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ON THE STRUCTURE AND ORIGIN OF "*CLADOPHORA* BALLS."

BY ELIZABETH ACTON, M.Sc.

[WITH FIVE FIGURES IN THE TEXT].

IN the early part of the year I had an opportunity of examining some " *Cladophora* balls " which had been collected from Loch Kildona, S. Uist, and several observations were made which had not previously been recorded. Prof. G. S. West suggested that, as these balls are not very well known in this country, a paper describing them might be of general interest.

" *Cladophora* balls " are of frequent occurrence in certain lakes in Scotland and Ireland. They are also widely distributed in other parts of Europe and their origin and structure have been carefully investigated by several authors. In 1902 Brand[1] published a paper dealing with the various members of the Aegagropilas and the anatomy of the single plants; the literature on the subject is also fully discussed. A few months later a paper appeared by Wesenberg-Lund[2] on *Aegagropila Sauteri* of Lac Sorö; this is devoted almost entirely to the origin and mode of formation of the ball-like stages which appear floating on the surface of the lake in the months of April and May. I am chiefly indebted to the works of Brand and Wesenberg-Lund for my knowledge of the *Cladophora*-Aegagropilas, but mention will be made later of other papers which have been consulted.

The " *Cladophora* balls " belong to the group of plants known as Aegagropilas. In the general sense this term has been used to describe loose masses of algæ rolled into irregular balls by the action of the waves and currents. In its systematic sense it has been

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[1] F. Brand. "Die Cladophora—Aegagropilen des Süsswassers." Hedwigia, Bd. 41, 1902.

[2] C. Wesenberg-Lund. "Sur les *Aegagropila Sauteri* du Lac de Sorö." Bull. Acad. roy. des sciences et des lettres de Danemark, 1903.

used to denote a certain section of the genus *Cladophora*, the members of which habitually form these conglomerate masses, and it is to this section that these balls belong. The specimens from L. Kildona have been identified as *Cladophora (Aeg.) holsatica* Kütz.

I. STRUCTURE OF THE BALL.

The balls examined were collected from the surface of Lake Kildona at the end of August, 1907 and had been standing since that time in a dish in the laboratory with water dripping on to them. They were dark green in colour and quite hard and firm in texture; they varied from 2–3 cm. in diameter (Fig. 1, A).
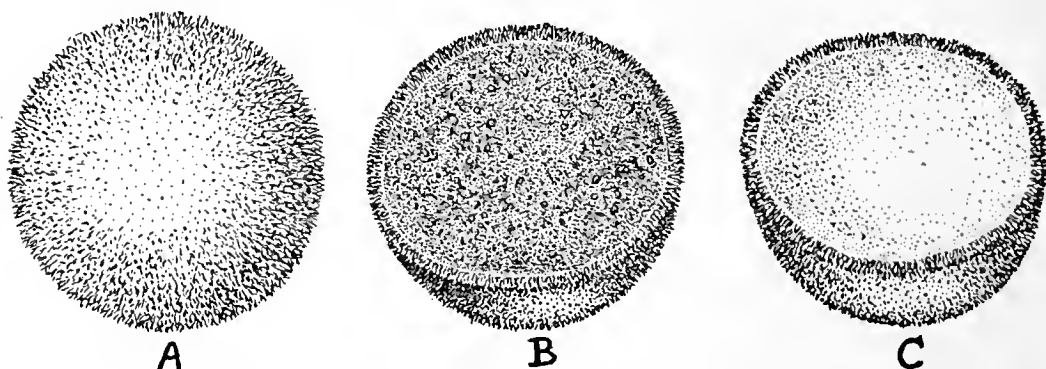
FIG. 1.   *Cladophora (Aegagropila) holsatica* Kütz.   A, whole ball; B, ball cut in half; C, mud removed shewing cavity of ball.   All natural size.[1]

The balls when cut open show a central cavity filled with débris consisting of dead *Aegagropila* cells, fine mud and various minute algæ in a more or less unhealthy condition.   The outer shell is about 3 mm. thick and consists of a very tightly interwoven mass of algal filaments (Fig. 1, B, C).   This tangled mass is made up of separate individuals which are tightly interlocked by special branches.   Fig. 2, A, shows a small individual plant.

Each plant shows a central axis of cells bearing tufts of branches (cf. Fig. 2, A).   Any cell can produce branches and in any direction according to external conditions; so that the branch system tends to become irregular.   Injury to the terminal cell has the effect of producing active lateral branching.

A very noticeable feature of the cell is the extreme thickness of the wall, which is conspicuously lamellated and has knob-like projections at the points where branches are about to form (cf. Fig. 3, D). The chloroplast is parietal and is made up of a large number of rounded plates.   The cells of the vegetative branches are cylindrical and slightly swollen at the upper end but they tend to become irregular in shape as they get older (Figs. 2, 3).

[1]   I am greatly indebted to Prof. G. S. West for this figure.

