiophores. This is traversed, firstly, by a small, unbranched free strand, running only to the base of a single sporangium ; next to this strand is a trace showing the A anomaly, and it seems doubtful whether these two belong to an unusually large bifascicular sporangiophore, or whether the lobe containing the small free strand represents a partially aborted sporangiophore. Beyond the trace showing the A anomaly are two free traces, clearly belonging to different, though almost completely concrescent sporangiophores. The traces of this complex run at different levels, but there are, nevertheless, levels at which the whole wide complex appears to possess no vascular strands. Coherent with this complex by the concrescence of the larger part of the surface of the stalk is :

6. Another complex consisting of two sporangiophores. The first sporangiophore is provided with a free trace and the other with a trace showing the B anomaly. This complex is followed by :

7. Four free, single and monofascicular sporangiophores. One of these possesses a free trace, two possess traces showing the A anomaly, and one a trace showing the B anomaly. The last of these sporangiophores is followed by :

8. A bifascicular sporangiophore which, though rather large, is clearly single in nature. One of its strands shows the A anomaly and the other the B anomaly. This sporangiophore is followed by :

9. A complex of three sporangiophores. The first and third members of this complex are supplied by traces showing the A anomaly, while the vascular supply of the middle member is a free trace. This complex is followed by :

10. A large complex of three or four sporangiophores. The first member of this complex is rather large and possesses two closely approximated vascular strands, one a free strand and the other showing the B anomaly. The second and third members each possess a free trace, and beyond the third is a considerable projection devoid of vascular strands, which might conceivably be regarded as the rudiment of a fourth sporangiophore.

This completes the analysis of the whorl.

Theoretical Considerations.

In a previous paper it has been argued that the distribution of the metaxylem of the cone of *E. maximum* indicated that in this species the vascular system of the cone had undergone reduction (Browne, 1915, pp. 235–7). As has already been pointed out the A, B, and C anomalies of

the sporangiophoric traces, anomalies which are found especially frequently in *E. maximum*, indicate a certain reduction of the protoxylem system. It is not possible in the present publication to deal at all fully with the course of the strands of protoxylem in the axis. It may, however, be stated that a study of the course of this system reveals the occasional existence of protoxylem strands unconnected with the main axial system of protoxylem. Two such detached strands have already been recorded from the cone of *E. sylvaticum* (Browne, 1921, p. 454). Such cases can hardly have arisen except by the poor development of the protoxylem system. Below the insertion of the lowest whorls both of Cones A and F of *E. maximum* one of the protoxylem strands supplying a normal trace was found to terminate blindly 300–500 μ lower down. Possibly a detailed study of Cones A and B would yield other examples of protoxylem strands ending blindly in a downward direction, though no others were found in Cone F. In the lower region of Cone G—the only part studied—there were five axial strands of protoxylem with blind basal endings. In two cases strands of protoxylem which appear, as we pass upwards, to arise *de novo* in the metaxylem are so clearly in the line of continuation of the protoxylem that died out 300–500 μ lower down that it is difficult not to regard them as separated from the main system by a local failure of certain cells to develop as tracheides. In neither of these cases did the main protoxylem strand pass out as a trace at the level of the next whorl of sporangiophores. On the contrary, these protoxylem strands were still pursuing their upward course when the series of sections came to an end and both had given off traces to members of several whorls. In another case a strand of this sort, which also persisted upwards beyond the end of the series of sections, passed through the level of two whorls before giving off a trace. Below the lowest whorl of this cone is a free strand of protoxylem, about 300 μ long. The position of this strand is such that it has the appearance of being the continuation of one of the branches arising by a supra-annular forking of the protoxylem. The distance between it and one of these protoxylem branches is about 250 μ. These and other cases leave a very strong impression that the free axial strands of protoxylem do not represent an increase of protoxylem development, but arise rather by local failure of certain stelar cells to develop as tracheides.

Summary.

1. The occurrence in cones of *Equisetum maximum* of traces of which the protoxylem, though entering the vascular bundle, fails to reach the axial protoxylem and dies out in the metaxylem ; of other traces of which the phloem only enters into connexion with the corresponding tissues of the axis ; and of yet other traces which die out completely in the cortex with-

out coming into contact with the axial stele, seems to point to the existence of a progressive reduction of the vascular system. Since the xylem of the sporangiophores consists practically exclusively of protoxylem this reduction has chiefly affected the latter, though in some cases the phloem is also affected.

2. Evidence that a reduction of the protoxylem system has occurred is supplied by the occasional presence in the steles of the cones of *E. maximum* of strands of protoxylem ending blindly in a downward direction.

3. Though apparently commoner in cones of *E. maximum* than in those of other species the anomalies described above are not confined to this species.

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The Origin of Species by Large, rather than by Gradual, Change, and by Guppy's Method of Differentiation.

BY

J. C. WILLIS, M.A., Sc.D., F.R.S.,

European Correspondent of the Botanic Garden, Rio de Janeiro.

'May not the births of new species, like the deaths of old ones, be sudden.'—LYELL.

IN the days of Special Creation it was of course assumed that each species was created *de novo* in the form in which it was found to occur most widely upon the surface of the earth, while varieties were supposed to owe their origin to subsequent change. Towards the end of this period, however, it began to be recognized, e. g. by Lyell and Hooker (8, p. 702, and 6, p. xxv, quoted in 'Age and Area', p. 3), that all existing species were not created simultaneously, but that new ones, which would usually (of necessity) occupy less area, were from time to time appearing. Once this was fully recognized, it is clear that the way was open for the acceptance of some scheme of evolution, so soon as something feasible should be proposed.

The great difficulty that lay before the 'special creationists' was to explain why species were so obviously grouped by affinities (as they were called), a fact which was perceived at a very early date, and was the underlying motive of the attempts that were continually being made to group plants and animals into genera and families in what was called the 'natural' system [1] of classification. If species were specially created, each for its own place, there seemed no reason why they should be so evidently capable of arrangement into genera and other groups, and why these groups should usually occupy continuous areas. Nothing, evidently, could explain affinity but some scheme of evolution. If we imagine species showing affinity to have descended from some common ancestor, and therefore suppose that on the whole the affinities of species will be more and more pronounced the less

[1] 'A genus is called natural not because it exists in nature, but because it comprehends species more naturally resembling each other than they resemble anything else' (Lindley (7), p. xvi).

'ancestral' their common ancestor may be, we obtain a simple and satisfying explanation. The present existing complex assortment of species must be conceived as having been evolved from a preceding and probably simpler one.

So obvious an explanation was evolution that it had already been put forward in very early times, but no one had been able to suggest any way in which it might be supposed to produce its results; and without a feasible mechanism no one was ready to accept it as an explanation, for it was not realized that its acceptance would make any difference to the progress of science.

At this stage Charles Darwin came upon the scene as the man of the moment, putting forward the simple mechanism of Natural Selection, a principle which when once stated was seen to be of axiomatic nature, and a principle which caught, and has held, the public fancy.[1] It seemed clear that results might be produced by aid of this mechanism, and upon this ground evolution was rapidly accepted, for at that time the laws of heredity were not understood, and it was supposed that all change, due to whatever kind of variation, fluctuating or not, was fully inherited, a supposition that later work has shown to be ill-founded.

Evolution, once adopted, was found to explain so enormous a range of facts, and its acceptance pointed the way to so many new lines of research, that it rapidly attained an unassailable position, quite independent of any support that might be given to it by the theory of Natural Selection. For a long period, however, Evolution and Natural Selection were not sharply distinguished from one another, and anything that gave evidence in support of the former was also supposed to uphold the latter, though the inherent logical weakness of this position was often pointed out. For a long time the current of opinion in favour of 'Darwinism' was too strong to take account of any obstacles. I have lately looked over some anti-Darwinian books in the Cambridge University Library, and have been struck with the frequency of such observations as the following:

'It follows, therefore, that if we accept the Evolutionists' view, every specialised chemical compound met with in some living beings only must fulfil the condition, that every approximation to the complete compound[2] must have been of advantage to the being in which it was produced in the struggle for life . . . unless these very substances existed in, and formed points of difference between, Mr. Darwin's few original forms' (9, p. 134).

Small steps in the production of chlorophyll, for example, could not be of any advantage (9, p. 168). On p. 188 Maclaren asks why a plant should

[1] Because, it has been suggested to me by Mrs. Arber, each man is pleased to think that he is one of those picked out by selection.

[2] A thing which does not occur in nature, it is pointed out.

elaborate so deadly a poison as aconite, when a simpler one would do ; and on p. 194 he points out that change of climate does not change the chemistry of a plant, so that there is no opening for Natural Selection in a change of conditions.

Coe (2, Ch. II) gives a very good series of extracts from Darwin, Wallace, and others, showing how they contradict themselves, e.g. in the 'Origin of Species', pp. 65, 146, Natural Selection is said to be always acting, while on pp. 85, 169, it only acts at long intervals and under certain favourable circumstances. On p. 25 he points out that it only comes into operation in case of *adverse* changes ; it is not wanted if the change is favourable. Also the changes that bring it into operation must not be too rapid, or the organisms would perish, nor too mild, or they would not involve a question of life and death. Just the right amount is wanted. Natural Selection must wait for favourable variations to turn up, and they may not turn up in time. Geometrical increase of a species does not occur. As the numbers keep roughly constant, though four give say eight, these do not give sixteen ; the number is again reduced to four every time ; the eight do not survive to propagate.

The great bulk of the research carried on was based upon Natural Selection, inasmuch as it seemed to give a satisfactory explanation of the facts of adaptation. The idea of adaptation was pushed to absurd extremes, and adaptations were found—as indeed was necessary if Natural Selection, which was essentially a theory of adaptation, were to hold its place as an active factor in evolution—in almost everything that was conceivable with the greatest stretches of imagination. In spite of desperate effort, however, in which one might say that the imagination was stretched beyond the limits of perfect elasticity, no one ever succeeded in finding adaptation in the enormous bulk of the characters that divide one species or genus from another, whether in plants or in animals. And not only so, but a more thoughtful analysis of the cases of adaptation actually described showed that nearly all of them involved *correlated* adaptation. For example, the possession of tendrils as climbing organs was always accompanied by flexibility of the stem, which was no longer able to stand by itself. It was therefore necessary to suppose that selection, picking out the very doubtfully advantageous beginning of tendrils, picked out also the less doubtfully disadvantageous weak stems. This difficulty has always been a fatal weakness in the explanation of adaptation by selection.

This same correlation difficulty repeats itself over and over again in other cases, and is a very formidable one to whose explanation the theory of Natural Selection has been unable to give any clue. Climbing plants occur over and over again among closely related erect plants, whether in the same genus or in closely allied genera, so that it is clear that the climbing habit has been independently acquired in hundreds of cases, and therefore

that it must be easy of acquisition. It is obviously inconceivable that all can be descended from a common climbing ancestor. Now in the case of herbaceous climbers, such as are common in the north, it may be just possible to conceive that weakness of the stem was gradually acquired, for there is but little woody tissue in any case; but how is this supposition to be applied to tropical lianas with large woody but flexible stems? Is it conceivable that the stems of the trees and large shrubs which make up much of the genus *Bauhinia*, for instance, were gradually selected for weakness till the lianas were produced? Some of the climbing *Bauhinias* have tendrils, and some twine. Did the latter wait till the utterly useless and dangerously disadvantageous weak stems had been selected down to the point where they became flexible, before they began to evolve the twining habit by selection? Yet they could not begin to select the twining habit till the weak stems had been arrived at, and a weak stem, with no means of support, in a tropical forest would stand a very poor chance. And why do some species twine and some climb by tendrils? One has only properly to realize the enormous difference between the stem of a tree and that of a woody climber, and the entire lack of use-value in any *small* change in strength or rigidity, together with the hopeless position of a weak stem with no means of support, to realize at the same time the total absurdity of the 'explanation' provided by Natural Selection. It is difficult to have long experience, in the tropics at any rate, without being convinced of its utter and pathetic inadequacy as an explanation of the facts, as I was convinced about 1899, after three years in Ceylon, and have tried to make clear on numerous subsequent occasions.

Though previous to about 1890 criticism had little or no effect, the reverence in which Natural Selection was held has since diminished, more especially with the rise of biometrical measurements and statistics, which showed that the universally occurring fluctuating variations upon which Darwin mainly depended were not fully hereditary, but exhibited regression to such an extent that improvement by their means was not possible beyond a certain point. Another strong bar to its acceptance was the growth of Mendelism, which was quite incapable of explanation upon this theory.

At the same time de Vries was elaborating the theory of Mutation (3), according to which progress in evolution took place by fixed and hereditary changes that at times appear suddenly, in the form commonly known as sports; and it is with de Vries that the credit of the change from the Darwinian view of very gradual alteration really lies. Much subsequent work has shown reason to believe that his mutations in *Oenothera* do not rest upon sufficient evidence, regarded as mutations. This, however, is but a locking of the stable door after the emergence of the theory of Mutation, which has come out to stay. It is steadily gaining ground, and at the present time the older theory of progress by means of fluctuating variations

has probably very few adherents. Even its most strenuous supporters now usually begin with a mutation, though they may improve it afterwards by Natural Selection of fluctuating variation.

Though by education an enthusiastic adherent of this latter theory, I found so many difficulties that were insoluble, and so often found simpler explanations, that I soon became a convert to Mutation (10, 2nd ed., p. 118; 3rd ed., p. 208). It struck me at once, however, that there was a logical unsoundness in the new theory, for there was not the least evidence to suggest that a sudden change or mutation in one direction could be followed by another in the same direction, if the first were selected; yet this was necessary if new structures of marked difference were to rise by small mutations, whether with or without Natural Selection.

This supposition was largely based upon my investigation of the flora of Ritigala Mountain (12) in Ceylon in 1905, and in May 1907 (i. e. a year later than Guppy, who will be mentioned presently) I published a paper (13) dealing with some of the implications of the subject. This paper was chiefly concerned, however, with bringing up a trenchant argument against the natural selection of fluctuating or infinitesimal variations, an argument which has been used in the controversy on several occasions, and has never been refuted.

It was shown that in the enormous majority of cases no use could even be suggested for the characters that mark specific or generic distinction, in spite of the great amount of work on adaptation. Also that, even if some use-value might conceivably be found in rare cases for the mature character, it could not be supposed to exist for the early stages of the same. The case of the weak stems that always accompany tendrils, and of the tendrils themselves, which was mentioned above, affords an excellent illustration of the practical impossibility of intermediate stages. This case occurs in hundreds of different places in the system of the flowering plants, so that all cannot be supposed to have a common ancestor that acquired the climbing habit, and combination of tendrils with weak stem, once for all.

The case was considered of *Coleus elongatus*, Trimen. This is a species peculiar to the summit of Ritigala, where it is accompanied by other peculiar species within a very limited area of not more than five acres. It was shown that there was not the least reason to suppose it (or them) to be of the nature of survivals. Consequently, even if the characters were ever useful or the reverse, they must have been so upon Ritigala summit, and nowhere else. This fact rendered unavailable the refuge to which the supporters of adaptation fly in such cases of difficulty—that the characters must have been useful somewhere else. Their other refuge—that they must have been useful at some time—was also rendered very precarious by the fact that geological and other evidence seemed to show that the conditions upon Ritigala had remained little altered since the Tertiary.

It was, moreover, shown that no use-value could conceivably be put to the two most important characters by which this *Coleus* was distinguished from other species of the genus—the equally toothed calyx and the different type of inflorescence. Even in the minor characters of difference (cf. 'Age and Area', p. 152, or below, p. 620) it was very difficult, if not usually impossible, to point out any way in which they could be of service or disservice. It was then shown that in the case of the two chief, and of some of the minor characters, evolution from the characters of the other Colei, or from some intermediate type, by gradual variation, was impossible, on account of the impassable gaps in the transition, only to be passed by sudden and rather 'large' variation. In the case of the calyx, for example, all sepals behave alike in ordinary variation, and a calyx of one large and four small sepals could never vary gradually in the direction of one with five equal sepals.

Further than this, evolution by Natural Selection demands variation in the same direction in large numbers, to avoid intercrossing, and on the summit of Ritigala there is not sufficient room for four local species to have evolved in this way—a *Coleus* on rocky spots, a *Trichomanes* in shady places, a *Bulbophyllum* living epiphytically, and a *Cyperus* in open grassy places, to say nothing of the endemic varieties. Finally, the *Coleus* was accompanied at the summit by its most nearly related species, *C. barbatus* (common in tropical Asia and Africa), living in similar spots, and just as common. This latter species is, upon my view, the probable ancestor of the former.

Many similar cases were then brought up from the floras of Ceylon and other countries to show that endemic species as a general rule were separated from the 'wides' that accompanied them (and were usually closely related) by differences which could only be passed over by mutations, often 'large'. To make a few quotations: '*Ranunculus sagittifolius*, confined to the high mountain region about Nuwara Eliya, differs widely from the only other Ceylon buttercup, *R. Wallichianus* (South Indian also), which occurs side by side with it, though in drier and sunnier places, but is closely allied to *R. reniformis* of the mountains of the Western Indian Peninsula, differing mainly in the petals, which are five in the Ceylon species, 12 to 15 in the Indian one. . . . Are we to suppose the conditions of life so different in the Ceylon and Indian mountains that a five-petalled flower will suit the one, a twelve-petalled the other? Or how is the one to pass into the other, or both to arise from a common ancestor, except by discontinuous variation?' 'Can it be supposed that the simple obovate-lanceolate leaf of *Acrotrema intermedium* fits it for the Kitulgala district, while the pinnate leaf with linear-lanceolate segments of *A. Thwaitesii* fits that species for the Dolosbage district, but a few miles away, a trifle higher up, and in a similar climate? . . . *A. lyratum*, characterized by very long peduncles, is found only on the summit of Nillowekanda, an isolated precipitous rock . . . is it

to be supposed that the long peduncles are any advantage, or that the struggle for existence upon the summit of Nillowekanda is so keen that they can have evolved there by infinitesimal variation? . . . What advantage can the two ovules of *Polyalthia Moonii* and *P. persicifolia* be against the one of the other species? *P. rufescens*, another species with two ovules, and closely allied to both, occupies the Cochin district of South India, and why should there be three species in so similar a country, especially as the Ceylon species live in the same district? And how did the one form get to the other, or both arise from a common ancestor, except by mutation? . . . Similar queries might be asked 800 times for the 800 endemics comprised in the Ceylon flora. . . .'

The characters that differentiate allied species are as a general rule of no importance one way or the other, and cannot have been the subject of selection. Only in rare cases do they even allow of intermediate stages. The only possible explanation to my mind was that provided by the 'parent and child' theory, that parent and child might, and very often did, exist side by side. To quote my paper again (p. 14): 'The general principle on which India and Ceylon have been peopled with the many species which they contain would seem to be that one very common species has spread widely, and, so to speak, shed local endemic species at different points, or else[1] that one species has spread, changing at almost every point into a local endemic species, which has again changed on reaching new localities.'

This view of evolution by mutations which in one step—or perhaps but less probably in two or more—transform one species by divergent variation into another, without in any way necessitating the death or destruction of the parent form (so that parent and child might survive together), had been published a year previously by Guppy (4),[2] whose book I had not then seen. It was clear that if one much-localized species were derived directly from another (usually of wider range) the mutation must almost certainly be large, and as there was no reason to suppose a mutation in any given direction to be followed by another in the same direction, the change from one species to another must probably be due to one or a few mutations, each one in the latter case probably changing completely one or more characters. Correlation being very common, one would incline to expect several or all of the characters of a species to change at once. In *Coleus elongatus*, for example, it was clear that nothing but a big mutation could alter calyx or inflorescence, though it was not absolutely necessary that both should be altered by the same mutation.

We have now to go back and consider the work of Guppy (4), in this same direction. His views were also derived from the study of local

[1] Meaning 'in other cases'.

[2] Priority in the idea of large variation is not claimed for either Dr. Guppy or the writer; it dates at least from Geoffroy Saint-Hilaire; cf. also the writings of Owen, Mivart, and others.

endemic forms, this time in the Pacific Islands. Dr. Guppy noticed that there seemed to be three principal stages in the development of local endemism. In the first stage the island was occupied, so far as a given genus was concerned, by one or more widely-ranging species usually of very variable nature, such, for example, as *Metrosideros polymorpha*. In the second stage the wide-ranger was accompanied, in some of the islands at any rate, by local endemic species more or less closely allied to it, and in the third stage there were only local endemics. He therefore imagined, just as I had done in the case of India and Ceylon, that the wide-ranging species had given rise to the local forms, and that it might—or perhaps in any case did—ultimately disappear. The following quotations will suffice to indicate the point of view which Dr. Guppy took up in 1906:

'One where the extremely variable or polymorphous species plays a conspicuous part, as represented in such genera as *Alphitonia*, *Dodonaea*, *Metrosideros*, *Pisonia*, and *Wikstroemia*, the general principle being that each genus is at first represented by a widely ranging, very variable species, which ultimately ceases to wander and settles down, and becomes the parent of different sets of species in the same groups' (4, p. 519).

'The rôle of the polymorphous species belongs alike to the plant and to the bird. A species that covers the range of a genus varies at first in every region, and ultimately gives birth to new species in some parts of its range. Then the wide-ranging species disappears, and the original area is divided up into a number of smaller areas, each with its own group of species' (p. 522).

Dr. Guppy was so kind as to give me a few notes upon his theory of Differentiation, from which I extract the portions shown in quotation marks. The theory of Differentiation involves the idea of variation that was always held, for example, by Hooker and Huxley, and which they pressed upon Darwin,to whom it was always a stumbling-block—that it involved a tendency to divergence (cf. Guppy in 'Age and Area', pp. 104–5).

'The same conception of divergent variation is attributed to Goethe by Geddes in his article on Variation in vol. 24 of the 9th edition of the "Encyclopaedia Britannica", p. 77. The German philosopher held a view which included, besides the centripetal force of heredity, that of a progressive or centrifugal tendency to adaptation to environment. . . . But there were other eminent investigators who seem to have got into the differentiation stride as soon as they tackled the subjects of variation and distribution. This is indicated in the "loi primordiale" of A. de Candolle in his "Géographie Botanique', ii, p. 1338, where the secondary modifications of the great plant groups are attributed to variations in conditions produced in the course of geological ages. Huxley, when he handled the gentians, got into the same stride, and in his letters to Hooker in Sept. 1886, where he characterizes

the primary group of these plants as the least differentiated, he alludes to the strange general parallelism in the northern hemisphere between the gentians and the crayfishes. It is the case of the tapirs over again, he also adds.'

'If we take the history of distribution of such families as the Menispermaceae and the Monimiaceae [cf. 'Age and Area', p. 172] we have the story of the dissociation of two family types into tribes, genera, species, and varieties, and as the parent type differentiates into tribes, and the tribes into genera, and the genera into species, varieties, and races, a subsidiary principle which has been termed "Rank and Range" comes into view. Here range goes with systematic rank, and since age goes with it as well, we have the Age and Area principle exemplified.

'But the whole story of the distribution of the flowering plants since their appearance in secondary times has been a story of differentiation of types and the dissociation of floras. From the migration standpoint this has long been studied by Mrs. Reid. But what I am concerned with here is the dissociation of the mixed floras of the Eocene ages, the elements of which are now separated in different climatic zones, and the breaking up of the synthetic pro-angiosperm types of the Cretaceous ages, types that combined characters that are now far apart both in a systematic and in a geographical sense. The phrase of "the age of palms and poplars" has been applied to the mixed Eocene floras, but the same forces of differentiation that in recent ages have placed palms and poplars in different climatic zones previously broke up the comprehensive pro-angiosperm types of the Cretaceous period, types that ushered in the appearance of the Dicotyledons, which now present the characters of the primary parent types in a dozen different families systematically and geographically separated from one another. The dissociation of mixed floras, and the differentiation of synthetic or comprehensive types, have worked together to bring about the present system of plant distribution.

'It is noteworthy that Weismann, after supplying the machinery for the building up of types, provided the machinery for breaking them up. Concomitant variation of many individuals in the same direction and under the same stimulus of changing environment furnished the type which under the influence of varying conditions would break down into lesser groups.

'After all, differentiation comes nearer home to us than the Darwinian machinery of evolution. It has influenced in the past, and is still in the present influencing, our amusements, our arts and sciences, and even our creeds. It is a more comfortable doctrine than that involved in the belief in the ruthless struggle of existence that is based on the triumph of the strong and the crushing of the weak.'

To return to my own work, in a later paper (15) I worked at the problem of the distribution of the Dilleniaceae, upon the assumption that mutation was the true explanation of evolution. I showed that there was a general tendency in the larger genera, here as in other families, for there to be one or two widely ranging species, accompanied at various spots by local endemics; and I pointed out that the easiest explanation was to suppose the former to *shed* the latter. On this supposition, it was clear that

there was no necessary reason why the whole tree of the descent of a family (descent and formation of its genera) should not actually exist upon the earth at the present time, and it was suggested that all the Dilleniaceae might be descended (directly, or indirectly through other genera) from *Tetracera*, the most widespread and probably the oldest genus.

In a later paper (16) it was pointed out that the fact that *Eugenia*, *Hedyotis*, and other genera were represented in Ceylon by different species upon different hill-tops was one which offered great difficulties to the theory of Natural Selection, and that 'it is more than doubtful if any given species is specially adapted to the exact local conditions in which it is found', other than is necessary from the fact that if not suited to them it will soon die out.

In (17) I devoted more attention to this subject, and suggested as a hypothesis 'that no specific change is too great to appear in one mutation', It was also pointed out that there was no reason to stop at the species, but that it was just as likely that larger units might arise at one step. This position was further elaborated in 'Age and Area', pp. 215–21.

It will be well to make clear at this point that up to now we have had only two suppositions about the origin of species that have been seriously accepted by the great majority of people, whether biologists or not. These are:

(*a*) Special Creation: species *created* showing the differences (commonly *large*) that actually exist, and that are usually discontinuous.

(*b*) Natural Selection: species *evolved* by the *gradual* selection of *small* differences between individuals.

Now, as usual, there appear to be portions of the truth in both, and to the latter we owe the enormous advance involved in the acceptance of evolution, an advance which could perhaps have come about in no other way so easily as in one that caught the popular fancy, as did Natural Selection. It is clear that differences between species must arise in one or the other way—gradually or suddenly. But (sudden) creation of them puts a barrier to further investigation, which is obviously unnatural in view of the affinities that exist, and evolution was bound to succeed it. The theory of Natural Selection *assumes* the *accumulation* of gradual change, and we have no evidence that such change can be accumulated. What is proposed here, and has been proposed for the last sixteen years, is a compromise between the two views above mentioned. And nothing but some compromise is possible, if one refuse to accept either as a whole, for change must be sudden or gradual, and there is now no possible doubt that evolution (or change) has gone on. There is little or no evidence for the accumulation of variations in one direction, or even for their occurrence except as up-and-down variation in respect of size, &c. There can be no doubt, after the

complete failure of the attempts to read adaptive values into the enormous majority of the diagnostic characters of living beings, that there is only rarely any handle for the operation of selection. Personally, I am inclined to think that change was generally effected by a single operation, but every compromise between the extremes is possible. But in view of the work done by Mr. Udny Yule and myself (19), showing that genera in their evolution follow very closely the rule of compound interest, it seems enormously more probable that the changes were single steps.

The current attitude of the Mendelians towards questions of evolution is one of an aggressive agnosticism. Since investigations upon Mendelian lines have not as yet been able to throw as much light upon the problem as had been at one time expected, they seem to think that no other line of attack upon the question will be any more likely to find a way that may possibly lead to something in the nature of a solution of the problem at some future date. They seem inclined to think that because they have not themselves seen a 'large' mutation, such a thing cannot be possible. But such a mutation need only be an event of the most extraordinary rarity to provide the world with all the species that it has ever contained. As I have pointed out ('Age and Area', p. 212), one large and viable mutation upon any area of a few square yards of the surface of the earth, and once in perhaps fifty years, would probably suffice.[1] The chance of seeing such a mutation occur is practically *nil*, whilst if the result were subsequently found it would probably be called a relic. Darwin's theory of Natural Selection has never had any proof except from *a priori* considerations, yet has been universally accepted, and has led to great advances in biology; and until the Mendelians show us how to control mutation (a thing that will evidently be some day possible), the proposition now put forward will presumably go without actual demonstration by verified fact. What I contend is that the facts brought up here and elsewhere go to show that neither of the extreme suppositions—Special Creation and Natural Selection—contains all the truth, and that therefore this, or some similar, compromise between them is rendered necessary by the present condition of our knowledge.

The small mutations that are all that the Mendelian school will allow are obviously in the highest degree unlikely to give rise to mutual intersterility, such as so commonly characterizes specific difference, and if they were to be accumulated it is difficult to see where the sterility would come in, for each would seem as likely to be fertile with its successor as with its predecessor—*A* with *B*, *B* with *C*, *C* with *D*, and so on. But let a big step, say from *A* to *M*, such as dropping of endosperm, be taken,

[1] Dr. Guppy has suggested that it is by no means unlikely that the many species once seen and never afterwards discoverable may often be such mutations. The case of *Christisonia albida*, described in Age and Area, p. 151, is almost certainly a case of a non-viable mutation, and it may be noted that Hooker, who was not a 'splitter', accepted it as a Linnean species.

and one would feel inclined to expect mutual intersterility as a matter of course.

If so large a difference as having, or not having, endosperm, rumination of endosperm, few or indefinite stamens, &c., &c., can occur, as it does occur, over and over again between genera which are obviously closely allied, we are evidently simply making difficulties for ourselves by supposing such differences to be gradually acquired. It must never be forgotten that gradual acquisition is *an assumption* of the theory of Natural Selection. Whether the differences were infinitesimal (or due to the universally occurring fluctuating variation), or whether they were more of the nature of sports, they were never supposed by Darwin and his followers to be anything but *small*, and evolution of new species was by their *accumulation*, whilst the larger groups were due to further accumulation and to destruction of the intermediate forms. Now the work which has been done to establish the theory of Age and Area goes to show that destruction of intermediates can no longer be invoked. There has been vast destruction of individuals, and probably of species which were only represented by a few individuals, but not of intermediates, unless these species which were destroyed were of intermediate type; and in that case it is difficult to see how they could give rise to the later and more successful forms. Even in the earliest known geological horizons that contain the group there can be recognized many families of flowering plants that exist to-day, and that cover a very large part of the systematic range at present existing. They are as well and as widely separated as those now existing, and into families that now exist, and if these gaps were due to destruction, then Natural Selection must have operated with great rapidity and decision in the earlier ages of the flowering plants. If the earliest known flowering plants already show such differences as that between Monocotyledons and Dicotyledons, then evolution upon the Darwinian plan must have been going on previously (*in flowering plants*) for an enormously longer period than has since elapsed, or selection and destruction must have been much more rapid.

The view that destruction of intermediate types was chiefly responsible for the differences between families might be more easily upheld were it not for the fact that one may find just as great differences occurring between closely allied genera, or even between species of the same genus, in which cases the time available, upon the Darwinian theory, must have been much less, and much less destruction was possible. Thus, for example, number of stamens is often a character of much importance in classification, yet, to take the first example that comes to hand, Lecythidaceae are marked by ∞ stamens, while the next family, Rhizophoraceae, show any number from 8 upwards to ∞ (and cf. below). Taking up Mr. Ridley's 'Flora of the Malay Peninsula', it opened at p. 380, and the first genus noticed was *Trigonochlamys*, endemic to the region, with two species. *T. Griffithii*, with 6 stamens,

occurs from Singapore to Pahang and Perak, or say along 300 miles of the peninsula, while *T. grandifolia*, with 3 stamens (a later and higher type of flower, by current acceptance), occurs upon Bukit Timah, a little hill in Singapore Island. Here was evidently a large mutation, for no destruction can be invoked to fill up a numerical gap like this; and the later-formed species is much more local than the other.

The fact that characters appear in a sporadic way in unexpected places, and in different places in families, is so well known that it has long been an axiom in taxonomic work that the use of single characters to differentiate species, genera, or families will inevitably give an artificial grouping, like the famous sexual system of Linnaeus. Even in this system, it was not practicable to keep the characters intact and still to classify all the species of any genus into that genus. From Lindley (7, pp. xiv, xvii) I take the following interesting remarks about the sexual system:

'Even the sexual system of Linnaeus could not be drawn up without splitting genera, if one desired complete agreement. Smith gives 173 genera of the British flora, and no less than 43 of these, and some in every class, contain species at variance with the characters of the classes and orders.

'It is a maxim of the Linnean school that the parts of fructification should be employed in characterizing classes, orders, and genera, to the exclusion of all modifications of the leaves or stem. This, although theoretically insisted upon, was practically abandoned by Linnaeus himself, and is to be received with great caution. The organs of fructification are only entitled to a superior degree of consideration when *found by experience* [1] to be less liable to variation than those of vegetation.'

We have now to go on to consider very briefly some of the more direct evidence in favour of 'large' mutations. An enormous amount of such evidence is available, thanks to the labours of generations of systematists, botanical and zoological; and it is proposed to give more, if necessary, in future papers.

What principle, if any, has governed the formation of the characters that divide one species, genus, or family from another has long been a great puzzle. The one general rule, *which has very many exceptions*, seems to be that as one goes higher up the scale from species to family the characters of the vegetative organs tend to be of less and less value as against those of the reproductive organs. This is usually explained as owing to the fact that the latter are less concerned with the performance of the ordinary daily functions of life. But though an everyday statement in teaching, this requires some qualification. In practice one finds that sometimes one, and sometimes another, character runs through a family, and it is only *experience*, as Lindley says, that can decide what are the most useful characters in any

[1] Italics mine.—J. C. W.

given case. If one were to decide that characters of embryo were the most important, and start upon the classification of the Orchidaceae, one would soon be in difficulty. One cannot take single characters, nor can one be sure, till one has actually worked with a family, what characters will prove to be of most importance in that family. Grasses, sedges, palms, duckweeds, aroids, rushes, gingers, bananas, buckwheats, water-lilies, pitcher-plants, sundews, begonias, &c., &c., are recognized by their leaves, others by the stem, by leaf-veining, or by other vegetative characters. One may find a character in one place a most important family diagnostic, like rumination of the endosperm, which is the only certain character of distinction between Anonaceae and Magnoliaceae; in another family, as in palms, it may occur over and over again in pairs of closely allied genera, one having it, the other not; whilst in some palms, like *Euterpe*, it may occur in some species and not in others. Asclepiadaceae can only be divided with certainty from some Apocynaceae by the occurrence of translators to the pollinia—what one would have thought, *a priori*, a character of trifling importance. There is almost no character of a family or genus that may not at times be generic or specific. So good an account of this fact, which is so well known that people have ceased to think about it, is given by Lindley (7, p. xxi) that it is worth quoting at some length:

'All Rubiaceae have opposite entire leaves . . . but in . . . *Fuchsia*, in which they are usually opposite, species exist in which they are not only alternate, but both one and the other upon the same plant . . . in Combretaceae and Leguminosae, orders usually having alternate leaves, they are occasionally opposite . . . in Aceraceae, Aurantiaceae, Geraniaceae, Rutaceae, and Sapindaceae, both simple and compound leaves are found. . . . Myrtaceae are distinguished by these glands (pellucid dots in the leaves) from Melastomaceae . . . in . . . Phytolaccaceae, Labiatae, &c., there are, however, genera with and without pellucid dots . . . The orders Cistaceae, Saxifragaceae, and Loganiaceae are among the . . . cases in which genera exist both with and without stipules.

'The number of sepals is sometimes a character of importance, as in Cruciferae, in which they are always 4 . . . in Malvaceae they are 3–4–5, in Guttiferae from 2 to 6 . . . Malvaceae have the calyx exclusively valvate . . . but in Penaeaceae both valvate and imbricate aestivation exists . . . it frequently happens that both regular and irregular calices coexist in the same order, as in Rosaceae, Labiatae . . . in Melastomaceae all degrees of cohesion take place between the calyx and the ovarium, and in Saxifragaceae this uncertainty of structure is still more remarkable.

'If the corolla is present, a plant is said to be dichlamydeous, and much importance is attached to this peculiarity; far more, I think, than it deserves. It constantly separates plants having much natural affinity . . . in the polypetalous orders of Crassulaceae, &c., &c., there are many monopetalous genera . . . Compositae are essentially distinguished by their valvate aestivation . . . an exception existing in the genus *Leptadenia* . . . *Echium* in Boraginaceae is irregular.

'Vitaceae, Gramineae, Cyperaceae . . . contain hermaphrodite and diclinous genera and . . . flowers of all kinds stand side by side in the Compositae.

'*Eschscholtzia* has decidedly perigynous stamens, and yet it is . . . a genus of Papaveraceae, the character of which is to have them hypogynous; and all kinds of gradations . . . are observable in Saxifragaceae . . . the stamens are . . . monadelphous in Malvaceae . . . but more commonly this character is unimportant, as in Malvaceae themselves, which have sometimes distinct stamens . . . in Solanaceae, the genera of which have usually their anthers bursting longitudinally, the genus *Solanum* itself opens by pores.

'Cases exist of both forms (apo- and syn-carpous ovary) being found in the same natural order . . . in Caryophyllaceae and Bruniaceae there are genera, the ovary of which contains several cells . . . in Pedaliaceae and Styraceae both erect and suspended ovules coexist . . . the genus *Conohoria* (*Alsodeia*) offers . . . an instance of three kinds of direction in as many species.

'Marcgraviaceae, Melastomaceae, Myrtaceae, Ranunculaceae, and Rosaceae, &c. . . . contain both baccate and capsular, dehiscent and indehiscent genera.

'I doubt very much whether presence or absence of albumen deserves much attention in orders . . . where the embryo and albumen are of nearly equal bulk . . . among Apocynaceae, which have solid albumen, it is ruminate in *Alyxia* . . . There are plants among Dicotyledons with only one cotyledon, as *Penaea* and some Myrtaceae, or several . . .'

The diagnostic characters of flowering plants, as regards their classificatory value, are often supposed to rank pretty much in the morphological order, embryo, seed, fruit, gynoecium, androecium, corolla, calyx, inflorescence, bracts, leaves, stem, and root. A glance at the characters that divide Monocotyledons from Dicotyledons is sufficient to show that this is only true, if at all, in a very general way, and this impression is soon confirmed if one take up the work of monographing any particular group. *Nothing but experience* can decide which is the most important character, or the most useful in classifying any family into its genera, &c. In the Lemnaceae, for example, the genera are divided by characters of the root; in the Cruciferae the hairs, in the Acanthaceae the pollen-patterns, in the Umbelliferae the ridges on the fruit, and the oil-passages in the walls of the fruit, are of the greatest possible importance in defining genera: and so on. The experience must be freshly gained for each family, and the characters that prove *by experience, in that family*, to be the most widespread and constant, and to differ in the most distinct way between genera that are recognized as such from experience of all their characters, and from continuous distribution, will be the most important in that family. If one were to sort out all characters in terms of the greatest frequency with which they proved to be of importance, one would be quite likely to find that the order above mentioned was adhered to in a general way, but that is all that can be said.

The essential characters that distinguish one form of specific or higher rank from another are rarely characters of mere size of organs, which might, had they any use-value, vary gradually. More usually they are distinct characters, with no use-value conceivable even in the mature stages, so that it is all but impossible to imagine them going gradually from one to another. As an illustration let us take the characters distinguishing the two Colei often mentioned :

	Coleus barbatus ('Bot. Mag.', t. 2318).	*C. elongatus* (Trimen's 'Ceylon Flora', t. 74).
1.	Stem cylindrical, tending to quadrangular in inflorescence.	Stem quadrangular.
2.	Stem pubescent with long hair.	Stem pubescent with short hair.
3.	Leaves oblong-oval, 1–2 in.	Leaves ovate-triangular, 1–2 in.
4.	Leaves closely pubescent.	Leaves finely pubescent.
5.	Leaves rather thick.	Leaves rather thin.
6.	Petioles rather short.	Petioles longer and slenderer.
7.	Inflorescence of condensed cymes, each about 5-flowered, forming false whorls of 10 flowers at each node.	Inflorescence of one-sided cymes, looking like racemes, about 1½ in. long, one at each side of each node.
8.	Flowers large.	Flowers small.
9.	Bracts large.	Bracts small.
10.	Calyx with long hairs.	Calyx with short hairs.
11.	Calyx of one large ovate upper tooth and four small lower.	Calyx of five almost exactly equal teeth.
12.	Corolla rich purple or white.	Corolla pale purple.
13.	Grows on rocky places.	Trails over rocks.