The majority of the branches are vegetative shoots, but modified branches with special functions also occur. Brand (1902, loc. cit.) has carefully studied the origin and nature of these branches and has divided them into "rhizoids," "cirrhoids," "neutral" or "indifferent" shoots and "stolons."
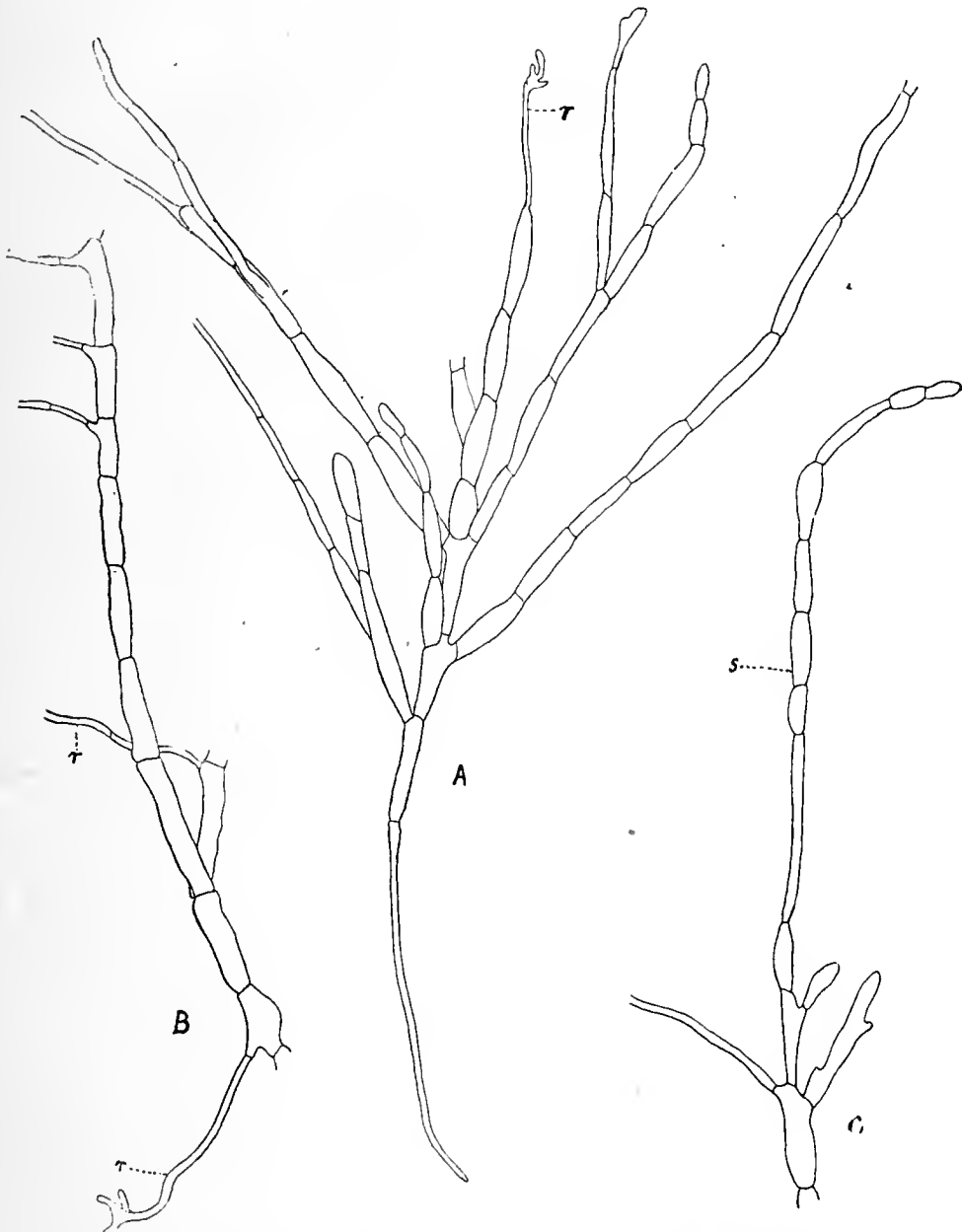
FIG. 2. A, isolated individual of *Cladophora* (*Aeg.*) *holsatica* Kütz, showing method of branching. B, "indifferent" shoot bearing "rhizoids." C, "stolon." × 31.

The "rhizoids" and "cirrhoids" are organs for interlocking the branches of the individual plants; the only difference being that in the rhizoids the end cell of the shoot alters its shape to fit into the conformation of a neighbouring cell or of any foreign body with which it comes in contact, while the cirrhoid simply coils round it without altering its shape (Fig. 3, B, C). The cells of

these shoots are relatively long and thin but they all contain a chloroplast.
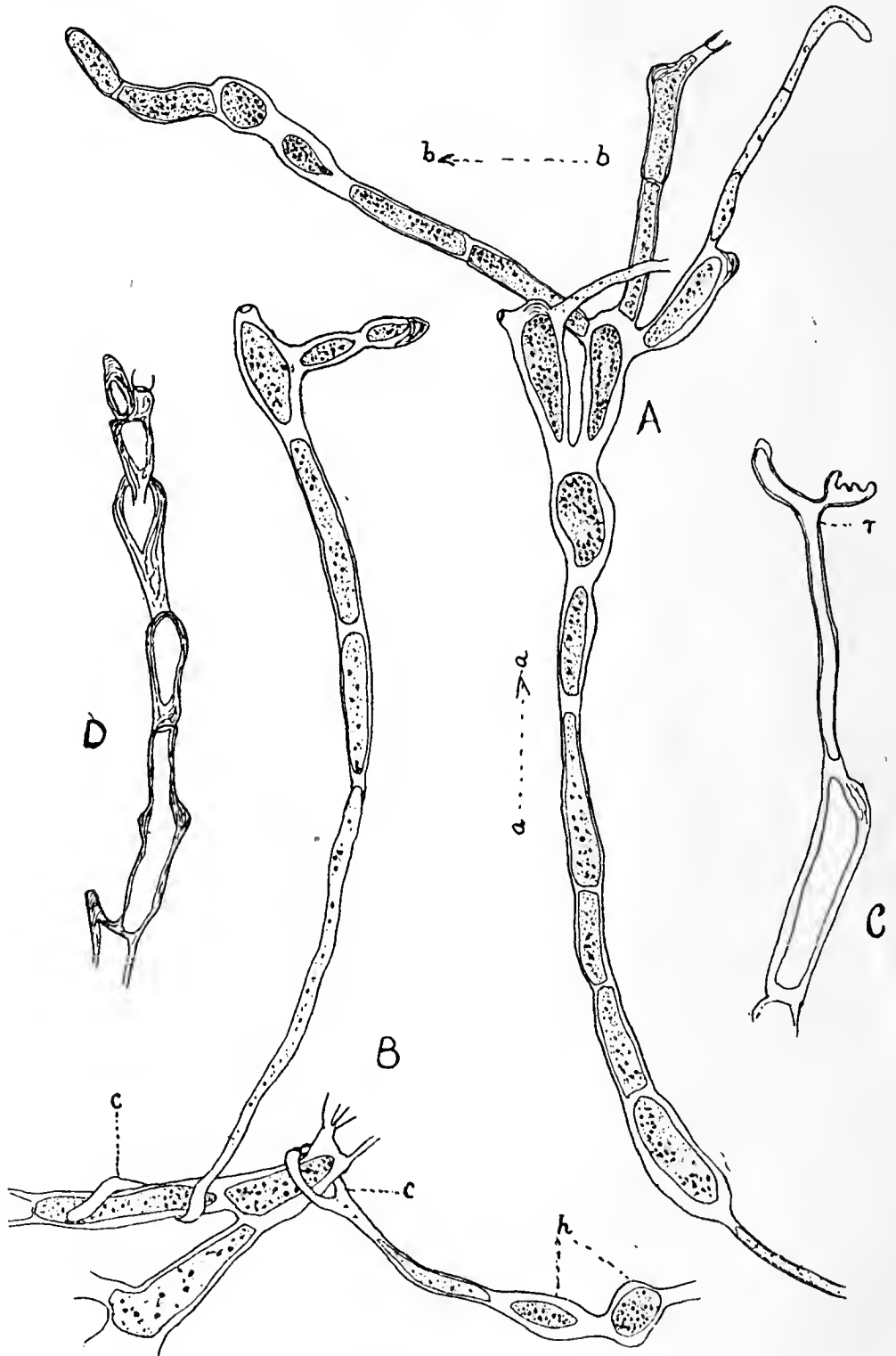
FIG. 3. A, small individual shewing change of polarity as described in text ; B, branches modified as " cirrhoids " (c) ; C, " rhizoid " (r) formed from apical cell of branch ; D, shoot illustrating the thickening of the cell wall at points where new branches are about to appear. A, B and D x 62 ; C x 96.

" Indifferent " branches are long thin shoots poor in chlorophyll which originally have no definite function but can develop, accord-

ing to the needs of the plant, into " rhizoids," " cirrhoids " or
" stolons." Fig. 2, B, shews a branch of this nature which is giving
off rhizoids from many of its cells.

" Stolons " are organs of propagation. They are in the nature
of long runners which form vegetative shoots at their ends either
directly as in Fig. 2, C, or indirectly by altering the shape of the
end cell and putting out branches.

The " rhizoids" are not rhizoids in the true sense, *i.e.*,
colourless hair-like structures developing from the lower end of the
plant. It is generally agreed that from its earliest stages the plant
has no distinction into rhizoidal and cauloidal parts. It has,
moreover, no polarity or rather it has a changeable polarity. This
is well illustrated in Fig. 3, A, where the plant has changed its
direction of growth through an angle of 90°, *i.e.*, from *aa* to *bb*.
The vegetative branches are putting out rhizoids from their terminal
cells and new vegetative shoots in an opposite direction. Changes
in external condition can alter the polarity of the main axis and
cause vegetative branches to develop from the older part of the
plant in a backward direction as well as a forward one.

Multiplication. According to Brand (loc. cit.) multiplication
only takes place in the Aegagropilas in vegetative ways. The cells
can perennate in unfavourable conditions. They are of exceedingly
slow growth and of great age. The oldest cells of the axial column
die off gradually in regular succession under normal conditions.
The death of these cells sets free the lateral branches in regular
acropetal succession. The plants have a limited size and the branches
when set free, form the main axis of a new individual which branches
and grows and in its turn forms new individuals by the death of the
cells of its main axis. In this way the plants resemble the
Sphagnaceæ and can be said to possess an unlimited duration of
life.

Brand (loc. cit.) does not agree with Kjellman[1] that there are
special resting cells or " basal gonidia." He states that in
Kjellman's plants there were an unusually large number of dead
intercalary cells and that the cells remaining were only old stem
cells which had survived adverse external conditions but which had
no special significance as reproductive bodies. It is certainly true
that, whether or not they have any special significance as reproduc-
tive bodies, these isolated old axial cells have the power of rejuvenating

[1] F. R. Kjellman. " Zur Organographie und Systematik der Aegagropilen."
Nova Acta Reg. Soc. Sc. Upsala, Ser. III, Vol. 17, 1898.

under the necessary conditions and producing new plants. They occurred fairly frequently in the material which I examined and seemed always to have very dense crowded contents and a thickened cell wall as described by Kjellman for his " basal gonidia." Fig. 4 shows one of these old cells putting out new branches.

In addition to these old axial cells, structures were present which undoubtedly have a definite reproductive significance. These are of the nature of hypnospores, and as far as I am aware, have not been mentioned elsewhere. These resting spores when mature may reach a diameter of 120μ though many are smaller. They are almost spherical in shape and have very thick walls. The cell contents are densely crowded and contain a large quantity of oil (Fig. 5).
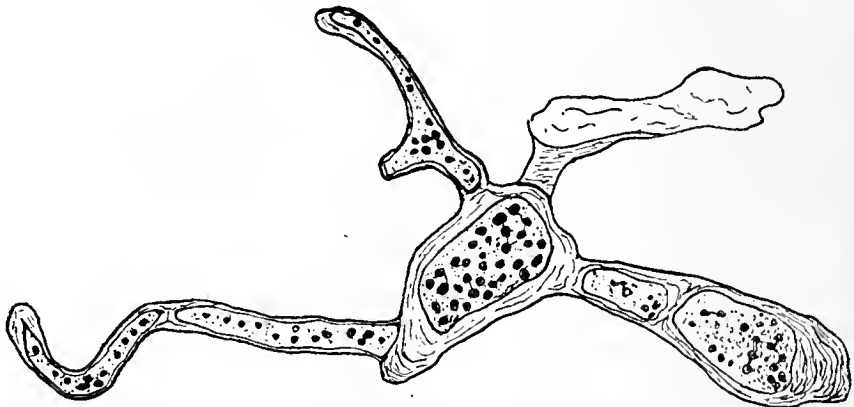
FIG. 4. Old cell from main axis which has become isolated and developed new branches. × 96.

It is probable that the contents of a single large vegetative cell break up into numerous resting spores though this has not actually been seen. The spores frequently occur in groups as in Fig. 5, A. The spores in such a group vary in diameter from 16μ to 46μ and have possibly just been liberated. Larger spores were generally isolated or in groups of two or three, as in Fig. 5, B. In many cases single spores were formed in the narrow cells of the " rhizoids " and " cirrhoids " and were seen escaping from the old cell. The narrow filament swells considerably in places and the protoplast concentrates at these points. Fig. 3, B, shows what is probably an early stage in the formation of resting spores from a " cirrhoid " branch.

On germination the outer coat of the spore is ruptured and is either completely thrown off as in Fig. 5, E, or remains for some time attached to the spore as in Fig. 5, D, F. The first stage in germination is a definitely marked thickening, similar to that which appears when a vegetative shoot is about to develop a branch at