Now, looking over these characters, it is at once clear that probably not even an enthusiastic 'splitter' would separate these two forms as more than varieties, if the differences in the characters 7 and 11 were not present. These differences, however, are so marked that the two plants are almost subgenerically separate. Intermediate stages, with gradual change, are conceivable in the case of characters 1, 2, 3, 5, 6, 8, 9, 10, and 12, but except in the case of 8 and 12, where *C. barbatus* has a larger and more brightly coloured flower, and so might be imagined more attractive to insects, no use-value can conceivably be put to them. But in the case of the two essential characters 7 and 11, not only is there no conceivable use-value, but it is impossible to have intermediate stages, were there even any reason why one should have them. The one could not vary gradually into the other. Nothing but 'large' mutation can explain the difference.

Whatever character one may take, one will find it to be sometimes constant throughout a family, sometimes appearing sporadically in one or more genera, sometimes even only in one or more species of a genus and not in the others. Its value, then, according to circumstances, may be family,

generic or specific. All this has been so long well known that it has become a truism, which people have accepted as an inexplicable fact, and ceased to think about. And, as in the case of other truisms, it is worth further investigation.

Thus, for example, in many families the fact that the ovary is superior or inferior is a character of the highest value, constant throughout the family, yet in at least 32 families, viz.:

Amaryllidaceae	Cunoniaceae	Lecythidaceae	Rhizophoraceae
Anacardiaceae	Ericaceae	Liliaceae	Rosaceae
Araliaceae	Flacourtiaceae	Loasaceae	Rubiaceae
Aristolochiaceae	Gesneriaceae	Melastomaceae	Santalaceae
Bromeliaceae	Goodeniaceae	Moraceae	Saururaceae
Bruniaceae	Haemodoraceae	Nymphaeaceae	Saxifragaceae
Campanulaceae	Hamamelidaceae	Pedaliaceae	Styracaceae
Chloranthaceae	Lauraceae	Phytolaccaceae	Vochysiaceae

there are exceptions to the rule, plants occurring with inferior ovaries in families that in general have superior, or vice versa. And in a few genera like *Saxifraga* one may find some species with superior, some with inferior ovary.

It is at once noticeable in this list that these families are in general *large* families, in which one would be inclined to expect, upon the Darwinian theory, more uniformity with respect to such a character. If it was settled by the early ancestors of Amaryllidaceae that an inferior ovary was the best, why did *Lophiola* in Atlantic North America, and *Tribonanthes* in South-west Australia, adopt a superior ovary? And one may ask such questions over and over again.

Or take, again, the presence or absence of endosperm, usually considered as one of the most important characters of all. It varies in at least the following 42 families:

Acanthaceae	Flacourtiaceae	Ochnaceae	Sterculiaceae
Anacardiaceae	Gesneriaceae	Oleaceae	Tamaricaceae
Apocynaceae	Gramineae	Onagraceae	Thymelaeaceae
Araceae	Icacinaceae	Plumbaginaceae	Tiliaceae
Cactaceae	Labiatae	Polygalaceae	Ulmaceae
Celastraceae	Leguminosae	Rhamnaceae	Urticaceae
Chenopodiaceae	Loasaceae	Rhizophoraceae	Verbenaceae
Connaraceae	Meliaceae	Rosaceae	Violaceae
Crassulaceae	Menispermaceae	Rubiaceae	Zygophyllaceae
Elatinaceae	Moraceae	Rutaceae	
Erythroxylaceae	Nymphaeaceae	Sapotaceae	

Again these are, it will be observed, large and well-known families, with few exceptions. And in such genera as *Erythroxylum* one may find species placed side by side by the most recent taxonomists, one possessing endosperm, the other not.

But space will not permit of giving long lists of families and genera that illustrate what we have said about the well-known (but little considered) fact that a character may at times be family, at other times only generic or specific. Whether leaves are alternate or opposite is a character that is frequently of great family or generic importance, yet in at least 87 families, and in numerous genera, both may be found. Intermediates are not possible, and neither arrangement has any use-value as against the other.

When the character is of great systematic importance it has in general no conceivable use-value as against the contrasted character, and cannot have been the subject of Natural Selection. It is impossible to conceive that it can matter in the struggle for existence whether the corolla is valvate or imbricate, the leaves alternate or opposite, the endosperm ruminate or equable, and so on, or whether, to take even more widespread characters, the embryo is mono- or di-cotyledonous, or the leaves net- or parallel-veined. As Guppy says ('Age and Area', p. 102), the Darwinian theory 'implies that the simpler, least mutable, and least adaptive characters that distinguish the great families are the last developed. This could never have been.' Further than this, they are not capable of change from one to the other, whether by gradual variation or by destruction of intermediates, or both. One cannot conceive of alternate leaves becoming gradually opposite, nor in fact does one find any fossil relics showing intermediate stages. The same may be said of the various aestivations of the corolla, of trimery, of porous opening of anthers, number and arrangement of the ovules, &c., &c. It is impossible to conceive, as I have maintained for twenty years, that Natural Selection can have produced such characters, and to argue that they are correlated with important characters due to Natural Selection is simply to invoke incomprehensibility, as did the special creationists. These latter characters cannot be external, or they would have been noticed and utilized by the taxonomists, and to argue that they are all internal is to ask too much of credulity; nor does it get over the chemical difficulty which was pointed out so long ago as 1877 (p. 606). If internal characters proceed by gradual selection, why does their external manifestation go in jumps, as must of necessity be the case with many of the most important characters? What intermediate types are conceivable between porous, valvular, longitudinal, and transverse opening of anthers—all at times family characters?

Or take 'smaller' variations, i. e. variations which we do not know to be in reality any smaller, but variations which experience has proved to be only available as specific or generic. In *Abrus* (Leguminosae, Papilionatae-Vicieae), in *Adenanthera* (do. Mimosoideae-Adenanthereae), in *Ormosia* (do. Papilionatae-Sophoreae), and in *Rhynchosia* (do. Papilionatae-Phaseoleae) some of the species have seeds which are sharply divided into a red end and a black end by a difference in the colour of the testa, which is

vividly red at one end and as vividly black at the other, the division following a perfectly straight line across the seed. Other species have simple bright red seeds. Incidentally, this is as good a case of 'mimicry' as those described in animals, and was at one time seriously advanced as such. No use-value can be suggested, and no intermediates are possible, nor is it conceivable that Natural Selection would work to such minuteness of detail as to divide the two colours by an exact straight line in all cases. There is nothing for it but to admit that it must have been acquired at one step. And the same step must have been taken by several different plants, for the four genera do not belong to the same division of Leguminosae, and one of them does not even belong to the Papilionatae.

We may proceed by giving one instance in some detail of the way in which a character that in one place is of family rank may in another become merely generic or specific. Whether the endosperm is or is not ruminate is the only general character of distinction between Anonaceae and Magnoliaceae, yet in *Alyxia* (Apocynaceae), in *Aralidium* and other genera (Araliaceae), in *Polysphaeria* (Rubiaceae), in *Tinospora* and others (Menispermaceae) rumination appears as merely a generic character, whilst in the palms, to give full details, it appears in the left-hand genus of each of the following pairs of genera that are placed side by side in the classification (numbers from Drude's classification in 'Die natürlichen Pflanzenfamilien', 1889):

Ruminate.	Not ruminate.
2. *Chamaerops* (sub-fam. I. 2)	3. *Trachycarpus*
20. *Copernicia* (I. 2)	19. *Screnaea*
23. *Medemia* (II. 1)	24. *Hyphaene*
30. *Raphia* (III. 2)	31. *Oncocalamus*
42. *Caryota* (IV. 1 a)	43. *Arenga*
58. *Catoblastus* (IV. 1 c)	57. *Iriartea*
69. *Reinhardtia* (IV. 1 d)	68. *Synechanthus*
73. *Prestoea* (IV. 1 e)	72. *Hyospathe*
78. *Iguanura* (do.)	77. *Linospadix*
82. *Hetcrospathe* (do.)	81. *Clinostigma*
87. *Phoenicophorium* (do.)	88. *Deckenia*
90. *Oncosperma* (do.)	89. *Acanthophoenix*
93. *Ptychandra* (do.)	94. *Cyphokentia*
101. *Ptychococcus* (do.)	100. *Cyrtostachys*
109. *Nenga*, p.p. (do.)	110. *Cyphophoenix*

It is impossible to escape the conclusion that all or most of these fifteen genera on the left must have acquired rumination independently. They belong to nearly every group of palms, and agree closely in other characters, each with the one opposite to it. If we suppose them to have descended from a pair of ancestors that agreed to separate on the question of endosperm, we must explain why they also disagree in other characters of

great importance, such as the number of the stamens, which varies from six to a very large number. Rumination has no use-value as against non-rumination, and there are no intermediates conceivable that would have any value. Either the same character has been acquired over and over again, or genera are not units of descent, but are polyphyletic, as has often been suggested (cf. 11, p. 446; 20; or 1, p. 119).[1] This latter supposition, however, does not seem to cover more than a very small proportion of the cases, and we are driven to the conclusion that the same indifferent, but systematically very important, character, which rarely admits of intermediate stages, and which therefore cannot have been acquired gradually or by selection, has been acquired over and over again. Nothing but sudden acquisition, or in other words 'large' mutation, seems capable of explaining this phenomenon, which is familiar to every worker in taxonomy.

But the acquisition of ruminate endosperm is not even a question of acquisition by one genus only and not by another. In the four following palms part of the genus shows it and part does not, so that it becomes merely a specific character, and it is clear that one species may acquire it and another not :

Ruminate.	Non-ruminate.
Euterpe oleracea	*Euterpe*, other spp.
Oenocarpus, § II	*Oenocarpus*, § III
Hydriastele, p.p.	*Hydriastele*, p.p.
Nenga, §§ I, II, III	*Nenga*, §§ IV, V

Or one may take evidence of a different kind by taking a single family and considering its range of variation. Take for example the Rubiaceae. None of the important characters of the family run unchanged throughout it, and one finds, for instance (the contrasted character is the usual one in the family) :

alternate leaves in *Didymochlamys* ;
whorled leaves in *Fadogia* ;
pinnate leaves in *Pentagonia* ;
gland-dotted leaves in *Rustia* ;
intrapetiolar stipules in many ;
leafy stipules in Galieae ;
dioecious flowers in *Kotchubaea* ;
zygomorphic flowers in *Capirona* ;
solitary axillary flowers in many ;
male and female inflorescences often very different ;
male and female flowers so different in *Melanopsidium* that they were formerly described as different genera ;
flowers united in pairs in *Morinda*, &c.
male flower 4–5-merous, female 8-merous, in *Thiicleodoxa* ;

[1] Sir N. Yermoloff (20), in his studies of the Diatomaceous genus *Navicula*, appeals to a principle of integration rather than to the differentiation of types, i. e. to the building up rather than to the breaking down of types.

calyx large and convolute in *Dictyandra*, large and imbricate in *Keenania*, breaking open irregularly in *Pelagodendron*, with calyculus in *Rctiniphyllum*, with one large sepal in *Mussaenda*, &c., 5-merous in ☿ and 2-merous in ♀ in *Phyllis*;
aestivation descending in corolla of *Posoqueria* and *Molopanthera*;
stamens united in *Capirona*, *Bikkia*, *Chiococcus*, unequal in *Hippotis*, 8–12 in *Praravinia*, opening by pores in *Rustia*, by valves in *Tresanthera*, two, with 5-merous corolla in *Sylvianthus*, &c.; anther multilocular in *Dictyandra*, with pollinia in *Randia acuminata*;
stamens often long and short (heterostyly);
ovary superior in *Gaertnera*, and in spp. of *Oldenlandia*;
ovaries united in pairs in *Morinda*;
ovary 1-locular in *Acranthera*, *Casasia*, &c., 3–5-locular in *Cuviera*, &c. 4-locular in *Euosmia*, 6–10-locular in *Praravinia*, ∞-locular in *Timonius*;
stigma 10-lobed in *Mesoptera*;
capsule septicidal or loculicidal; schizocarp in *Diodia*, *Richardsonia*, *Xanthophyllum*, &c.; several with circumscissile capsule; some with berry;
no endosperm in *Abbottia*, *Guettarda*, &c.; ruminate endosperm in *Polysphaeria*;
radicle of embryo sometimes curved;
no cotyledons in embryo of *Guettarda*.

There is no escape from the conclusion that characters of all kinds, however important they may be in classification, may be acquired over and over again by single genera, and therefore that they can be easily acquired without needing an immense period for the acquisition.

It is also very striking that characters are more constant in small families than in large. This of course is well known, and I shall doubtless be accused of talking platitudes in bringing forward such a point. But it is not what one would expect upon the Darwinian theory. If the common ancestor of the Rubiaceae had the characters that are most widespread throughout the family, such as opposite leaves, regular flowers, free stamens, inferior ovary, capsule, endosperm, straight embryo with cotyledons, &c., why are members of the family found that depart from all these characters, when this is a large and 'successful' family, that presumably owes its success to its characters? These 'abnormal' characters do not occur in the most nearly related families to the Rubiaceae, so that to imagine them as survivors when the Rubiaceae were isolated from others by destruction of the intermediates, will not help the matter. If they connect the Rubiaceae to any families, it is not to the families usually regarded as their allies, such as Caprifoliaceae. Further, why do the small families, so often supposed to be relics, show such constancy? One would incline to expect more variety in them.

In what has been said so far I have left entirely out of consideration what seems to me the greatest proof of all for the probability that evolution

has taken place by sudden mutations covering the whole gap between forms. This is the work of Mr. G. Udny Yule and myself upon the statistics of evolution. In that paper (19) we have shown that the evolution of new genera out of old follows with quite astonishing exactness the rule of compound interest. The close approximation of the logarithm curves to straight lines (an exact straight line would mean an exact following of the law of compound interest) leaves no room for doubt that this is the rule which evolution has followed. After a given time one genus has become two, and after another period of time two have become four, and so on. Now if genera and species are formed upon such a rule, it would seem all but inconceivable that they should have been formed by gradual steps, or in any other way than by sudden change. Not only does this appear, but it is also clear that the large genera must be the ancestors of the small; the genera cannot have arisen from common parents, now extinct, as is demanded by the Darwinian theory. The general result of the work is to show that evolution has proceeded upon a very definite plan; 'the manner in which it has unfolded itself has been relatively little affected by the various vital and other factors, these only causing deviations this way and that from the dominant plan.' If one accept the idea of gradual development of species and genera, in view of this work, then one must suppose that all the stages in that gradual development proceeded upon definite preordained lines.

In later papers the thesis which has been briefly indicated here will be worked out with greater profusion of illustration. The object now is simply to show that the evolution of species from one another by means of large mutations is a highly probable occurrence. One species may thus be the parent—direct or indirect—of a number of others, found upon the same area, or upon portions of it, as both Guppy and myself have shown with regard to local endemics. That variation consequently has a tendency to be centrifugal is thus rendered extremely probable, and this question will be dealt with later in more detail.

Summary.

This paper attempts to set forth some of the arguments in favour of the origin of species by large mutations, rather than by the gradual accumulation of small variation (whether infinitesimal or mutational) that is *assumed* in the Darwinian theory, and for which no proof whatever has yet been given. Some compromise between the two extreme suppositions—Special Creation, by which species were *created* with the existing *large* differences, and Natural Selection, by which they were *evolved* by *accumulation* of *small* differences—is needful, and it is suggested that the best is the evolution of

species by 'large' differences that suddenly appear. Neither of the extremes can now command general acceptance, and it is suggested that they should, so to speak, exchange partners.

Natural Selection is essentially a theory of progressive adaptation, and it is shown that adaptation usually involves correlation, and there is no reason to suppose that gradual stages, always correlated, can be picked out, whilst often the one half of the selection could not be made till the other half was complete, and the result of the latter only would be fatal, as in tropical lianas, for example, where the twining stem could not begin to be selected till the weak and flexible stem had been produced, though the latter, with no means of support, would be at a hopeless disadvantage.

A brief account is given of my former investigations of the endemic species of Ritigala, and it is shown that the chief distinguishing characters of *Coleus elongatus* (to take a definite example) do not allow of intermediate forms, nor can they be the subject of Natural Selection. The only reasonable explanation is that they were formed by large changes. And the same is the case for a vast proportion of important distinguishing characters between species generally.

Guppy's Differentiation theory, which is upon much the same lines, is then touched upon, with some notes by Dr. Guppy himself.

Other published work is then described in brief, all pointing to the same general conclusion, and it is shown that destruction of intermediates, usually called in upon the Darwinian theory, will not explain the facts, nor, in the majority of cases, is there any reason for it.

It is then pointed out that nothing but experience can decide what are the most useful distinguishing characters in any given case. To make up one's mind beforehand to use chiefly characters *A*, *B*, and *C*, for example, would only be to court disaster.

Instances are given of characters sometimes of great importance as family diagnostics and in other cases only generic or specific; and the characters of the 'abnormal' members of Rubiaceae are given in full detail to show how in a single large family no character whatever need necessarily be steadfast throughout the family. The incidence of rumination of endosperm, sometimes (e.g. in the palms) a generic or even only a specific character, is described in more detail, but such detailed work must in general be left for later papers.

Finally, the very conclusive evidence in favour of large mutations that is given by the work of Mr. Yule and the author is touched upon. If genera follow in their evolution the rules of compound interest, then it is practically impossible to suppose them formed in any other way than by sudden mutations. All the evidence that is produced in this paper points to the same conclusion.

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The Effect of Boric Acid and Borax on the Broad Bean and certain other Plants.[1]

BY

KATHERINE WARINGTON, M.Sc.

(*Rothamsted Experimental Station.*)

With Plate XIII and six Figures in the Text.

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I. Introduction.

THE action on plant life of chemical elements, other than those usually considered nutritive, is of such fundamental importance that a large volume of work has been done on the subject. Many of these substances have been found to occur naturally in plant tissues, boron being first discovered by Wittstein and Apoiger (37) in 1857, and since that date many investigations have been made on the distribution of boron compounds and their influence on plant life.

Bertrand (4 (*b*)) has made a wide study of these plant poisons, including boric acid, and has drawn special attention to their beneficial effect when presented in very small quantities: Pellet (29), however, holds that no favourable action results from the use of manganese, aluminium, or boron compounds in the cultivation of sugar beet; but Agulhon's work with boron compounds is of particular interest, on account of his exhaustive treatment of the subject, and the large variety of plants studied. Most of the other important work prior to 1914 has been dealt with by Brenchley (11 (*b*)).

More recent investigations have been chiefly American, dealing mainly with the toxic effect of boron compounds present in fertilizers used in the field. Experiments of this type have been carried out by Conner and Fergus (14, 15), Schreiner and his collaborators (33), Plummer and Wolff (30), Proulx and others (31), Brown (12), Blair (6), and Blackwell and Collins (8).

The results of these large-scale experiments show clearly the complexity

of the subject, since the reaction of the plant is seen to depend on the crop, the nature of the soil, the type of boron compound and the method of its application. In addition, the rainfall and other seasonal conditions are of the greatest importance. Though the injurious effect of boron on plants has been emphasized by the American work, cases of possible stimulation are mentioned. However, there is nothing comparable to the extraordinary beneficial results obtained by the earlier French work of Bertrand (4 (*b*)) and Agulhon (1 (*a*)); recently Bruno (13) has put forward a criticism of the methods employed in the U.S.A. as a possible explanation of the inconsistent results obtained in the two countries. But since so little is at present known about the part played by such substances as boron compounds in the plant, and further the reaction of the plant is dependent on so many external factors, it is hardly surprising that all experimental results should not fall into line.

In some experiments carried out by Dr. J. Davidson at Rothamsted in 1920 in connexion with the bean aphis, broad bean plants in water-culture solution supplied with a small quantity of boric acid were strikingly superior to the rest of the series. Accordingly, the present investigation was undertaken in order to determine more fully the action of boric acid on the broad bean and certain other plants.

II. Experimental Work.

The methods employed were as follows:

(1) Water culture.
(2) Pot culture.
(3) Field experiments.

1. *Water Cultures.*

Experiments were carried out chiefly with broad beans and barley.

Germination was effected in damp sawdust, the seedlings being placed in their respective solutions when they had developed roots of 1 to 2 in. in length, usually about ten days after sowing.

The Rothamsted food solution, with composition as follows, was used in every case:

KNO_3	1 grm.
KH_2PO_4	0·5 „
NaCl	0·5 „
$CaSO_4$	0·5 „
$MgSO_4$	0·5 „
$FeCl_3$	0·04 grm.
Distilled water to make up 1 litre.	

This solution was tested for the presence of boron, but the result was negative.

The nutrient solutions were renewed frequently. Three weeks usually elapsed before the first change, but later fresh solution was supplied at fortnightly or weekly intervals. The bottles were kept well filled with distilled water between the renewing of the solutions. Sufficient aeration of the roots seemed to be effected by this means. The plants were grown in separate bottles, each of 600 c.c. capacity.

A. *Broad Beans.*

The variety of broad bean used throughout this work was Sutton's Prolific Longpod, and the seed was graded by weight for each experiment.

a. *The Effect of Different Concentrations of Boric Acid in the Nutrient Solution.*

In the first three series the effect of a wide range of concentrations of H_3BO_3 was tried, viz. from 1 : 5,000 to 1 : 100,000,000.

Five or ten plants were taken as the unit.

The experiments were carried out at three different seasons of the year, spring, early summer, and autumn respectively. Consequently, the plants were grown under totally different conditions of light, temperature, and humidity. However, in spite of this, and the corresponding variation in such vital plant processes as transpiration, carbon assimilation, and respiration, the main results in all three cases were consistent, showing that seasonal changes cannot in any way be responsible for the remarkable effects attributed to the action of boric acid.

(*a*) *Development of the root.* Differences between the plants were first evident in the root system, being noticeable after only five to seven days' growth in their respective solutions.

H_3BO_3 1 : 5,000.

The root development was poor, but on the whole better than that of the controls, though in a comparison of their dry weights little difference was seen (Table I). The first-formed laterals also showed a strong tendency to arise in the upper portion of the primary root, above the solution, and to grow out at a wide angle as if trying to avoid entering the liquid (Text-fig. 1). Later the rootlets were more evenly distributed and altogether appeared quite normal. A few isolated cases of curled root-tips were noticed, but it was uncertain whether this was due to the poisonous nature of the solution or to some mechanical factor.

H_3BO_3 1 : 25,000 and 1 : 50,000.

These two sets showed up very strikingly after a week's growth. The laterals were more numerous than in any other set, and there was only a slight tendency shown for them to be at first localized in the upper portion

of the root (Text-fig. 1). Of the two, the 1 : 25,000 set more nearly resembled the 1 : 5,000, while in the plants grown with 1 : 50,000 H_3BO_3 but few of the characteristics of the roots grown in the strongest solution were evident; thus it would seem that the high concentration of the boric acid was the factor responsible for any peculiarities in growth in the latter solution.

H_3BO_3 1 : 100,000, 1 : 500,000, and 1 : 2,500,000.

The roots of the plants in these concentrations were slower in developing than those receiving a somewhat larger quantity. None of the character-

TEXT-FIG. 1. Typical broad bean seedlings after five days' growth in a nutrient solution with or without boric acid.

istics of the 1 : 5,000 set were seen, but, on the other hand, growth was not so good as in the case of plants treated with 1 : 50,000 H_3BO_3.

H_3BO_3 1 : 12,500,000 and 1 : 100,000,000.

As might be expected, the plants in the most dilute solution of boric acid showed little difference in their root development from the untreated plants, but those in 1 : 12,500,000 H_3BO_3 made decidedly more growth than either the 1 : 100,000,000 set or the controls, and the dry weight figures bear this out (Table I).

Control: no Boric Acid supplied.

At first the development of the root compared favourably with the other sets, but very soon fell behind (Text-fig. 1). In the majority of cases the

type of root was most distinctive, being short and thick with a stunted appearance [1] (Pl. XIII, Fig. 1). However, the individual variation was very great and a few isolated cases occurred with the long thin type of root, which was that universally met with among the plants supplied with boric acid (Pl. XIII, Fig. 2), but in no case was a good root system developed.

While the contrasts between the root development of the individual sets supplied with different concentrations of boric acid were much more strongly marked in the early stages of growth, the difference between the controls and all the boron-treated plants became more evident as growth went on, suggesting that it was the presence or absence of boron that was the important factor, rather than the actual concentration of H_3BO_3 supplied. That this was the case will be more clearly seen after development of the shoot has been considered.

(*b*) *Development of the shoot.* Differences between the various sets became noticeable in the development of the shoot slightly later than in the case of the roots; however, a clear contrast was evident after about ten days' growth in their respective solutions.

H_3BO_3 1 : 5,000.

These plants offered a marked contrast to all the others, both in colour and appearance of the shoot. From the first they were rather less forward than those receiving smaller doses of boric acid, but were well ahead of the controls. Besides presenting a flaccid appearance, many were pale yellow, and although they became a better green colour later, the leaves frequently turned brown and withered along the margins. The lower leaves were the first to become affected, but towards the end of the experiment quite a number of the upper leaflets showed it too. A similar condition of the leaf has been described by Brenchley (11 (*a*)) for peas grown in high concentrations of boric acid. In the experiment carried out in the spring the plants were much more severely injured than those receiving exactly the same treatment during the summer months. This was probably due to the fact that both the rate of growth and the vitality of the plant would be less vigorous in the early part of the year than during the summer, and consequently the plant would be less able to resist the influence of the poison. A similar relationship between the season and the degree of toxicity of a given amount of poison has been more fully described by Brenchley (11 (*a*)) in the case of the action of both zinc sulphate and boric acid on peas and barley.

H_3BO_3 1 : 25,000, 1 : 50,000, and 1 : 100,000.

These three sets soon ran ahead of all the others, and for some time were decidedly the tallest plants, though this distinction in height gradually disappeared towards the close of the experiment.

[1] See note at end.

Poisoning effects were not much seen, being usually confined to the lower leaves.

H_3BO_3 *1 : 500,000, 1 : 2,500,000 and 1 : 12,500,000.*

Good shoot development was obtained in all these concentrations of boric acid, though the rate of growth was rather slower than where a slightly larger quantity had been given.

H_3BO_3 *1 : 100,000,000.*

As was seen in the case of the root development, such a small quantity of boric acid failed to create any apparent difference between these plants and the controls : in fact their growth was so similar that this concentration was omitted in the last of these three experiments.

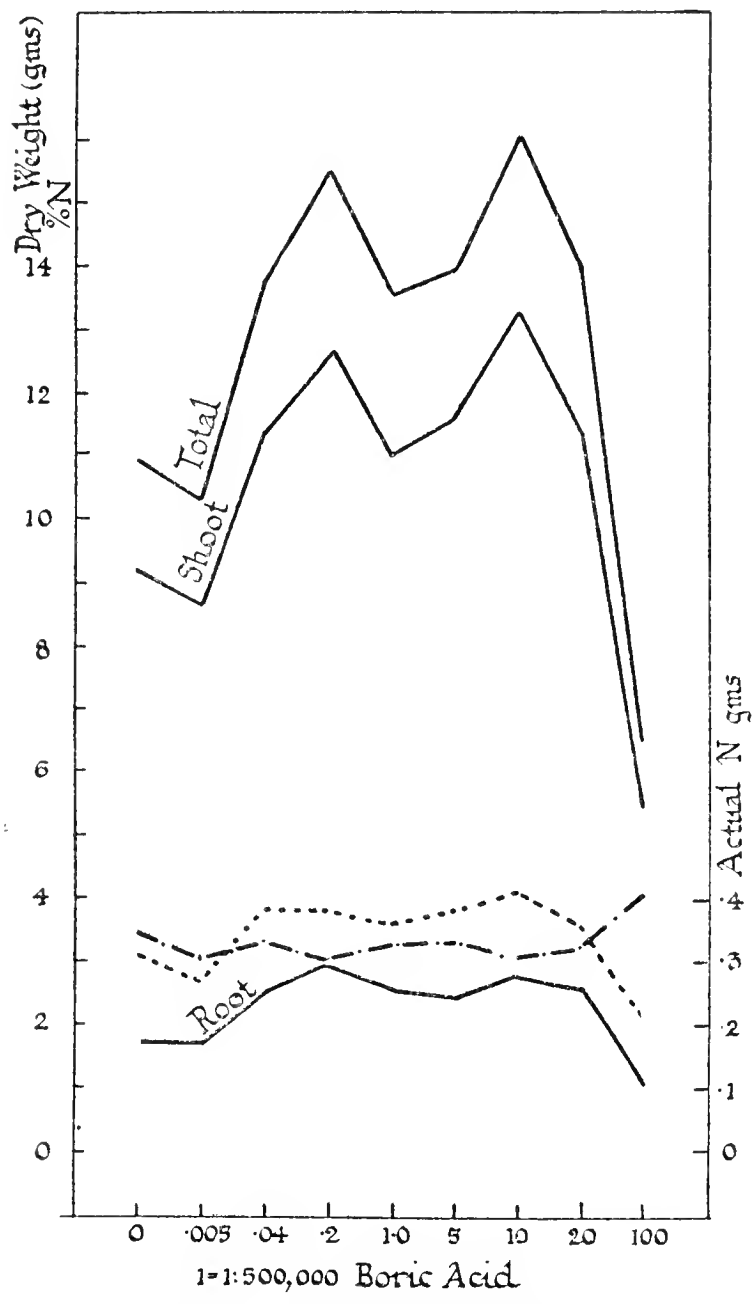
Control : no Boric Acid supplied.

For the first three weeks growth was apparently normal, and though the plants were far behind those treated with boric acid, yet they seemed to be healthy. But when the rest of the series were beginning to flower (usually after four or more weeks) the control plants presented a very abnormal appearance. They were small, stunted, and dark green in colour ; the flower buds in many cases withered and fell off, while only a few plants bloomed. The apex of the shoot also withered, and the stem at this point became blackened (Pl. XIII, Fig. 3). This blackening started at the apex and travelled slowly down the stem, visible to the eye as black streaks. At the same time the leaves were affected. Their texture was leathery compared with the leaves of the boron-treated plants, and in many cases had the appearance of being covered with small yellow dots. They also showed a strong tendency to fall off, the base of the petiole being cut off quite clean. Mason (22) has described a somewhat comparable phenomenon in Sea Island cotton, attributing the boll-shedding to a retardation in the rate of production of assimilates. Macroscopic examination of the petiole showed that the tissue was blackened in a similar way to the stem. A microscopical investigation of the anatomical changes is now being made and will be described in a later paper.

All the control plants did not show this withering at the same time, and at the conclusion of one experiment several untreated plants remained apparently healthy, though this was unusual. Two such exceptional plants were allowed to continue growth for several weeks ; three plants of similar age, but which had been continuously supplied with boric acid, were also kept on under the same conditions. At the end of a few weeks the untreated plants showed the typical 'dying off', while those receiving boric acid showed not the slightest indication of it, even after four months' growth, though by this time they had begun to die normally, the lower leaves

turning yellow and falling off, death proceeding from below upwards. It would seem, therefore, that the period when the withering appears varies with the individual plant, but given sufficient time the 'dying off' phenomenon inevitably occurs.

The contrast between the shoots of plants treated with different concentrations of boric acid (except in extreme cases) was hardly evident towards the close of the experiment, while the difference in the growth of the controls from all the others was more clearly emphasized (Pl. XIII, Fig. 5). This gave support to the idea that the presence of boric acid was of fundamental importance, though the actual concentration within certain limits, approximately 1 : 25,000–1 : 12,500,000, was of little consequence.



TEXT-FIG. 2. Broad beans grown in water culture solution containing different quantities of boric acid. March 10–May 17, 1921 (average of five plants) : —— Dry Weight ; —.—.— per cent. Nitrogen ; - - - - - - Actual N.

(*c*) *Discussion of dry weights.* The dry weight figures for these first three experiments on broad beans substantially support the conclusions which have already been drawn by inspection of the plants.

Consideration of Table I and Text-fig. 2 shows clearly that such a minute quantity of boric acid as 1 : 100,000,000 does not exert any appreciable influence on growth, but from 1 : 12,500,000 up to 1 : 25,000 a considerable increase over the figures for the control plants is seen. Above 1 : 25,000 a toxic effect is apparent in the case of the preliminary experiment carried out in the spring, though in the two remaining series the same quantity of boric acid appears to have been beneficial. This varying effect of a similar amount of the poison has already been shown to be dependent on seasonal conditions.

It is evident that though on the whole the addition of 1 : 50,000 boric acid yielded the best results, such widely varying quantities as 1 : 12,500,000 or 1 : 25,000 were able to exert a beneficial effect of much the same order.

(*d*) $\frac{Shoot}{Root}$ *ratio.* A decided drop in the $\frac{\text{shoot}}{\text{root}}$ ratio below that of the

TABLE I.

Mean Dry Weight and $\frac{Shoot}{Root}$ *Ratios of Broad Beans grown in Water Culture.*

Concentration of H_3BO_3.	*Mar.* 10–*May* 17, 1921. *Average of* 5 *plants.*				*April* 28–*June* 13, 1921. *Average of* 10 *plants.*				*September* 3–*October* 17, 1921. *Average of* 10 *plants.*			
	Root.	*Shoot.*	*Total.*	$\frac{Shoot}{Root}$.	*Root.*	*Shoot.*	*Total.*	$\frac{Shoot}{Root}$.	*Root.*	*Shoot.*	*Total.*	$\frac{Shoot}{Root}$.
	grm.	grm.	grm.		grm.	grm.	grm.		grm.	grm.	grm.	
Control	1·72	9·18	10·90 [1]	5·34	0·89	4·41	5·30 ± 0·17	4·95	0·51	2·57	3·08	5·03
1 : 100,000,000	1·70	8·63	10·33	5·08	1·04	5·20	6·24 ± 0·21	5·00	—	—	—	—
1 : 12,500,000	2·45	11·33	13·78	4·62	1·56	6·35	7·91 ± 0·36 [2]	4·07	0·94	5·20	6·14	5·53
1 : 2,500,000	2·97	12·50	15·47	4·21	2·40	8·60	11·00 ± 0·27 [3]	3·58	0·98	4·97	5·95	5·07
1 : 500,000	2·57	10·98	13·55 [1]	4·27	2·43	8·01	10·44 ± 0·41	3·30	1·01	5·25	6·26	5·20
1 : 100,000	2·45	11·50	13·95 [1]	4·69	2·51	8·60	11·11 ± 0·48	3·43	0·87	4·31	5·18 [3]	4·96
1 : 50,000	2·75	13·22	15·97	4·81	2·65	8·90	11·55 ± 0·28	3·36	1·04	4·95	5·99 [3]	4·76
1 : 25,000	2·58	11·37	13·95	4·41	2·12	8·20	10·32 ± 0·44	3·87	0·94	5·28	6·22	5·62
1 : 5,000	1·03	5·48	6·50 [1]	5·32	1·41	6·66	8·07 ± 0·48	4·72	0·60	3·51	4·11	5·85

The probable errors are given in a single typical case.

[1] 4 plants only. [2] 8 plants only. [3] 9 plants only.

controls occurs in all those concentrations of boric acid which are favourable to growth, viz. 1 : 12,500,000–1 : 25,000 approximately (Table I). A similar drop was found by Agulhon (1 (*a*)) in the case of wheat grown in water culture. When the concentration is sufficient to be toxic, the ratio rises, bringing the value up to much the same level as in the controls. Thus it would seem that it is primarily the root that is influenced by boric acid. In this connexion it is of particular interest that Agulhon (1 (*a*)) and later Vinson and Catlin (35) have shown unquestionable stimulation of the root system of the radish with small applications of boric acid, since the root systems of the broad bean, radish, and wheat are so distinct from one another.

β. The Effect of adding Boric Acid at Different Stages of Growth.

A large number of broad bean plants were set up in the normal water-culture solution; five additional plants received 1 : 50,000 and five 1 : 2,500,000 H_3BO_3 respectively, besides the ordinary nutrient salts; these concentrations of boric acid being chosen as they were both near the limits of its favourable action.

The solutions were renewed every ten days, and at each change five[1] more plants were supplied with the stronger dose of H_3BO_3 and five with the smaller quantity; but all plants, when once treated with boron, were kept continuously supplied with it throughout the experiment.

Those which received boric acid from the start soon made more rapid growth than the untreated plants, all flowering well, and the set grown without H_3BO_3 for only ten days appeared very similar. However, twenty or thirty days' growth in the absence of boron had a decidedly bad effect. Three of these plants showed the typical 'dying off' of the main shoot, previously described as characteristic of broad beans grown without boron, and the remainder were poor and backward. In one case, 'dying off' occurred a few days after 1 : 2,500,000 H_3BO_3 had been supplied, which suggested that the physiological condition of the plant, of which this withering was the outward expression, had already set in before treatment. After forty days' growth without boric acid, only three of the ten plants were still healthy, and after fifty days a single plant remained normal in appearance, while *all* those deprived of boron for sixty days were characteristically withered (Pl. XIII, Fig. 6).

It was noticeable that few plants flowered while still in the normal culture solution, and that though the 'dying off' phenomenon was of general occurrence, the time of its appearance was quite irregular. In two plants, signs of withering set in within twenty-four days, while in others it did not appear until forty-six days' growth. However, in all cases it occurred before the plants were harvested, unless boric acid was supplied in time.

[1] The unit was originally 10, but owing to the death of numerous seedlings in a spell of hot weather it had to be reduced to 5.

The remarkable feature, however, in this experiment, was the recovery of the plant subsequent to the addition of boric acid. In every case (except a few of those deprived of boron for sixty days), though withering had apparently taken a complete hold of the plant, some renewal of growth took place, whether it was merely the production of a few new roots, or the development of a large secondary shoot and an almost entirely new root system (Pl. XIII, Figs. 3 and 4). It would seem, therefore, that boron is able to benefit the plant even at a late stage in its growth, though its effect is certainly less pronounced than when supplied before the injury due to its absence is too far advanced.

(*a*) *General type of recovery subsequent to the addition of boric acid.* The individual history of one of the plants showing recovery is interesting and may be taken as typical of the majority of others (Pl. XIII, Fig. 4).

i. The plant was grown in normal solution for twenty days and then placed in a similar solution which contained 1 : 2,500,000 H_3BO_3.

ii. Four days later, signs of 'dying off' in the shoot were evident. (Possibly the preliminary stages of withering had been overlooked when the plant was transferred, or the disorganization may conceivably have already set in, though not have been visible.)

iii. Fourteen days later the shoot was still very poor, but fresh growth was taking place in the root. The short, thick roots, typical of plants grown in the absence of boron, began to elongate into long, fine ones, and entirely new laterals were also produced. At an early stage in this renewal of growth, the line of demarcation between the old and new portion of the root was clearly defined, and presented a most curious appearance.

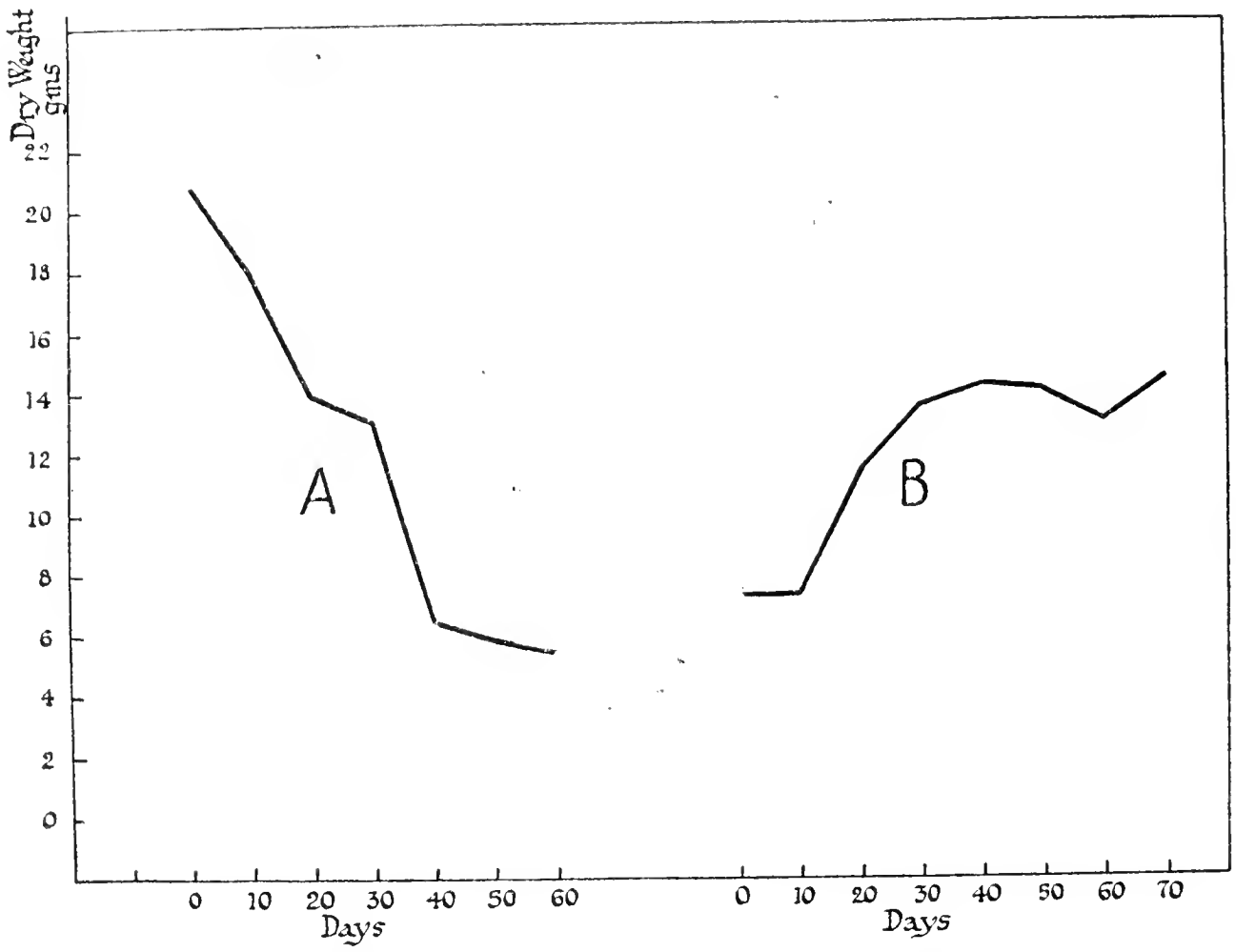
iv. Nineteen days later, though the main shoot still remained withered, a healthy secondary shoot was in flower. Besides being considerably larger than the original shoot, this secondary branch was different in appearance from the main axis. The leaves were rather lighter in colour, thin and not leathery in texture, giving the whole shoot an almost succulent appearance. By this time the root system was composed of a dense mass of laterals, though formerly there had been only a few short and thickened roots.

In some cases apparently healthy tillers were produced by plants grown without boron. At first sight, this appeared to be conflicting with the view that it was the presence of boric acid that caused the renewal of growth. However, such tillers never flowered, always remained stunted, and eventually 'died off' in a similar manner to the main axis.

In a few isolated cases, another type of recovery took place. The plant did not show any definite signs of 'dying off' when transferred to the solution containing boric acid, although it had been grown for a very considerable time without boron and was in a poor condition. After thirty days' treatment, a number of fresh green leaves were noticed at the apex of

the main shoot, though the lower leaves remained yellow and unhealthy. In two of these cases a new supply of flowers was produced at the apex, although the former ones had withered before maturing and had fallen off. It seems probable, therefore, that as these striking recoveries were of such universal occurrence throughout the experiment, the assumption that the addition of boric acid was responsible for the renewed growth was justifiable.

(*b*) *Effect of the concentration of boric acid supplied.* On the whole there was but little difference noticed between the plants treated with the



TEXT-FIG. 3. Total dry weight of broad beans (average of five plants).
A. Grown without boric acid for different periods; 1 : 50,000 then added. May 21–July 26, 1922.
B. Grown with boric acid for different periods; 1 : 50,000 then removed. Aug. 9–Oct. 18, 1922.

larger and smaller quantity of H_3BO_3 respectively. However, there was one distinct exception, viz. the set of plants grown for thirty days before treatment with boron. In this case those receiving 1 : 2,500,000 H_3BO_3 were clearly poorer than the corresponding set treated with 1 : 50,000 H_3BO_3; 'dying off' was also more prevalent among the former. It seems probable that at this time, and under these conditions, a critical stage in the growth of the plants had been reached owing to the deficiency of boron, and that consequently the plants were particularly sensitive to its action. The application of a large quantity would naturally remedy the deficiency more rapidly, and possibly even prevent the appearance of the withering; a small application, however, such as 1 : 2,500,000, though eventually able to

bring about recovery, would conceivably be insufficient to check the 'dying off', and the appearance of this phenomenon in several plants can be thus accounted for.

The dry weight values (Table II and Text-fig. 3, A) are in a more or less strict downward sequence from those plants which were continuously supplied with boric acid to those which were treated for a few days only. Allowing for individual variation, the actual concentration of boric acid supplied is seen to have had little effect on the dry weight, except in the case to which attention has already been drawn, and possibly in this case also individual variation may have caused the result to be unduly accentuated.

TABLE II.

Mean Dry Weight of Broad Beans supplied with Boric Acid at Different Stages of Growth.

May 21–July 26, 1922. Average of 5 plants.

Days without H_3BO_3,	*followed by*	*Days with* H_3BO_3.	1 : 50,000 H_3BO_3. *Root.*	*Shoot.*	*Total.*	1 : 2,500,000 H_3BO_3. *Root.*	*Shoot.*	*Total.*
			grm.	grm.	grm.	grm.	grm.	grm.
0		66	3·94	16·83	20·77	3·32	14·16	17·48
10		56	3·18	14·84	18·02	3·22	15·15	18·37
20		46	2·67	11·18	13·85	2·26	9·41	11·67
30		36	2·60	10·40	13·00 [1]	1·28	4·11	5·39 [1]
40		26	1·12	5·30	6·42 [1]	1·15	5·42	6·57 [1]
50		16	1·16	4·65	5·81 [1]	1·00	5·03	6·03 [1]
60		6	0·80	4·65	5·45 [1 2]	0·51	2·42	2·93 [1, 2]

γ. *The Effect of removing Boric Acid at Different Stages of Growth.*

On transferring a few plants grown for twenty days in a nutrient solution containing 1 : 50,000 H_3BO_3 to ordinary culture solution, it was evident that a deficiency of boron was felt, as 'dying off' set in after about three weeks. Accordingly, an experiment was set up to see the result of depriving plants of boron after being supplied with H_3BO_3 for different periods, since apparently the boron absorbed in twenty days was inadequate for the whole growth period.

Besides the usual nutrient salts, half the plants were treated with 1 : 50,000 H_3BO_3 and the other half with 1 : 2,500,000 H_3BO_3. In addition, ten plants received no boric acid at all. All the solutions were renewed every ten days, five plants hitherto treated with one or other of the two strengths of boric acid being placed in ordinary culture solution at each change, and not

[1] Average of three plants only, as the remainder were carried on longer for observations on the recovery.

[2] These plants may be regarded as controls, as the application of boric acid had no apparent effect during the 6 days.

again supplied with boric acid throughout the experiment. In order to prevent the transference of small traces of boron after the change, the roots were washed with distilled water, and as long as the size of the plants allowed of it clean bottles were used.

The untreated set soon fell considerably behind all the others; the short, thick type of root previously described for plants grown in the absence of boron was less evident, though the whole root system was poor. Seasonal conditions probably account for this, and the question is more fully discussed farther on.

Another peculiarity of the plants receiving no boron was the outgrowth of numerous secondary branches. None of these tillers flowered, and in all respects they closely resembled the type of growth of the main axis. Brenchley (11 (*a*)) has described a similar bushiness of growth in peas supplied with toxic doses of boric acid; but in the case of broad beans it would seem that the absence of an essential element was the factor concerned.

(*a*) *Death subsequent to the removal of boric acid.* Each set as it was in turn deprived of boric acid gradually showed the characteristic 'dying off', usually from three to six weeks after the transference into the normal culture solution had been made.

Five weeks from the start of the experiment all the untreated plants, and several of those treated for ten days only, had begun to wither, while those which had been supplied with boric acid for thirty or more days were in flower and still growing rapidly. Although treatment for ten days was hardly beneficial, twenty or thirty days' growth in boric acid caused a marked improvement, and a further superiority was seen in those supplied with H_3BO_3 for longer periods.

After seventy days, all the plants which had been treated with 1 : 50,000 H_3BO_3 were harvested. At this time, all such plants deprived of boron for forty days or longer were 'dying off', but the remainder, with a single exception, were still healthy whether they had already been transferred to the normal solution or were still supplied with boric acid. The 1 : 2,500,000 sets, however, were carried on for twenty-six days longer, no further change in their solutions being made, all except five of the plants being already in the ordinary culture solution.

At the end of this period *every* plant was either severely withered or at least showing signs of dying at the apex of the shoot, except the set of five which were still receiving boric acid; the latter were perfectly healthy and even still flowering (Pl. XIII, Fig. 7).

It is clear, then, that a continual supply of boron enables the broad bean to make normal development, and that a comparatively large quantity given during the first few weeks of growth is less effective than a small amount supplied continuously.

(*b*) *Effect of the concentration of boric acid supplied.* For the first week

or so, little if any difference was evident between the plants treated with the two concentrations of boric acid, viz. 1 : 50,000 and 1 : 2,500,000 ; but a contrast became apparent later, being especially marked in the sets grown for twenty days in the two solutions (Table III). Three weeks after the removal of H_3BO_3 all the plants previously treated with the smaller quantity were 'dying off', while those grown in the stronger solution were perfectly healthy and still in flower. In general 'dying off' set in about three to five weeks after the removal of boron where 1 : 2,500,000 H_3BO_3 had been supplied, while withering was not apparent until four and a half to six weeks after the plants were transferred from a solution containing 1 : 50,000 H_3BO_3, so that, on the whole, the larger quantity of boron was of more value to the plant than the smaller application, when the treatment lasted for a limited period only.

The upward sequence of the dry weights (Table III and Text-fig. 3, B) as the treatment with boric acid was prolonged corroborates what has already been described. It is evident that but little dry matter is laid down after 'dying off' occurs, for of the plants which were carried on for the additional twenty-six days, only those which did not show signs of withering at the beginning of this period, i.e. those supplied with H_3BO_3 for at least forty days, were able to continue growth.

TABLE III.

Mean Dry Weights of Broad Bean Plants deprived of Boric Acid at Different Stages of Growth.

Average of 5 plants.

Aug. 9–*Oct.* 18, 1922. 1 : 50,000 H_3BO_3.					*Aug.* 9–Nov. 13, 1922. 1 : 2,500,000 H_3BO_3.			
Days in H_3BO_3.	*Days without* H_3BO_3.	*Root.*	*Shoot.*	*Total.*	*Days without* H_3BO_3.	*Root.*	*Shoot.*	*Total.*
		grm.	grm.	grm.		grm.	grm.	grm.
0	70	0·95	6·21	7·16	96	0·81	5·86	6·67
10	60	1·26	6·08	7·34	86	1·00	6·53	7·53
20	50	1·88	9·46	11·34	76	1·53	7·20	8·73
30	40	2·26	11·22	13·48	66	1·66	9·46	11·12
40	30	2·53	11·60	14·13	56	2·55	13·89	16·44
50	20	2·49	11·46	13·95	46	2·90	14·70	17·60
60	10	2·38	10·71	13·09	36	3·71	15·74	19·45
70	0	3·04	11·23	14·27	0	3·15	16·08	19·23

From the foregoing experiments it is clear that a small quantity[1] of boron supplied some factor essential to the growth of the broad bean plant that was lacking in the usual nutrient solution.

Mazé (23), in a somewhat similar manner, has claimed that among other elements boron is essential to the development of *Zea Mays*.

[1] The total quantity of boric acid supplied varied with the duration of the experiment from 1·20 to 2·64 mg. H_3BO_3 where the concentration was 1 : 2,500,000. On an average each plant would have access to 0·2 mg. boric acid per week, but it has not yet been ascertained whether the whole of the quantity is taken up.

The presence of boron in a large number of plants in nature has been frequently described, and in the present instance it has been detected in garden-grown broad beans, a larger proportion being present in the pods than in the stems or leaves; Cook (16), also, has drawn attention to the occurrence of boron in plants grown as controls to those on boron-treated plots. But in these experiments boron was detected in the dried shoots of broad beans grown entirely in ordinary nutrient solution. The question at once arose as to the origin of the boron in this case, and also as to why such considerable growth was possible before the deficiency made itself evident.

Three possible sources of boron to the plant grown in water culture are:

(1) Atmosphere.
(2) Nutrient solution.
(3) Seed.

(1) The possibility of the plant obtaining boron from the atmosphere may be regarded as quite unlikely.

(2) Qualitative analysis of the nutrient solution showed that it was not responsible for any supply of boron.

(3) The seed, therefore, remained the probable source of the element. Accordingly a large number of broad bean seeds, such as had been used for the water-culture experiments, were ground up into a fine meal and a chemical analysis made, the method used being that described by Bertrand and Thomas (5). The free end of the turmeric paper turned a bright red colour, indicating the presence of boron. The reaction was so definite that in all probability a comparatively large quantity of boron was present; this would explain the great length of time during which healthy growth was maintained by the broad bean before the deficiency became apparent.

δ. *The Effect of the Removal of the Cotyledons on Subsequent Growth in Water Culture.*

Since boron is apparently of such fundamental importance to the broad bean plant, it seemed probable that removal of the cotyledons, in themselves a source of boron, before growth in water culture, would show up more clearly any difference between plants treated with H_3BO_3 or supplied with the usual nutrient salts only.

Many authors, from quite early times, have shown that removal of the cotyledons or endosperm from the embryo resulted in impaired growth and vigour. Both Bonnet (9) and Sachs (32) described this in their work on *Phascolus multiflorus*. More recent work on the subject has been done by Andronescu (2) on *Zea Mays* and Duggar (18) on Canada field peas and field corn.

Difficulty was experienced at first in growing the dissected out plantlets, the method tried at the outset being as follows: the seeds were

soaked in water overnight, and the plumule and radicle dissected out the next morning and placed on a porous pot standing in nutrient solution. Slight growth was made, but all died after a few days. The use of a nutrient solution with the addition of organic matter such as glucose or mannite gave rather better results, but fungal and bacterial growth made this method unsuitable.

Finally, it was decided to allow germination to start in damp sawdust and not to attempt to grow the young plants until the radicle was about an inch long. At this stage the plumule was in most cases quite yellow, but had sometimes turned green. It was found almost impossible to obtain seedlings uniform in their development, but care was taken to put the strongest of them in the control solution, so that any error thus introduced should be in favour of those deprived of boric acid. Eight of these de-cotyledoned seedlings were set up in ordinary nutrient solution, four of which received in addition 1 : 50,000 H_3BO_3. As a further contrast a seedling still retaining its cotyledons was set up in each kind of solution.

From the first the seedlings which had been divested of their cotyledons fell very much behind the untreated plants, and they never attained anything approaching normal development. Growth was so slow that even after twenty days in the solution only a few laterals had been developed, and after forty-two days there was but little difference to be seen between those supplied with H_3BO_3 and the control plants. However, a week later, when some of the plants were beginning to flower, the controls fell behind, and in some cases signs of 'dying off' were evident. In time, all the plants deprived of boric acid showed this phenomenon, but in no case was there the slightest indication of withering among those supplied with boric acid, either in the untreated or de-cotyledoned plants.

Qualitative analyses of soaked or germinated seeds showed that not only the cotyledons but also the plumule and radicle contained a relatively large proportion of boron. Removal of the cotyledons did not, therefore, completely deprive the plantlets of the element, and this would account for the considerable growth made by the untreated plants. Some food materials also would have been absorbed by the plantlets from the cotyledons during germination, and it is possible that a transference of boron took place at the same time, thus further augmenting the supply. It is also probable that, under these conditions of extremely slow growth, a quantity of boron which under ordinary circumstances would have been entirely inadequate for so long a period, was sufficient to meet the demands of the plant.

B. *Barley.*

In 1914 Brenchley (11 (*a*)) described a series of water-culture experiments on the action of boric acid on barley. The present investigation was carried out on somewhat similar lines to obtain a comparison both with the

earlier work and also with the experiments on the broad bean here described. In general, the results are in close agreement with those of Brenchley, in spite of the fact that in the 1914 work the nutrient solutions were never renewed and the plants were harvested at a comparatively early stage, so that the action of boron is apparently similar whether in the presence of a plentiful or restricted food supply. The barley seed used in 1921 was an unnamed pure strain from Professor Biffin, but in the following season the variety was pure line Goldthorpe from Leamington. In each case the seed was graded by weight to within 0·01 grm.

a. The Effect of Different Concentrations of Boric Acid in the Nutrient Solution.

In the preliminary experiment the effect of a wide range of concentrations was tried, viz. from 1 : 5,000 to 1 : 100,000,000. Five plants were taken as the unit in this case.

(*a*) *Development of the root.* The plants treated with 1 : 5,000 H_3BO_3 showed the injurious effect of boron after as little as three days' growth in the solution. The roots were thick and short, and curled root-tips were noticed in several cases. Later a number of long, fine laterals were produced, resulting in a bushy appearance. A gradual improvement in the root development was evident as the concentration of boric acid was reduced, no bushiness being noticeable when less than 1 : 50,000 H_3BO_3 was given, while the 1 : 2,500,000 and weaker sets closely resembled the untreated plants.

(*b*) *Development of the shoot.* The 1 : 5,000 set soon showed the toxic effect of boron. The leaves turned yellow at the tip, and later brown spots appeared, beginning at the apex and margins and gradually covering the entire leaf-blade. As fresh growth was produced it was rapidly affected in the same way, the injury always appearing first in the lower leaves. The nature and mode of progression of the poisoning action were exactly similar to that previously described by Brenchley (11 (*a*)). The leaves were small and narrow, and in every case the plants were poor and scarcely tillered and never came into ear. The flecking of the leaves gradually disappeared as the quantity of boric acid supplied was decreased, and in the plants grown with 1 : 500,000 and 1 : 2,500,000 the discoloration was very slight; both these sets also came into ear. The plants grown in 1 : 2,500,000 and 1 : 12,500,000 H_3BO_3 were the best in this series. They were taller and a darker colour than the controls and appeared particularly healthy. This dark-green coloration is apparently typical of plants treated with non-toxic doses of boric acid, and has also been previously noticed.

No stimulating effect, however, was apparent in the plants grown with 1 : 100,000,000 H_3BO_3; they in all ways closely resembled the controls, the latter being healthy and well grown.

β. Comparison between the Effect of Boric Acid and Borax in the Nutrient Solution.

Borax and boric acid were used in such quantities that an equivalent amount of boron was supplied in each case; i. e. 1 grm. H_3BO_3 = 1·54 grm. $Na_2B_4O_7$. 10 H_2O. It was uncertain whether boron in the form of the salt would prove more or less toxic than when presented as the acid.

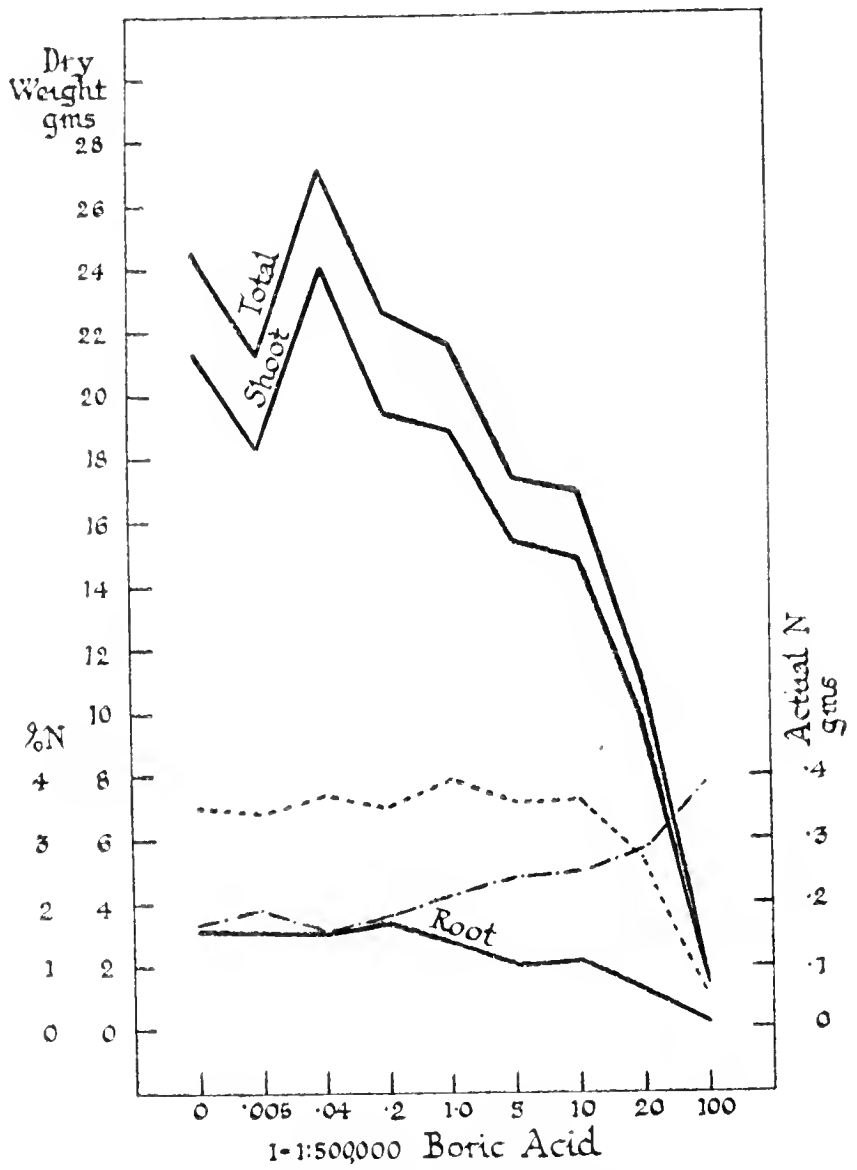
The range of concentrations in this case was from 1 : 50,000 to 1 : 100,000,000 H_3BO_3, since any heavier dose was obviously toxic. Ten plants were taken as the unit.

As might be expected from the results of the previous experiments, no very toxic action was noticeable with any of the concentrations of boric acid used, except in the 1 : 50,000 set. In this case, however, a distinct bushiness of the root resulting from an outgrowth of fine laterals from short and thickened roots was shown, and the leaves also were badly spotted. However, in the plants receiving an equivalent amount of boron in the form of borax no bushiness of the root was apparent, though the development was poor. This was the only definite indication that the action of borax was less toxic than that of boric acid, for in other respects the plants appeared similar, e. g. the leaves were affected in much the same degree, and even the dry weights of the roots were of the same order. All the remaining sets showed a gradual improvement corresponding to the reduction in the concentration of boric acid or borax. As in the previous experiment, the discoloration of the leaf was practically negligible at a concentration of 1 : 2,500,000 H_3BO_3. The 1 : 12,500,000 H_3BO_3 and the corresponding borax set were decidedly the best of the whole series; the controls, though perfectly normal and healthy, lacked the luxuriant growth and the dark colour of these plants (Pl. XIII, Fig. 8). On the whole, the borax-treated plants seemed slightly superior to the corresponding sets supplied with boric acid, and, though no actual measurements were made, the transpiration rate was apparently greater in the former, which would support this view; however, a comparison of typical individuals showed that there was no appreciable difference (Pl. XIII, Fig. 9).

Discussion of dry weights. From a comparison of the dry weight figures (Tables IV and V and Text-fig. 4) it is evident that concentrations as low as 1 : 2,500,000 H_3BO_3 exerted a definitely toxic effect, which was more accentuated as the quantity of boric acid increased. Below 1 : 2,500,000, however, no injurious action was apparent, and in one experiment there was some slight indication of stimulation in the dry weight, but this may possibly be rather attributed to individual variation.

The absence of any increase over that of the controls in the dry weights of plants supplied with 1 : 12,500,000 H_3BO_3 was however unexpected, since to the eye the untreated plants were distinctly the smaller. In all probability these larger plants contained a higher percentage of water and

an increase in the green weight might have been realized. (The fresh weight of plants in water culture is difficult to determine with any accuracy, owing to the wet condition of the roots.) These plants also seemed to be less mature than the controls, the boron possibly exerting some retarding effect. Consequently, if the plants had been harvested at a later stage, some increase in the dry weight might reasonably have been expected to be shown. Should this suggestion be justifiable, the slight indication of stimulation which was obtained in the preliminary experiment, where the plants were



TEXT-FIG. 4. Barley grown in water-culture solution containing different quantities of boric acid. Feb. 21–May 24, 1921 (average of five plants): —— Dry Weight; —·—·—· per cent. Nitrogen; - - - - - - Actual N.

not harvested until in ear, may possibly be of real import. However, the evidence is too incomplete for any definite conclusions to be drawn. The difference between plants treated with equivalent amounts of boric acid and borax is not significant, but on the whole the borax-treated plants gave a slightly higher dry weight figure than the corresponding sets treated with boric acid.

No definite relationship was shown between the $\frac{\text{shoot}}{\text{root}}$ ratios of the variously treated barley plants, as was noticed in the case of the broad bean. It would seem, therefore, that in the case of barley the root and shoot are affected more or less equally by the boron compound.

TABLE IV.

Mean Dry Weights and $\frac{Shoot}{Root}$ *Ratios of Barley grown in Water Culture.*

(*Average of 3–5 plants.*[1])
Feb. 21–*May* 24, 1921.

Concentration of H_3BO_3.	*Root.*	*Shoot.*	*Total.*	$\frac{Shoot}{Root}$.
	grm.	grm.	grm.	
Control	3·12	21·33	24·45 ± 0·17	6·84
1 : 100,000,000	2·91	18·31	21·22 ± 1·32	6·29
1 : 12,500,000	2·99	24·00	26·99 ± 0·15	8·03
1 : 2,500,000	3·19	19·43	22·62 ± 0·71	6·11
1 : 500,000	2·70	18·85	21·55 ± 0·51	6·98
1 : 100,000	1·94	15·44	17·38 ± 0·96	7·96
1 : 50,000	2·14	14·82	16·96 ± 0·23	6·93
1 : 25,000	1·22	9·72	10·94 ± 1·47	7·97
1 : 5,000	0·15	1·42	1·57 ± 0·17	9·46

TABLE V.

Mean Dry Weights and $\frac{Shoot}{Root}$ *Ratios of Barley grown in Water Culture with Boric Acid or Borax containing equivalent quantity of Boron. (Concentrations given as* H_3BO_3.)

March 1–*May* 11, 1922 (*average of 5 plants*).
Plants in the centre of the Greenhouse.[2]

Concentration of H_3BO_3.	*Boric Acid.*			$\frac{Shoot}{Root}$.	*Borax.*			$\frac{Shoot}{Root}$.
	Root.	*Shoot.*	*Total.*		*Root.*	*Shoot.*	*Total.*	
	grm.	grm.	grm.		grm.	grm.	grm.	
Control	1·53	9·37	10·90	6·1	1·53	9·37	10·90	6·1
1 : 100,000,000	1·60	8·92	10·52	5·6	1·76	9·74	11·50	5·5
1 : 12,500,000	1·61	9·08	10·69	5·6	1·64	9·47	11·11	5·8
1 : 2,500,000	1·44	7·30	8·74	5·1	1·73	8·55	10·28	4·9
1 : 500,000	1·68	7·73	9·41	4·6	1·55	8·16	9·71	5·3
1 : 100,000	1·40	6·88	8·28	4·9	1·31	7·47	8·78	5·7
1 : 50,000	0·99	5·85	6·84	5·9	0·80	5·19	5·99	6·5

Plants at the side of the Greenhouse.
(*Average of 5 plants.*)

Concentration of H_3BO_3.	*Boric Acid.*			$\frac{Shoot}{Root}$.	*Borax.*			$\frac{Shoot}{Root}$.
	Root.	*Shoot.*	*Total.*		*Root.*	*Shoot.*	*Total.*	
	grm.	grm.	grm.		grm.	grm.	grm.	
Control	1·90	9·78	11·70	5·1	1·90	9·78	11·70	5·1
1 : 100,000,000	1·75	8·62	10·37	4·9	2·06	9·35	11·40	4·5
1 : 12,500,000	1·62	8·27	9·89	5·1	1·58	7·33	8·91	4·6
1 : 2,500,000	1·33	5·92	7·25	4·5	1·54	8·02	9·56	5·2
1 : 500,000	1·35	5·98	7·33	4·4	1·75	7·70	9·45	4·4
1 : 100,000	1·16	5·87	7·03	5·1	1·18	6·10	7·28	5·2
1 : 50,000	0·83	4·72	5·55	5·7	0·88	4·57	5·45	5·2

[1] Reduction in the average number was unavoidably due to death or abnormally poor development of plants.

[2] Owing to lack of space in the greenhouse with similar conditions of illumination, the experiment was divided into two equal parts and the dry weights considered separately.

γ. Comparison between the Effect of Boric Acid on Broad Beans and Barley.

From the results of the water-culture experiments on these two plants, it is evident that barley is less able to stand high concentrations of H_3BO_3 than the broad bean, for the latter made excellent growth in a solution which was highly poisonous to barley, viz. 1 : 50,000 H_3BO_3, and further 1 : 12,500,000 H_3BO_3, which was just below the toxic limit for barley, was barely enough to ensure healthy growth of the broad bean.

Barley, on the one hand, can apparently make approximately optimum growth in the absence of any boron compound, and would therefore seem more or less indifferent to its presence, provided that the quantity is not sufficient to be toxic; on the other hand, the ordinary nutrient solution has been shown to be inadequate for even normal development of the broad bean, but the addition of a small amount of boron is able to supply some factor which is lacking.

Since boron had been detected in the broad bean seed, that of the barley was tested in a similar manner, but in this case practically no boron was found. Some explanation is therefore afforded for the different reaction of the two plants towards the presence of boric acid in the nutrient solution, for the occurrence of an element in the reserve food of the seed might possibly indicate the importance of that element to the plant in question.

C. *Miscellaneous Plants.*

Since the addition of small quantities of boric acid to the nutrient solution caused such striking results in the growth of the broad bean plant, a number of other plants were tested in a similar manner. Although Agulhon (1 (*a*)), in particular, has shown that the importance of boron is by no means confined to the Leguminosae, it is evident from several workers, Cook (16) and Morse (26) for example, that certain members of this Natural Order readily respond to its influence. Accordingly the majority of the plants chosen were of this group.

In each case fifteen plants were set up in ordinary culture solution at the beginning of August 1922; five receiving in addition 1 : 2,500,000 H_3BO_3 and five 1 : 100,000 H_3BO_3. All the seeds were graded, with the exception of the clovers.

(*a*) *Phascolus multiflorus* (Runner Bean—Sutton's Prizewinner).

From the start the plants supplied with boric acid made better growth than the controls, the difference being first noticeable in the better development of lateral roots. Later a distinct difference in the type of root was evident, those in the control solution being short and thick, while the boron-treated plants had long fine roots.

After the plants had been set up in their respective solutions for about a fortnight, the shoots of all the plants receiving boric acid began to elongate rapidly, as is characteristic of the runner bean. No sign of this was seen in any of the five controls. Eventually the boron-treated plants attained a height of several feet and flowered profusely, but the controls remained stunted until the close of the experiment and never even formed flower buds (Pl. XIII, Fig. 10).

Some indication of a toxic influence was evident in the plants grown with 1 : 100,000 H_3BO_3 in spite of the marked beneficial effect, for the lower leaves were inclined to turn yellow and some brown patches were seen. Apart from this slightly unfavourable appearance of the plants treated with the larger quantity of H_3BO_3, there was little if any difference shown between the plants grown in the two concentrations of boric acid respectively.

(*b*) *Phaseolus vulgaris* (Dwarf Bean—Sutton's Canadian Wonder).

The effect of boric acid on the dwarf bean was not seen so quickly as was the case with the runner bean. For some time all the plants were rather poor, probably owing to seasonal conditions, and it was impossible to note any appreciable difference between the sets, owing to the great lack of uniformity. However, after about three weeks' growth, the boron-treated plants ran ahead of the controls, both shoot and root being better developed, though no striking contrast between the type of root was apparent.

The control plants made very little growth indeed, and eventually all died, yet at first they were even a better green colour than the treated plants; the latter were decidedly yellowish, but grew to fair-sized plants, flowered, and in some cases fruited.

(*c*) *Trifolium incarnatum* (Crimson Clover).

This plant gave particularly striking results which were confirmed by further experiments. Even after two days' growth in their respective solutions a difference was noticeable in the root development, those without boric acid showing a curious bending at the tip with a tendency to a constriction behind the bend, while the treated plants did not show this, or at most in a very small degree. Later the lateral roots were distinctly better developed in both sets of plants grown with boric acid than in the controls. Ten days from the start of the experiment the untreated plants had developed a short and stunted root system, while those supplied with boric acid had long, fine roots, the difference becoming more accentuated as growth went on. The time when this abnormal type of root became apparent was evidently dependent upon seasonal conditions, for in the case of some *Trifolium incarnatum* plants, set up in ordinary nutrient solution in September, the roots for a considerable time closely resembled those which

were grown with boric acid; after several weeks, however, a contrast was clearly marked, the stunted appearance being quite distinct. In all probability, since growth was so slow at this time of the year, a comparatively long interval was necessary before the deficiency of boron became apparent.

Differences between the shoots of the treated and the control plants were not noticed until after eight weeks' growth even in the case of plants set up in August; but at this time the plants without boron were decidedly the smaller, and the leaves showed a tendency to turn reddish in colour. By December 6 two of these plants were dead and the remainder very poor, no signs of flowering being shown even by the end of February.

The plants supplied with boron, however, grew luxuriantly, those receiving the smaller quantity being of a particularly healthy green colour; in other respects there was apparently but little difference between the effect of the two concentrations of H_3BO_3, and by the middle of February both these sets showed signs of flowering.

A similar series of plants was set up, also in August, but in this case the solutions were not renewed. The results were in close agreement with the former series, but there was no flowering and the untreated plants succumbed rather more quickly.

(*d*) *Trifolium repens* (Wild White Clover).

Very much less difference was seen between the controls and the boric-acid-treated plants than was the case with the crimson clover.

The controls were certainly inferior, both in the root and shoot, though the development in each case was perfectly normal. The plants grown with the larger quantity of boric acid were inclined to be yellowish, though those receiving the smaller dose were a good colour and, as regards both the shoot and root development, quite the best of the series.

(*e*) *Pisum sativum* (Peas).

(1) *Harbinger*. For some time no obvious difference was seen between the three sets, but later it was clear that the root system was poorer in the untreated plants; the shoots also were inferior, no flowering occurring in even a single case. On the other hand, the plants supplied with boric acid both flowered and fruited. This lack of flowering may have been due to seasonal conditions, as usually this pea flowers freely in ordinary water culture.

(2) *Pioneer*. This variety of pea was evidently more susceptible to the influence of boron than the Harbinger, for the contrast between the controls and the plants treated with H_3BO_3 was much more marked. In this case also the controls failed to flower, although the treated plants both flowered and fruited. The two concentrations of boric acid had apparently much the same effect.

(*f*) *Winter Vetch.* (*g*) *Rye.*

Hardly any difference was noticeable in either of these plants between those treated with boric acid and the controls. In the case of rye, growth was apparently satisfactory in all the sets, though no ears were formed—probably on account of the unfavourable season of the year. The vetch did not flower either.

Although these last few tests have only been carried out on quite a small scale, it seems probable that boron is able to supply some need in the case of several other plants besides the broad bean. *Phaseolus multiflorus* and *Trifolium incarnatum* in particular appear to derive especial benefit from treatment with a small quantity of boric acid.

In the earlier work on boron Brenchley (11 (*a*)) has stated that lupins were particularly difficult to grow in water culture, since they tended to drop their leaves for no apparent reason. This is somewhat suggestive of the leaf-fall noticed in broad beans deprived of boric acid, and it is possible that lupins may be another instance of a plant benefiting from a continual supply of a minute quantity of boron.

It is hoped to repeat these experiments at the first opportunity, with the addition of several other plants, including yellow and white lupins, in order to investigate the matter more completely.

2. *Pot Cultures.*

Experiments were carried out with both broad beans and barley. Tall, narrow, glazed pots containing $22\frac{1}{2}$ lb. of soil were used in every case. Owing to the heavy nature of the Rothamsted soil, which renders it difficult to handle in pot-culture work, 10 per cent. sand was added, this mixture being found quite satisfactory.

All the pots received a basal dressing of 5 grm. superphosphate, 1 grm. potassium sulphate, and 1·0–1·5 grm. sodium nitrate, whether or not they were further treated with boron compounds.

Five or six pots were taken as the unit.

A. *Broad Beans.*

Sutton's Prolific Longpod was used in every case and graded for each experiment, two plants being grown per pot.

a. *The Effect of adding Various Quantities of Boric Acid to the Soil.*

The effect of a wide range of concentrations of H_3BO_3 was tried in the two preliminary experiments; in the first case it was mixed throughout the whole depth of the pot, but in subsequent series it was applied as a top-

dressing,[1] since this mode of application more closely resembled field methods.

(*a*) *Boric acid mixed throughout the soil* (22½ lb.). 4 grm. of H_3BO_3 were strongly toxic; besides being so injurious to the plant, a grey deposit was noticed on the surface of the soil, giving it a most unhealthy appearance. This was thought to be due to a surface concentration of a boron compound, since it gave a very strong boron reaction on testing with the turmeric method.

Germination was severely retarded in these pots and the plumules on emerging were frequently yellow, this chlorotic condition remaining for a considerable time in some cases. The retarding influence of boron compounds on the germination of seeds was noticed by Heckle (21) as early as 1875. Since that date, similar results with a large variety of plants have been obtained by a number of workers on the subject, including Archangeli (3), Morel (25), Agulhon (1 (*a*)), Voelcker (36), Sherwin (34), Blair and Brown (7), and Neller and Morse (27).

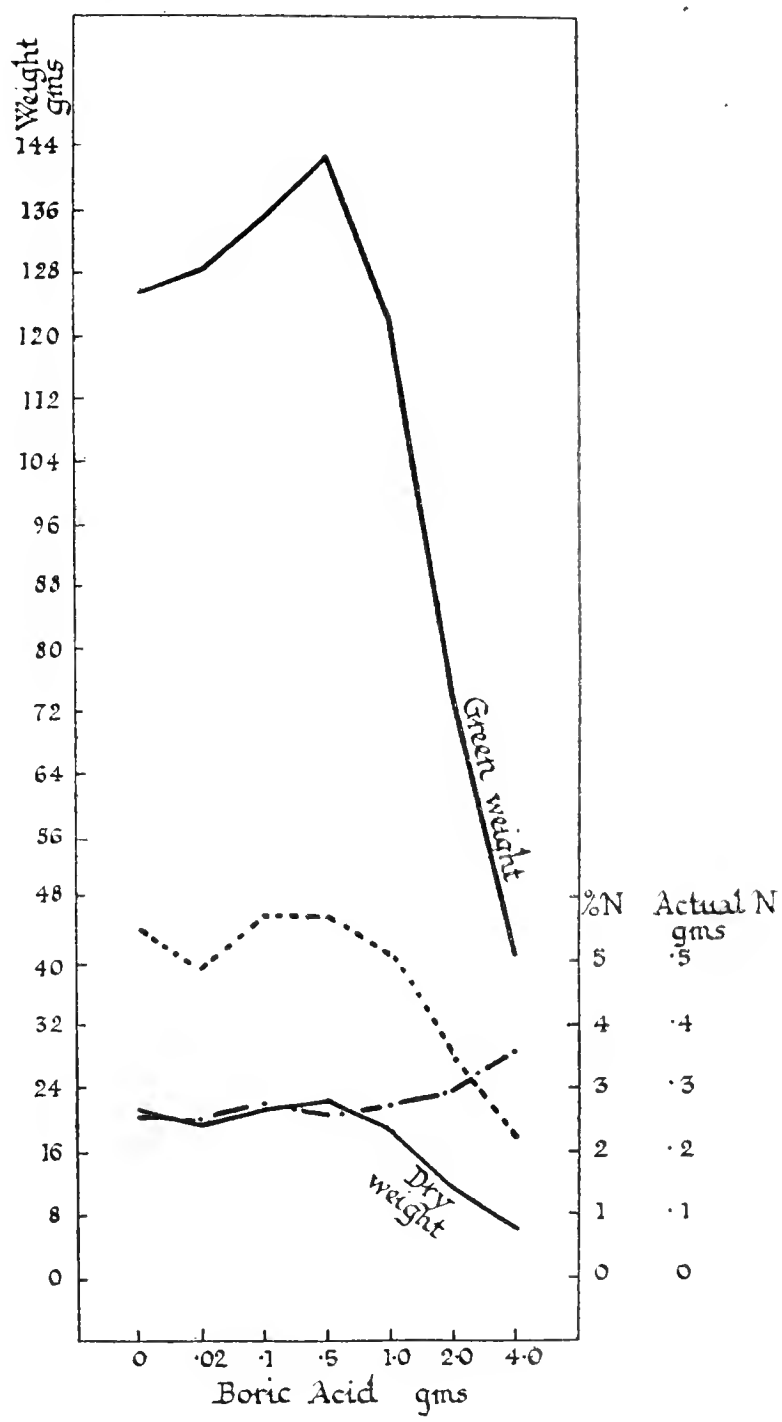
As growth went on, the plants became light green, but the leaves turned brown along their margins in a manner similar to that described in the water-culture experiments. Spotting of the leaves, as was seen in barley, never occurred. The injury was at first apparent in the lower leaves, but nearly all of them showed it later. Growth was very slow, but eventually several of the plants flowered.