some point on the inner wall of the spore. It is probably at this point that the outer coat is broken. This thickening increases until a distinct peg-like process, sometimes reaching a length of 20μ, is formed. This process eventually develops into a " rhizoid " and part of the protoplast passes into it (Fig. 5, F). It was not possible to trace the development of the spore beyond this stage. It has been mentioned before that the material had been in the laboratory for eight years and the balls were slowly dying owing to being completely covered by a layer of minute diatoms which come from the tap water. Examination of the balls introduced various fungus spores and the rapid development of these hastened the decay of the alga.
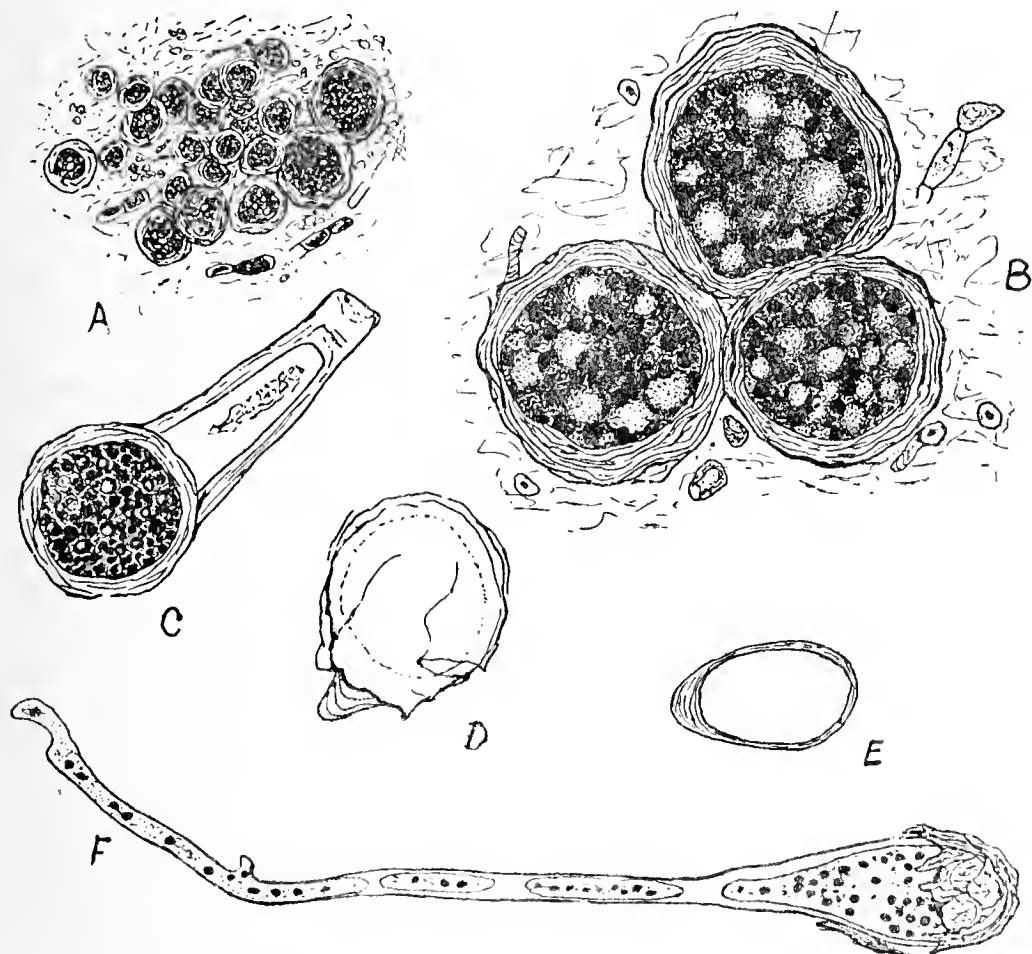
Fig. 5.   A, group of small resting spores : B, spores shewing oil drops ; C, spore which has remained attached to the old cell wall ; D, germinating spore throwing off outer coat. E and F, germinating spores. A, C, E and F, × 150. B and D, × 370.

Resting spores could not be found in balls which had been fixed in formalin as soon as they were collected.   Possibly they are only formed under very adverse conditions and in balls which are about to break up. Balls which have been partially destroyed by the action of too strong light at the surface of the water may sink to the

bottom and there gradually form resting spores before breaking up. But in freshly collected material, balls of this nature would probably be discarded or, at any rate, not examined ; and so the resting spores would be overlooked.

II. Origin of the Ball-like Forms.

Lorenz[1] in 1901 was the first to show that these ball-like forms are developed from isolated individuals and later Brand (loc. cit.) shewed that each species of *Aegagropila* could, given the necessary external conditions, exist in the different forms of thallus described as " Rasen," " Polstren " and " Ballen" so that specific characters should be sought in the anatomy of the single plant rather than in the form of the thallus which is of quite secondary importance.

The different forms of thallus and their mutual dependence on one another have been described in detail for *Aeg. Sauteri* by Wesenberg-Lund (loc. cit.) and since his account will probably apply in essential points to other species, a short summary of it is given here.

In one of the creeks of Lac Sorö the floor is covered with a thick felt-like layer composed of small separate individuals of *Aeg. Sauteri*. These individuals have a tufted radiating structure. The creek is not deeper than four metres in any part and has a soft muddy floor.

A slight undulatory movement is communicated to the superficial layer of this felt by strong winds, causing the individuals to hook on to each other. In this way irregular packets are formed. The number of these packets increases towards the edge of the creek. The balls first appear at the edge of the felt where the action of the waves is strongest and the mud of the floor is somewhat coarser in texture. Near the edge of the creek where the depth is about one metre they can be found in quantity. These globular forms owe their existence to the mechanical action of the waves acting on a single individual of the felt or on packets of several individuals hooked together. The spherical form and radiating structure have been produced by the rolling and friction against the sandy bottom in the shallower parts where the action of the waves is more strongly felt.

The close texture of the peripheral part is explained by the tendency which the alga has of replacing broken terminal joints by active lateral branching just below the injury, and by the

[1]   J. R. Lorenz.   Ergänzungen zur Bildungsgeschichte der sogen. " Seeknodel " (*Aeg. Sauteri* Kg.).   Verh. d. kais., königl. zool. bot. Ges. Wien, Bd. 51, 1901.

presence of specialised filaments which firmly bind together the vegetative branches.   Growth is slow and takes place in a tangential as well as a radial direction.   This tangential growth and probably also the successive destruction of the older branches, causes a cavity to form in the centre of the ball.   Usually this cavity is filled with water, but during the months of May and April they enclose a considerable quantity of gas which enables them to float on the surface while they remain at the bottom during the remainder of the year.[1]

The presence of the gas in the interior of the ball is explained by the fact that as the intensity of the light is increased the cells on the inner part of the ball take part in assimilation as well as those in the exterior, and the carbon-dioxide from these escapes into the interior of the ball.   The close texture of the peripheral part prevents the rapid escape of the gas and in time sufficient is collected to float the ball.   The majority of these floating balls probably perish owing to the strong illumination which is inimical to the growth of the plant.

The regular periodic appearance of the balls at the surface during April and May is explained as follows.   In normal conditions the zone occupied by the balls would be too intensely lighted for the growth of the plant.   But during the greater part of the year the lake is extremely rich in plankton which acts as a screen and prevents the intense light from reaching the floor.   It is only during April and May that the water is transparent and so phytosynthetic activity is increased sufficiently to float the balls.

It is possible that the Aegagropilas are more widely distributed than is supposed, for their primary condition is a felt-like mass of isolated individuals living where the water is a few metres in depth. This can only be found by dragging.   It is only under very special conditions that the absolutely regular ball forms are produced as instanced by the regular periodic occurrence of balls of *Aeg· Sauteri* at the surface of Lac Sorö but not elsewhere, although the plant has been collected by dragging from other lakes.   The isolated individuals must come under the rolling action of the waves which means that they must find their way to shallower parts of the water either naturally or by accidental means.   But since less depth of water usually means greater intensity of light, it is only when the intensity of the light is lowered, either by presence of plankton as in Lac Sorö or by other means, that the balls can exist.