2 grm. H_3BO_3 per pot were decidedly less toxic than 4 grm. (Table VI), germination being less retarded and the plants flowering better; however, the leaves were inclined to be yellowish and showed signs of quite severe poisoning; where only 1 grm. H_3BO_3 was applied, toxic effects were rare, brown leaf-margins being seen in isolated cases only, and the green and dry weights being only slightly depressed (Table VI). Plants receiving lower dressings were quite similar in colour and general appearance to the controls, though some superiority in height was evident where 0·5 or 0·1 grm. had been applied. This apparent stimulation was also noticeable in the green weight, an increase of 14·4 per cent. being obtained with the larger quantity; the rise in dry weight, however, was practically negligible (Table VI and Text-fig. 5). Neller and Morse (27) have noticed a similar indication of stimulation to the eye in the case of *Phaseolus* in pot culture, but here also no increase in the dry weight was realized, even the beneficial appearance having disappeared at the close of the experiment.

The untreated plants were in every way perfectly normal: the phenomenon of 'dying off', of universal occurrence in broad bean plants grown in water culture without boron, did not occur in a single instance; in fact the pot-culture control plants closely resembled the sets in water culture which were supplied with low concentrations of boric acid.

[1] The H_3BO_3 was sprinkled evenly over the surface of the soil and the pots watered.

Since small quantities of boron compounds are common constituents of the soil, and traces have been detected in the soils used in this pot-culture work, it is probable that the plant is able to absorb sufficient of the element from the untreated soil to make normal development: if this were not so, it would be difficult to account for the success with which broad beans are usually cultivated in garden soil, though it is still an open question whether growth might not be improved on occasions by the addition of further small quantities of boron compounds.



TEXT-FIG. 5. Broad beans grown in pot culture with various quantities of boric acid mixed throughout the soil. Mar. 4–May 31, 1921 (average of five pots): —— Weight; —·—·—· per cent. Nitrogen; - - - - - Actual N.

(*b*) *Boric acid applied as a top-dressing.* Applied as a top-dressing, boric acid was more toxic than when the same quantity was mixed throughout the soil, and this is borne out by the green and dry weight figures (Table VI). The application was made twenty-four days after sowing. All the plants died when treated with 4 grm. or 2 grm. H_3BO_3 per pot, and even 1 grm. proved very injurious, only a few plants surviving for a short time.

The exceptional drought conditions of 1921, in spite of regular watering of the pots, may have caused this result to be unduly accentuated, but it is to be expected that the boric acid would have a more marked effect on the plant when concentrated in the upper soil layers where the young roots were developing. 0·5 grm. H_3BO_3 per pot was also decidedly injurious, the plants showing the typical leaf poisoning; but 0·1 grm. or 0·02 grm. caused no toxic effect, the plants in the former set being even slightly taller than the controls, while in both cases a small increase over the untreated plants was obtained in the green and dry weights (Table VI).

β. Comparison between the Effect of Boric Acid and Borax in the Soil.

In this series $Na_2B_4O_7 + 0{\cdot}10\ H_2O$ was tested simultaneously with

boric acid, equivalent amounts of boron being supplied in each case (1·0 boric acid = 1·54 borax). The top-dressings were applied at the time of sowing.

1 grm. H_3BO_3 was the heaviest dressing given in this experiment, as any larger quantity was strongly toxic. Germination was decidedly retarded and the plants were yellowish green with brown-edged leaves. The corresponding borax set were also very poor plants, though they apparently suffered rather less injury than those treated with the equivalent quantity of boric acid. Some indication of the more poisonous nature of H_3BO_3 is evident from the green weight value, but no contrast is shown in the dry weights (Table VI).

Haselhoff (20) found that borax is less injurious to *Phaseolus vulgaris* in soil culture than a similar quantity of boric acid, but in the present case an even larger amount has proved less toxic.

0·5 grm. boric acid per pot was again slightly more toxic than the corresponding amount of borax, but the injury in both cases was much less severe than where the heavier dressings had been supplied. Still lower quantities of boric acid or borax caused no toxic effect at all, the drop in the green and dry weight of the borax set being most likely due to individual variation (Table VII). However, no beneficial effect was apparent either, both sets of plants closely resembling the controls in colour, height, general appearance, and green and dry weight.

The difference in the degree of injury caused by a dressing of 1 grm. boric acid in this case and the previous experiment seems to require some explanation (Tables VI and VII). The boric acid was applied at different stages in the growth of the plant in the two cases, but a subsequent experiment has shown that under similar conditions no great difference, at least in the dry weight, results from an alteration in the time of application. The great contrast in the seasons 1921 and 1922 more probably accounts for the discrepancy between the results. From this it seems probable that, though borax may be slightly less toxic, there is little if any real differerence between the action of borax and boric acid; which points to the fact that the element boron is the important factor in both cases, as Peligot (28) showed in 1876.

In order to determine whether these dressings had any marked effect on the root system as well as on the shoot, several roots were washed out from the soil, typical plants being chosen. It was found that in the case of plants treated with either 1 grm. of H_3BO_3 or the corresponding quantity of borax, the roots were long and not at all plentiful, being especially scarce in the surface layers, but tubercle formation was apparently normal. On the other hand, plants receiving a small dose of H_3BO_3, e.g. 0·1 grm., had a large root system similar to the untreated plants. A striking contrast between the root growth of the control plants and that of the heavily

treated sets was seen, for in the former case only were the roots well developed near the surface, though as regards total length the two cases were very similar. This is quite in accordance with what might be expected, for the roots in the heavily treated pots would natually make most growth in the less toxic region, i. e. other than the surface soil which had received the dressing. A similar result has been described by Breckenridge (10), but as he applied the borax below the seed, the roots developed mainly at the surface, which was in this case the less toxic soil layer.

γ. Comparison between the Application of Boric Acid at two Different Stages of Growth.

In the preliminary experiment *α* (*b*) the dressing was applied some time after sowing, but in the succeeding case *β* the soil was treated before germination had taken place.

Since the time of application of manurial dressings is of such importance in agricultural practice, a series of pots were set up to ascertain if there

TABLE VI.

Mean Green and Dry Weights of Broad Beans grown in Pot Culture.

Average of 5 or 6 pots. 2 plants per pot.[1]

H_3BO_3 per pot.	*Mar. 4–May 31, 1921. Mixed throughout the soil.*		*April 15–June 29, 1921. Top-dressed 25 days after sowing.*		*July 4–Sept. 23, 1922. Top-dressed directly after sowing.*		*17 days later.*	
	Green Wt.	*Dry Wt.*	*Green Wt.*	*Dry Wt.*	*Green Wt.*	*Dry Wt.*	*Green Wt.*	*Dry Wt.*
grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
4·0	41·10	6.20	0·0	0·0	—	—	—	—
2·0	74·10	11·69	0·0	0·0	—	—	—	—
1·0	122·37	18·49	0·0	0·0	—	—	—	—
0·5	143·35	22·03	65·32	11·92	125·18	15·81	117·54	14·93
0·1	135·63	21·16	118·84	21·76	152·56	20·70	114·85	19·60
0·02	128·05	19·70	122·32	21·88	155·83	20·59	160·36	21·23
Control	125·34	21·33	114·58	20·95	159·16	21·28	159·16	21·28

was any difference in the effect of the boric acid dressing when added immediately after sowing or when the plants were several inches high, both sets of plants being grown under strictly comparable conditions.[2]

Accordingly 0·5 grm., 0·1 grm., and 0·02 grm. H_3BO_3 per pot was applied as a top-dressing—

(1) At the time of sowing.

(2) Seventeen days later.

In the first case, toxic effects such as retardation of germination and leaf injury were noticeable where 0·5 grm. H_3BO_3 had been applied, and even 0·1 grm. proved slightly unfavourable (Table VI). Where the dressing was

[1] Pots containing only single plants were omitted in the mean.

[2] Infestation with aphis was successfully checked by spraying with 0·15–0·2 per cent. nicotine in soap solution of 1 oz. per gallon. This had no apparent effect on experimental results.

not applied until considerable growth had been made, 0·5 grm. H_3BO_3 per pot quickly caused signs of poisoning and a marked retardation in growth, the plants changing from a healthy green to a yellowish colour, and the leaves showing the typical brown margins. 0·1 grm. H_3BO_3 added at this stage, however, had only a very slight toxic effect.

At the close of the experiment there was no apparent difference between the plants treated with boric acid at the two stages of growth, though the dry and especially the green weights of the latter treated sets were slightly lower than those which received the dressing before germination (Table VI).

General conclusions.

(1) Applications of over 1 grm. of boric acid were injurious when mixed throughout the soil, though 0·5 grm. proved toxic if applied as a top-dressing.

(2) In two instances a possible increase in the green weight was obtained with small quantities of H_3BO_3.

(3) Borax was possibly slightly less toxic than an equivalent amount of boric acid.

TABLE VII.

Mean Green and Dry Weights of Broad Beans grown in Pot Culture with Boric Acid and Borax containing equivalent Boron. (Quantities given in terms of H_3BO_3.)

Average of 6 *pots.* 2 *plants per pot.*
Top-dressed immediately after sowing, Mar. 18–*June* 16, 1922.

	Boric Acid.		*Borax.*	
H_3BO_3 per pot.	*Green Weight.*	*Dry Weight.*	*Green Weight.*	*Dry Weight.*
grm.	grm.	grm.	grm.	grm.
1·0	76·10	12·21	81·80	12·18
0·5	113·61	18·71	116·25	18·31
0·1	133·18	21·83	128·28	20·59
0·02	134·18	21·05	129·59	22·16
Control	133·43	21·54	133·43	21·54

B. *Barley.*

The seed for these pot-culture experiments was the same as that used in the water-culture work; it was graded for each series, three plants being grown per pot.

a. *The Effect of adding Various Quantities of Boric Acid to the Soil.*

A wide range of concentrations of H_3BO_3 was tried: (*a*) Mixed throughout the soil; (*b*) applied as a top-dressing.

(*a*) *Boric acid mixed throughout the soil* (22½ lb.). From the outset

barley was clearly more susceptible to boric acid injury than the broad bean, for the toxic effect was so severe where 4 grm. H_3BO_3 per pot was applied, that plants survived in two cases only. The injury was first evident in a strongly marked retardation in germination; the young shoots were yellow or even pink when they first appeared, but though they turned slightly green after about ten days, the majority of them failed to recover. The few survivors were extremely poor and scarcely tillered, and their leaves were badly spotted in a manner similar to that described for barley in water-culture experiments. Agulhon (1 (*a*)) has stated that this peculiar reddish tinge is a typical symptom of boron-poisoning in cereals.

The crystalline substance deposited on the evaporation of the droplets of liquid which were exuded from the hydathode at the tip of the leaf-blade was collected from these chlorotic seedlings for several days in succession, the crystals from the control plants being collected separately at the same time. These two crystalline deposits were tested for boron by the following method, both a minute quantity of H_3BO_3 and distilled water being similarly tested as checks.

The crystals were dissolved in a drop of 95 per cent. alcohol; two or three drops of acetic acid and alcoholic turmeric were added, and with the further addition of a few c.c. of distilled water the whole was evaporated to dryness on a water-bath. The residue in the case of the crystals from the chlorotic seedlings was reddish-brown in colour, and turned blue-black on the addition of a drop of NaOH, indicating the presence of boron. However, the residue from the crystals from the control plants remained yellow-brown in colour.

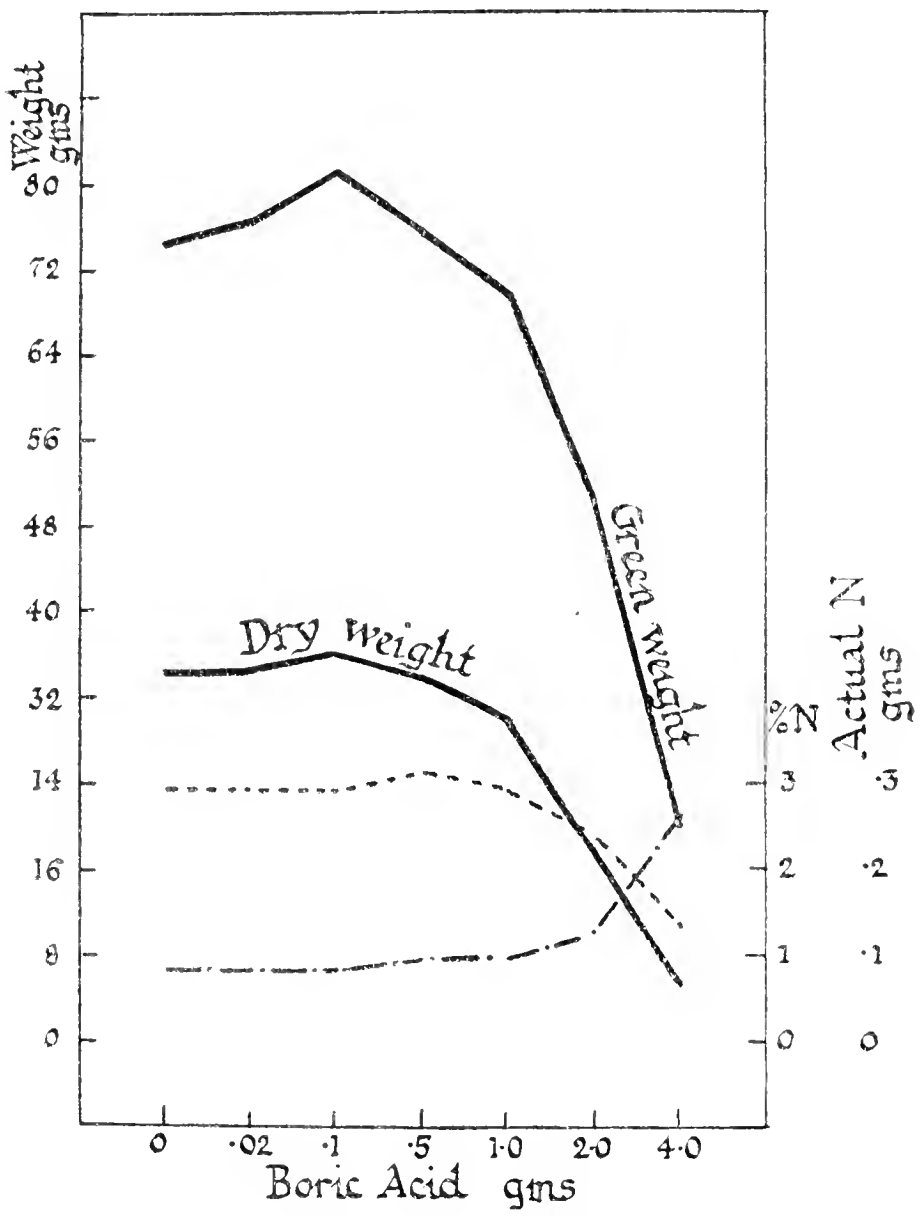
It would seem, therefore, that the boron added to the soil was taken up by the roots and passed up through the plant by the transpiration stream, as has been described by Free (19) in the case of *Pelargonium*; consequently the injury evident in these plants is most probably due to the presence of boric acid in the plant tissues.

Morse (26) has described a similar exudation of droplets from the leaf-margins of potatoes grown in pot culture in his experiments on borax in fertilizers. However, he makes no mention of testing such droplets for the presence of boron, though he detected it in the brown parts of the injured leaves. The grey deposit on the surface of the soil similar to that seen in the broad bean pot cultures was also noticed here.

An application of 2 grm. H_3BO_3 per pot was not nearly so injurious as 4 grm. (Table VIII), though germination was distinctly retarded and three of the fifteen plants died. Many of the young shoots were yellow or pink in colour, but they all turned green later and showed the typical brown flecking of the leaves. As fresh growth was made it rapidly became affected in the same way. All the plants tillered and came into ear: but the most striking feature of this set was their lateness in ripening, for when the

controls and most of the other sets were fully mature, these plants were quite green. Voelcker (36) has described a similar retardation in his experiments on the action of boron on wheat and barley.

1 grm. H_3BO_3 was also injurious, though the poisoning effects were less evident than where heavier dressings had been given (Table VIII); only slight injury in the early stages of growth was apparent in plants grown with 0·5 grm. H_3BO_3, and lower dressings produced no harmful effect at all; in fact 0·1 grm. seemed slightly beneficial, and a decided increase in the



TEXT-FIG. 6. Barley grown in pot culture with various quantities of boric acid mixed throughout the soil. Feb. 24–July 8, 1921 (average of five pots): —— Weight; —·—·—·— per cent. Nitrogen; - - - - - - Actual N.

green weight was obtained, though the rise in the dry weight was only very slight (Table VIII and Text-fig. 6). These plants, however, matured slightly later than the controls and showed a few isolated cases of leaf spotting in the early stages. The untreated plants were in every way healthy and well grown, closely resembling the sets treated with the smallest quantities of boric acid in appearance and dry weight (Table VIII).

(*b*) *Boric acid applied as a top-dressing fifteen days after sowing.* Though barley suffered more injury than broad beans when the boric acid was mixed throughout the soil, in the top-dressing experiments the reverse occurred. Whereas even 1 grm. per pot completely killed all broad bean

plants, barley was able to make some growth where a 2 grm. dressing had been applied (Table VIII). Difference in seasonal conditions cannot in any way be responsible for this result, as the corresponding experiments with beans and barley were set up on approximately the same dates. The duration of the experiments naturally differed owing to the longer growth period of barley, but since the injury was always most severe in the early stages of growth, this has probably little to do with the results. The toxic effects were exactly similar to those previously described for barley grown in pot culture.

A dressing of 4 grm. H_3BO_3 per pot completely killed all the plants, and though application of 2 grm. or 1 grm. per pot was not sufficient to prevent growth, yet the plants were severely injured (Table VIII), no ears being formed in the former sets, and the ripening off being noticeably retarded in both cases. Where 0·5 grm. H_3BO_3 was applied, this lateness in maturing was still seen and the leaves were badly spotted; however, a rise occurred in the green weight, though the dry weight was decidedly depressed (Table VIII). The lower dressings caused no injury, leaf spotting being evident in isolated cases only; in fact the effect was slightly beneficial, a distinct rise in the dry weight being obtained with the 0·1 grm. application (Table VIII), and both this set and the plants treated with 0·02 grm. appeared to the eye rather better than the controls, though similar in degree of maturity.

β. Comparison between the Effect of Boric Acid and Borax in the Soil.

The dressings of boric acid and borax were made immediately after sowing in this case, 1·0 boric acid being equivalent to 1·54 borax.

1 grm. boric acid and the corresponding borax application caused a marked retardation in germination, and the shoots were of the characteristic yellow-pink colour; boron was again detected in the liquid exuded from these chlorotic seedlings.

Contrary to what occurred in the case of the broad bean, borax seemed more injurious than boric acid to barley, a result similar to that which Voelcker (36) obtained in 1915. For example, fewer plants died in the set grown with 1 grm. of H_3BO_3, and the survivors made an earlier recovery and remained considerably better for some time than the corresponding borax-treated plants. However, at the close of the experiment there was little to choose between them, ears being formed in all cases and a similar retardation in maturing being apparent; both the green and dry weights also were very similar (Table IX). This may possibly be explained on the ground that the more soluble borax would permeate the soil more quickly, and therefore affect the plant more severely at first, but, since the quantity of boron in both cases was the same, the ultimate effect of the two compounds would be similar. The dark blue-green colour of these sets was

suggestive of plants supplied with excessive nitrate, and the analogy was carried further in their lateness in maturing.

0·5 grm. H_3BO_3 was also decidedly injurious, and the equivalent dressing of borax again proved more toxic, being in this case accompanied by a slight decrease in dry weight (Table IX). The injury was most apparent in quite the early stages of growth; the retardation in ripening was only very slight. Where 0·1 grm. H_3BO_3 or the equivalent quantity of borax was applied, toxic effects were rare, and at the close of the experiment these plants were slightly superior both to those receiving lower dressings and the controls; however, no stimulation was apparent from either the green or dry weight figures as in the previous experiments (Table IX). This discrepancy may be due to the seasonal conditions under which the experiments were respectively carried out, or possibly the beneficial effect may only be realized in the weight of the plant at a certain stage in its growth; in the one case, the plants may have been harvested before that stage had been reached, and further, such a stage may itself depend on seasonal conditions, being reached sooner in some years than in others.

TABLE VIII.

Mean Green and Dry Weights of Barley grown in Pot Culture.

Average of 5 *pots.* 3 *plants per pot.*[1]

	Mixed throughout soil. *Feb.* 24–*July* 8, 1921.			*Top-dressed* 15 *days after sowing.* *April* 15–*Aug.* 6, 1921.		
H_3BO_3 per pot.	*Green Wt.*	*Dry Wt.*	*% Dry in Green.*	*Green Wt.*	*Dry Wt.*	*% Dry in Green.*
grm.	grm.	grm.		grm.	grm.	
4·0	20·15	5·10	25·30	0·0	0·0	—
2·0	50·44	18·34	36·37	13·89	5·18	37·29
1·0	69·70	29·99	43·03	26·68	9·99	37·44
0·5	75·92	33·86	44·61	41·56	16·00	38·50
0·1	81·26	35·90	44·18	39·83	26·16	65·67
0·02	76·74	34·39	44·81	32·35	22·07	68·21
Control	74·73	34·30	45·90	34·29	21·82	63·64

Agulhon (1 (*a*)) has attributed the increase in the green weight value of plants grown with even slightly toxic doses of boric acid to an increase in water-holding capacity resulting from a state of over-mineralization, induced by the absorption of more than normal quantities of boron. Such an increase in water-content is shown up well by the values for the percentage of the dry in green weight in the various sets (Tables VIII and IX). In the case of the heavily treated plants which were so strikingly late in maturing, the value is very little more than half that of the controls, but the figure approximates to normal in those sets which show definite injury with a comparatively light dressing of boric acid.

[1] Pots containing fewer than three plants were included in the mean, as they only occurred in heavily treated sets and were not caused by normal failure of germination.

General conclusions.

1·0 grm. or more H_3BO_3 per pot caused a depression in the dry weight, though 0·5 grm. was harmful if applied as a top-dressing. Possibly 0·1 grm. was slightly beneficial, though lower dressings were ineffective.

2. Borax was slightly more toxic than an equivalent amount of boric acid.

The probable errors have been worked out in typical cases for both barley and broad beans grown in pot culture. With barley treated with a top-dressing of boric acid (Table VIII), the error for the green weight ranges from ± 1·56 to ± 0·24, and for the dry weight from ± 0·67 to ± 0·28.

Table IX.

Mean Green and Dry Weights of Barley grown in Pot Culture with Boric Acid or Borax containing equivalent Boron. (Quantities given in terms of H_3BO_3.)

Average of 6 pots. 3 plants per pot.[1]
Top-dressed immediately after sowing. Mar. 15–Aug. 5, 1922.

	Boric Acid.			*Borax.*		
H_3BO_3 per pot.	*Green Wt.*	*Dry Wt.*	*% Dry in Green.*	*Green Wt.*	*Dry Wt.*	*% Dry in Green.*
	grm.	grm.		grm.	grm.	grm.
1·0	34·63	13·08	37·70	34·68	13·48	38·87
0·5	35·01	18·65	53·20	35·25	16·42	46·58
0·1	33·65	21·49 [2]	63·86	32·71	21·35	65·27
0·02	30·48	21·64	70·99	33·37	22·71	68·00
Control	35·64	— [2]	—	35·64	— [2]	—

Table X.

Nitrogen in Dry Matter. Broad Beans grown in Water Culture.

Concentration of H_3BO_3.	*Mar. 10–May 17, 1921.*		*April 28–June 13, 1921.*		*Sept. 3–Oct. 17, 1921.*	
	% N.	*Actual N.*	*% N.*	*Actual N.*	*% N.*	*Actual N.*
		grm.		grm.		grm.
1 : 5,000	4·04	0·22	2·94	0·20	4·55	0·16
1 : 25,000	3·19	0·36	2·61	0·21	4·31	0·23
1 : 50,000	3·07	0·41	2· 6	0·21	4·41	0·22
1 : 100,000	3·31	0·38	2·61	0·22	4·23	0·18
1 : 500,000	3·27	0·36	2·61	0·21	4·24	0·22
1 : 2,500,000	3·02	0·38	2·72	0·23	4·11	0·20
1 : 12,500,000	3·31	0·38	2·93	0·19	4·30	0·22
1 : 100,000,000	3·08	0·27	3·42	0·18	—	—
Control	3·41	0·31	3·48	0·15	4·35	0·11

[1] Pots containing fewer than three plants were included in the mean, as they only occurred in heavily treated sets and were not caused by normal failure of germination.

[2] Figures not available or mean of two pots only through accident.

Table XI.

Nitrogen in Dry Matter. Broad Beans grown in Pot Culture.

H_3BO_3 *per pot.*	*Mixed throughout the soil.* *Mar.* 4–*May* 31, 1921.		*Top-dressed* 25 *days after sowing.* *April* 15–*June* 29, 1921.		*Top-dressed directly after sowing.* *Mar.* 18–*June* 16, 1922.			
					Boric Acid.		*Borax.*	
	% N.	*Actual N.*	*% N.*	*Actual N.*	*% N.*	*Actual N.*	*% N.*	*Actual N.*
grm.		grm.		grm.		grm.		grm.
4·0	3·59	0·22	—	—	—	—	—	—
2·0	2·95	0·35	—	—	—	—	—	—
1·0	2·74	0·51	—	—	3·46	0·42	3·32	0·40
0·5	2·57	0·57	3·22	0·38	3·28	0·61	3·13	0·57
0·1	2·67	0·57	2·51	0·55	2·58	0·56	2·92	0·60
0·02	2·50	0·49	2·62	0·57	2·77	0·58	2·95	0·65
Control	2·59	0·55	2·61	0·55	3·00	0·65	3·00	0·65

Table XII.

Nitrogen in Dry Matter. Barley grown in Water Culture.

Concentration of H_3BO_3.	*Feb.* 21–*May* 24, 1921.		*Mar.* 1–*May* 11, 1922.			
			Boric Acid.		*Borax.*	
	% N.	*Actual N.*	*% N.*	*Actual N.*	*% N.*	*Actual N.*
		grm.		grm.		grm.
1 : 5,000	3·87	0·06	—	—	—	—
1 : 25,000	2·80	0·27	—	—	—	—
1 : 50,000	2·42	0·36	4·26	0·23	4·25	0·21
1 : 100,000	2·36	0·36	3·70	0·24	3·39	0·23
1 : 500,000	2·09	0·39	3·54	0·24	2·93	0·23
1 : 2,500,000	1·78	0·35	3·34	0·22	3·02	0·25
1 : 12.500,000	1·54	0·37	3·10	0·27	2·99	0·25
1 : 100,000,000	1·88	0·34	3·01	0·26	2·60	0·25
Control	1·63	0·35	2·59	0·25	2·59	0·24

Table XIII.

Nitrogen in Dry Matter. Barley grown in Pot Culture.

H_3BO_3 *per pot.*	*Mixed throughout the soil.* *Feb.* 24–*July* 8, 1921.		*Top-dressed* 15 *days after sowing.* *April* 15–*Aug.* 6, 1921.	
	% N.	*Actual N.*	*% N.*	*Actual N.*
grm.		grm.		grm.
4·0	2·52	0·13	—	—
2·0	1·24	0·23	3·37	0·18
1·0	0·96	0·29	2·87	0·29
0·5	0·90	0·31	2·30	0·37
0·1	0·82	0·29	1·48	0·39
0·02	0·84	0·29	1·51	0·33
Control	0·83	0·29	1·51	0·33

In the case of broad beans, however, the individual variation is very great, and the probable error for the series treated with boric acid mixed throughout the soil (Table VI) ranges from ± 7·35 to ± 1·92 for the green

weight, and for the dry weight from ± 1·09 to ± 0·37. The only conclusions that may be considered justifiable are therefore those relating to the boron injury; beneficial effects in pot culture, though apparent to the eye in some cases, must be regarded as questionable.

3. *Field Experiments.*

A. *Broad Beans.*

Three plots, each of area $\frac{1}{50}$ acre, were laid out in triplicate. All received a basal dressing of 3 cwt. superphosphate, 1 cwt. sulphate of potash, and $\frac{3}{4}$ cwt. sulphate of ammonia per acre. In addition, three plots received 20 lb. H_3BO_3, and three 8 lb. H_3BO_3 per acre; the manures were applied as a top-dressing at the time of sowing. Owing to unfortunate weather conditions and a lack of uniformity in the seed sowing and the soil, the results regarding possible beneficial effects from the dressings were inconclusive, though no injury was apparent. However, in the three plots which were strictly comparable, a 10 per cent. increase over the control was obtained in the yield of pods, harvested when green, from the plots receiving 8 lb. boric acid per acre.

B. *Barley.*

A similar series of plots were sown with barley. The mineral dressings were the same as for the broad bean plots, except that $1\frac{1}{2}$ cwt. per acre of sulphate of ammonia was supplied. The manures were applied as a top-dressing just before sowing. No injurious or beneficial effects were evident from either of the dressings, and any differences in the yields from the various plots were well within the limits of experimental error.

III. General Discussion.

As boron is of such importance to the broad bean, the question naturally arises as to the part played by the element in the plant metabolism. It may be regarded on the one hand as an element similar in function to C, H, O, N, S, P, &c., the only difference being the very small quantity of boron required; or, on the other hand, it may be classed as a catalytic agent not of itself directly useful, but able to aid some plant function in an indirect manner. Such a catalyst may even be essential for the normal functioning of the plant; for example, Bertrand (4 (*b*)) has shown that the manganese in laccase is an oxygen carrier necessary for the functioning of the enzyme.

Agulhon (1 (*a*)) recognizes a class of elements which he terms 'particuliers'; these he considers are characteristic of certain groups of individuals or of life under certain conditions, and may be subdivided into two groups, (1) of nutritive, (2) of catalytic function; boron he regards as an example

of the latter. But since a catalyst is essentially a substance not itself used up during the reaction upon which its presence depends, and since it is evident from the present work on the broad bean that the supply of boron must be continual in order to be effective, it would seem more probable that the action of the element is nutritive, and that it is in some way fixed by the plant and not in a state of circulation.

It has been shown by a number of workers on the subject, that certain of the Leguminosae readily respond to the action of boron compounds, yet even in this comparatively small group of plants a great difference in the degree of response is shown. Boron is apparently essential to the broad bean, and, as far as preliminary evidence goes, probably also to several other plants, such as the runner bean, dwarf bean, and crimson clover; while peas, wild white clover, and winter vetch appear to be more or less independent of its presence, though even in these cases some slight beneficial effect is evident from the addition of small quantities of H_3BO_3 to the nutrient solution.

The importance of the conditions under which the plant is growing on the reaction towards boron has been frequently seen during this investigation. For example, both the degree of toxicity of the same quantity of boric acid and the type of root developed in water culture by the untreated plants were found to vary with seasonal conditions, though the main results were independent of any such changes. High concentrations of boric acid were, for instance, considerably more injurious to broad beans grown in early spring or autumn than during the summer months, due probably to the fact that at such seasons growth was less vigorous and the plants less resistant to poisonous agents.

The roots of the control plants both in broad beans and crimson clover were less abnormal in appearance when grown in the late autumn than when set up earlier in the year, the stunted growth being hardly noticeable in the former series or at most only after a long time. In this case also, the slow rate of growth probably accounted for the delay in the appearance of the effects of boron deficiency, the roots being able to develop normally for a considerable time.

Whatever the function of boron, it is to some extent at least independent of the food supply, whether limited or abundant, for in Brenchley's (11 (*a*)) work on the action of boric acid on peas and barley no renewal of the nutrient solution was made during the course of the experiment, yet the present results are in close agreement with this earlier work though the solutions were regularly renewed.

Nitrogen plays such a leading part in the nutrition of all plants that the effect of boron on the quantity of nitrogen absorbed is of importance. Consideration of Tables X–XIII and Text-figs. 2, 4, 5, 6 shows that the application of excessive amounts of boric acid was correlated with an

increase in the percentage of nitrogen, though the actual nitrogen absorbed was either unaffected or considerably depressed. Cook and Wilson (17 (*b*)) in field experiments found a higher percentage of N both in the straw and grain of wheat treated with boron than from the control plots; in fact it is a common experience that the presence of some injurious factor is accompanied by a rise in the percentage of nitrogen found in the plant. However, since the actual nitrogen absorbed is not increased by the presence of toxic agents, the rise in the percentage of nitrogen must be attributed mainly to a reduction in the output of dry matter of which the chief constituents are the carbohydrates, and not solely to the direct influence of the injurious factor on the intake of nitrogen. In the case of barley grown in water culture most of the concentrations of H_3BO_3 were toxic and the nitrogen percentage gradually rose as the quantity of boron was increased (Text-fig. 4). With the broad bean the majority of the concentrations were beneficial and the percentage of nitrogen remained approximately constant, irrespective of the strength of boric acid supplied, but where the dose became toxic, i. e. 1 : 5,000, a rise was obtained as in the case of barley (Text-fig. 2).

However, the unhealthy condition due to a deficiency of boron, which was seen only with the broad bean in water culture, apparently had no effect on the percentage of nitrogen, although the actual amount was considerably lower than in the well-grown plants, in some cases falling even below that of the poisoned sets.

The right interpretation of the term 'stimulation' is still an open question, for it is equally difficult to define 'normal' growth. Peas in ordinary nutrient solution, for instance, apparently develop quite normally, but when the addition of a small quantity of boron causes a still better growth, the question arises as to whether the so-called 'normal' development was not really abnormal owing to the omission of some necessary element. And also, it is equally uncertain whether the plant might not be further improved by the addition of some other substance as yet undetected.

With broad beans the case is perhaps simpler, as it is clear that growth in the so-called 'complete' nutrient solution is abnormal; but here, again, it has not yet been shown whether other substances might not be able either to supply the same need or to still further benefit the plant.

The possibility of boric acid acting merely as an antiseptic agent has been shown by Agulhon (1 (*a*)) to be quite unlikely, and in support of this, in the present experiments, moulds have occasionally developed on the roots of plants grown with toxic concentration of H_3BO_3. The acidifying action he also considered negligible, and this is confirmed by the fact that 1 : 5,000 boric acid is less acid than the normal nutrient solution used in this water-culture work, as determined by pH values. After analysing a large number of plants and plant organs for the presence of boron,

Agulhon (1 (*a*)) has put forward the idea that, as the element occurs in comparatively large quantities in the wood, it may be of especial importance in the formation of the vascular system. This view is supported by the fact that a disintegration of the vascular bundles takes place, proceeding from the apex downwards, in broad bean plants deprived of boron: this anatomical change is at present under investigation and will be described in a later paper.

Another noticeable feature in plants suffering from a deficiency of boron is that the injury is first apparent in the meristematic regions. Whereas death under normal conditions occurs first in the lower parts of the plant, 'dying off' progresses from the apex and travels downwards, so that the activities of boron appear to be closely connected with the healthy development of permanent from meristematic tissue.

The action of boron is presumably of a specific nature, since it appears to function in a different manner in different plants, possibly being in some cases an essential element, and in others of comparatively little importance.

The relationship of boron to plant life is somewhat suggestive of that between vitamines and animals, and in various ways the resemblance appears to be very close. The main lines of agreement are:

1. The comparatively small quantity of the substance required.
2. The unhealthy condition resulting from a deficiency of the substance.
3. The prevention of, or recovery from, the unhealthy condition by the addition of the substance.
4. The need for the supply of the substance to be maintained throughout life.

Accessory food factors or vitamines are utilized in small quantities quite out of proportion to the importance of their function, and similarly even 1 : 2,500,000 boric acid has been shown to be adequate for healthy growth of the broad bean. Vitamines are generally regarded as a class distinct from the better known animal food-stuffs, and it is conceivable that a similar category of accessory plant foods would afford a convenient means of classifying such substances as boron. A class of this nature was foreshadowed by Agulhon (1 (*a*)) in his 'particuliers' elements, but he was not able to demonstrate a specific case where death resulted from the deficiency of such an element.

The function of boron in the life of the plant is still an unsettled question, though it seems more probable that its action is in some way nutritive rather than catalytic; similarly it is uncertain whether vitamines 'prove to be structural components of living tissues of which a supply is essential even though quantitatively unimportant, or whether (as it is equally possible)

they are found to act rather as catalysts in certain normal processes of metabolism' (24).

But it must here be emphasized that no suggestion is made that boron is in any way analogous to a vitamine, but rather that their respective *effects* afford a striking parallel.

Summary.

1. In water culture a continual supply of boric acid appears to be essential to the healthy growth of the broad bean plant, concentrations of 1 : 12,500,000–1 : 25,000 H_3BO_3 being beneficial.

In its absence, death occurs in a characteristic manner, and the apex of the shoot becomes withered and blackened, though the addition of boric acid after these symptoms have set in results in a renewal of growth by means of new lateral shoots and roots. This type of dying never occurs in broad bean plants grown in pot culture, and it is concluded that sufficient boron is present, as a trace has been detected in the soils used.

2. The absence of boron does not cause death in barley, growth being healthy in ordinary culture solution.

3. Excess of boric acid is poisonous to the broad bean, injury being apparent with 1 : 5,000 H_3BO_3 in water culture and with 0·5 grm. or over per 22½ lb. of soil in pot culture, according to the method of application. Smaller quantities added to the soil are either without effect or cause an increase in the green weight only.

4. Boric acid is more poisonous to barley than to the broad bean; in water culture a concentration of 1 : 2,500,000 H_3BO_3 and in pot culture 1·0 grm. or 0·5 grm. per 22½ lb of soil is injurious, according to the method of application. Smaller quantities are either ineffective or slightly favourable, though the benefit is usually evident to the eye only and not shown in the dry weight.

5. Injury is marked by (1) retardation of germination; (2) first chlorosis and later brown markings of the leaves; the barley leaf becomes spotted, but that of the broad bean shows a band of brown along the margins; (3) retardation in maturing in the case of barley in soil culture.

6. Preliminary experiments show that several other plants, and especially *Phascolus multiflorus* and *Trifolium incarnatum*, appear to derive benefit from the addition of small quantities of boric acid to the nutrient solution, though rye behaves similarly to barley, and is apparently indifferent to low concentrations.

7. Boron is found to be present in considerable quantity in the dried shoots of broad bean plants grown in a nutrient solution containing no boron, and also in the seed. In garden-grown plants a larger proportion of boron was present in the pods than in either the stems or leaves. A trace

was the most detected in the barley seed or in the dried shoots of untreated barley grown in water culture.

8. The function of boron in the case of the broad bean appears to be probably nutritive rather than catalytic, since a supply is required throughout the life of the plant. A parallel is drawn between the action of boron on plants and the vitamines on animal life.

In conclusion, I wish to express my thanks to Dr. W. E. Brenchley for her ready advice and helpful suggestions throughout this investigation. I am also indebted to Professor Biffen, Mr. Martin Sutton, and Captain Hunter for the supply of seeds used in the experimental work.

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EXPLANATION OF PLATE XIII.

Illustrating Miss Warington's paper on the Effect of Boric Acid and Borax on the Broad Bean and certain other Plants.

All plants have been grown in water culture in nutrient solution.

Photograph 1. Typical root of broad bean grown without boric acid.

Photograph 2. Typical root of broad bean grown with a small quantity of boric acid.

Photograph 3. Broad bean shoot showing new growth (on left) subsequent to the addition of boric acid after the characteristic death of the main axis (on right).

Photograph 4. Broad bean showing the comparative size of shoots before and after the addition of boric acid. The small main axis (on left) was the only shoot before treatment with boric acid.

Photograph 5. Broad beans grown with various concentrations of boric acid; (left to right) 1 : 5,000; 1 : 50,000; 1 : 100,000; 1 : 500,000; control with no boric acid.

Photograph 6. Addition of boric acid to broad beans after increasing periods before treatment; (left to right) 0, 10, 20–60 days.

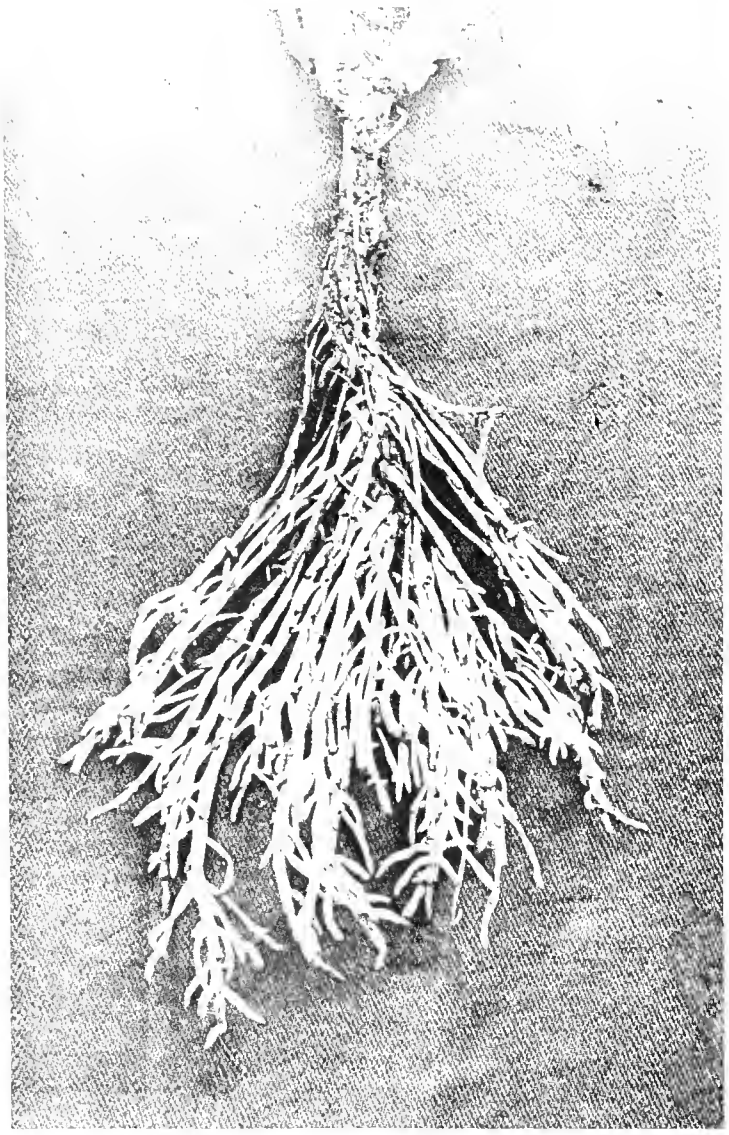
Photograph 7. Removal of boric acid from broad beans after increasing periods of treatment; (left to right) 0, 10, 20–70 days.

Photograph 8. Barley grown with various concentrations of borax; (left to right) control with no borax; 1 : 100,000,000 increasing to 1 : 50,000 (terms given as boric acid).

Photograph 9. Comparison between barley grown with boric acid (1 : 12,500,000) and borax (on right) containing equivalent boron.

Photograph 10. Preliminary result of the effect of boric acid on the runner bean. Control with no boric acid (on left); 1 : 2,500,000 (on right).

NOTE.—Since this paper went to press attention has been drawn to a paper by Addoms (Amer. Journ. Bot., x, 1923) in which the author shows that a short, stubby, branched root system (as described above on p. 634) is associated with high concentrations of potassium di-hydrogen phosphate and the consequent high hydrogen-ion concentration. However, although the nutritive solution used in these experiments contained a relatively large amount of this salt, yet it is not entirely responsible for the distinctive root growth here described, as a very similar type of root system has since been obtained when part of the phosphate was supplied in the form of the mono-hydrogen salt, thereby considerably reducing the hydrogen-ion concentration.



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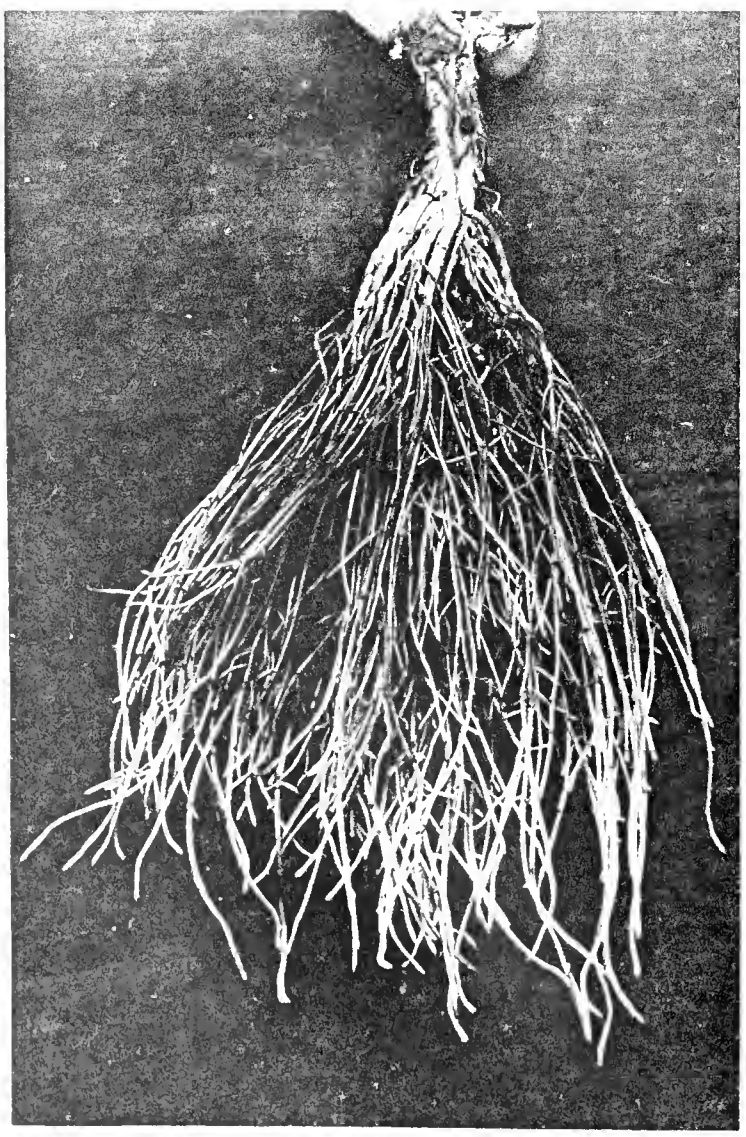

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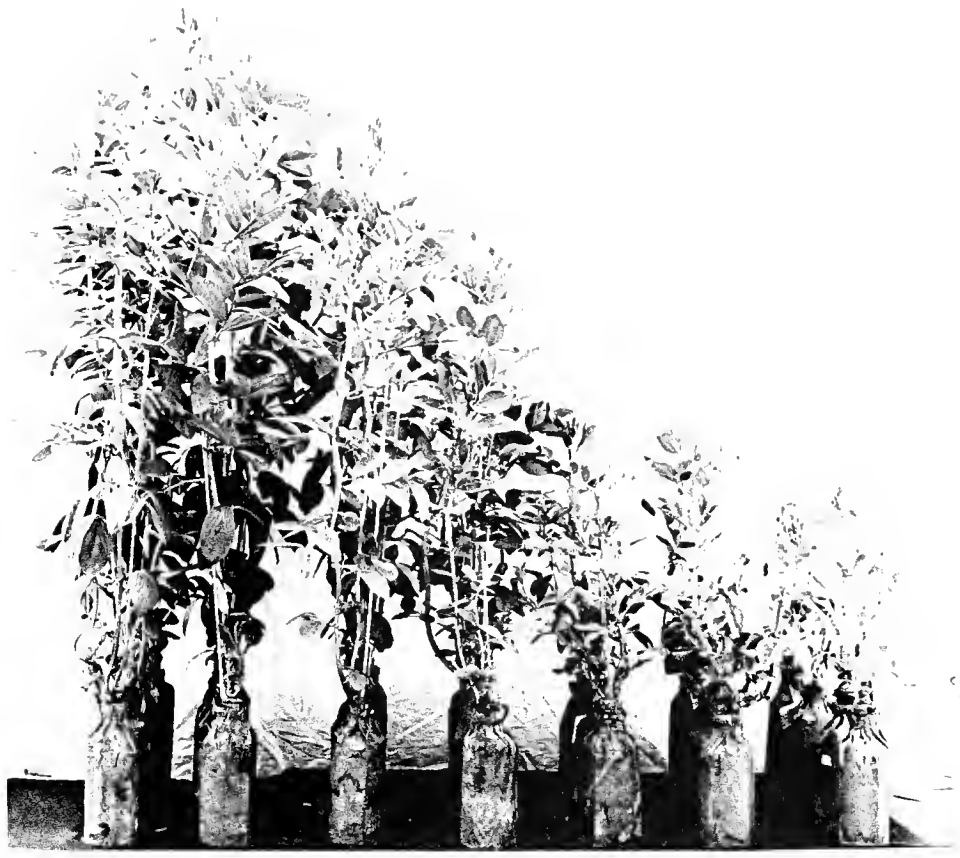

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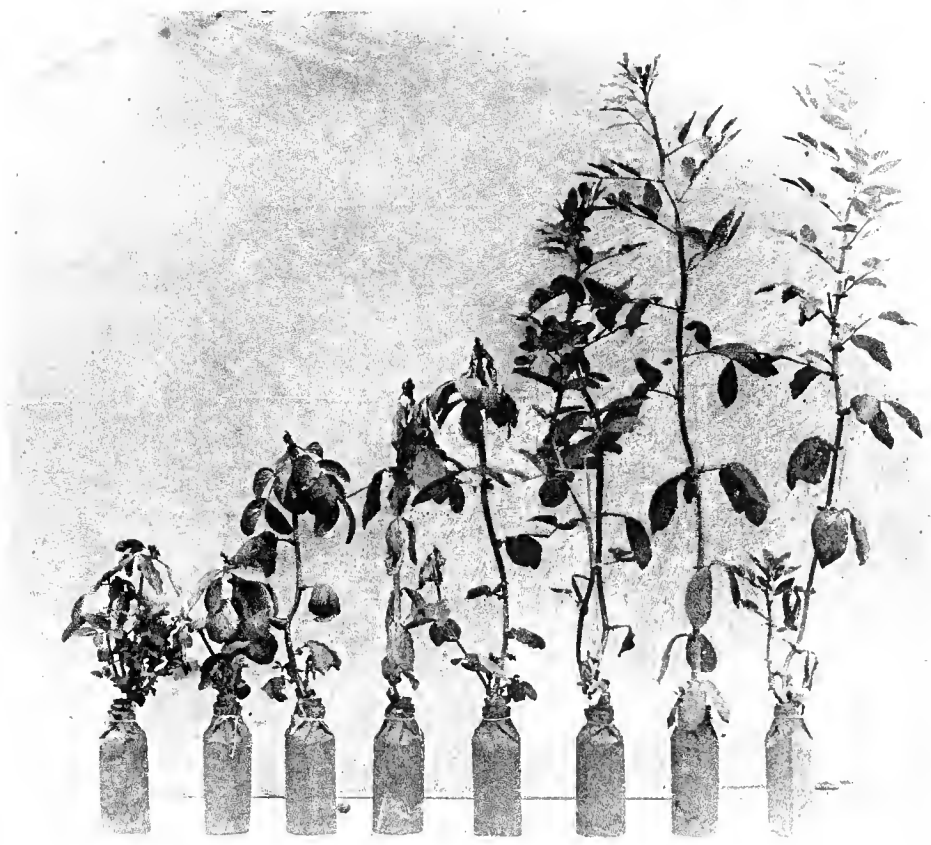

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Diphasic Liquid Systems and Bio-electrical Phenomena.—A Reply to Criticism.

BY

R. BEUTNER.

IT is generally recognized that an attempt should be made to apply physical and chemical laws to all biological phenomena. It has been a strange feature of the special field of electro-physiology that an application of this kind has been almost wanting. A number of peculiar theories were extensively discussed, viz. the 'pre-existence theory' (E. du Bois-Reymond), the 'alteration-theory' (R. Herrmann), the 'theory of ionic selective permeability' (W. Ostwald, Bernstein, and others), the 'lipoid theory' (M. Cremer), &c. Little was contributed from physical and chemical experimental work, so that a gap really existed between electro-chemistry on the one side and electro-physiology on the other.

This was partly due to the failure of older physiologists (du Bois-Reymond, Herrmann) to furnish precise quantitative measurements relating to biological electric currents. *It is principally to the merit of an English physiologist, J. S. Macdonald, to have given, for the first time, such quantitative data.* Based on an extensive series of measurements of the current in a frog's nerve, he found that a regular relation existed between the electric current of the nerve and its salt content. One of his chief conclusions may be fully quoted: '*There is no sign of any critical point marking the separation of two possible phenomena, one a function of the condition of life, and the other a physical phenomenon dominated by the salt-content of the nerve and capable of continuation after its death*' (Proc. Roy. Soc., lxvii. 310).

This most important work was one of the starting-points for the present author's investigation of the physico-chemical nature of biological currents. In the laboratory of the well-known American biologist, J. Loeb, extensive electric measurements on plants were made by him which revealed the same phenomena in a more precise manner; later the author succeeded in imitating closely these electrical phenomena by means of synthetic organic substances.[1]

[1] A description of all results obtained is found in the book of the author on Entstehung elektrischer Ströme in Geweben und ihre künstliche Nachahmung, Stuttgart, 1920.

As a detailed description of these experiments is not possible here, it may be stated that the conclusions of Macdonald, cited above, were completely confirmed. Further, it was possible to make clear the nature of the physiological law which he discovered. The logarithmic relation, between the salt content and the electromotive force, observed by him in the nerve, was traced as a property of certain systems composed of well-known organic substances (immiscible with water) and aqueous solutions.

It is very unfortunate indeed that the work of Macdonald has been entirely neglected by electro-physiologists, apparently because it contained little theoretical discussion of well-known hypotheses; it merely presented the results of a large number of careful measurements.

The majority of physiological workers were not inclined to accept such experimental results as a basis for further progress. It seemed more important to them to find new arguments *for* or *against* any of the existing theories. Macdonald's work was published in 1900 in the most prominent English scientific journal; hardly any quotation of it is found in later research work, however. No one of the contemporary physiologists pointed out the great importance of the success he had achieved. The attempt to continue the line of research inaugurated by Macdonald—which was undertaken by the author as described above—was doomed to meet the same disregard. This is clearly manifest in a criticism published by Dr. Dorothy Haynes in this Journal, a short time ago.[1]

Dr. Haynes begins her criticism by pointing out the importance of the statement 'that it is the salt content of a cell which determines its electric behaviour'. 'Such a view, if substantiated, is of fundamental importance for physiology, and no further justification will be needed for a careful review of the evidence upon which it is based.' Referring to the statement of Macdonald cited above, it is obvious that the evidence in question was brought forward by him 23 years ago; since the statement of Macdonald is based on experiments which admit of no other interpretation it is hardly possible to speak of a 'view' in this case. No priority in regard to this statement should be attributed to the present author, as is done by Dr. Haynes, who apparently was not cognizant of the work of Macdonald.

The aim of the present author was to imitate artificially Macdonald's '*phenomenon dominated by the salt content*', which he describes as not being separated from the '*function of the condition of life*'. *Some* of the numerous systems which were found to exhibit an analogy with this phenomenon contain salicylic acid. Dr. Haynes tries to prove that in these cases the effect of the salt content is *really an effect of an acid as the adding of a neutral salt is assumed to increase the acidity.* This would mean that adding 1/10 mol. NaCl (or KCl) should decrease the pH value of a saturated

[1] Vol. xxxvii, p. 96, 1923.

solution of salicylic acid from about *3* (which is roughly the pH of saturated salicylic acid) to at least *1*, since the electromotive force of the system

saturated aqueous solution of salicylic acid without KCl	oil intermediate conductor, saturated with salicylic acid	saturated aqueous solution of salicylic acid containing KCl

is higher than 0·12 volt (compare table on page 147 and 148 of the book of the author), and since each 0·057 volt corresponds to a decrease of the pH value by one unit, as is well known. A decrease of pH from 3 to 1 would mean an increase of the H-ion concentration of about 1/1000 normal HCl to 1/10 normal HCl, and yet such a concentration is brought about by the mere addition of KCl. Although the impossibility of this assumption is apparent, the author has performed a measurement of the pH of a saturated solution of salicylic acid (= 2, 8) which appeared not to be *affected to more than* 0·1 by the adding of 1/10 mol. KCl.[1]

No experimental evidence relating to salicylic acid, nor any quantitative calculation of the pH-values, is found in the criticism of Dr. Haynes. She asserts, however, that the author 'has altogether failed to substantiate his statement that salts rather than acids play the predominating rôle in the systems he investigates'. 'A careful review of the experimental evidence shows the differences of potential which he obtains can be correlated with a difference of H-ion concentration whenever free acid is present in the oil and that the theory of salt action is derived from a misinterpretation of the complex chemical systems with which he [the author] deals.'

In connexion with these assertions another experimental conclusion of Macdonald may be quoted here: 'Solutions of NaOH, HCl, NaCl, or KCl mainly affect the demonstrable value of the demarcation source according to their concentration.' This shows the impossibility of potential differences correlated with the H-ion concentration being present in tissues, since pH is, of course, affected by HCl-concentrations in the opposite sense to that by NaOH-concentrations.[2]

No further details of the physio-chemical objections raised by Dr. Haynes need be discussed here, part of them being due to ascribing assumptions to the author which he has never put forward, e. g. the assumption of

[1] In an article in the Biochem. Journal, xv. 440-61 (1921), Dr. Haynes describes measurements on the action of salts upon buffer solutions and finds that the adding of N/2·5 KCl decreases pH from 6.88 to 6.7 (with a phosphate mixture instead of salicylic acid). It is obvious that in this case, also, the decrease of pH is far too small to account for such powerful electromotive effects as may be produced by the salt content.

[2] Macdonald's statement is based on experiments with animal nerve. A similar experiment on plants was described by J. Loeb and the author (Biochem. Zeitschr., lxi. 15, 1912). It was found that the potential difference on the cuticle of a plant was not changed if HCl or NaOH was added while the salt content was kept constant. The large range of pH between 3 and 11 was found to be without any influence. It is very striking to compare these results with the statement cited above, that 'the potential difference can be correlated with a difference of H-ion concentration'.

the non-existence of diffusion potentials in 'oils'.[1] Many details relating to the book of the author are described by Dr. Haynes as 'curious mis-interpretation', 'faulty interpretation', or as 'being very hard to understand', for all of which she appears to think the author is to be blamed.

In order to solve the problem of the origin of electric currents in tissues it seemed of prime importance *to find substances which actually reproduce electromotive phenomena observed with a tissue.* After years of experimental work the author succeeded in finding some such substances. This could only be done by *experiments*; the detailed questions about the physical nature of the electromotive action of those substances could be solved only after they had been found. In the old electro-physiological theories, however, no definite substances were known which might have been responsible for electro-physiological effects. Instead, hypotheses were put forward—e. g. in the 'membrane theory'—about the possible properties of the 'plasma membrane', the composition of which was entirely unknown.

The author is well aware of the need of further developing the results obtained so far. Every criticism would be welcome which—on the basis of experimental evidence—would indicate what substances might serve as better models for the reproduction of biological currents. It is hard to understand, however, how a justified criticism can be based on the statement that the new method 'cannot take us very far in the interpretation of the cellular mechanism of the cell.'[2]

Such a statement indicates the influence of the old alteration-hypothesis of L. Herrmann and his followers, to the discussion of which many volumes have been devoted, especially in Germany. As a matter of fact, nothing definite is known so far about cellular mechanism and little can be expected from further discussion of the matter, or by a prophecy that any experimental method holds no possibilities of future developments.

'Apple skin with its coating of wax and specialized nature is very far removed from the plasma membrane of the living cell' is another criticism of Dr. Haynes. Macdonald, however, has shown that *the nerve* also exhibits an effect 'dominated by the salt content'. Further experiments described in the older literature show that all kinds of other tissues (muscle, &c.) exhibit a similar effect. Also, the effect of the salt content is *of the same kind in all cases,* and it certainly is, therefore, quite a general biological phenomenon. The author holds that on the biological side no criticism of his experiments is possible without due reference to the most important work of Macdonald, who was the first to observe the electro-physical effect of the salt content.[3] Since Dr. Haynes does not even mention

[1] A whole chapter in the book of the author (pp. 72–86) is devoted to this question. On the basis of experiments it is found that diffusion potentials do exist in oils.

[2] Dr. Haynes, loc. cit., at the end of p. 101.

[3] Similar experiments were described by Biedermann (1880) and others, who found that water has

Macdonald it seems doubtful whether her statement cited above is justified, as well as the following ones: 'On the biological side Beutner lays himself open to serious criticism.' 'In his theory of the current of injury . . . Beutner assumes a simplicity and uniformity of structure of the living organism to which no physiologist can give countenance.' As a matter of fact the experiments of the author on the current of injury in plants point to a more complicated structure than assumed by any of the former theories, since the possibility of an asymmetry of the membranous constituents, giving rise to an electromotive force, had never been suggested before. The assertion of Dr. Haynes that the author 'postulates for the fruit a homogeneous flesh' is entirely unfounded and contradicted by the experiments described on pp. 140–3 of the book of the author.

It must be stated, finally, that Dr. Haynes even denies the existence of statements which are manifestly to be found in the book of the author. Concerning the higher content of fatty acid in the outer skin she declares 'that he [the present author] does not state whence his information is derived'. Although the author is doubtful whether his arguments would carry conviction to Dr. Haynes, yet he begs to say that the source of his information is his own book, where one full page (p. 145) and a table are devoted to explaining that the above statement is derived from the greater electromotive effect of the salt content on the outer skin.

a positivizing affect on the muscle, which proves that the effect of the salt content is a general phenomenon. None of these investigators, however, thought of establishing quantitative relations between salt content and electromotive force. This was first done by Macdonald.

Diphasic Liquid Systems and Bio-electrical Phenomena.

BY

DOROTHY HAYNES,

Department of Plant Physiology and Pathology, Imperial College of Science and Technology.

THE writer is glad to have the opportunity of replying to Dr. Beutner's paper in this Journal (pp. 673-7). It is felt that certain points have been left somewhat obscure, and it is desired to revise one or two statements which as they stand are incorrect. Dr. Beutner complains of various misrepresentations; the writer much regrets if she has misunderstood any material point, but as Dr. Beutner avoids all detail of these it is impossible to ascertain the grounds of his complaint. In dealing with the specific questions which he has brought forward it has been thought useful to summarize briefly the points at issue.

The difficulty in dealing with Dr. Beutner's systems lies in the fact that they are not in true equilibrium. It had been assumed in the original criticism that in the case of non-acid oils equilibrium is rapidly reached at the surface, but beyond this there exists a small zone into which soluble salt diffuses. In a recent review of some part of Dr. Beutner's work Gardner[1] interprets his results on the assumption that the penetration of electrolytes is strictly confined to the surface layer; this accounts for the rapid establishment of equilibrium which Dr. Beutner observed as well as for the numerical results he obtained, but it is difficult to reconcile the assumption with a distribution by diffusion, and it is interesting to notice that Baur,[1] working on similar lines, has adopted an adsorption formula. It may be suggested, however, that rapid diffusion may quite possibly be confined to a thin film into which water penetrates, since Dr. Beutner's measurements of potential appear to have been carried out on dry oils, while his estimates of concentration were deduced from changes of conductivity in these oils after they had been shaken up with aqueous solutions. It should be remarked that it was not intended to suggest that Dr. Beutner denied

[1] Fourth Report on Colloid Chemistry, p. 116, Brit. Assoc.

the possibility of a diffusion potential in oils—criticism was directed to the fact that he regards it as negligible throughout his systems.

In the previous paper criticism was mainly directed to those systems which contain free acid in the oil phase. It is felt that sufficient emphasis was not laid upon the fact that all Dr. Beutner's measurements of solubility depend upon changes of conductivity in oils saturated with water, and that where chlorides or salts of other mineral acid react with organic acid present in the oil phase, much of the increased conductivity may be due to increase in the concentration of hydrogen ions. For this reason it is unlikely that Dr. Beutner's measurements afford any true indication of the concentrations of sodium ions in the 'oil' phase. At boundaries where the sodium salt of an organic acid is present this difficulty does not arise, and Dr. Beutner's assumption that the change of conductivity is principally due to increase of sodium ions may well be justified. Since sodium ions are present in large excess, the interphasic potential will be determined by the solubility of the sodium salt; the writer's previous criticisms on this point are therefore not valid. In reversible conditions the interphasic potential will be measured by the distribution of the sodium ion, since this is common to the two phases; therefore, given reversibility, the formula holds good at this boundary; but it is hardly admissible to regard such systems as reversible, and diffusion potentials probably contribute to the total E.M.F.

In the original criticism it was suggested that the E.M.F. of reversible systems of the type

$$\begin{array}{c|c|c} KCl & Acid & KCl \\ C_1 & oil & C_2 \end{array}$$

may be due to a difference of hydrogen-ion concentration on either side of an oil phase in which hydrogen-ion concentration is constant. Dr. Beutner attempts to disprove this suggestion by comparing the E.M.F. of the system

$$\begin{array}{c|c|c} \text{salicylic acid} & \text{oil saturated with} & \text{salicylic acid} \\ \text{saturated solution} & \text{salicylic acid} & KCl \end{array}$$

with the pH values of the two aqueous solutions. He has, however, left out of consideration the fact that the equilibrium

$$KCl + C_6H_4(OH)COOH \rightleftarrows C_6H_4(OH)COOK + HCl$$

will be disturbed by the introduction of the oil phase, which will not only act as a reserve of acid but will also tend to dissolve undissociated potassium salicylate. On both accounts the equilibrium will be displaced to the right, and it is probable that a very considerable increase of hydrogen-ion concentration will result. This, however, cannot account for the whole difference of potential observed in the above system, and a consideration of this system has drawn attention to the fact—the importance of which has been overlooked both by Dr. Beutner and the present writer—that potassium

ions may very probably be maintained in the oil at a nearly constant concentration, while transference of potassium ions takes place mainly as undissociated salt. If this be an adequate explanation, Dr. Beutner may fairly claim justification for the view that the solubility of salts is the predominating factor in the production of differences of potential in his systems, although the differences of hydrogen-ion concentration which arise must also be taken into account; but his quantitative results must in this case be entirely invalidated, since changes of conductivity will be mainly due to the production and solution of hydrogen chloride. Moreover, as has been already remarked, there appears in many cases to be no adequate reason for assuming the absence of a diffusion potential.

On more general questions there is little to be added to what has already been said—the value of an adequate model of the cell in its electrochemical relations cannot be doubted, but until we know more of cell mechanism the attempt to construct such a model would appear premature and liable to mislead. This is said with no desire to underrate the value of attempts to draw analogies between physical and biological systems; such analogies constitute the chief hope of advance upon the biological side, but it must be remembered that their interpretation is beset with all the difficulties which arguments from analogy admittedly encounter. Dr. Beutner attaches great importance to Macdonald's experiments on dissected nerves. It may well be true that the nerve-sheath, which is known to contain much lipoid substance, plays a part similar to that of the waxy cuticle of the apple, and that Dr. Beutner's acid-oil systems are typical of both. This is undoubtedly important, but since both of these are very special structures it is hardly a justification for the assumption that the differences of potential at all cell surfaces are solely conditioned by differential solubility in a lipoid layer which contains free acid, especially in view of the fact that the lipoid theory has been universally rejected by physiologists after a very large amount of investigation. Dr. Beutner's treatment of the 'current of injury', and the fact that he deduces the distribution of fatty acid in the apple from this phenomenon, are evidence of the large claim of universal validity which he makes for his hypothesis. It should perhaps be added that the statement as to a homogeneous 'flesh' to which Dr. Beutner objects was based on his treatment of the 'flesh' as a separate phase.

The Moisture-relations of Terrestrial Algae.[1]

II. The Changes during Exposure to Drought and Treatment with Hypertonic Solutions.

BY

F. E. FRITSCH

AND

F. M. HAINES.

With eight Figures in the Text.

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INTRODUCTION.

IN the first paper of this series (Fritsch, 1922) it was shown for a number of common terrestrial algae that a considerable amount of moisture is retained within the cells in the air-dry condition, so that only relatively small quantities are requisite to replace that lost by the protoplasts in drying. It was suggested that this marked retention of moisture might be due to concentration of the sap (loc. cit., p. 19). The observations

[1] From the Botanical Department, East London College. Some of the results of the present investigations have already been communicated in abstract (Journ. of Ecol., x, pp. 229–31, 1922).

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communicated in the present paper are the outcome of an attempt to study the mechanism involved. The problem was first approached by an investigation of the effect of various strengths of plasmolysing solutions. For this purpose Tidman's sea-salt was selected, so as to avoid possible inaccuracies due to the toxic action of single salts on the protoplasm.

The material investigated was primarily the same as that employed in the first paper (Fritsch, 1922, p. 2), but relatively little of the *Hormidium*-stage of *Prasiola crispa* was obtainable during the course of the work, and it has consequently not been dealt with as fully as some of the other forms. Apart from these, investigations were also made on (*a*) a form of *Hormidium flaccidum* occurring in some quantity in different parts of the Redlands woods in Surrey; (*b*) *Cystococcus humicola*, Naeg., growing on wooden palings in Dorking; and (*c*) moss protonema (probably belonging to the moss *Hypnum cupressiforme*, L., var. *filiforme*, Brid.) from the Redlands woods. A practice was made of collecting the material for the different successive experiments from as small an area (often only a few square feet), as possible, so as to ensure relative uniformity (cf. p. 697), but this was not always feasible, and for some of the forms there were two or more collecting grounds.[1] There is, however, no reason to suppose that these habitats differed at all essentially from one another. Most of the experimental work was carried out at East London College, but some at North Holmwood, Surrey. The bulk of the observations were made during the winters of 1922 and 1923 and the early spring of 1922, since suitable material was not easily obtainable during the dry summer weather.

In the course of our investigations numerous problems have been approached, a detailed solution of which lay outside the scope of the present paper (cf. especially section E). We propose to deal with some of these in later communications.

A. The General Behaviour towards Plasmolysing Solutions.

It is noticeable that, if material of these terrestrial forms is placed in plasmolysing media, some of them require solutions of very considerable

[1] The following is a list of these habitats and of the experiments in which the respective materials were used: (*a*) For *Zygogonium ericetorum*: (i) little-shaded pathway (Expts. I, III); (ii) unshaded pathway (Expts. IV, V, IX, X, XII, XVI, XX); (iii) somewhat shaded pathway (Expts. XXXI, XXXV, XXXVI). (*b*) For *Hormidium flaccidum*: (i) on sandy vertical bank at side of pathway (Expt. II); (ii) bare clayey soil, in great part shaded by a large holly (Expts. VI, XI, XXXII, XXXVI); (iii) sandy path, fully exposed to the light (Expts. XIV, XXVI, XXIX). (*c*) Moss protonema (all from same habitat as (ii) for *Hormidium*). (*d*) For *Pleurococcus*: (i) wooden palings on outskirts of Dorking (Expt. VII); (ii) wooden palings, South Holmwood (Expts. XVII, XXIV, XXVIII, XXX, XXXVI).

strength before plasmolysis is to be observed in any of the cells (cf. also Fritsch, 1922, p. 14). To this category belong *Pleurococcus Naegelii*, *Cystococcus humicola*, and under certain conditions the filamentous stage of *Prasiola crispa*,[1] all three of which are unaffected or only very slightly affected by immersion in a 10 per cent. solution of sea-salt;[2] as a matter of fact, to obtain decided plasmolysis in a majority of the cells, solutions of a strength of 20 per cent. and upwards have had to be employed. Not all the forms investigated, however, require solutions of such a strength to bring about pronounced plasmolysis. In many cases a 5 per cent. solution of sea-salt suffices to plasmolyse markedly many of the cells of *Zygogonium ericetorum*,[3] *Hormidium flaccidum*, and of the protonema of the moss studied.

A second striking fact relating to the plasmolysis of these forms is the great inequality in the behaviour of the cells within one and the same mass of material, collected from the same spot and at the same time. In the case of the filamentous forms this is often observable within a single filament; the unplasmolysed cells are scattered singly in quite an erratic manner in filaments, most of whose cells are plasmolysed, and vice versa. In the case of the moss protonema the cells at the tips of the branches were very commonly, though not invariably, unaffected by the strength of sea-salt used (5 per cent.). By contrast with these terrestrial forms aquatic algae (*Spirogyra*, *Mougeotia*, *Oedogonium*) usually exhibit a uniform reaction of the cells throughout whole threads, although in the case of *Cladophora* the older cells of the thick main axes are usually not plasmolysed by the same strength of solution as affects the cells of the finer branches.

There is a minimum concentration of sea-salt which will produce any plasmolysis at all in the terrestrial forms studied, but with this strength the shrinkage is only very slight, and usually only to be observed in quite *an insignificant percentage of the cells.* Using solutions of progressive strengths above the minimum one not only observes an increase in the extent of plasmolysis in the individual cells, but a more or less steady increase in the numbers of plasmolysed cells (cf. Table I). As a general rule, however, it is necessary to employ strengths far above the minimum to bring about plasmolysis of the majority of the cells of a given mass of material. Thus, in the case of material of *Zygogonium ericetorum*,[3] examined immediately after collection, the minimum concentration proved to be 2·25 per cent. sea-salt; even with 10 per cent. solutions, however, there were occasional cells that were not plasmolysed. In the case of

[1] The statement made in the first paper (Fritsch, 1922, p. 14) that the cells of the filamentous stage of *Prasiola* are markedly plasmolysed by a 10 per cent. solution of sea-salt was probably due to investigation of recently inundated threads and has not been confirmed.

[2] Similar high concentrations of sugars are necessary to produce plasmolysis. Thus very few cells of either *Pleurococcus* or *Prasiola* plasmolyse in 40 per cent. sucrose.

[3] This is the same as the *Zygnema ericetorum* of the earlier paper.

Hormidium flaccidum the minimum was 2·5 per cent., but with a 5 per cent. solution contraction was little marked in the granular cells (cf. below).

TABLE I.

Comparison of plasmolysis of identical material of various terrestrial forms in different strengths of sea-salt.

Material.	*Expt. No.*	*Cells counted.*	*Strength of sol.*	*Strongly plasmolysed.*[1]	*Slightly plasmolysed.*[1]	*Unaffected.*
			%	%	%	%
Zygogonium	XVI	1,104	3·0	1·9	60.6	37·6
,,	,,	1,185	4·0	12·9	61·7	25·2
,,	,,	1,226	5·0	71·8	16·7	11·4
Hormidium	II	423	3·0	0·7	37·3	61·9
,,	,,	757	5·0	20·9	68.6	10.6
,,	,,	1,023	3·0	45·8	32·7	21·6
,,	,,	1,019	5·0	91·2	3·6	5·2
Prasiola	XXVII	382	10·0	7·6	54·2	38·2
,,	,,	1,449	20·0	74·6	11·0	14·4
Protonema	XV	811	3·0	17·8	60·1	22·2
,,	,,	851	4·0	62·8	24·7	12·6
,,	,,	764	5·0	70·0	17·8	12·2
				Plasmolysed.		*Unaffected.*
				%		%
Pleurococcus	VII (1)	687	15·0	27·1		72·9
,,	,,	661	17·5	39·0		61·0
,,	,,	669	20·0	39·9		60·1
,,	,,	643	22·5	50·1		49·9
,,	,,	645	25·0	50·2		49·8
,,	VII (2)	1,002	15·0	19·9		80·1
,,	,,	1,007	17·5	22·5		77·5
,,	,,	1,004	20·0	26·9		73·1
,,	,,	1,003	22·5	33·6		66·4
,,	,,	1,502	25·0	45·7		54·3

Many of the cells of these terrestrial algae include larger or smaller numbers of highly refractive granules, which appear in part at least to be fatty in nature (cf. p. 721). The feature in question is often so marked that one can broadly classify the cells in a given mass of material into 'granular' and 'non-granular'; a classification often employed below. In the filamentous forms whole threads may be granular, non-granular, or mixed. The granular cells, as found in nature, differ somewhat among one another in character, some containing more or less uniform, rather evenly distributed granules, others such as are of very unequal size and often very uneven shape. But one of the most distinctive types of granular cells—apparently especially prevalent during mild periods of drought in

[1] In many cases it has been found desirable to distinguish between 'strongly plasmolysed' and 'slightly plasmolysed' cells, the latter being those in which only a slight shrinkage of the protoplasts away from the corners of the cells was recognizable. The distinction is somewhat arbitrary, but in practice not difficult to draw. It was, however, impossible to make the distinction in the unicellular forms owing to the small dimensions of the cells.

nature—is that in which the granules exhibit an essentially peripheral disposition (cf. Fritsch, 1916, p. 143); in such cases they are not uncommonly rather small and often so densely arranged as to appear like a fine moniliform thread in optical section and a coarse pitting in surface view. Granular cells have been observed in all the terrestrial algae examined, but are on the whole more striking in the filamentous than in the unicellular forms. Nothing of this kind has been seen in the moss protonema.

Particularly when working with strengths of solutions not far above the minimum, one often observes a striking correlation between the occurrence of plasmolysis and the granular or non-granular character of the cell. With these lower strengths it is in the main the non-granular cells or those with very few granules that are markedly plasmolysed (cf. Table II), and appreciable numbers of granular cells only begin to be strongly plasmolysed when we come to use the higher strengths. This is by no means without exception, but nevertheless expresses the general state of affairs. Thus, even in relatively weak solutions, one often finds a certain number of cells with plentiful granules strongly plasmolysed[1] (cf. Table II). Especially cells with prominent peripheral distribution of fine granules fail to contract until very strong solutions are used, and in *Pleurococcus* granular cells are often the ones to remain unaffected even by a 25 per cent. solution of sea-salt.

TABLE II.

Relation of plasmolysis at lower strengths of sea-salt to character of cells.

(*a* = cells with abundant granules; *b* = non-granular cells.)

Material.	*Expt. No.*	*Cells counted.*	*Strength of sol.* %	*Strongly plasmolysed.*[1] %		*Slightly. plasmolysed.*[1] %		*Unaffected.* %	
				a	*b*	*a*	*b*	*a*	*b*
Zygogonium	I	1,798	3·0	4·7	2·1	28·3	6·8	55·3	2·8
Hormidium	II	423	3·0	0·7	—	3·8	33·5	56·9	5·0
,,	VI	2,100	5·0	1·1	90·6	5·0	1·4	1·6	0·3
Prasiola	XXVII	382	10·0	—	7·6	23·0	31·1	38·2	—
,,	,,	1,449	20·0	2·2	72·4	10·6	0·4	14·4	—

It has, however, become clear that the presence of granules in itself is not by any means the sole determinant of the plasmolysing qualities of the cells. This has been established by keeping material of the different forms investigated well moistened for a fortnight or more, one set being exposed to the full light of the greenhouse, the other being screened by a covering of slightly translucent brown paper (Table III). Except in the case of

[1] See foot-note on p. 686.

Pleurococcus, which in this respect proved to be as indifferent as to other treatment described later, the material grown in the dark showed a much larger percentage of plasmolysing cells. This, except in the *Hormidium*, is due to a great increase in the numbers of strongly plasmolysed[1] cells. The *Zygogonium* used was granular throughout, and remained so in both cases; that which had been kept in the dark, however, was found to present a majority of cells with very fine uniform granules, whilst the threads exposed to the light partly consisted of cells with large coarse granules. In the material that had been in the dark there were very occasional threads with relatively scanty granules, but on the whole one was struck by their apparent stability; if they constitute a food-reserve, they do not appear to be easily used during a prolonged period in which photosynthesis is impossible (cf. Piercy, 1917, p. 533). The *Hormidium* employed contained relatively few granular cells, and here too there was no sensible alteration in this respect in either lot of material at the end of the experiment. The percentage of dead cells was considerably greater in the algal mats that had been in the dark. In the case of the protonema there was likewise no obvious difference in the microscopic characters of the two sets of material.

TABLE III.

Comparison of plasmolysis of identical material kept in the light and dark respectively (Expts. VIII and XXXVI).

(*l.* = light; *d.* = dark.)

Material.	*Cells counted.*	*Strength of sol.* %	*Time of exposure (days).*	*Strongly plasmolysed.* %		*Slightly plasmolysed.* %		*Unaffected.* %	
				d.	*l.*	*d.*	*l.*	*d.*	*l.*
Zygogonium	900, 971	5·0	14·0	69·3	12·5	22·7	43·7	8·0	44·0
Hormidium	1,022, 1,060	3·0	29·0	39·1	42·4	43·8	28·6	17·1	29·0
Protonema	1,000, 1,000	5·0	25·0	75·3	58·7	19·4	20·3	5·4	21·0

Material.	*Cells counted.*	*Strength of sol.* %	*Time of exposure (days).*	*Plasmolysed.* %		*Unaffected.* %	
				d.	*l.*	*d.*	*l.*
Pleurococcus	1,000, 1,000	25·0	14·0	86·0	82·5	14·0	17·5

The results just given may be ascribed to two possible causes. The larger number of plasmolysing cells in material kept in the dark may be a result of decreased permeability of the protoplasmic membrane. At the same time it seems probable that the differences are partly caused by the presence of abundant soluble products of photosynthesis in the cell-sap, and that the frequent absence of plasmolysis in strongly granular cells is due to such cells being rich in products of assimilation.