[1] Wesenberg-Lund and Brand have shown by experiments in the laboratory that the balls rise to the surface in the daytime when the illumination is strong and sink to the bottom in the evening and remain there on a dull day, *i.e.*, the rise to the surface is due to active photosynthesis in strong sunlight.

According to Wesenberg-Lund (loc. cit.) the formation of the balls is primarily due to the incessant destruction of the terminal branches directed towards the exterior which causes the growth of new adventitious filaments. Where no external factor hinders the growth of terminal filaments the thallus is tufted with free branches. But the motion of the waves against a sandy floor supplies this factor and the harder the floor the more regular will be the shape of the ball. The greater irregularity of the balls of *Aeg. Sauteri* described by Lorenz (loc. cit.) from the Zeller See, and the absence of irregular forms from Lac Sorö is due to the difference in the substratum in the two cases. In the Zeller See the floor is a soft lime and that of Lac Sorö is sandy.

According to Lorenz, light plays an important part in the formation of the balls. The more intense light in shallow water produces a more active growth and the motion of the waves exposes all parts to the light in turn and induces a radiating structure. He also thinks that a *soft* bottom favours the formation of the balls since it offers less resistance to the rolling of the balls. This explanation does not account for the death of the terminal cells which is a very noticeable feature in the branches directed towards the exterior.

Wesenberg-Lund thinks that light is quite a secondary factor. Brand (1906),[1] on the contrary, thinks that light is the chief factor, but operating in an exactly opposite way to that described by Lorenz He thinks that the death of the terminal cells and consequent increased formation of lateral branches is brought about by their successive exposure to light and not, as Wesenberg-Lund supposes, by friction against the sandy floor. The cells are too elastic and thick-walled to be injured to any great extent in this way. He shows that in balls exposed to a strong light in tanks, the outer part of the ball died and the branches started to grow towards the centre the inner part of the ball being a bright green.

It is obvious, however, that whatever external conditions are are necessary for the formation of the balls, the peculiar character of the alga itself must play a very important part, for the balls of *Aeg. holsatica* which had been living for eight years in a small dish, exposed to unnatural conditions, still kept their spherical shape.

In conclusion, I wish to express my thanks to Prof. G. S. West for suggesting this work and for advice during the investigation.

[1] F. Brand. " Uber *Cladophora crispata* und die Sektion *Aegagropila*." Hedwigia, Bd. 45, 1906.

CARBON ASSIMILATION.

A Review of Recent Work on the Pigments of the Green Leaf and the Processes connected with them.

By Ingvar Jörgensen and Walter Stiles.

(Continued from Vol. XIV, p. 294).

E. Simple Laboratory Experiments on the Leaf Pigments.

As Willstätter truly points out, the experiments described in text-books of plant physiology for the demonstration in class work of the properties of chlorophyll are quite inadequate.

In preceding sections of this chapter, we have described Willstätter's methods for the extraction in quantity of the leaf pigments. It is quite obvious, however, that the length of time and large amount of material and reagents required for these extractions, render their use scarcely possible for ordinary class work. We have therefore brought together in this place a number of easily performed experiments which amplify the collection of examples given in Willstätter and Stoll's book.

The performance of these experiments will not only lead the student to clearer ideas about the chemistry of the leaf pigments, but will also give an opportunity for that chemical and physical manipulation which is becoming increasingly necessary to the plant physiologist, but for the practice of which there is no great opportunity in most other parts of a plant physiology course.

Preliminary. It will be found of great convenience for class work to collect in the summer every year a quantity of nettle leaves. These are dried at air temperature ; they are spread out on sheets of paper and a sheet of paper placed on top of them to prevent dust from falling on them, and to prevent undue exposure. They are then ground up finely and dried completely for several days at a temperature of 30^0 to 40^0C., in an incubator.

The powder so obtained is kept in a stoppered bottle. So prepared, the powder retains for a long time the leaf pigments unaltered in quantity and quality.

Any further drying required must be carried out by placing the leaf powder in a vacuum desiccator over sulphuric acid. This procedure is necessary for instance when it is required to show that pure solvents do not extract the pigments from dry leaf powder. In this case it is also necessary that the solvents should be as water-free as possible. Ordinary solvents may have to be redistilled over quick-lime, calcium filings, etc.

Experiment 1. Extraction of the pigments.   Required: small Buchner funnel with flask and a water pump; 20 c.c. 85% acetone or 90% alcohol.

Two grams of leaf powder are sucked to a filter paper on the Buchner funnel and a small quantity of the solvent added. This is allowed to soak into the powder for a few minutes. The fluid is then sucked through with the pump. The operation is repeated until all the 20 c.c. of solvent has been added, when the powder is sucked dry. A deep blue green solution, with red fluorescence, is obtained which contains all the four pigments from the leaf. Usually the powder will still be coloured green as the extraction is not generally complete.

Experiment 2. Transfer of the pigments from an acetone solution to an ether, or to a petrol ether, solution.   Required: 1 separating funnel; about 10 c.c. ether and 10 c.c. petrol ether; 5 c.c. acetone extract of leaves.

Five c.c. of the acetone extract obtained in Experiment 1 are poured into double the quantity of ether contained in a separating funnel. An equal quantity of distilled water is added, this being poured gently down the side of the funnel in order to avoid the formation of emulsions. In the course of a few minutes, the ether layer separates out and now contains the pigments. The lower layer, which is slightly green, is run off. The addition of distilled water and subsequent removal of the lower layer is repeated about four times, in order completely to remove the acetone from the ether solution. If the ether solution should have become at all emulsified, it can be cleared by shaking with anhydrous sodium sulphate and filtering.

A petrol ether solution may be obtained in the same way by using 10 c.c. of petrol ether in place of ether.

Experiment 3. Demonstration of the two green pigments.   Required: 10 c.c. petrol ether solution of mixed pigments; 10 c.c. 92% methyl alcohol; 2 separating funnels.

The petrol ether solution from the last experiment is shaken with 10 c.c. 92% methyl alcohol. Two layers are formed of which the petrol ether layer contains chlorophyll a and the methyl alcohol layer chlorophyll b. The solution of chlorophyll a is blue green while that of chlorophyll b is a purer green, but the colour difference between them is diminished owing to the presence of the yellow pigments, of which carotin is in the petrol ether, and xanthophyll in the methyl alcohol.

A characteristic difference between the two green pigments is to be found in the phase which appears on saponification with methyl alcoholic potassium hydroxide. This phase-test is best carried out in ethereal solution. The methyl alcoholic solution is therefore poured from the separating funnel into another and, as described in Experiment 2 transferred to an ethereal solution. The petrol ether solution and ether solution are then used for phase tests as described in Experiment 4.

The difference in the absorption spectra of the two chlorophylls is not easily observed unless the solutions are very pure.

Experiment 4. Saponification of the green pigments. Required: 5 c.c. of an ether solution containing the pigments. The petrol ether and ether solutions containing chlorophylls a and b obtained in Experiment 3; 10 c.c. methyl alcoholic potash.

An ether solution of chlorophyll does not react with weak alkali as being an ester it is without acid properties. If however, strong alkalis are used, a brown colouration appears which changes back later to green.

Pour a little of the ether solution from Experiment 2 into a test-tube and in a pipette take a little strong solution of potash in methyl alcohol (obtained by dissolving 30 gms. potassium hydroxide in 100 c.c. methyl alcohol). Place the lower end of the pipette at the bottom of the test-tube and allow the potash to run in below the chlorophyll solution. At the interface between the solutions there appears immediately a brown coloured layer which diffuses on shaking. In about ten minutes it changes back through an olive green colour to pure green. The chlorophyll has been saponified to the potassium salt of the acid chlorophyllin. This salt is insoluble in ether, so if water is added to bring about a separation of the two layers, the green colour is no longer present in the ethereal layer.

The brown phase produced in this saponification of a mixture of the two chlorophylls is the resultant of a yellow phase produced by chlorophyll a and a brown-red phase produced by chlorophyll b. The phase test should therefore also be carried out separately with the petrol ether solution containing chlorophyll a and the ether solution containing chlorophyll b obtained in Experiment 3.

It should be observed that if water is added directly the brown phase appears, the greater part of the green pigment is soluble in ether, and will again give the brown phase on treatment with alkali.

Note Willstätter's theory of the lactam ring to explain the appearance and disappearance of the brown phase.

The phase test also applies to the chlorophyllides and phæophytin and to the phæophorbides. It is not given with allomerised chlorophyll.

This allomerisation takes place in alcoholic solution particularly when water-free (see Section B of this chapter). The chlorophyllides are also very easily allomerised and lose thereby their power of crystallisation. Small quantities of water and of acids protect the substances against allomerisation, while alkalis increase the velocity of the reaction.

Experiment 5. Allomerised chlorophyll does not give the brown phase test.

Dissolve a little crude chlorophyll, obtained by evaporating an ether solution, in absolute alcohol. Add a little alkali, and perform the phase test from time to time till at last the brown phase no longer appears.

Experiment 6. Separation of the green and yellow pigments. Required: 5 c.c. ether solution of pigments; 2 c.c. 30% potassium hydrate in methyl alcohol; 5 c.c. ether.

Shake 5 c.c. of the ether solution of the pigments with 2 c.c. of the strong alkali. After the green colour has reappeared, slowly add 10 c.c. water and then add a little more ether. On shaking the test-tube two layers are produced of which the lower watery-alkaline one contains the saponified green pigments, while the carotin and xanthophyll are contained in the upper ethereal layer.

This test is employed in the examination of the purity of a chlorophyll preparation. If all the yellow pigments have been removed, the ethereal layer in this experiment should remain colourless after saponification of the chlorophyll.

Experiment 7. Separation of the two yellow pigments. Required: The ethereal solution of yellow pigments from Experiment 6; 10 c.c. petrol ether; 30–50 c.c. 90% methyl alcohol; 1 separating funnel.

The ether layer obtained in the last experiment is washed with water in a separating funnel and evaporated down to 1 c.c. It is then diluted with 10 c.c. petrol ether and next mixed with 10 c.c. 90% methyl alcohol. The methyl alcoholic layer is removed and the petrol ether layer is again treated with methyl alcohol and the methyl alcoholic layer again removed. This process is repeated until the methyl alcohol is no longer coloured. The methyl alcohol contains the xanthophyll, the petrol ether the carotin.

It may be recalled that the yellow pigments in solution greedily absorb oxygen. Some observers, either unaware of this or assuming that the chlorophyll they used was free from yellow pigments without applying tests to prove it (Experiment 6), have mistakenly stated that chlorophyll greedily absorbs oxygen.

In solution the two yellow pigments appear very similar. They can, however, be distinguished by means of their absorption spectra. (See section C of this chapter).

Experiment 8. Phytochlorin and Phytorhodin. Required : 5 c.c. ether solution containing both chlorophyll components (Experiment 2) ; 3 c.c. 30% potash solution in methyl alcohol ; hydrochloric acid of various concentrations ; separating funnel.

Five c.c. of an ether solution containing both chlorophylls a and b are evaporated to dryness in a test-tube, and the residue treated with 3 c.c. of boiling, concentrated potash solution in methyl alcohol, and boiled gently for half a minute. A liquid with red fluorescence is produced, which consists of a solution of the potassium salts of *isochlorophyllins.* The solution is diluted with double its volume of water and concentrated hydrochloric acid is added until the solution is just acid. The liquid is then shaken with ether in a separating funnel ; the dissociation products produced by the previous treatment go over to the ether solution which thus acquires an olive-brown colour.

The ether solution is shaken twice, each time with 10 c.c. 4% hydrochloric acid, and the green-blue acid layer is separated and neutralised with ammonia and shaken with more ether, which then contains in solution phytochlorin e, the derivative of chlorophyll a The phytochlorin e gives to the ether an olive-green colour.

The ether layer remaining in the funnel after the separation of the green-blue acid layer is now extracted with 10 c.c. 12% hydrochloric acid. The green acid solution so obtained is diluted with water and shaken with ether which then becomes coloured red and contains phytorhodin g, the derivative of chlorophyll b.

It should be noted that saponification with hot alkali as in this experiment, produces changes in the chlorophyll compounds different from those produced by saponification in the cold.

The potassium salts of the chlorophyllins which are produced by gentle saponification in the cold are not fluorescent, and under the action of acids pass over into the weakly basic phytochlorins f and g and phytorhodins k and i.

By saponification with hot alkali isochlorophyllins are formed which are fluorescent. They are complex magnesium compounds of phytochlorin e and phytorhodin g. On the addition of acid to the isochlorophyllins, these two important dissociation products are themselves formed.

The process can also be effected by addition of acid first and subsequent saponification with hot alkali.