[1] Cf. foot-note on p. 686.

B. Description of Methods adopted in the Subsequent Investigations and Discussion of Possible Sources of Error.

In view of the facts which have just been detailed it will be evident that little can be gleaned from comparative estimations of the minimum concentrations necessary to effect plasmolysis, since such concentrations are only to be related to a small percentage of the cells. A better comparative method of obtaining some conception of the relation to plasmolysing solutions seemed to be to employ a solution rather above the minimum and to determine by counting under the microscope the percentages of plasmolysed and unaffected cells. In order to obviate changes of concentration during the estimations a ring of marine glue dissolved in xylol was run round the edge of the cover-glass. Usually at least 1,000 cells were counted at each estimation, but occasionally this was impossible owing to lack of time; in some cases the result seemed so even that a smaller number of cells was deemed sufficient. As a general rule from fifteen to forty-five minutes were occupied in an estimation. The estimations were made with the help of a mechanical stage, and usually the material under the cover-glass was counted exactly as it appeared with the movement of the stage, thus obviating any personal bias.

The sources of possible error in this method of estimation are: (1) lack of uniformity of material, (2) personal error in the counting, and (3) effect of the xylol in the sealing medium on the permeability of the material. The error under items (1) and (2) was determined by repeated estimations of *different* mounts of supposedly identical material, and the full results are epitomized in Table IV. In general the amount of error will be found to be small in relation to the percentages upon which any stress has been laid in the subsequent matter. It will be evident that more reliance can be placed on the bigger totals than on the smaller. The only serious discrepancy among them is that shown by the first count for *Hormidium*, but we doubt very much whether an error of this magnitude has often entered into our estimations. In making the latter it was noticed again and again how the totals for the different categories maintained the same relative proportions during successive periods of the count (cf. also the analogous results often obtained with the same alga from the same habitat at different times; see Table VII). A very striking uniformity is shown by the data for *Pleurococcus*, which is no doubt due to the possibility of getting a better assortment of such a unicellular form than can be done in the case of the filamentous types.

The possible error introduced by the presence of xylol in the sealing medium was investigated by comparing the counts for material sealed with melted marine glue and with marine glue dissolved in xylol respectively.

The results are given in Table IV A, and they show quite clearly that the presence of xylol brings about no difference that is outside the limits of the ordinary experimental error above considered.

TABLE IV.

Calculation of possible error.

Material.	*Cells counted.*	*Sol.* %	*Strongly plasmol.*[1] %	*Deviat. from mean.*	*Error.* %	*Slightly plasmol.*[1] %	*Deviat. from mean.*	*Error.* %	*Unaffected.* %	*Deviat. from mean.*	*Error* %
Zygogonium	1,035	5·0	76·9	−2·6	3·4	14·4	+4·9	34·0	8·7	−2·3	26·4
,,	1,000	,,	83·6	+4·1	4·9	6·4	−3·1	48·5	10·0	−1·0	10·0
,,	1,046	,,	86·3	+6·8	7·9	4·0	−5·5	137·5	9·7	−1·3	13·4
,,	1,051	,,	71·2	−8·3	11·6	13·2	+3·7	28·0	15·7	+4·7	30·0
Average			**79·5**			**9·5**			**11·0**		
Zygogonium	1,093	3·0	68·6	+2·8	4·1	19·4	−0·5	2·6	12·0	−2·3	19·2
,,	1,262	,,	64·1	−1·7	2·7	19·6	−0·3	1·5	16·4	+2·1	12·8
,,	1,023	,,	64·7	−1·1	1·7	20·7	+0·8	3·9	14·6	+0·3	2·1
Average			**65·8**			**19·9**			**14·3**		
Hormidium	1,023	3·0	45·8	−13·0	28·4	32·7	+3·2	9·8	21·6	+9·9	45·9
,,	1,008	,,	65·8	+7·0	10·6	26·6	−2·9	10·9	7·6	−4·1	54·0
,,	1,012	,,	67·8	+9·0	13·3	23·7	−5·8	24·5	8·5	−3·2	37·6
,,	1,004	,,	55·7	−3·1	5·6	35·2	+5·7	16·2	9·1	−2·6	28·6
Average			**58·8**			**29·5**			**11·7**		
Protonema	1,010	5·0	76·1	−3·1	4·1	14·3	+1·0	7·0	9·7	+2·1	21·6
,,	1,000	,,	83·0	+3·8	4·6	10·7	−2·6	24·3	6·3	−1·3	20·6
,,	1,000	,,	75·2	−4·0	5·3	17·2	+3·9	22·7	7·6	—	—
,,	1,000	,,	82·4	+3·2	3·9	11·0	−2·3	20·9	6·6	−1·0	15·2
Average			**79·2**			**13·3**			**7·6**		

			Plasmol. %	*Deviat.*	*Error.* %	*Unaffected.* %	*Deviat.*	*Error.* %
Pleurococcus	1,000	25·0	87·5	+2·1	2·4	12·5	−2·1	16·8
,,	,,	,,	83·9	−1·5	1·8	16·1	+1·5	9·3
,,	,,	,,	86·2	+0·8	1·0	13·8	−0·8	5·8
,,	,,	,,	84·1	−1·3	1·5	15·9	+1·3	8·2
Average			**85·4**			**14·6**		

TABLE IV A.

Investigation of error due to xylol in the sealing medium.

(5 % sea-salt solution.)

Material.	*Method of sealing.*	*Cells counted.*	*Strongly plasmolysed.* %	*Slightly plasmolysed.* %	*Unaffected.* %
Zygogonium	(in xylol)	1,056	94·7	2·0	3·3
,,	(melted)	1,208	92·7	3·3	4·0
Protonema	(in xylol)	949	83·2	12·4	4·4
,,	(melted)	1,000	84·7	7·6	7·7

[1] Cf. foot-note on p. 686.

C. The Behaviour when exposed to Drought.

An appreciable alteration in the behaviour towards plasmolysing solutions, in most of the forms investigated, is to be observed during a spell of drought. Using the same strength of sea-salt solution, the number of plasmolysed cells and the extent of plasmolysis in the affected cells gradually diminish day by day till ultimately very little plasmolysis is to be observed. In the experiments in question, a large number of which were performed, the initial material was usually obtained during damp weather and a strength of salt solution selected sufficiently concentrated to bring about marked plasmolysis in a large majority of the cells. The material was then allowed to dry exposed to the air of the laboratory in the earlier experiments, but in the later ones was dried in a desiccator over suitable strengths of sulphuric acid. Except in a few cases, in which a constant temperature was maintained, the experiments were exposed to ordinary room temperature. Relatively small quantities of material were employed, all collected under the same conditions, on the same day, and from the same habitat—usually not more than could be placed in an ordinary watch-glass. The material was left on the patch of soil as collected. The experiments were almost always started on the same day or on the day after collection. As far as possible daily estimations were made of the drying material until it appeared that no further appreciable change was taking place.

The results of a number of these experiments are epitomized in Table V. They are essentially uniform as regards the three filamentous algae, and fall far outside the limits of our experimental error. Only in the case of Experiment VI with *Hormidium flaccidum* was there any appreciable percentage of strongly plasmolysed cells at the end of the period of drought, and even in this case the very considerable reduction as compared with the original material is quite obvious.[1] It will be noticed that, whilst in all these cases there is a great increase in the percentage of unaffected cells after drought, there is no such uniformity as regards the slightly plasmolysed ones, which sometimes show a decided decrease and sometimes an increase. We are inclined to think that this depends partly on the condition of the original material, and on the duration and intensity of the drought. Attention must also be drawn to the fact that, after prolonged drought, a certain number of the cells of all the filamentous forms are found to show a permanent slight contraction of the protoplasts, visible even when material is mounted in ordinary tap-water (cf. below, p. 716). This fact was not realized until a considerable number of the estimations had been carried out,

[1] The presence of strongly plasmolysing cells in this case is probably due to the high humidity of the air during this experiment (cf. p. 696).

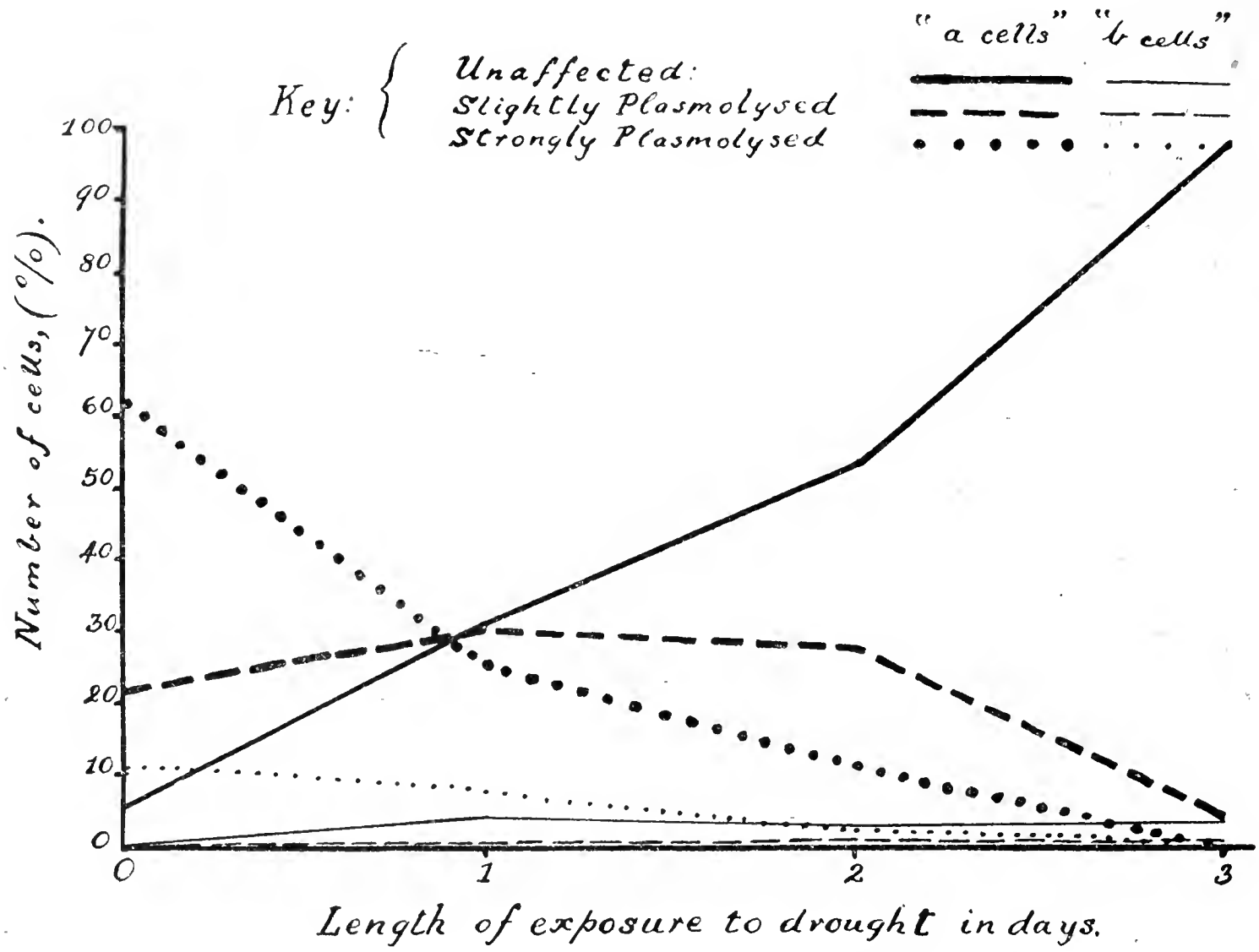

FIG. 1. Influence of drought on plasmolysis of *Zygogonium ericetorum* (Expt. XII). *a* = granular, *b* = non-granular cells.

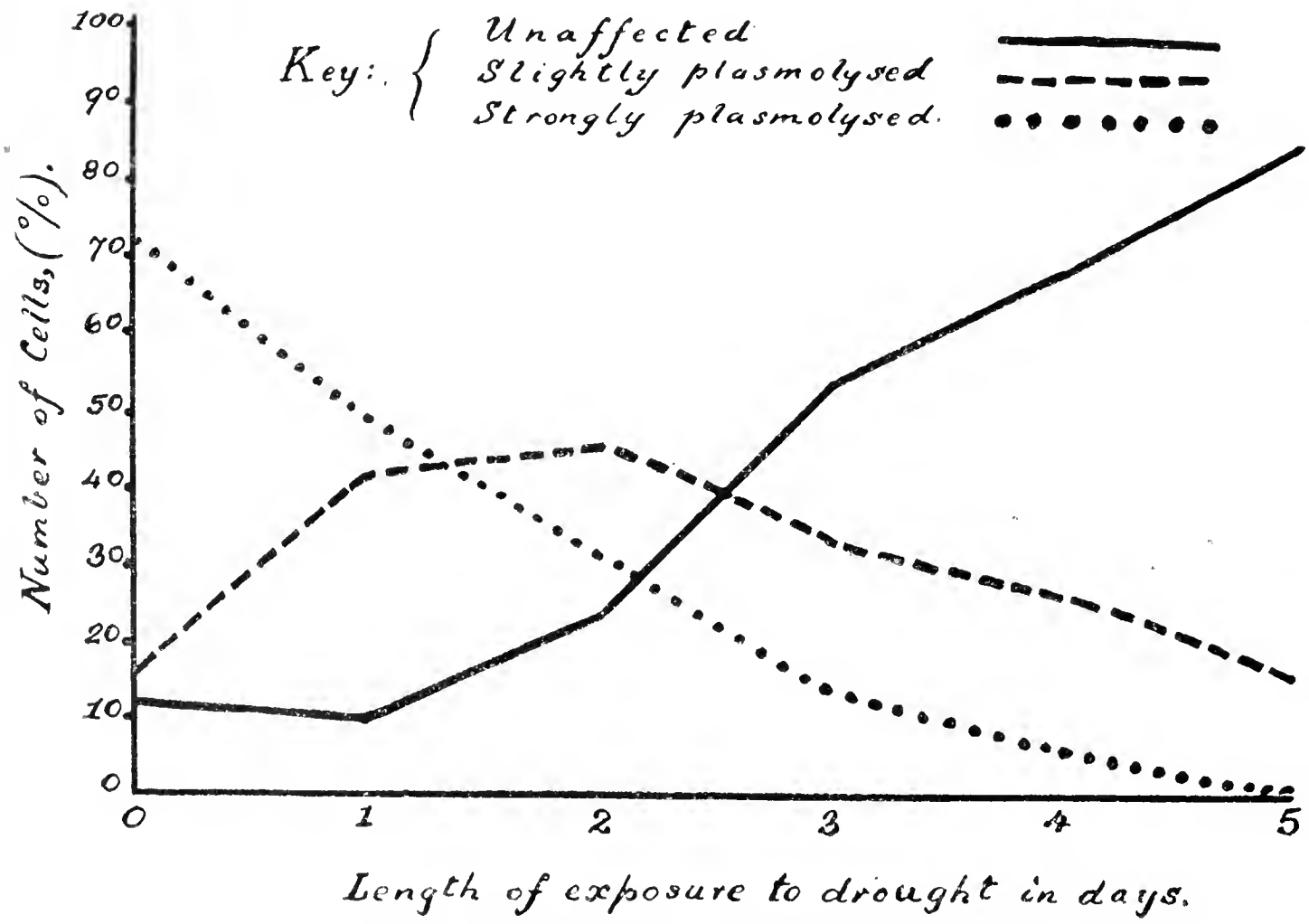

FIG. 2. Effect of drought on *Zygogonium* (Expt. XVI).

TABLE V.

Percentages of plasmolysed and unaffected cells in material of various terrestrial forms before and after a period of drought.

(Under each experiment the first line gives the original condition, the second the final condition.)

Material.	*Expt. No.*	*Cells counted.*	*Strength of sol.*	*Strongly plasmolysed.*	*Slightly plasmolysed.*	*Unaffected.*	*Duration of drought.*	*Nature of drought.*
			%	%	%	%		
Zygogonium	I–III	1,798	3	6·8	35·1	58·1		
,,	,,	990	3	—	2·6	97·4	28 days	air
,,	IV–V	2,008	5	12·5	38·0	49·3		
,,	,,	1,839	5	0·1	7·2	92·7	14 ,,	air
,,	IX	1,987	5	69·9	12·2	17·9		
,,	,,	636	5	0·3	50·5	49·2	4 ,,	pure H_2SO_4
,,	XII	1,886	5	71·8	21·7	6·5		
,,	,,	926	5	—	5·1	94·9	3 ,,	,,
,,	XVI	1,226	5	71·8	16·7	11·5		
,,	,,	841	5	0·5	15·1	84·4	5 ,,	34·5 % H_2SO_4
Hormidium	VI	2,100	5	91·7	6·4	1·9		
,,	,,	1,663	5	6·6	53·1	40·3	7 ,,	air
,,	XIV	1,870	5	41·0	48·4	10·6		
,,	,,	1,400	5	—	12·9	87·1	2 ,,	45 % H_2SO_4
,,	XXVI	1,503	5	56·0	39·0	3·0		
,,	,,	626	5	—	46·2	53·8	7 ,,	34·5 % H_2SO_4
Prasiola	XXVII	1,449	20	74·6	11·0	14·4		
,,	,,	905	20	—	28·3	71·7	2 ,,	pure H_2SO_4
Protonema	XV	1,215	5	58·0	27·9	14·1		
,,	,,	655	5	2·9	83·8	13·3	$5\frac{1}{2}$,,	34·5 % H_2SO_4
,,	XXXIV	2,006	5	82·8	12·3	4·5		
,,	,,	519	5	5·2	63·9	30·9	4 ,,	,,
Pleurococcus	XVII	1,852	25	77·9		22·1	11 ,,	pure H_2SO_4
,,	,,	904	25	68·2		31·8		
,,	XXVIII	1,000	20	67·0		33·0	45 ,,	,,
,,	,,	641	20	61·8		38·2		
,,	XXX	2,034	25	88·5		11·5	9 weeks	,,
,,	,,	994	25	80·5[1]		19·5		
Cystococcus	XXII	818	25	87·2		12·8	4 days	34·5 % H_2SO_4
,,	,,	613	25	81·6		18·4		

[1] Cf. foot-note on p. 699.

and under the heading of 'slightly plasmolysed' cells in the final drought condition there are therefore comprised a more or less considerable percentage of cells which are in this permanently contracted state. Probably, therefore, a considerably larger percentage of cells than is shown in the alternate lines of Table V is to be regarded as unaffected by the plasmolysing solution. In the case of the protonema, which otherwise falls into line with the filamentous algae, the great apparent increase in slightly plasmolysed cells seems due to a relatively larger number of the cells being in this permanently contracted state than in the algae investigated.

TABLE VI.

Successive estimations of material of Zygogonium (habitat II) exposed to drought over 34·5 per cent. sulphuric acid.

(Experiment XVI, 5 % sea-salt.)

Date.	*Cells counted.*	*Strongly plasmolysed.*	*Slightly plasmolysed.*	*Unaffected.*	*Dead*[1] *cells.*
		%	%	%	%
April 1[2]	1,226	71·8	16·7	11·5	16·3
,, 2	913	49·5	41·0	9·5	15·0
,, 3	860	30·8	45·6	23·6	18·3
,, 4	900	13·8	32·3	53·9	24·7
,, 5	823	6·6	25·6	67·8	34·7
,, 6	841	0·5	15·1	84·4	20·7
Material at this stage of drought transferred to damp air (cf. p. 708).					
,, 7	571	0·2	14·4	85·4	28·0
,, 8	398	0·3	16·1	83·6	25·6
,, 19	1,042	1·1	51·2	47·7	35·3
,, 28	518	0·2	48·2	51·5	31·9
May 3	495	0·8	59·6	39·6	27 9
,, 10	520	1·7	70·9	27·3	29·9
,, 10 (10 % sol.)	264	37·5	53·8	8·7	—

In most of the drought experiments repeated estimations were made during the period of desiccation—daily in the case of the short-period experiments. The progressive effects of drought could thus be studied, but the results are too numerous to be reproduced in full here. In illustration we give one table (VI) and a number of figures (1–5). When the estimations are made at daily intervals, and provided a large percentage of the cells is strongly plasmolysed in the original material, one observes, in the case of the filamentous algae, for one or two days a more or less considerable increase in the number of slightly plasmolysed cells (cf.

[1] Where given, the dead cells are stated as a percentage calculated on the total number of living cells, instead of on the total number of cells, so that the percentages of plasmolysed cells recorded should represent a percentage of the living cells, i. e. of those still having the capacity to plasmolyse.

[2] The first line gives the condition of the material on the day of collection.

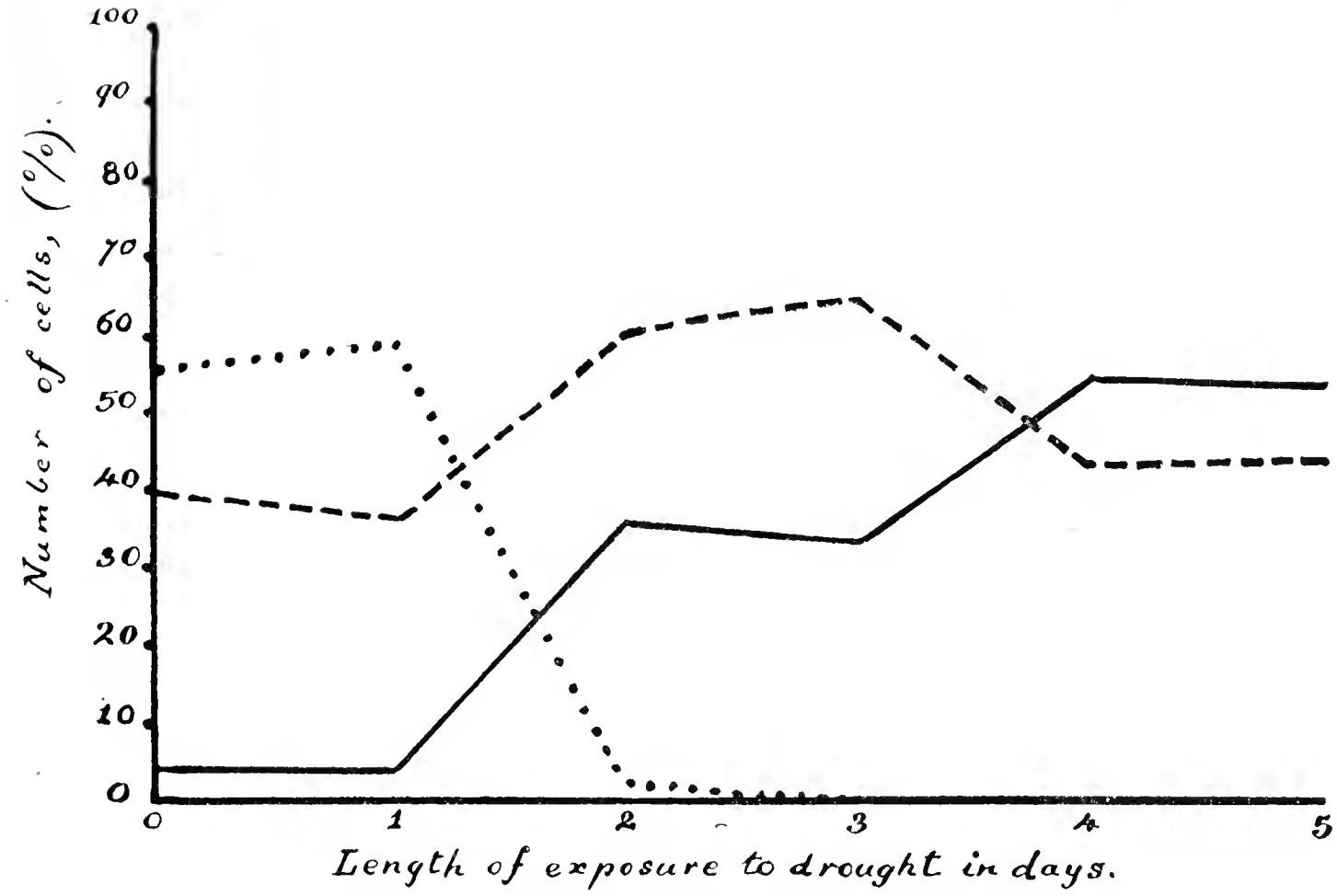

FIG. 3. Effect of drought on *Hormidium flaccidum* (Expt. XXVI).

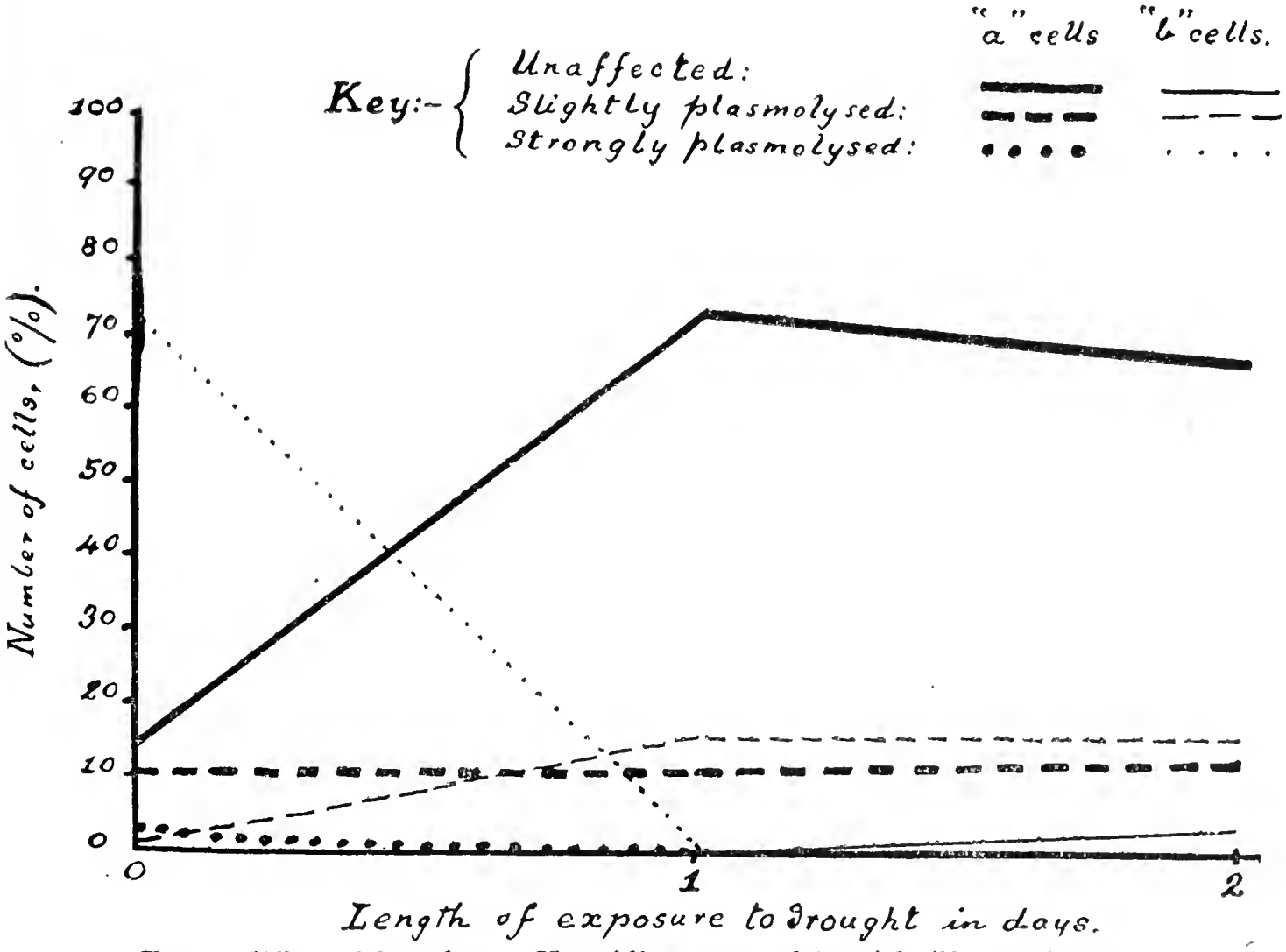

FIG. 4. Effect of drought on *Hormidium*-stage of *Prasiola* (Expt. XXVII).
a = granular, *b* = non-granular cells.

especially Figs. 2 and 3), whilst the percentage of unaffected cells for the first day or two increases but little. Subsequently the number of slightly plasmolysed cells again decreases, and that of the unaffected cells rises rapidly (cf. Figs. 1–3). In the case of moss protonema the same relation holds good (Fig. 5), but here the appearance of permanent contraction in many of the cells (cf. p. 694) ultimately leads again to an apparent increase in the numbers of slightly plasmolysed and a decrease in the number of unaffected cells. In these forms, therefore, there is evidently a gradual loss of the tendency to plasmolyse, many of the formerly

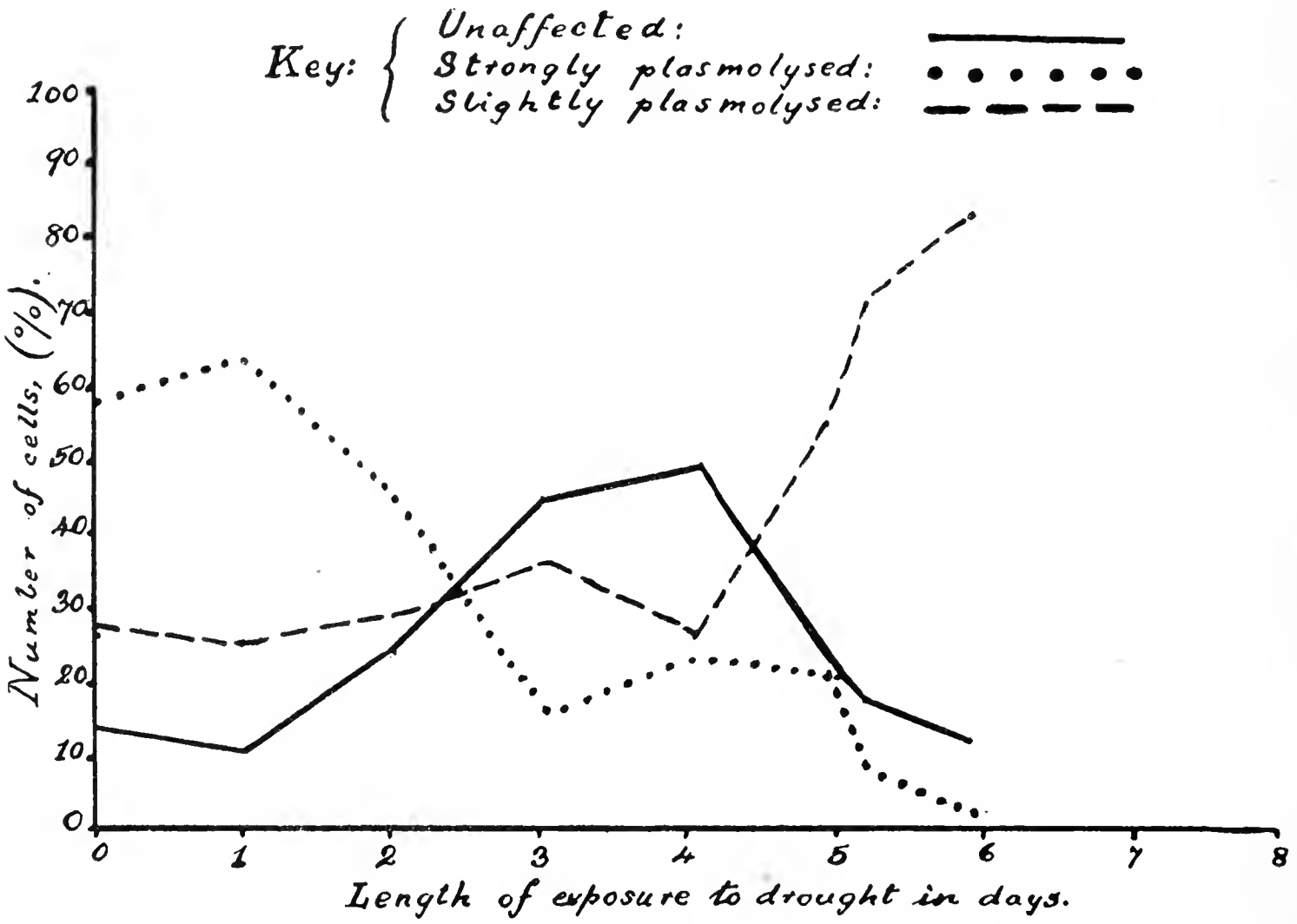

FIG. 5. Influence of drought on plasmolysis of moss protonema (expt. XV).

strongly plasmolysed cells, during the first days of a period of drought, still exhibiting slight plasmolysis. In the final stages, however, the majority show no plasmolysis.

In the case of the filamentous forms the behaviour during drought nearly always follows the sequence just described and irregularities were rarely noticed. In some of the earlier experiments, where the material was merely dried by exposure to the air of the laboratory, certain variations were, however, noticed which appear to be related to changes in the humidity of the air. Thus, after a week's drying in the laboratory, the *Hormidium* in Experiment VI gave on Feb. 20: 6·6 per cent. strongly plasmolysed, 53·1 per cent. slightly plasmolysed, and 40·3 per cent. unaffected cells; on Feb. 27, however, the figures were: 19·4 per cent. strongly plasmolysed,

75·4 per cent. slightly plasmolysed, and 5·2 per cent. unaffected cells. The air showed a very high relative humidity between the 20th and 27th, and this probably accounts for the result. Somewhat similar figures were obtained in an experiment with *Zygogonium* under the same conditions at the same time, and it was after these experiences that desiccation over sulphuric acid was substituted for mere drying in air.

Table VII.

Comparison of plasmolysis in material of terrestrial algae obtained from the same habitat on different occasions.

Material.	*Habitat.*	*Date.*	*Cells counted.*	*Strength of sol.* %	*Strongly plasmolysed.* %	*Slightly plasmolysed.* %	*Unaffected.* %
Zygogonium	II	1922 Feb. 1	2,008	5	12·5	38·0	49·2
,,	,,	1922 ,, 26	1,987	5	69·9	12·2	17·9
,,	,,	1922 Mar. 20	1,886	5	71 8	21·7	6·5
,,	,,	1922 Apr. 1	1,226	5	71·8	16·8	11·4
,,	III	1923 Feb. 4	1,544	5	66·8	16·7	16·5
,,	,,	1923 ,, 11	4,132	5	79·5	9·5	11·0
Hormidium	II	1922 Feb 13	2,100	5	91·7	6·4	1·9
,,	,,	1922 Mar. 1	2,053	5	62·1	33·1	4·8
,,	III	1923 Mar 27	1,870	5	40·9	48·5	10·6
,,	,,	1923 May 1	1,503	5	56·0	39·0	5·0

It is evident that an analogous alteration in behaviour towards plasmolysing solutions is to be observed also in a state of nature. In Table VII are given some data that bear on this point. The most striking feature about the four estimations of *Zygogonium* from habitat II is the astonishing uniformity of the last three. The first one, on Feb. 1, was made just after a dry spell, whilst the remaining three all fell within a period of intermittent wet weather. The two estimations of this alga from habitat III were likewise made during a spell of wet weather. In the case of the *Hormidium* from habitat II the first estimation was made after wet weather, the second after a few days' lull. It is hoped in a later communication to produce more data with reference to the changes in terrestrial algae under varying conditions of humidity in nature, the data just offered not having been definitely collected with this object in view.

In all of the experiments described above sea-salt was used as the plasmolysing medium, but in one experiment with *Hormidium* (XXIX) 40 per cent. sucrose was employed. In the fresh material (1,000 cells counted) there were 88·7 per cent. strongly, and 11·3 per cent. slightly, plasmolysed cells, none being unaffected. After two days in a desiccator over 34·5 per cent. sulphuric acid the result of the count (378 cells) showed 4·6 per cent. slightly plasmolysed and 95·4 per cent. unaffected cells; none were strongly plasmolysed and about three-quarters of the cells were dead. This may be compared with the result of Experiment XIV.

The question still arises as to how far changes might take place in material kept damp and merely exposed to the conditions extant in the laboratory. There is no doubt that under these circumstances the filamentous forms in some cases tend to exhibit a reduction of the number of strongly plasmolysed cells, perhaps as a result of favourable conditions for photosynthesis. Thus, in Experiment XII, an estimation of some of the original material which had been kept damp afforded on the day of the final drought determination 18·2 per cent. strongly plasmolysed, 70·0 per cent. slightly plasmolysed, and 11·8 per cent. unaffected cells; but this is quite a different result from that obtained with the drought material (cf. Table V). In other experiments, however, no such alteration of the original material was found; in Experiment I, for example, an estimation, after 28 days, of part of the original material that had been kept damp gave 11·3 per cent. strongly plasmolysed, 46·7 per cent. slightly plasmolysed, and 42·0 per cent. unaffected cells, a result which does not differ appreciably from that given by the material at the outset of the experiment (cf. Table V).

TABLE VIII.

Successive estimations of material of Pleurococcus (habitat II) exposed to drought over pure sulphuric acid.

(Experiment XXVIII, 20 % sea-salt.)

Date.	*Cells counted.*	*Plasmolysed.*	*Unaffected.*	*Dead cells.*[1]
		%	%	%
May 15	1,000	67·0	33·0	25·4
,, 16	1,000	83·7	16·3	11·6
,, 17	1,151	69·5	30·5	9·6
,, 18	922	76·0	24·0	18·0
,, 19	1,015	88·2	11·8	8·0
,, 22	1,030	86·2	13·7	9·1
,, 23	1,095	91·3	8·7	8·2
,, 30	582	85·9	14·1	9·5
June 12	840	45·5	54·5	33·6
,, 29	641	61·8	38·2	71·8

The results so far obtained with *Pleurococcus* and *Cystococcus* appear quite anomalous and difficult to understand (cf. Tables V and VIII and Fig. 6). It must remain doubtful whether in these forms there is any alteration in the behaviour towards plasmolysing solutions comparable with that above described for the filamentous forms, since no really decisive results have been obtained. If analogous changes do occur, they certainly only ensue after very prolonged and intensive drought over pure sulphuric acid. It would seem that in some cases death of the cells intervenes before any decisive response to the conditions of drought has taken place (cf.

[1] Cf. foot-note on p. 694.

Table VIII). A marked feature, moreover, of our consecutive estimations of *Pleurococcus* subjected to drought has been the peculiar variability of the figures at successive periods, of which both Table VIII and Fig. 6 afford testimony. Yet Table IV demonstrates that the error in the case of *Pleurococcus* is remarkably small, and one can only conclude that other factors come into play which have at present not been recognized. In Experiment

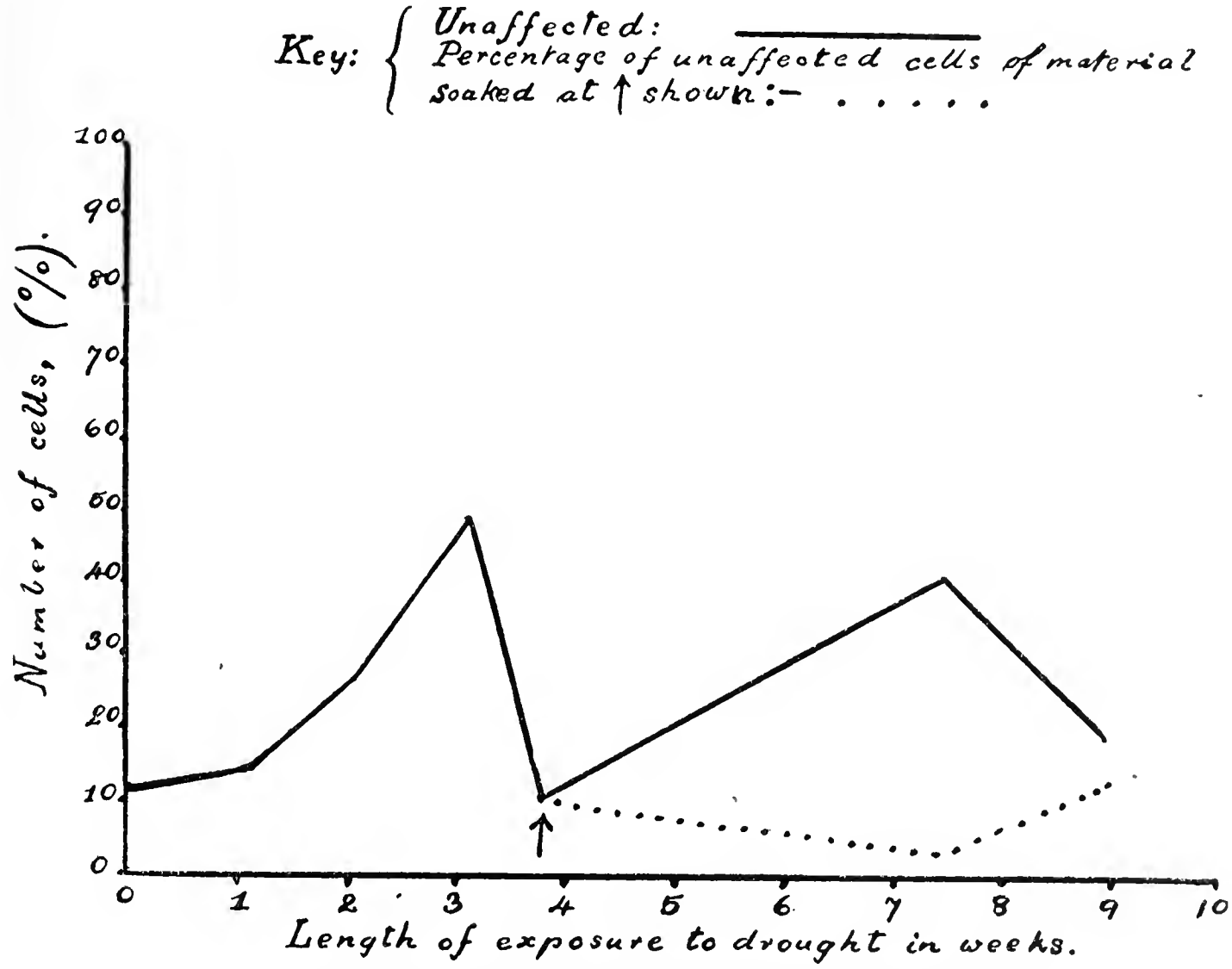

FIG. 6. Effect of drought on *Pleurococcus Naegelii* (Expt. XXX). Material soaked with water after nearly four weeks.