The following scheme may help to make clear the relations between these various derivatives in the case of chlorophyll a. An exactly similar scheme may be made for the case of chlorophyll b.

$$[Mg\,N_4C_{32}H_{30}O](COOCH_3)(COOC_{20}H_{39}) \longrightarrow [N_4C_{32}H_{32}O](COOCH_3)(COOC_{20}H_{39})$$

With acid

chlorophyll a   phæophytin a

With hot alkali   ↓   With hot alkali ↓

$$[Mg\,N_4C_{32}H_{30}O](COOH)(COOH) \longrightarrow [N_4C_{32}H_{32}O](COOH)(COOH)$$

With acid

isochlorophyllin a   phytochlorin e
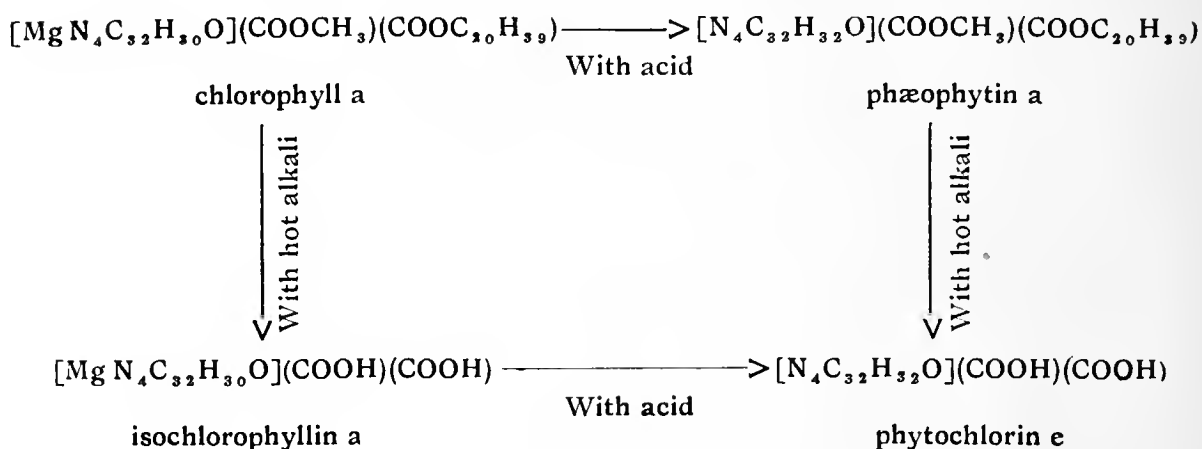
The relation between chlorophyll and isochlorophyllin is not as simple as that expressed in the above scheme as the alkali not only saponifies the two ester groups but also produces an alteration in the lactam ring grouping, as indicated by the appearance of the brown phase.

Another difference between saponification with cold alkali and with hot alkali is that during the latter process the yellow pigments are destroyed. If water is added after the saponification and the solution shaken up with ether, the ether should remain colourless.

We have gone into the explanation of the changes taking place in this experiment in some detail, because importance attaches to the two dissociation products phytochlorin e and phytorhodin g. It was the formation of these substances that led Willstätter to the discovery that phæophytin, and also chlorophyll, is a mixture of two components. The experiment, moreover, is also of importance as a modification of the method is used in the quantitative estimation of the green pigments in the leaf.

Experiment 9. Substitution of other metals for the magnesium in chlorophyll.

Two c.c. of an ether solution of chlorophyll are shaken with a

little 20% hydrochloric acid and then washed with water in a separating funnel. In this way is produced in ether solution, a magnesium free chlorophyll derivative, phæophytin. The solution is evaporated down on a water bath and the residue dissolved in 5 c.c. alcohol. Note the olive green colour of the solution. This is heated and a grain of copper acetate is added. The colour changes back to a brilliant green, but without the chlorophyll fluorescence. A copper compound of chlorophyll has been produced very similar to the magnesium compound, but much more stable.

For spectroscopic examination of this substance, see Experiment 15.

Phæophytin combines very easily with salts of some metals to form intensely coloured stable compounds. Ferric salts give, even in the cold, a greenish blue solution with a weak fluorescence. Zinc acetate gives a blue green solution with strong fluorescence.

Not only phæophytin but all the chlorophyll derivatives devoid of magnesium, such as phæophorbide, phytochlorin, phytorhodin and the various porphyrins behave similarly towards the salts of certain metals (copper, zinc and iron) and form complex compounds all very stable in acid and alkaline media. The formation of these complex compounds is accompanied by such noticeable changes in colour that even the smallest traces of certain metals can be discovered in this way. Hence it is very difficult to prepare the magnesium free chlorophyll derivatives absolutely pure, as even the zinc from the walls of glass vessels may disturb the molecule ; for the same reason spatulas of ignoble metals must not be used.

Also solvents may disturb the molecules of these derivatives owing to the impurities in them. Thus ' pure ' methyl alcohol often contains a small quantity of copper which would be sufficient to affect the magnesium free derivatives. Willstätter uses this property in order to test the purity of methyl alcohol as regards copper, by dissolving some phytochlorin e in the methyl alcohol. After standing for some time, the chlorophyll derivative is carried over into ether and the excess of phytochlorin removed by washing with 10% hydrochloric acid. As the copper compound of phytochlorin e is stable in presence of this strength of acid, it remains in the ether layer to which it gives an intense blue-green colouration.

The spectrum of these derivatives is also quite distinct from the metal free substances and more like the chlorophyll spectrum. See Experiment 15, b and c.

The compounds formed by the magnesium free derivatives with other metals are unstable towards acids, and require other conditions for their formation. Thus phytochlorin e in methyl alcoholic solution gives, with water-free barium hydroxide in excess, a barium compound. Even compounds with the alkali metals can be formed in a similar way, the compounds with potassium being the least stable of them all.

The magnesium compound occupies a place in the middle of the series as regards stability, the two extremes being the copper and potassium compounds.

Experiment 10.   The action of chlorophyllase.

Fresh leaves of a species rich in chlorophyllase (Heracleum, Galeopsis) are finely divided and put in a 70% acetone solution, 3 c.c. of solution being used for every gram of leaf powder. The chlorophyll, by means of the chlorophyllase, is dissociated into phytol and the acid chlorophyllide. This can be demonstrated after about a quarter of an hour if the solution is diluted with water, transferred to ether and shaken with 0·05% sodium hydroxide. The sodium hydroxide takes up more colouring matter the further the enzyme action has progressed.

Experiment 11.   Destruction of chlorophyllase.

If fresh leaves of a species rich in chlorophyllase are first steeped in boiling water for a few minutes before they are placed in the acetone solution, unaltered chlorophyll is extracted which does not react with dilute alkali.