XXX, the results of which are reproduced in Fig. 6, the material was kept throughout in the dark and at a constant temperature of 18° C., but nevertheless the figures are as erratic as in the other cases.[1] It remains to mention that the cells of *Pleurococcus* show none of that permanent contraction of the protoplast during the later stages of drought, such as has been recorded above for the filamentous forms; whenever the material was examined in tap-water the living cells looked quite normal. Although few experiments have been performed with *Cystococcus* there is every reason to believe that its behaviour is similar to that of *Pleurococcus*.

[1] An average of 2,000 cells was counted at each estimation (except for the last two) in this experiment. A further estimation made after sixteen weeks' exposure to pure sulphuric acid produced no different result, 75·8 per cent. of the cells still plasmolysing.

D. The Effect of Prolonged Action of the Plasmolysing Solution.

In a considerable number of cases material, with the edge of the cover-glass sealed as described above (p. 689), was estimated at daily intervals; between the successive determinations the slide was usually exposed to room temperature and to a moderate illumination. Some of the results are epitomized in Table IX (cf. also Fig. 7). It will be seen that in the filamentous forms there is invariably a recovery from plasmolysis, analogous to but not always as marked as the final response on the part of drought material to plasmolysing solutions. In both cases there is a very appreciable reduction in the number of strongly plasmolysed cells, and the usual increase in the number of slightly plasmolysed cells in the final stage is here, too, no doubt due to the fact that a larger or smaller number of the cells have their protoplasts in a permanently contracted condition (cf. pp. 691, 716); that this was the case could naturally only be established at the end of each experiment. The moss protonema again stands out more strikingly in this respect than do the filamentous algae. Attention may be drawn to the fact that a larger percentage of dead cells was invariably found at the end of the experiment in the filamentous forms. Whilst we do not attach much importance to the estimations of dead cells, since the liability to error is much greater here, the results are so uniform that they can scarcely be due to chance.

With the unicellular forms the results are almost as difficult to understand as in the case of the drought experiments considered in the preceding section. Thus, whilst in Experiments VII and XXVIII with *Pleurococcus* an obvious recovery was noted, Experiment XVII and others no tincluded in Table IX showed no alteration in the material that could be regarded as beyond the limits of experimental error, a similar conclusion being reached in the case of *Cystococcus*. In Experiment XXVIII there was no appreciable recovery during the first four days, but after that a rapid change took place, so that on the eighth day practically no plasmolysed cells were to be found. It thus appears that *Pleurococcus* is usually capable of remaining unaffected by the unfavourable conditions in a sealed slide for a much longer time than the filamentous forms, in which the recovery is very rapid (cf. below), and the duration of its resistance may well depend on the condition of the original material. This would explain the discrepancy between the different experiments. In this connexion attention may be drawn to the fact that in Experiment XVII, in which no recovery was noted, there is no difference between the percentages of dead cells at the beginning and end of the experiment, implying considerable power of resistance on the part of the material. In Experiment XXVIII, on the other hand, there were far more dead cells at the end of the experiment than at the outset.

TABLE IX.

Recovery from plasmolysis, during prolonged exposure to the plasmolysing solution, on the part of various terrestrial forms.

(In the case of each experiment the first line gives the original, the second the final condition.)

Material.	*Expt. No.*[1]	*Cells counted.*	*Strength of sol.*	*Strongly plasmolysed.*	*Slightly plasmolysed.*	*Unaffected.*	*Dead.*[2]	*Duration of experiment (days).*
			%	%	%	%	%	
Zygogonium	IX, X	1,987	5	69·9	12·2	17·9	2·1	1
,,	,,	864	5	23·6	39·6	36·8	2·4	
,,	XVI	1,226	5	71·8	16·7	11·5	16·3	5
,,	,,	756	5	2·8	25·6	71·6	32·7	
,,	XX	884	6 (cold)	55·3	19·7	25·0	14·1	2
,,	,,	887	6 (cold)	7·9	33·1	59·0	18·1	
,,	XX	882	6 (warm)	45·6	28·4	26·0	17·0	2
,,	,,	759	6 (warm)	0·1	12·0	87·9	25·5	
Hormidium	XI	2,053	5	62·1	33·0	4·9	2·5	2
,,	,,	503	5	—	86·3	13·7	4·2	
,,	XXVI	1,503	5	56·0	40·0	4·0	9·9	4
,,	,,	524	5	—	43·7	56·3	14·7	
Prasiola	XXVII	1,449	20	74·6	11·0	14·4	1·3	4
,,	,,	455	20	—	1·3	98·7	2·4	
Protonema	XV	1,215	5	58·0	27·9	14·1	—	6
,,	,,	406	5	22·2	74·4	3·4	—	
,,	XXIII	887	5	53·7	30·9	15·4	—	4
,,	,,	618	5	25·1	64·7	10·2	—	
Pleurococcus	VII	1,301	25	57·1		42·9	—	3
,,	,,	632	25	32·1		67·9	—	
,,	XVII	1,852	25	77·9		22·1	8·8	7
,,	,,	1,028	25	77·7		22·3	8·0	
,,	XXVIII	1,000	20	67·0		33·0	25·4	8
,,	,,	1,008	20	0·8		99·2	43·9	
Cystococcus	XXII	818	25	87·2		12·8	—	4
,,	,,	613	25	81·6		18·4	—	

The recovery in the filamentous forms is as a general rule very rapid. Thus, in Experiment XVI on *Zygogonium* the estimation of the original material (cf. Table IX) was made at 4.30 p.m.; at 9.30 p.m. on the same day the figures obtained (866 cells counted) were: 19·2 per cent. strongly plasmolysed, 61·6 per cent. slightly plasmolysed, and 19·2 per cent. unaffected. During the subsequent days (cf. Fig. 7) there was quite a gradual diminution of the strongly plasmolysed cells, whilst the number of slightly plasmolysed ones remained practically constant till three days later, when a sudden rapid decrease was noted. Perfectly similar results were obtained in all the estimations of the filamentous algae, but in the case of the protonema the change is always more gradual and a relatively large number of the strongly plasmolysed cells fail to recover (cf. Table IX and Fig. 7).

[1] In many cases a drought series and a recovery from plasmolysis estimation were carried out in the same experiment, the determination of the original material serving both as the starting-point of the drought estimations and as the first estimation of the material whose recovery from plasmolysis was to be studied.

[2] Cf. foot-note on p. 694.

It may be that the sudden drop in the number of slightly plasmolysed cells, which is to be observed in the filamentous forms after the experiment has been going on for some days, marks the death-point of a large number of the cells.

The rapidity of recovery would seem to depend to some extent on the temperature. In Experiment XX with *Zygogonium* the second estimation carried out on a sealed slide kept in a warm room shows a much higher total of unaffected cells in the final condition than in any of the other similar experiments made with this alga. The experiment with *Prasiola*

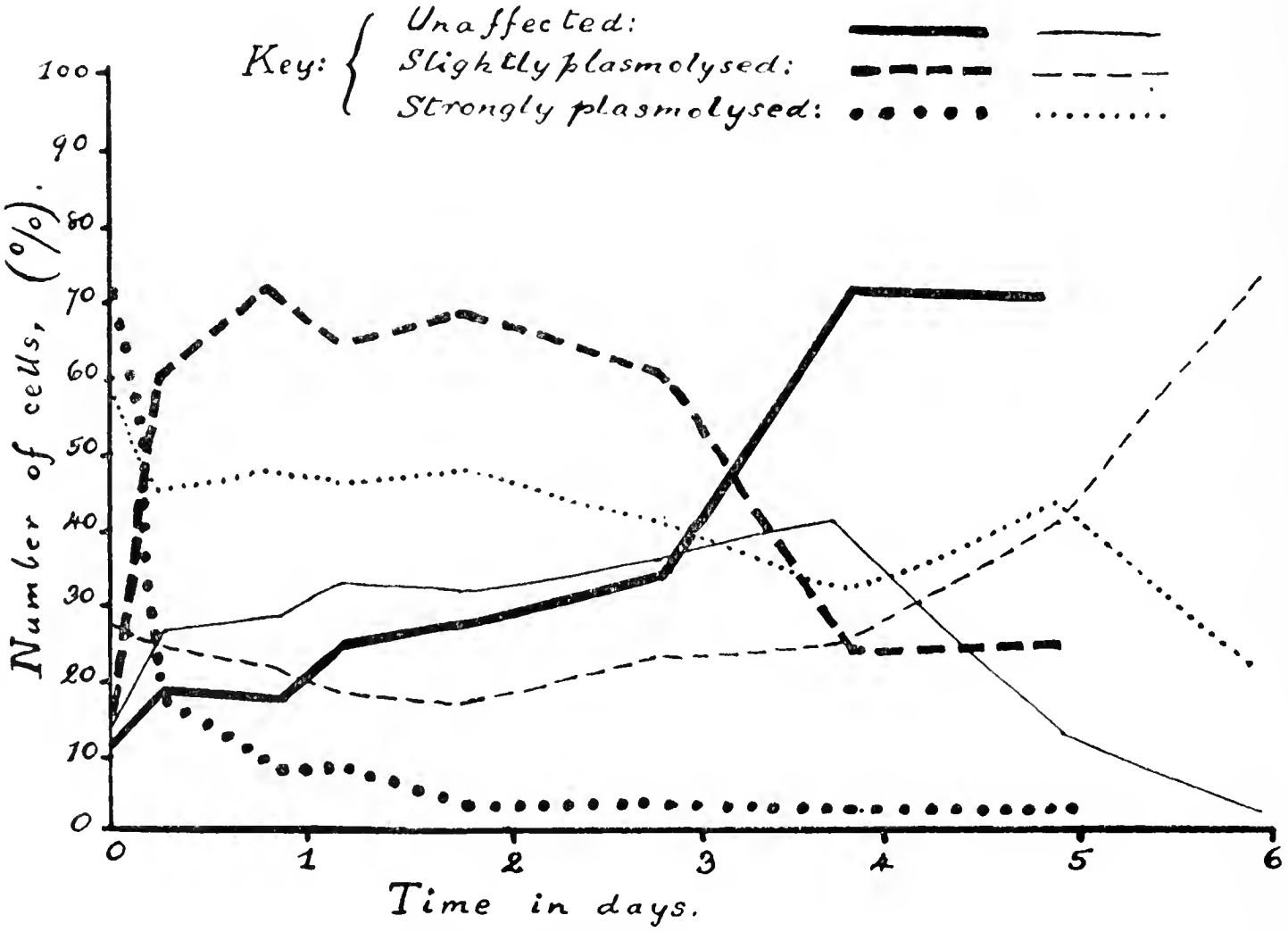

FIG. 7. Recovery from plasmolysis in a sealed slide. The heavy lines show the results for *Zygogonium* (Expt. XVI), the thin lines those for moss protonema (Expt. XV).

was also conducted in May, whilst the others were performed in March or April.

It has already been indicated that in *Pleurococcus*, if recovery occurs at all, it usually sets in only after some days. In Experiment VII, however, some results were obtained which seem to indicate that, when the material is in a suitable condition, some at least of the cells may recover very rapidly.

These results are set out in Table X and were all obtained on the same day with the same mass of material. They are so uniform, without any exception, that they can scarcely be due to experimental error, yet on other occasions (e. g. in Experiment XVII) nothing of the kind was to be observed. It is, however, to be noted that in the latter case there was no recovery even after seven days (cf. Table IX), whereas in Experiment VII recovery was evidently continued.

TABLE X.

Estimation at half-hour intervals of percentages of plasmolysed cells of Pleurococcus (Experiment VII).

Strength of sol. %	*Cells counted.*	*Percentage plasmolysed.* 1*st half-hour.*	2*nd half-hour.*
15·0	500, 502	20·8	19·1
17·5	502, 505	24·7	20·4
20·0	502, 502	30·5	23·3
22·5	502, 501	37·3	29·9
25·0	501, 401	48·7	44·4

In another case (Experiment XXIV) estimations of 1,000 cells were made at two-hourly intervals on a sealed slide with *Pleurococcus* kept in a warm room. There were some indications here of a daily cycle, the percentage of plasmolysed cells sinking towards midday and rising again in the evening and, during the two days of the experiment, showing a slight downward tendency. The results are not at all conclusive, but owing to the large amount of time consumed in such experiments it has not been possible to repeat them up to the present.

Recovery from plasmolysis was also established in a very large number of cases by direct microscopic observation of small groups of initially plasmolysed cells over a period of several days. In the case of *Zygogonium* such recovery was repeatedly noted in 3, 5, and 10 per cent. solutions, being complete in about three days; the recovery of granular cells was almost invariable, that of non-granular cells not so frequent, especially in the stronger solutions. *Hormidium* showed frequent complete recovery both in 5 and 10 per cent. solutions. Moss protonema usually fails to recover, but in one set of cells complete recovery was observed in a 5 per cent. solution, never in a 10 per cent. solution; in most cases the extent of plasmolysis was found to increase at first and then to decrease again, although, as just mentioned, this only led to complete recovery in one thread. Analogous observations were also carried out on the cells of the leaves of a moss (*Hypnum cupressiforme*, L., var. *filiforme*, Brid.) and gave results quite identical with those just recorded for the protomena, recovery being only noted in one case in a 5 per cent. solution. In a few cases cells of a narrow species of *Spirogyra* showed recovery both in 5 and 10 per cent. solutions; more usually, however, they gave a negative result (cf. also Table XI); cells of *Cladophora* and *Chaetophora* in a 3 per cent. solution showed no decrease in shrinkage of the protoplasts during a period of ten days. The epidermal cells of *Saxifraga sarmentosa* lastly, though occasionally recovering in 3 per cent. solutions, failed to show any recovery either in 5 or 10 per cent.

solutions; here again, as in the protonema, a slight increase in plasmolysis was often observed at first, to be followed by a subsequent slight decrease. These results bring out very clearly the great capacity for recovery on the part of the two terrestrial algae studied, by contrast with the moss (protonema and leaf), aquatic algae, and the flowering plant (see also Table XI).

Table XI.

Behaviour of the protoplasts of various forms when exposed to prolonged action of the plasmolysing solution in a sealed slide.

(The figures give the areas of the protoplasts in percentages of the total areas of the cells.)

Material.	*Strength of sol.*	*Time in days.*					
		1	2	3	4	5	7
	%						
Zygogonium (granular)	5	82·2	88·3	100·0	—	—	—
	5	90·1	100·0	—	—	—	—
	10	82·2	82·4	87·5	95·8	—	—
	10	72·0	93·6	100·0	100·0	—	—
	5 % NaCl	85·1	85·3	98·6	99·6	—	—
Zygogonium (non-granular)	5	57·0	78·5	94·5	—	—	97·3
	10	52·2	70·1	50·8	60·8	—	—
	10	45·9	60·2	96·8	100·0	—	—
Hormidium	5	85·2	87·9	99·2	—	—	100·0
	5	81·7	—	81·9	90·9	94·1	—
	10	82·2	100·0	100·0	100·0	—	—
	10	76·0	85·5	80·1	81·8	—	—
	5 % NaCl	81·4	92·8	94·3	87·7	—	—
Protonema	5	75·8	—	100·0	80·2	85·3	—
	5	71·7	—	65·2	66·3	—	—
	5 % NaCl	83·0	80·4	82·9	85·2	—	—
Spirogyra (narrow sp.)	5	88·7	—	90·5	92·1	93·7	—
	5	47·9	—	100·0	100·0	100·0	—
	10	66·1	100·0	100·0	100·0	—	—
	10	91·3	72·3	43·5	43·5	—	—
Spirogyra (broad sp.)	10	96·6	92·7	92·3	92·8	—	—
	5 % NaCl	67·5	56·6	55·5	64 7	—	—

The data given in the preceding paragraph were in part obtained merely by daily observation, but in a considerable number of cases more accurate means were employed in order to follow the stages in recovery. The method most usually employed, and that by which the results given in Table XI were obtained, consisted in making camera lucida drawings of the outlines of the protoplasts of selected cells on successive days and estimating the degree of contraction by finding the areas of the protoplasts as represented in the drawings with the help of a planimeter. In Table XI these results are expressed as percentages of the total area of the interior of the cell as seen on the same drawings. Whereas the results epitomized in Table IX and Fig. 7 indicate the aggregate behaviour of the cells of a given mass of material, those in Table XI indicate the behaviour of individual cells. The most striking feature in the latter case is again the usually very rapid

recovery, if recovery is to occur at all. Another fact of some interest is that the extent of the initial plasmolysis bears no relation to the occurrence or non-occurrence of a subsequent recovery, nor to the rapidity with which the latter sets in. There are indications that recovery does not so easily take place in a 5 per cent. sodium chloride as in the balanced solution. Attention should finally be drawn to the fact that the conclusions detailed in the preceding paragraph were drawn from a much larger number of observations than are shown in Table XI, which would have become too unwieldy if all had been included.

Some rather peculiar results were obtained in analogous experiments in which sugars were used. If threads of *Zygogonium* are placed in a 20 per cent. sucrose solution, no plasmolysis at all is to be observed at first, although such a solution has an osmotic pressure exceeding that of a 3 per cent. sea-salt solution which plasmolyses many of the cells of the alga. A day later, however, slight plasmolysis is to be seen in many of the cells and remains apparent for some days, though ultimately mostly disappearing. The same results were obtained with 10 per cent. glucose, whilst 30 per cent. glucose causes immediate violent plasmolysis, from which the cells in a sealed slide recover in about two days. This matter has so far not been further followed up.

In most of the cases described above, in which recovery was established by direct microscopic observation, a more or less marked central aggregation of the chloroplast or chloroplasts, as well as of the granules when present, was noted, the whole periphery of the newly expanded protoplast appearing clear, opaque, and whitish. Frequently, too, a change in the character of the granules was established, those in the final condition being large and irregular (cf. p. 716 on condition of the cells during drought). On the whole this condition was more evidently seen when recovery was rapid than when it was slow.

It is probable that recovery from plasmolysis in a sealed slide is partly to be regarded as a pathological phenomenon, implying a state of decreased vitality on the part of the material, chiefly perhaps as a result of interference with respiration. There are two possible methods by which such a pathological condition could lead to the observed recovery. Firstly, it might be expected to bring about increased permeability of the cells, thus allowing penetration of the surrounding solution. Secondly, the protoplast might swell by simple imbibition, this process being augmented by degradation-products such as acids produced as a result of the unhealthy condition of the cell. Emphasis must, however, be laid upon the fact that, if such factors come into play, their action is different in the case of terrestrial and aquatic algae and also different in the moss and the flowering plant (cf. p. 703). Moreover, the very marked similarity in the response to drought and in the reaction to a continued exposure to the plasmolysing solution,

upon which stress has already been laid, appears very indicative of a capacity on the part of these terrestrial forms to accommodate their cells to extreme conditions of their environment.

The possibility of the xylol present in the sealing medium being responsible for the results recorded in this section was disproved by placing two mats of *Zygogonium* respectively in 5 per cent. sea-salt solution and a similar solution to which a small amount of 5 per cent. sea-salt, previously shaken up with xylol, had been added. The dishes containing the solutions were covered over with glass plates and estimations of the two sets of material were made two days later in the usual way. The results were :

Solution.	*Cells counted.*	*Strongly plasmolysed.*	*Slightly plasmolysed.*	*Unaffected.*
		%	%	%
5 % sea-salt	1,021	22·9	23·4	53·7
Ditto + xylol	966	31·5	37·4	31·0

It is seen that the effect of the xylol is in the opposite direction to that noted in the above experiments, and its presence cannot therefore be responsible for the observed results.

The frequent recovery of the cells of the terrestrial forms studied from plasmolysis in hypertonic solutions led us to attempt their cultivation in such solutions. With this end in view, small patches of soil bearing mats of *Zygogonium*, *Hormidium*, and protonema were placed in wide crystallizing dishes in solutions of sea-salt of various strengths (usually 5 per cent.), partly out of doors and partly in a warm greenhouse; the dishes were covered with glass plates and exposed to varied illumination. Except in one experiment with *Zygogonium* in a 5 per cent. solution, however, no further growth of any of the forms was noticed. In the one case small tufts of threads arose from the edge of the mat and trailed out into the surrounding fluid. Such growth was, however, limited to a fortnight, and some weeks later a large number of the constituent cells proved to be dead, whilst others (nearly always granular cells) were perfectly healthy and green, though mostly plasmolysed.

The general result of the remaining experiments of this kind may be briefly summarized as follows. A large number of the cells of the three forms examined remain in a healthy condition for periods of six weeks to three months and upwards, the duration of resistance probably depending *inter alia* on the condition of the original material. Those of the cells that suffer from the conditions of the experiment die in a relatively short space of time, probably usually within the first week, and show brown, often uncontracted contents. The surviving cells are very commonly the granular ones, and in the case of protonema, where the mortality is much greater, especially those situated at the tips of the branches. A variable number of the surviving cells are found to be plasmolysed, and this is often the condi-

tion of the majority; as a general rule the plasmolysis is not very extreme and its extent appears to undergo no appreciable alteration after the experiment has been in progress for some weeks. That these plasmolysed cells are otherwise perfectly healthy is shown by their capacity for immediate and complete recovery, even after weeks in the salt solution, when placed in tap-water. It should be especially emphasized that cells with uncontracted though quite healthy protoplasts were always to be found in the threads, even after many weeks' sojourn in the salt solution, and this again implies on the part of a larger or smaller number of the cells a marked capacity for resistance to the high concentration of the surrounding fluid. Such cells may obviously be the starting-points for growth, and that this has only been observed in one instance may well be due to other conditions of the experiment being unsuitable. We hope to return to this problem at a later stage.

The observations just detailed indicate that material permanently immersed in hypertonic solutions shows a failure on the part of many of the initially plasmolysed cells to recover. This conclusion is obviously at variance with the results obtained in the case of algal threads kept in a sealed slide which were considered in the earlier part of this section. It is for this reason that we regard these results as probably in part due to pathological causes. There is no doubt, however, that even in open solutions a gradual recovery takes place during the first days (cf. Table XII), but the recovery is probably again in great part pathological and followed by the death of the cells in question. Thus, whilst in the first days there is

TABLE XII.

Successive estimations of material of Zygogonium immersed in 5 per cent. sea-salt solution (Experiment XXXV).

Date.	*Cells counted.*	*Strongly plasmolysed.*	*Slightly plasmolysed.*	*Unaffected.*
		%	%	%
Feb. 5	1,544	66·8	16·7	16·5
„ 6	1,021	22·9	23·4	53·7
„ 7	1,000	27·2	10·7	62·1
„ 9	1,030	47·3	21·8	30·9

a very obvious decrease in the numbers of plasmolysed cells, the percentages of such cells tend subsequently to increase again in the direction of a maximum. This increase is, however, a deception and due to the fact that there is a great mortality among the unaffected cells, the plasmolysed ones which constitute the main mass of the healthy cells thus, although actually remaining constant in number, appearing to undergo a great relative increase. Whilst the majority of the healthy surviving cells are often plasmolysed, there are, as already mentioned above, always a number of healthy unaffected cells. It is probable that these are cells which were

unaffected from the very first. In Experiment XXXV all the surviving cells had small granules, whilst the moribund unaffected cells were distinguished by the possession of coarse irregular granules.

In concluding this section attention may be drawn to the most important conclusion reached, viz. that the cells surviving prolonged treatment with hypertonic solutions are either those which are impermeable to the solution as implied by the persistence of plasmolysis or more rarely such as are possessed of a mechanism inhibiting the occurrence of plasmolysis as implied by their remaining unaffected. The close correspondence between the reaction to drought and the reaction to hypertonic solutions would seem to make it probable that the surviving cells are of the same kind in both cases, a matter which is further considered in the next section (cf. p. 717).

E. Recovery from the Drought Condition and the State of the Material during Drought.

In several cases, after the material had been subjected to more or less intense drought, it was placed either in saturated air or was actually soaked with water.

Table XIII.

Comparison of plasmolysis in drought material of various terrestrial forms and in the same material some time after exposure to moisture.

(The first line in each case shows the drought condition,[1] the second that after exposure to moisture.)

Material.	*Expt. No.*	*Cells counted.*	*Strength of sol.*	*Strongly plasmolysed.*	*Slightly plasmolysed.*	*Unaffected.*	*Duration of exposure to moisture (days).*
			%	%	%	%	
Zygogonium	V	1,839	5	0·1	7·2	92·7	
,,	,,	715	5	—	81·3	18·7	3
,,	XII	926	5	—	5·1	94·9	
,,	,,	827	5	1·2	22·5	76·3	3
,,	XVI	841	5	0·5	15·1	84·4	
,,	,,	1,042	5	1·1	51·2	47·7	13
,,	III	990	3	—	2·6	97·4	
,,	,,	960	3	0·4	68·8	30·8	12
Hormidium	XIV	1,400	5	—	12·9	87·1	
,,	,,	503	5	—	13·7	86·3	2
Prasiola	XXVII	905	20	—	28·3	71·7	
,,	,,	921	20	45·8	27·3	26·9	21
Protonema	XV	655	5	2·9	83·8	13·3	
,,	,,	931	5	32·3	37·6	30·1	14
,,	XXXIV[2]	887	5	—	62·3	47·7	
,,	,,	1,105	5	80·7	14·3	5·0	1
,,	,,	640	5	—	34·2	65 8	
,,	,,	565	5	68·7	28·8	2·5	1

[1] Regarding the nature and duration of the previous drought, cf. Table V.

[2] In Experiment XXXIV patches of soil with protonema were removed from the desiccator on successive days and soaked with water.

Its relation to the same strength of plasmolysing solution as had been used for the previous drought determinations was investigated a few days later. Some of the results are epitomized in Table XIII (cf. also Table VI and Figs. 6 and 8). Although in most cases some alteration in the behaviour of the material was to be recorded, a pronounced recovery was only observed when the duration of the previous drought had been short, as in the case of the *Prasiola* and protonema. In such cases, moreover, the recovery is rapid, and one can feel sure that it is actually due to an alteration of the cells that had previously been affected by the drought. On the

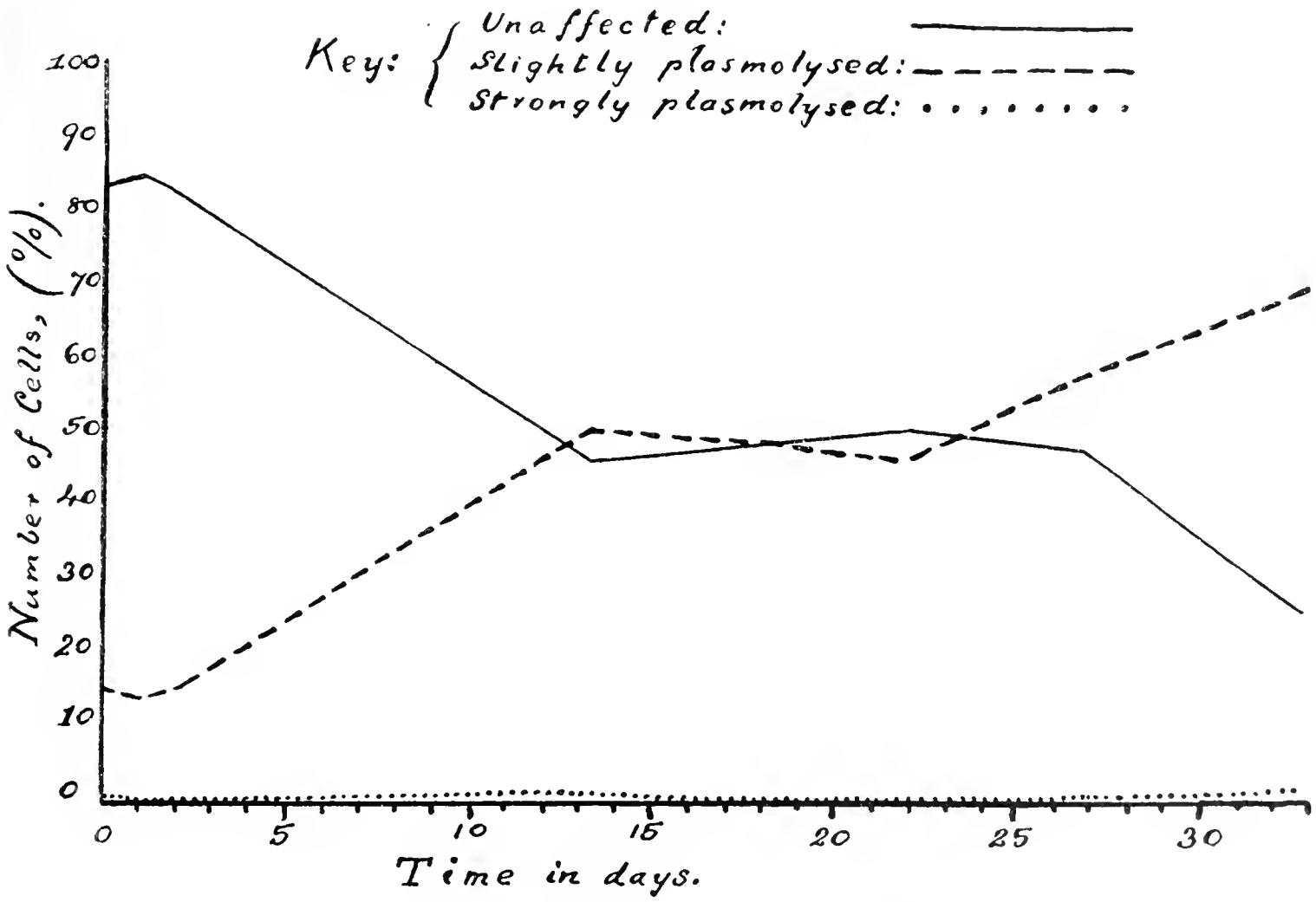

FIG. 8. Recovery from drought in the case of *Zygogonium* (Expt. XVI).

other hand, if material during exposure to drought has once reached the state in which a large number of the cells exhibit the slight permanent contraction of the protoplast mentioned on p. 691, recovery is very slow, and one is in some doubt as to whether increasing plasmolysis is due to an actual recovery of the tendency to plasmolyse or to the gradual formation of new threads by some of the surviving normal cells.[1] Those recovering from drought, however, are generally distinguished by microscopic characteristics (cf. p. 715) from those due to new growth. That there is an actual recovery, even in such cases, is indicated by observations made in Experiment XVI (cf. Table VI and Fig. 8), where the gradual increase in the number of slightly plasmolysed cells proved to be due to an actual increase in the

[1] Moreover, when recovery is slow, the deleterious effect of laboratory conditions (cf. p. 716) may be just as responsible for the state of the protoplast as the original drought.

capacity of the cells to plasmolyse. This was shown by the fact that on May 10 a 10 per cent. sea-salt solution produced far more plasmolysis than a 5 per cent. one. The frequent failure to recover, or the slow recovery noted in many of our experiments, is no doubt to be ascribed to the fact that the intensity of the drought to which the material was subjected was in general rather great, probably far greater than ordinarily occurs in nature. That the difference between drought and wet material is not confined to the filamentous forms is illustrated by Fig. 6, which gives the results of experiments with *Pleurococcus*.

The question may now be approached as to the actual nature of the change that takes place in the cells of the forms investigated during a period of drought. There are obviously various explanations that could be advanced to account for the gradual disappearance of the tendency to plasmolyse. Thus, there may be an increase in the permeability of the protoplasts, production of additional osmotic material may take place, or the viscosity of the protoplasts may increase to such an extent that the latter become sufficiently rigid to resist the tendency to undergo contraction. In the case of recovery of material kept in hypertonic solutions there is the further possibility that the protoplasts are slightly permeable from the beginning, but this is unlikely to apply to more than a minority of the cells, in view of the persistence of plasmolysis in the majority in open hypertonic solutions.

(*a*) *Observations on Permeability to Stains.*

1. *Zygogonium.* In the case of *Zygogonium* (material from habitat III) experiments have been conducted with a number of different stains, dissolved in small quantity in a 5 per cent. solution of sea-salt, in order to investigate a possible relation between the penetration of the stain and the occurrence of plasmolysis. After treatment for twenty minutes with such a solution to which a little methyl violet had been added, the dead and unplasmolysed cells of the alga showed deep staining of the walls and the protoplasts, whilst in the plasmolysed cells the latter were practically unaffected. After five minutes in a 5 per cent. solution with four drops of aqueous 1 per cent. erythrosin the unaffected cells were deeply stained and the majority of the plasmolysed cells unstained; treatment for half an hour afforded the same result, though the staining was much more pronounced. When a little eosin was dissolved in the plasmolysing solution, only the unaffected cells were stained as a general rule; the result was apparent already after five minutes, but much more marked after half an hour.

On the other hand, methyl green, nigrosin, and Bismarck brown afforded no such results, even after an hour's treatment; in the first two cases there was no evident penetration of the stain into any of the cells, whilst with Bismarck brown there was faint staining of the chloroplast in all cases.

A solution made up of one drop of 1 per cent. Congo red in 1 c.c. 5 per cent. sea-salt produced a very deep coloration of the wall after three-quarters of an hour in all the dead cells, but only a very slight one, if any, in the living cells; there was no penetration into the protoplast of the latter whether plasmolysed or not. It may be added that neutral red does not differentiate between different cells of this alga, as it does in *Hormidium* (cf. below); the stain is taken up very rapidly, even from a very dilute solution, and appears in the chloroplasts, which become deeply tinted.

The cells of *Zygogonium* are thus permeable to different stains and, in the case of several of these, the degree of permeability varies, the stain penetrating readily into some of the cells and not into others. Eosin, as giving the most pronounced results and apparently not staining the wall, was selected for further investigations. The number of cells becoming stained after half an hour's treatment differs very much (cf. the first column of Table XV) and evidently depends on the condition of the material.

Table XIV.

Relation between penetration of eosin and response to 5 per cent. sea-salt solution on the part of fresh material of Zygogonium.[1]

(Habitat II, Experiment XXV.)

	Strongly plasmolysed.		*Slightly plasmolysed.*		*Unaffected.*	
	Stained.	*Unstained.*	*Stained.*	*Unstained.*	*Stained.*	*Unstained.*
	%	%	%	%	%	%
Immediately	3·2	35·0	9·2	33·6	19·0	—
After one hour	15·4	2·1	42·1	2·5	37·9	—

The rate of penetration of the stain varies. In Experiment XXV (cf. Table XIV) the unaffected cells stained immediately, and so did some of the plasmolysed ones. The majority of these, however, required a varying time to take up the stain, but it had penetrated into most of them at the end of an hour. This was, however, an extreme result, partly due perhaps to the experiment being performed in a very warm room, and in other cases many of the cells resisted the penetration of the stain for a much longer time. There is often, as mentioned above, a considerable degree of parallelism between the permeability to the stain and to a 5 per cent. sea-salt solution, as indicated by the occurrence or non-occurrence of plasmolysis. It may be pointed out, however, that the degree of correspondence between the two sets of phenomena will of course depend on the strength of plasmolysing solution used and the condition of the material. Threads which had been for a few days immersed in 5 per cent. salt solution (cf. p. 706) gave very definite results,

[1] Slightly over 200 cells counted at each estimation. Since the experiment was performed in a very warm room the material shows recovery at the second estimation.

the contents of the plasmolysed cells all being unstained after half an hour, whilst the majority of the unaffected cells were stained. In later stages of such experiments, however, the few unaffected cells are not stained, whilst a certain number of the strongly plasmolysed ones are.

Some of the drought material of Experiment XVI (cf. Table V) was subjected to the same treatment. The contents of all the dead cells and of the non-granular ones were immediately deeply stained, whilst the numerous cells with large irregular granules (cf. below) required but little longer to stain. The protoplasts of the few perfectly healthy green cells with fine granules, on the other hand, remained unstained, although it was very noticeable that in these last the wall became distinctly coloured, much more so than in the other cells or in the original fresh material. Similar results were obtained with the material that had been in a sealed slide for five days (cf. Table IX, Experiment XVI), the protoplasts of the majority of the cells being immediately stained, whilst the few healthy green cells with fine peripheral granules remained unstained.

TABLE XV.

Percentages of cells[1] *stained with eosin* (*treatment as described in text*) *in Zygogonium during drought and subsequent recovery.*

(Experiment XXXI, habitat III.)

	Original material.	*Days of drought.*					*Days after soaking.*		
		1	2	3	4	5	2	3	5
Series 1	11·0	83·7	99·1	—	—	—	72·5	—	46·6
,, 2	2·5	5·4	5·4	87·4	99·2	100·0	—	96·4	97·1
,, 3	0·0	15·6	94·2	99·7	—	—	83·2	—	—

There can hardly be any doubt that there is an appreciable alteration in the degree of permeability to eosin on the part of the cells of *Zygogonium* during a period of drought.[2] This has been established by keeping material over 34·5 per cent. sulphuric acid in a desiccator, in the dark and at a constant temperature of 18° C., and making daily estimations of the numbers of stained and unstained cells. At each estimation some of the filaments, well teased apart, were placed for half an hour in a watch-glass in 0·2 per cent. eosin in tap-water, and the material was then mounted in ordinary water. The results are reproduced for three separate series in Table XV. A marked feature of all three series is the suddenness with which the change in per-

[1] 2,000 or more cells were counted at each estimation in this experiment, no dead ones being taken into account.

[2] The only other explanation that could be advanced for the phenomena about to be described is that the increased tendency to take up the stain is due to a change in the reaction, and therefore in the electrical condition of the protoplasmic substance, rather than to an increased permeability. Such an explanation, whilst possibly applying in the case of *Hormidium* (cf. p. 713), is not established for *Zygogonium* by the available data.

meability to the stain sets in. It will be seen that, if the drought is sufficiently prolonged, all the cells may ultimately take up the stain (series 2). A further noteworthy point is that soaking of the material with water, after a brief period of drought, leads to a more or less rapid decrease in the percentage of stained cells (cf. series 1 and 3), whilst there is no such decrease if the drought has been prolonged (series 2). This falls into line with the observations detailed above on the recovery of drought material when placed in damp air (cf. p. 709), where in the vast majority of cases the previous drought was as intense or even more intense than in series 2. Dilute erythrosin gives quite similar results to those described for eosin, but no such alteration in the behaviour of the protoplasts with respect to neutral red, such as is recorded below for *Hormidium*, was found in the case of *Zygogonium* ; ten minutes' treatment with 0·2 per cent. neutral red stained all the cells, and even after three days' drought there was no change in this respect.

Table XVI.

Percentages of cells[1] *stained and unstained by neutral red (treatment as described in text) in Hormidium during drought and subsequent recovery.*

(Experiment XXXII, habitat II.)

	Series 1.			*Series 2.*		
	Whole stained.	*Protopl. stained.*	*Whole unstained.*	*Whole stained.*	*Protopl. stained.*	*Whole unstained.*
Original material	25·5	8·3	66·2	25·6	36·0	38·4
1 day's drought	11·3	5·2	83·5	—	—	—
2 days' ,,	0·0	1·5	98·5	4·9	3·1	92·0
3 ,, ,,	—	—	—	1·2	9·4	89·4
5 ,, ,,	—	—	—	0·2	10·0	99·8
3 days after soaking	—	—	—	7·5	19·2	73·0

2. *Hormidium.* This alga gives results with 0·2 per cent. eosin and dilute erythrosin which are identical with those obtained for *Zygogonium*, the healthy cells of fresh material altogether excluding the stain, whilst nearly all the cells of drought material take it up rapidly. Neutral red affords different results. With this stain the cells fall into three groups, viz. those which are unstained, those with the protoplast but not the chloroplast stained, and those in which the whole contents are stained.

The effect of drought is rather contrary to expectation (cf. Table XVI). The material in this case was stained for 10 minutes in 0·4 per cent. (series 1) or 0·2 per cent. (series 2) neutral red dissolved in tap-water, the estimation again being made in tap-water. The conditions of drought were the same as in the experiments above described for *Zygogonium*. The progressive change in the material is again quite obvious, but in this case it is in the direction of a decrease to a minimum of the number of stained cells.

[1] 2,000 cells counted at almost every estimation ; dead cells ignored.

The result could be explained as due to a decrease in the permeability of the protoplasts, but this is not very likely in the face of other conclusions reached in the present paper. It seems more probable that it is to be ascribed to a change in the reaction of the protoplast, so that the stain is not accumulated. Evidence for this view is afforded by the fact that stains of opposite sign (eosin and erythrosin on the one hand, neutral red on the other) are respectively only capable of accumulation when the material is in a condition in which it is unaffected by the opposite stain. *Zygogonium*, as already mentioned, does not show this change in behaviour towards neutral red during drought, which is indicative of the diversity and complexity of the phenomena involved. Soaking of the drought material with water, as in *Zygogonium*, leads after some days to a recovery in the original direction (cf. series 2).

3. *Moss protonema.* Treatment of the original material of Experiment XV (cf. Table V) with 5 per cent. sea-salt, to which a very small quantity of eosin had been added, gave results analogous to those above described for *Zygogonium*. The vast majority of the unaffected cells were deeply stained, the slightly plasmolysed cells stained after a little time, and most of the strongly plasmolysed ones were unstained, although a few here and there were deeply coloured. The drought material of this experiment treated in the same way showed the majority of the unaffected cells unstained, the slightiy plasmolysed ones mostly stained, whilst a few of the strongly plasmolysed cells were stained, though most of them were not. Since the statistical observations in Experiment XV (cf. p. 693) indicate that some at least of the originally strongly plasmolysed cells can recover, and since direct microscopic observation of protonema sealed in a 5 per cent. sea-salt solution (cf. pp. 703, 704) shows that some of these cells are incapable of recovery, it would appear that there are in the protonema used two types of strongly plasmolysing cells differing in their degree of permeability. Some are altogether impermeable and remain plasmolysed, others are slightly permeable, so that, though plasmolysed at first, they ultimately recover. It should be emphasized that there does not appear to be any great difference in the behaviour towards eosin on the part of the strongly plasmolysed cells in the fresh and the drought material, the principal difference lying in the unaffected cells.

Material that had been for four days in 5 per cent. sea-salt in a sealed slide (Experiment XXIII, Table IX) showed, after twenty minutes' treatment as above, practically all the slightly plasmolysed cells stained, whilst the unaffected and strongly plasmolysed ones were each about half stained, half unstained (cf. with the results for drought material). In another experiment (XV, Table IX), with six days' exposure to the solution, all the cells stained deeply in a short time, although some of the strongly plasmolysed ones more slowly than the others.

Two separate investigations (Experiment XXXIII) on the progressive changes in staining of the cells of protonema with eosin during drought (34·5 per cent. sulphuric acid in the dark at 18° C.) have been undertaken.[1] In the first series the percentages of stained cells on successive days were: 17·5 (original material), 20·3, 87·5, 18·5, 17·2; in the second series they were: 29·4 (original material), 38·7, 99·8, and, after four days' drought, approximately half the cells stained (actual count not made). In both series the result, for the first two days of drought, was as in *Zygogonium*, but after that there was in each case a great decrease in the percentage of stained cells, a fact for which there is at present no satisfactory explanation. In the second series parts of the protonema mat were removed at various stages of drought and soaked with water, though otherwise left under the same conditions. Material thus removed after two days' drought (then showing 99·8 per cent. cells stained) gave one day after soaking 81·7 per cent. and two days after soaking 79·3 per cent. stained cells, after three days' drought and one day's soaking with water 86·4 per cent. stained cells. It is evident, therefore, that with reference to the capacity to exclude the stain there is very little recovery on the part of drought material in this case. Only one investigation of the effect of dilute erythrosin has been undertaken, and this afforded results which were practically identical with those obtained with *Hormidium* and *Zygogonium*; after five days' drought the majority of the cells stained deeply in a short space of time.

4. *Pleurococcus and Cystococcus.* No clear results have been obtained with either of these algae. If cells of *Pleurococcus* are mounted in 0·2 per cent. eosin, only very few take up the stain, and simultaneous treatment with a 25 per cent. sea-salt solution shows that the strongly plasmolysed cells never stain, the dead cells invariably, whilst of the unaffected cells roughly half are stained. Nor is there any sensible difference in drought material; thus, in Experiment XXX (cf. Table V) an estimation, made after ten weeks' drought, showed that only 6·4 per cent. of the cells stained, which corresponds to about one-third of the unaffected cells present. In the same experiment, material that had been subjected to four weeks' drought and had then been soaked with water for six weeks gave 6·0 per cent. cells stained, corresponding to about one-half the unaffected cells. Examination of material which had been in a 25 per cent. solution in a sealed slide for seven days (Experiment XVII, Table IX) afforded identical results, the ratio of stained to unstained unaffected cells being 41 : 59. In the case of *Cystococcus* the unaffected cells on the whole stain more strongly with eosin than the plasmolysed ones, but this is not an invariable rule. No difference could be detected in this respect between fresh and drought material.

The general conclusions to be drawn from the above observations are considered in the next section.

[1] Method as above described for *Zygogonium*; 1,000 cells or more counted at each estimation.

(*b*) *Microscopic Characters of Drought Material.*

The cells to be found in drought material can be broadly divided into three groups: (*a*) the dead cells which have discoloured, though not necessarily shrunken, contents; (*b*) living cells with a slightly contracted and apparently rigid protoplast, since the contraction does not disappear in ordinary water and plasmolysis does not ensue even after treatment with very strong solutions (25 per cent. sea-salt); and (*c*) healthy pure green cells with a quite uncontracted protoplast and not affected by similar strong plasmolysing solutions. In *Pleurococcus* and *Cystococcus*, as far as our observations go, the second type of cell is not found and many of the cells remain capable of plasmolysis even after very prolonged drought. In the filamentous forms the relative numbers of the different types of cells present depend on the intensity and duration of the drought; at first there are relatively many of the third type, later the second type predominates, and under extreme drought the majority of the cells may die. What extent of drought may be necessary to lead to the death of all the cells in a given mass of terrestrial alga or moss protonema we are unable to say (cf., however, Schroeder, 1886), but we are inclined to think it would have to be very intense or very prolonged.

The cells of the second type are not dead (cf. section (*c*)), although a large number of them may be moribund, if the drought has been intense or prolonged. These cells, when granular, invariably contain large coarse granules of unequal size and showing an irregular distribution, some parts of the periphery of the protoplast being crowded with them, others practically free. Non-granular cells, especially in *Zygogonium*, often have a peculiar whitish opaque look about the edge of the protoplast. Material that has been in a sealed slide presents similar features, but cells of either type are rarely found in material out of doors and then only in small numbers. In *Hormidium* during drought a large spherical granule is often to be seen in these cells in the clear area outside the chloroplast.

It is evident, however, that this rigid state of the protoplast can arise also as a result of conditions other than drought. In the first place it would seem to develop after some weeks' sojourn in the laboratory, even in material that has been kept continuously moist. Further, it has been observed to appear in a large number of the cells of *Zygogonium* as a result of exposure to bright light. Thus, in one of our experiments on the influence of darkness and illumination on the plasmolysing capacities of the cells of this alga, in which the experiment had been inadvertently allowed to continue for four weeks, the cells of the 'light' material were found to be mainly in this condition, whilst those of the 'dark' material showed it to a much lesser extent. It may be added that in the former the cells were nearly all purple

as a result of the development of phycoporphyrin, whereas in the latter they were pure green.

The only cells which after drought can be regarded as really healthy are those whose protoplast is quite uncontracted, which possess a healthy green colour, and in the filamentous algae usually have a very regular layer of fine, even granules at the periphery of the protoplast. These cells, which after prolonged drought are not very numerous or may be completely absent, are the ones which prove to be impermeable to eosin in the case of *Zygogonium* (cf. p. 712) and the protonema (p. 714), whilst the slightly contracted cells are all readily permeable, although not generally as rapidly as the dead cells.

In the case of material kept in hypertonic solutions (cf. p. 706 et seq.) there are likewise usually a certain number of cells with uncontracted protoplasts, and, from the similarity in microscopic characteristics, it is probable that they are the same cells as appear quite unaffected at the end of a drought experiment. Side by side with the unaffected cells in material kept in hypertonic solutions there are, however, far more numerous strongly plasmolysed cells. Some at least, if not the majority, of these must correspond to the numerous cells with permanent slight contraction of the protoplast in drought material. These cells are therefore more resistant to a prolonged sojourn in a hypertonic solution than to drought, since the great permeability of these cells in the latter case is probably an indication of their being in a more or less unhealthy condition, leading sooner or later to death, and from which a recovery is perhaps only possible in early stages (cf. p. 706). Moreover, although plasmolysed, the protoplasts of the cells in open hypertonic solutions are not rigid, since they recover in tap-water (cf. p. 707).

The healthy uncontracted cells remaining after drought and prolonged treatment with hypertonic solutions, in view of their practical impermeability to stains, are probably also impermeable to the salt solution. Since they remain unplasmolysed, the concentration of their sap in osmotic material would seem to be at least as great as that of the 5 per cent. sea-salt solution used. In fresh material, however (cf. Table XIV), it is the plasmolysed and not the unaffected cells that fail to take up stains. To account for the presence of unplasmolysed and non-staining cells in the final condition, therefore, there are two possible explanations: These cells may be the unaffected ones of the original material (which might be supposed to have an osmotic strength greater than 5 per cent. sea-salt solution) which, at first readily permeable, have become impermeable. Alternatively these cells may be the original strongly plasmolysed ones which have remained relatively impermeable but have met the conditions to which they have been subjected by the formation of additional osmotic material. The failure of both suggestions to cover the facts completely, since the cells in question are often also unaffected by solutions far stronger than 5 per cent., hints at the

possibility of imbibition phenomena being also concerned. In the case of *Prasiola*, *Pleurococcus*, and *Cystococcus* such an explanation would appear to be the only feasible one to account for the absence of plasmolysis in a considerable number of the cells in solutions of a strength of 20 per cent. and upwards.

(*c*) *Observations with Dark-ground Illumination.*

For these investigations the illuminant used was an electric arc taking from fifteen to twenty-five amperes, in conjunction with a Watson paraboloid condenser. Fresh material of *Spirogyra* was always examined first with the object of obtaining an idea of the condition of the normal algal protoplast. This shows complete milkiness and vigorous Brownian movement throughout the cytoplasm.

When fresh material of *Zygogonium* is examined, vibratory Brownian movement of minute particles in the protoplasm is distinct in all except the dead cells. Non-granular cells are seen to have but a thin cytoplasmic lining beneath the cell-wall, with a wide vacuole between it and the central chloroplast, whilst in granular cells there is a much wider cytoplasmic lining and a smaller vacuole.

Examination of the drought material of *Zygogonium* from Experiment XII showed a general appearance of milkiness in the granular cells, but no Brownian movement was recognizable. Diffraction lines were observed around the granules, but were definitely limited to them, and the milky appearance of the protoplast throughout demonstrated that no coagulation had taken place. In none of the granular cells of drought material could a vacuole be recognized, an observation which was confirmed on other occasions using other material. Similarly in the case of *Zygogonium* that had been for two months in a solution of 5 per cent. sea-salt, the protoplasts of the healthy cells, though mostly contracted (cf. p. 706), were milky throughout, showed no Brownian movement, nor could vacuoles ever be distinguished. After shorter periods of exposure to drought or hypertonic solutions some of the cells still show Brownian movement.

In the healthy cells of fresh *Hormidium*-material observation with dark-ground illumination again shows general milkiness of the protoplast, vigorous Brownian movement of the minute particles present, and a distinct vacuole. In the drought material from Experiment XIV and others, the milkiness was less marked, no Brownian movement could be recognized, and no vacuole was discerned. The cells showed progressive degrees of decreasing milkiness, but in no case were they as milky as in the fresh material; the slight diffraction discernible again proved to be due to the granules rather than to coagulation of the protoplast. The healthy cells of filaments that had been immersed in 5 per cent. sea-salt for two months presented just the same

features as the cells of drought material. The obviously dead cells in all cases looked quite different from the others.

Only fresh material of *Prasiola* has been examined with this type of illumination. The cells showed pronounced milkiness of the protoplast, no Brownian movement, and usually no indication of vacuoles. In some of the longer cells, however, there appeared to be very small vacuoles around the periphery of the protoplast, which is contrary to what is usually described for this alga (cf. Fritsch, 1922, p. 14), and may be due to the fact that the material examined had been completely submerged during the previous days as the result of heavy rains.

A comparison of fresh and drought material of protonema showed the same contrast as above described for the two filamentous algae: Brownian movement and marked vacuoles in the fresh cells, no movement and no vacuoles in the drought cells, which, however, appeared quite healthy. Material that had been submerged for two months in 5 per cent. sea-salt solution showed in the healthy cells near the tips of the branches (cf. p. 706) a very well-defined cytoplasmic lining which was typically milky throughout ; there was a clear vacuole in this case.

In *Pleurococcus*, lastly, one recognizes no vacuoles, even in fresh material (cf. Fritsch, 1922, p. 14) ; the protoplasts have a general milky appearance, but the large granules in the cells make it difficult to say whether there is any Brownian movement or not. It is probable, however, that there is none.

The general conclusions to be drawn from these observations are that the granular cells of drought material still possess a healthy protoplast, which has, however, undergone a marked increase in viscosity, so that Brownian movement can no longer be recognized in it. It would appear, too, that an increase in the area occupied by the protoplast takes place at the expense of the vacuole. In all of these respects *Pleurococcus* and *Prasiola*, in the normal condition as found in nature, resemble the drought condition of the other forms, and to this many of their peculiarities and their great power of resistance may be due. Extreme viscosity of the protoplast may well account for the failure of the majority of the cells of drought material to undergo plasmolysis. On the other hand, the recovery from plasmolysis of strongly plasmolysed cells that have been immersed for long periods in hypertonic solutions, when mounted in tap-water (cf. p. 707), shows that the protoplasts of these cells, though otherwise usually showing similar characteristics to the protoplasts of cells in the drought condition, are not as viscous. The same remarks also apply to the fresh protoplasts of *Pleurococcus* and *Prasiola*, since plasmolysis of these takes place when high concentrations are used.

(*d*) *Investigation of Centrifugalized Material.*

As a further method of investigating the supposed change in viscosity of the protoplasts (cf. above), the effect of centrifugalizing at a speed of 2,500 revolutions per minute on the position of bodies within the cells was examined. If fresh material of *Spirogyra*, *Zygogonium*, *Hormidium*, or moss protonema, placed together with a little water in the bottom of the centrifuge tube, be subjected to the centrifugal force mentioned for 15 minutes, a pronounced displacement of nucleus and chloroplasts to the side of the cell takes place. In the case of the two terrestrial algae the granules 'cream' in the opposite direction to the chloroplast, thus affording additional evidence of their fatty nature. On the other hand, in *Pleurococcus* and *Prasiola* no displacement of the cell-contents was to be observed, even after one hour's centrifugalizing at the above speed.

A similar test was applied to material of *Zygogonium*, *Hormidium*, and moss protonema, which had been exposed to four days' drought over 34·5 per cent. sulphuric acid in the dark at 18° C. Before centrifugalizing, the material was thoroughly soaked and was then placed in water in the centrifuge tube. In this case neither one-quarter hour nor one hour's centrifugalizing served to bring about any alteration in the cell-contents, which appeared in the same position as in uncentrifugalized material. The mats were then thoroughly soaked and some of the material subjected to the same treatment three days later, but no difference as compared with the drought material was to be noticed. It should be added that, at the time when the drought material was centrifugalized, parts of the same mats which had been kept moist in the thermostat under otherwise similar conditions to those to which the drought material was exposed were also centrifugalized, and after a quarter of an hour showed the same kind of displacement as the original material. This proved that the features observed in the drought material are not a result of exposure to the conditions extant in the laboratory.

The observations made on centrifugalized material thus afford a striking confirmation of the view, derived from the examination with dark-ground illumination, that as a result of drought there is a great increase in the viscosity of the protoplasm in *Zygogonium*, *Hormidium*, and the moss protonema. In *Pleurococcus* and *Prasiola*, on the other hand, such high viscosity of the protoplasm is evidently the normal condition. This feature accounts for the inert character of drought material and, in the case of the two forms last mentioned, even of the fresh condition.

The effects of centrifugalizing on material which had been immersed for some time in sea-salt solutions was only tried on *Zygogonium* two days after Experiment XXXV had been in progress. After a quarter of an hour's centrifugalizing most of the cells did not show any displacement, but in a few cases it was quite marked.

F. The Nature of the Granules found in the Cells of Terrestrial Algae.

We have been able to obtain evidence which shows that in the cells of several of the terrestrial algae examined the granules are of at least two kinds. Those of the one kind are minute, relatively uniform in their size and distribution, which is frequently peripheral, and are characterized by the fact that, whereas they fail to give any of the usual fat reactions, they stain a dark colour after a longer or shorter treatment with silver nitrate without exposure to light. In the case of *Zygogonium* half an hour's treatment with the reagent suffices, whilst in *Hormidium* and *Pleurococcus* a much longer period is requisite and it has been found necessary to add glycerine to the reagent in order to facilitate its penetration. In some cases there are relatively few of these granules (especially *Pleurococcus*). They have not been observed in *Prasiola*.

The granules of the other kind are larger and irregular in distribution and appear to differ somewhat in their reactions in the different forms. In *Zygogonium* they give the normal reactions for fats, both with Sudan III and osmic acid; in *Hormidium* they stain after some time in the former, but appear unaffected by the latter; in *Pleurococcus* a certain number of these granules stain with osmic acid, but Sudan III has no effect. Possibly, however, these differences are only due to differences in the ease of penetration. In practically all cases the majority of the larger granules are soluble in ether, chloroform, and acetone, although in *Prasiola* solution is only attained after a long time (cf. Piercy, 1917, p. 526). Alcohol dissolves them in *Zygogonium* and to some extent in *Prasiola*, but apparently not in the other cases. The large granules are evidently of the nature of fats and are possibly the only kind present in *Prasiola*.

Ether and acetone appear quite often to remove all the granules in the cells, whilst with chloroform, at least in the case of *Zygogonium*, the small ones remain behind, and, after such treatment, can be brought out clearly by subsequent staining with silver nitrate. In the case of *Hormidium* and *Pleurococcus* we have been unable to arrive at any definite conclusion on this point.

G. General Conclusions.

The methods adopted in the present investigations are described on pp. 683, 689–91, 700, 704, 706, 712, 718, and 720, where also the extent of error involved is considered. The most important facts that have been established are:

1. With a given strength of sea-salt solution there is great inequality in the behaviour of the cells of the terrestrial algae investigated, as well as of

moss protonema. Some of the cells become strongly plasmolysed, others only slightly, whilst a varying percentage are totally unaffected. In order to bring about plasmolysis of a majority of the cells, very high concentrations are often necessary, the outstanding forms in this respect being *Prasiola*, *Pleurococcus*, and *Cystococcus*. With the latter two even 25 per cent. solutions of sea-salt leave about 10–20 per cent. of the cells unplasmolysed. In both of these respects aquatic algae afford a marked contrast, the cells plasmolysing with low strengths of solution and usually showing an almost uniform reaction.

2. With increasing concentrations of sea-salt solution there is a progressive increase, both in the numbers of cells plasmolysed and in the extent of plasmolysis in the individual cells, a fact which implies that in the terrestrial forms investigated there is a more or less complete grading of the cells as regards their response to plasmolysing solutions.

3. When subjected to concentrations not far above the minimum, the majority of the plasmolysed cells are those which lack the granules which are so characteristic of terrestrial algae, whilst with high concentrations the unaffected cells are almost invariably such as are rich in these granules. The granular or non-granular character of the cell is, however, not the only determinant of its plasmolysing qualities, as shown by the fact that far more plasmolysed cells are to be found in material grown for some days in the dark than in similar material that has remained exposed to the light.

4. During a period of drought there is a progressive daily decrease in the degree of plasmolysis in the filamentous forms, as well as in the numbers of plasmolysed cells, culminating after some little time, as a general rule, in a complete absence of strongly plasmolysed cells and a majority of unaffected cells. There is thus evidently a gradual loss of the tendency of the cells to plasmolyse.

5. In *Pleurococcus* (probably also *Cystococcus*), on the other hand, there is no such definite response to drought. If the plasmolysing tendencies of the cells change at all, they do so only after very prolonged and intensive drought, and apparently death of the cells may often intervene before any decisive response has occurred. In our numerous experiments with *Pleurococcus* very erratic, fluctuating results have been obtained which are at present inexplicable.

6. If threads of the filamentous forms are permanently mounted in sea-salt solutions of varying strength in a sealed slide (cf. p. 689), the cells show a recovery from plasmolysis which is rapid at first, but subsequently slower, until after some days no decided plasmolysis is to be found. This fact has been established both by means of comparative estimations and direct microscopic observation. The rate of recovery depends on the temperature.

7. If material of *Pleurococcus* or *Cystococcus* is sealed in this way in a 25 per cent. sea-salt solution, there is often no change for several days.

The data given in this paper indicate that at certain times these two algae are capable of remaining unaffected for a long time by the unfavourable conditions prevailing in such a sealed slide.

8. The recovery referred to in 6 and 7 is probably in part pathological, but emphasis must be laid on the similarity of the response to that observed during drought, and on the fact that aquatic algae, as well as the epidermal cells of *Saxifraga sarmentosa*, show a very much smaller capacity for recovery under such conditions. The cells of the protonema and leaves of mosses seem in this respect to occupy a somewhat intermediate position.

9. If small patches of soil bearing mats of the filamentous forms are placed in open vessels containing various concentrations of sea-salt (usually 5 per cent.), a large number of the cells remain alive for weeks or even months. In the first days after such treatment a considerable number of the cells recover from plasmolysis, but the majority of these at least die within a week or so. The remainder are of a healthy green colour, but usually most of them are plasmolysed, though not very strongly. Even after weeks of the treatment these cells recover instantaneously if the threads are mounted in tap-water. Side by side with the plasmolysed cells there are always to be found a smaller or larger number of cells with quite uncontracted, healthy green protoplasts. Such might serve as the starting-points for growth, which has, however, so far been observed in one case only (*Zygogonium*).

10. Recovery from the effects of drought on subsequent access of moisture takes place only very slowly, unless the previous drought has been of short duration or relatively mild. In *Pleurococcus* it appears to be more rapid than in the others (cf. Fig. 6).

11. The cells of the forms studied show marked differences among one another in their degree of permeability to certain stains. In the case of *Hormidium* treated with neutral red some cells are altogether unstained, others exhibit staining of the protoplasts except for the chloroplasts, whilst in a third group the entire contents are stained.

12. In *Zygogonium* and the protonema the permeability to eosin (and other stains) runs more or less parallel with the behaviour of the cells towards a 5 per cent. sea-salt solution, the majority of the unaffected cells staining almost immediately, whilst the plasmolysed ones remain unstained for a much longer time. In *Pleurococcus* only the unaffected cells (using 25 per cent. sea-salt solution) take up the stain, and only about half of these.

13. During the progressive stages of drought a great increase in the permeability of the cells of *Zygogonium*, *Hormidium*, and the protonema to various stains is observable, the number of stained cells ultimately reaching nearly 100 per cent., although subsequently falling off again in an inexplicable manner in the protonema (cf. p. 715). In the final drought condition of the algae the only unstained cells are the few perfectly healthy ones. If the

period of drought has not been too prolonged, there is, in the case of the algae, an increasing percentage of unstained cells during the days succeeding access of moisture.

14. In *Hormidium* there is a progressive decrease in the number of cells stained with neutral red during a period of drought, the decrease being evident in both the categories above distinguished (cf. 11). If the material is wetted before the drought has lasted too long, there is a reversal. There is no obvious difference in the staining reactions with eosin of fresh and of drought material of *Pleurococcus*.

15. Material of *Zygogonium* and protonema that has been in a sealed slide shows staining properties similar to those of drought material.

16. Three kinds of cells are distinguishable in drought material of the filamentous forms, viz.: (*a*) dead cells; (*b*) living, though perhaps in part moribund, cells which have a rigid, slightly contracted protoplast and, in the algae, contain coarse granules; (*c*) healthy green cells, in the algae commonly provided with numerous fine peripheral granules, with an uncontracted protoplast which, like that of the cells under (*b*), is not affected even by strong (25 per cent. sea-salt) plasmolysing solutions. The same types of cells are recognizable in material that has been for some days in a sealed slide. The second kind of cell also appears at times in material that has been for a prolonged period in the laboratory or exposed to strong illumination. Such cells have not been observed in *Pleurococcus*.

17. Examination with dark-ground illumination shows that the (*b*) and (*c*) cells possess a living protoplast which is not coagulated, but no Brownian movement is to be recognized within it, and, by contrast with fresh material of *Zygogonium*, *Hormidium*, and the protonema, the cells fail to show any vacuoles. Even fresh material of *Prasiola* and *Pleurococcus* exhibits these characteristics, which in the other forms are only to be seen in the drought condition.

18. If fresh material of these different forms is centrifugalized for a quarter of an hour at 2,500 revolutions to the minute the chloroplasts and nuclei are found to be displaced towards one side of the cell in all the filamentous forms except *Prasiola*, the granules, when present, creaming in the opposite direction. Drought material is, however, unaffected even by an hour's centrifugalizing. Fresh material of *Pleurococcus* and *Prasiola* behaves just like drought material of the other forms—that is to say, one hour's centrifugalizing produces no effect.

19. The granules found in the cells of these terrestrial forms probably in large part consist of fats, which, however, appear to vary somewhat in their solubilities in the usual fat solvents. At the same time in all the algae investigated, except *Prasiola*, we have obtained evidence for the presence of a second type of granule, characterized by small size, usually peripheral distribution, and the assumption of a dark, almost black colour after treat-

ment with silver nitrate in the absence of light. These granules are apparently the ones that are particularly prevalent in the healthy cells surviving drought.

The general conclusion to be arrived at is that, in fresh material of the terrestrial forms investigated, the cells are in diverse states as regards drought resistance, resistance to hypertonic solutions, and permeability to stains. There would, however, probably be no means of grouping these cells in sharply delimited categories, since in all these respects there appears to be a practically complete grading. The numbers of cells consequently that are killed or become unhealthy during a period of drought or exposure to hypertonic solutions will depend on the condition of the original material, and in this respect *Pleurococcus* and *Cystococcus* differ from all the others in possessing a far larger number of resistant cells.

The experiments with material placed in open vessels in hypertonic solutions show that a certain, and no doubt varying, percentage of the cells, which are characterized by rapid recovery from plasmolysis, are little resistant to these conditions and die in a short space of time, whilst a relatively large percentage, though in great part remaining plasmolysed, continue in a healthy condition for many weeks or even months. Under conditions of drought or in a sealed slide (cf. p. 689), on the other hand, practically all the cells in the final stage exhibit no plasmolysis, though a very large number of them show the permanent slight contraction of the protoplast which does not disappear even in tap-water. These cells, which are readily permeable to such stains as eosin and erythrosin, are apparently in an unhealthy state though not dead, and their abundant occurrence both in drought material and in a sealed slide indicates that, in both these cases, the conditions are more deleterious than in open hypertonic solutions. In all probability these cells correspond to those which remain plasmolysed under the latter treatment, since the numbers of perfectly healthy unaffected cells, always rather small, are approximately the same in all three cases. The cells with slightly contracted protoplasts are probably in a moribund condition, from which recovery is either very slow or altogether impossible ; recovery may depend on the intensity and duration of the previous treatment. There are some data that indicate that a relatively slight drought produces a condition that is reversible, a more pronounced one an irreversible condition. In any case it may be well to point out that the drought to which these forms have been subjected in our experiments was either more prolonged or more intense than anything that is likely to occur in nature.

With all kinds of treatment, however, there usually remain a certain number of healthy green cells, not plasmolysed by even high concentrations (25 per cent.), generally impermeable to the various stains used, and in the filamentous algae usually characterized by the possession of numerous fine peripheral granules. Even supposing that all the cells above described as

moribund really die, the few healthy surviving cells would probably serve as a sufficient basis for new growth when conditions again became favourable. During the prolonged drought of 1921 many of the filamentous forms disappeared altogether (as far as macroscopic observation went) from their normal habitats. Yet, in the autumn or later, they appeared again in quantity in the old situations.

Whatever the treatment may have been, the surviving cells, whether healthy or unhealthy, are characterized by the possession of a more or less rigid, highly viscous protoplast, absence of vacuoles, and in the algae usually abundant granular contents. Most of these features, distinctive only of drought material in other cases, are exhibited already by the fresh cells of *Pleurococcus* and, to a less extent, of *Prasiola*. The very marked powers of resistance of the unicellular alga, a familiar fact of observation in nature and strikingly exemplified in our experiments, is no doubt to be related to the peculiar characteristics of its protoplasts. This form is, so to say, permanently in the drought condition. It is probable that *Cystococcus* altogether resembles it in these respects.

The problem as to the mechanism of water retention, which we set out to investigate, can be regarded as only partially solved. It is plain that a high osmotic concentration of the sap, if it plays any rôle at all, is not a fundamental part of the mechanism.[1] The gel-condition of the protoplast, with absence of all large vacuoles, indicates the possibility of imbibition phenomena being principally concerned. That such a gel would retain a certain quantity of moisture on drying under any ordinary atmospheric conditions is undoubted, but it is open to question whether the amount would be as large as has been found to be the case. Since the protoplasts of *Pleurococcus* are apparently always in this state, the behaviour of this form is of particular interest, for we know that its cells will resist drought for months without apparently losing any of their normal vital properties. This alga, however, exhibits a very small moisture-content (cf. Fritsch, 1922, p. 16) and a low capacity for absorbing moisture when dry (loc. cit., p. 4). Moreover, it shows a higher permanent loss of moisture after heating to 100° C. than either *Zygogonium* or *Prasiola*. All these facts tend to show that the moisture is imbibed within the gel-like protoplast, that it is held tenaciously against dry air, and that it is only after heating that its imbibitional efficiency is destroyed. On the other hand, *Prasiola*, whose protoplast seems to be in much the same condition, does not appear to possess as efficient a mechanism.

In all the other forms, where the gel-condition is only reached during drought, a majority of the cells at these times are probably in a far less healthy state than in *Pleurococcus* and their imbibitional efficiency may

[1] The observations made since the writing of the article on terrestrial algae (in Journal of Ecology, vol. x, 1922) have led us to modify the view expressed on p. 230 of that paper.

be more or less impaired. At the same time the fact that the really healthy surviving cells are apparently always those that are provided with fine peripheral granules of unknown nature hints at the probability of still other factors coming into play. It is not impossible that moisture-retention may be partly due to a change in the cell-walls, since alterations in staining reactions, sometimes shown by the walls during drought (cf. p. 712), indicate that they also are capable of some modification under these conditions.

H. Summary.

The present paper deals with the changes occurring in terrestrial algae and moss protonema during drought and when exposed for long periods to hypertonic solutions. During drought there is a gradual diminution in the tendency of the cells to plasmolyse with a given hypertonic solution until finally there is no appreciable plasmolysis at all. At the same time there is a marked alteration in the permeability of the cells to stains. Subsequent access of moisture brings about changes in the reverse direction, unless the drought is severe or very prolonged. If material is sealed in a hypertonic solution and investigated day by day, a similar gradual disappearance of plasmolysis is noted, an effect which is probably partly pathological. In all these respects, however, *Pleurococcus* shows far less tendency to change than the filamentous forms examined.

In material exposed to hypertonic solutions, but unsealed, only a certain number of the cells recover from plasmolysis and the majority of these subsequently die ; the rest, often a large percentage, remain plasmolysed, though retaining their vitality for weeks or months. At the same time there is usually a small number of healthy cells with uncontracted protoplasts.

The cells surviving after a period of drought or of prolonged treatment with hypertonic solutions have a moreor less rigid, highly viscous protoplast without obvious vacuoles. This has been demonstrated especially by the use of dark-ground illumination and the examination of 'centrifugalized' material ; it is the normal state of the cells of *Pleurococcus* and *Prasiola*. It is suggested that this gel-condition of the protoplast may in part explain the marked retention of moisture by such terrestrial forms in the air-dry condition, although there are some indications that other factors also are concerned.

East London College,
March 22, 1923.

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NOTES.

MICROSCOPICAL TECHNIQUE.—The series of processes and technical methods employed between the fixation of plant-materials for cytological investigation and the completion of the preparations are, in many instances, open to considerable improvement and call for full inquiry on physical lines.

It is proposed to consider in short notes, of which the following is a first instalment, certain difficulties which have arisen in the course of cytological work and to suggest methods by which they can be overcome.

The Stretching of Paraffin-ribbons on Glass Slides.

Considerable difficulty is often encountered in securing perfect stretching of paraffin-ribbons on the slides on which they are being mounted. This difficulty can, however, be easily overcome if the slide on which mounting is to be conducted be uniformly heated to a temperature closely approaching the melting-point of the paraffin concerned, and maintained at that temperature until perfect stretching is secured. A method found successful is as follows:

An earthenware pot with a concave bottom (such as a 'shrimp pot') of about 3 in. in diameter and $1\frac{1}{2}$ in. in depth is employed. It is inverted in a metal dish (say, 7 or 8 in. diam.) containing water. There should be sufficient water to completely fill the pot and to allow the inverted base of the pot to emerge from the surrounding water by about $\frac{1}{4}$ in. only. The water can be easily raised to a temperature just below the melting-point of the paraffin employed, and maintained at this temperature by a small Bunsen flame or by a spirit-lamp. The slide on which the ribbon is to be mounted is flooded with albumin-water, or either Land's gum-arabic-chromate or albumin-chromate fixative, on which the ribbon is floated.[1] The slide is then placed on the inverted concave base of the pot, which now represents a shallow dry basin, on the rim of which the slide is resting. The shallow basin is now flooded by means of a pipette with cold water until the whole of the under surface of the slide is in contact with the water. The slide gradually acquires a temperature approaching the melting-point of the floating paraffin-ribbon, which is gradually and uniformly stretched as heating continues. When no further stretching is observable, the slide is removed from the rim of the basin and most of the surplus fixative is drained away and the ribbon stranded on the slide. Any surplus fixative can then be removed with blotting-paper before the slide is laid aside to dry.

In dealing with very thin ribbons, in which wrinkling is commonly pronounced, it

[1] Bot. Gaz., vol. lix, p. 398, 1915.

is essential to secure a very gradual stretching. This can be secured almost invariably by the above method if the water employed in flooding the basin is initially very cold. The danger of permanent adhesions of opposite sides of folds in the ribbon is thus avoided, and a perfect stretching secured for ribbons of the thickness of 1 μ. A sufficiency of albumin-water or other liquid fixative should in all cases be employed to avoid the stranding of the ribbon at any point on the slide before stretching is completed.

In dealing with hard materials, an examination with a hand-lens sometimes shows that, even after stretching of the ribbon has continued for two or three minutes, that of the embedded materials is incomplete. In such cases a complete stretching is secured, with apparently no harmful effects by raising the temperature of the water in the containing vessel to the melting-point of the paraffin.

The slide should be allowed to cool in every case before surplus fixative is removed, and the slide should be replaced on the rim of the basin and in contact with the water before the last portion of the fixative is removed. If this is attended to, full contact between ribbon and slide is secured.

The main surplus of fixative should be drained away by allowing the slide to remain for a short period in an almost vertical position, or by means of blotting-paper which is not allowed to touch the sections. The last traces of unnecessary fixative are removed by three strips of blotting-paper, which are laid over the ribbon and lightly rubbed with the finger.

A more efficient apparatus than the one above described can be made from a brass disc of $2\frac{3}{4}$ in. diameter and $\frac{3}{4}$ in. thickness. The top of this is recessed in the lathe to a depth of about $\frac{3}{16}$ in., leaving a rim $\frac{1}{8}$ in. in width. The hollow thus prepared corresponds to the concave base of the shrimp pot. Three lugs fitted with levelling-screws are fixed below the disc, and serve to raise the latter well above the bottom of the water-bath and to secure a levelling of the slide and an even distribution of the fixative fluid whereby any local stranding of the ribbon, during stretching, may be avoided. Both the disc and the three lugs can be made of lead, instead of brass; it serves admirably and is easier to work in a laboratory lathe. The metal dishes used as water-containers can be obtained at any hardware shop at a cost of a few pence.

W. HORTON.

HARTLEY BOTANICAL LABORATORIES,
THE UNIVERSITY, LIVERPOOL.

LIFE-HISTORY OF RHYTISMA ACERINUM (PRELIMINARY ACCOUNT).—The needle-shaped ascospores of *Rhytisma acerinum* are one-celled, uninucleate, and furnished with a massive sheath. From experiments carried out in the laboratory the spores conveyed by air currents appear to fall exclusively on the upper leaf surface. They readily germinate in a weak decoction of prunes, first becoming septate into two, rarely three, cells. The germ-tube almost invariably arises in a lateral position from the blunted half. The mycelium is exceedingly fine, the nuclei being very minute; it develops rapidly within the upper epidermal cells, completely filling up the latter. Infected areas first appear as yellow patches which very early begin to blacken at the centre; these areas form stromata which first give rise to pycnidia. Increased development of the mycelium in the epidermal cells of specified areas in the stroma causes these cells to split in half tangentially, the upper half forming a dome-shaped roof and the lower remaining as the floor of the future pycnidium. Here the mycelium gives rise to simple conidiophores which abstrict small, uninucleate conidia in enormous numbers. The conidia appear at the ostiole of the pycnidium in a viscid exudate; their function is not known. After the disappearance in late summer of the conidia the persistent conidiophores either become concrescent and blackened or, if the pycnidium is to be converted into an apothecium, they are raised up by growth of the cells below them and become a part of the future apothecial roof. Usually, however, apothecia arise *de novo* at the margin of the stroma, in which case the roof is formed by the addition of cells abstricted from the mycelium below. When the process of roof thickening is over there is an appreciable space for further development. Among the apothecial cells there now appear cells conspicuous by their larger size and contents, which, from their structure and orientation, are highly suggestive of archicarps consisting of a basal oogonium bearing a trichogyne above as a separate cell; the trichogyne abuts on another larger cell, possibly an antheridium. The cytology of these structures is still under investigation. No fertilization was observed, but the cells of the archicarp were seen in some cases to break down, suggesting a scolecite formation; it is, however, probable that these structures are abortive. The paraphyses developed from large basal apothecial cells are straight, with pointed tips, and linked together with well-marked H-connexions. A transverse section of an apothecium with young developing asci shows the latter arising in groups at definite intervals and emanating from a complex of cells. At this stage changes are seen to take place within the dome of the apothecium in preparation for dehiscence of the latter. A tangential slit is developed in the roof, and this gradually widens, forming a cavity filled with gelatinous material. The gelatinization is not progressive, but the cavity gradually gets bigger, probably owing to absorption of water causing the inner part of the roof immediately below the cavity to protrude into the apothecium. This would bring about increase of pressure within the closed apothecium, and, owing to the convexity of the roof, increase of pressure from below, together with absorption of moisture into the cavity, would ultimately bring about rupture of the outer wall of the roof. In

newly opened apothecia we find the cracks occupied by a yellowish exudate. The exudate dries on exposure to the air and pulls up the protruding inner wall of the roof, which finally becomes broken. The apothecia are now fully open, and the ascospores are liberated in enormous quantities. The young ascus shows a well-defined nucleus with a well-marked nucleolus. The needle-shaped spores are grouped in the ascus, a central spore being surrounded by the remaining seven, each with a well-marked sheath. The ascus opens in an irregular manner and the spores are shot out of the apothecia periodically.

S. G. JONES.

University College of Wales,
Aberystwyth.

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EDITED FOR THE
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By A. G. TANSLEY

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