The action of the enzyme chlorophyllase consists in either an alcoholysis (in alcoholic media) or hydrolysis (in aqueous media). For instance, in methyl alcoholic media, the phytol group is replaced by a methyl group according to the equation

$$(C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOC_{20}H_{39})\ +\ CH_3OH\ =$$
$$C_{20}H_{39}OH\ +\ (C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOCH_3).$$
Phytol.   Methyl chlorophyllide.
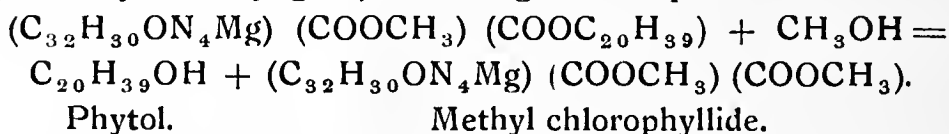
In aqueous solutions hydrolysis takes place with the formation of free acid chlorophyllide

$$(C_{32}H_{30}ON_4Mg)(COOCH_3)(COOC_{20}H_{39})\ +\ H_2O\ =\ C_{20}H_{39}OH$$
$$+\ (C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOH).$$
Chlorophyllide.
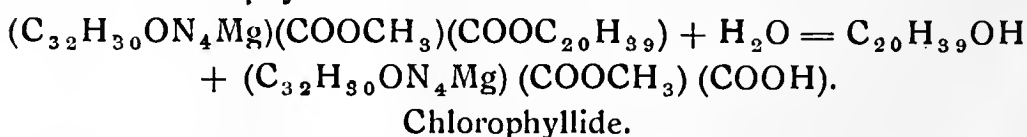
Experiments 10 and 11 demonstrate the hydrolysis of chlorophyll and also indicate that it is an enzyme action. Chlorophyllase is a

very stable enzyme ; it is not even destroyed by boiling in alcohol for a short time. But if leaves are boiled in water the enzyme is destroyed.

It should be noted that the enzyme on account of its insolubility remains in the leaf when this is extracted with solvents.

By treatment with acids, magnesium is removed from the chlorophyllides with production of the corresponding phæophorbides. Thus methyl chlorophyllide a $(MgN_4C_{32}H_{30}O)(COOCH_3)(COOCH_3)$ gives methyl phæophorbide a $(N_4C_{32}H_{32}O)(COOCH_3)(COOCH_3)$.

The relations of these various substances may be made clear by the following scheme taken from Willstätter.

phæophytin a $(N_4C_{32}H_{32}O)(COOCH_3)(COOC_{20}H_{39})$

— with HCl and methyl alcohol →
methyl phæophorbide a $(N_4C_{32}H_{32}O)(COOCH_3)(COOCH_3)$

— with concentrated acid →
phæophorbide a $(N_4C_{32}H_{32}O)(COOCH_3)(COOH)$

chlorophyll a $(MgN_4C_{32}H_{30}O)(COOCH_3)(COOC_{20}H_{39})$ — with dilute acid → phæophytin a

— with enzyme →
methyl chlorophyllide a $(MgN_4C_{32}H_{30}O)(COOCH_3)(COOCH_3)$ — with concentrated acid → methyl phæophorbide a

— with enzyme →
chlorophyllide a $(MgN_4C_{32}H_{30}O)(COOCH_3)(COOH)$ — with dilute acid → phæophorbide a

with concentrated acid → (with dilute acid)

with enzyme →

Experiment 12. Microscopic examination of ethyl chlorophyllide.

Prepare sections of fresh Heracleum leaves and mount them n a drop of 90% alcohol. Leave the slide under a bell jar containing a dish of alcohol. The section slowly dries in the course of half a day or a day. It is then examined under the microscope when there will be observed the characteristic triangular and hexagonal crystals of ethyl chlorophyllide (crystalline chlorophyll).

Experiment 13. Production of methyl chlorophyllide in the leaf.

Sections may be used as in the preceding experiment, or a piece of a leaf may be employed. In the latter case a test-tube with 4 c.c. 75% methyl alcohol is taken and 1 gram of fresh leaf is added to it. The leaf first becomes a darker green and then during the course of a few hours becomes yellowish. On holding the leaf to the light there can be observed with the naked eye a number of black points. If sections of the leaf be cut and examined under the microscope, these spots appear as aggregates composed of rhombohedral crystals, occurring only in certain cells.

Experiment. 14. Extraction of ethyl chlorophyllide.

Two grams of dry Heracleum leaf powder is left for a day in a test-tube containing 6 c.c. 90% alcohol. The extract is then filtered through a small Buchner funnel and the powder on the filter washed with a little acetone. The filtrate is washed with the same quantity of ether, and then with water. The ether solution is transferred to a separating funnel and washed with water, and then concentrated on a water bath to $\frac{1}{2}$ or 1 c.c., and 3 c.c. petrol ether is added. On standing, the ethyl chlorophyllide is precipitated in the form of crystalline aggregates. It is freed from yellow pigments by shaking with a *little* ether, and can be further purified by redissolving in ether and precipitating again with petrol ether.

Experiment 15. Spectroscopic examination. Required: small spectroscope and glass vessel with parallel sides of about 1 cm. in width; source of light (incandescent burner or Nernst lamp or sunlight).

The following absorption spectra may be examined :—
a. Chlorophyll spectrum from acetone extract obtained in Experiment 1. The extract is diluted with about five times its volume of 85% acetone. The spectrum shows a main absorption in the red at the Fraunhofer line C. Then follow, towards the violet, three absorption bands decreasing in intensity, and the end absorption in the

blue to violet parts of the spectrum.

b. Phæophytin. By adding a drop of strong hydrochloric acid to the extract used in *a*, the magnesium is removed from the complex containing it and phæophytin formed.

There is now an intense absorption in the green just before the line E.

c. Copper compound with phæophytin (see Experiment 9). The intense absorption in the green disappears and the spectrum is very similar to the chlorophyll spectrum.

d. Carotin and xanthophyll. The absorption spectra of the yellow pigments has been described in section C. There is one band in the blue, another in the indigo blue and the end absorption in the violet. Unless the correct concentration is used there will either be complete absorption or none. By altering the concentration it should be possible to obtain the correct strength of solutions for observing the bands.

Spectroscopic analysis is, of course, very useful in work with chlorophyll and its derivatives, as most of the pure substances have characteristic spectra. But in class work where it is difficult to obtain even moderately pure substances, it will scarcely be possible to go much further in this matter than we have indicated in the preceding experiment. It should however be noted that one of the crucial tests for chlorophyll is its spectrum, as the breaking down of the magnesium-containing complex alters this.

Experiments on the state of aggregation of chlorophyll. Experiment 16. Formation of a colloidal solution of chlorophyll.

Evaporate down 10 c.c. of the acetone extract as obtained in Experiment 1 to about 2 c.c. A colloidal solution of chlorophyll is then made by pouring this acetone solution into a large volume of distilled water (20 to 100 c.c.) the liquid being continually stirred. This operation can be most conveniently done by taking the acetone solution in a pipette and allowing it to run out of the pipette while the latter is used as a stirring rod in the water. Note the change in colour to a purer green, and the disappearance of fluorescence.

The principle involved in this method of preparation of colloidal chlorophyll consists in the replacement of the solvent (acetone) by a medium (water) in which the solute (chlorophyll) is insoluble.

Thus a colloidal solution of sulphur can be similarly made. Sulphur is slightly soluble in warm alcohol, but insoluble in water.

If an alcoholic solution of sulphur is poured into a large volume of distilled water, a sulphur sol is produced.

Experiment 17. To show the difference between a true and a colloidal solution of chlorophyll.

Evaporate 10 c.c. of the acetone extract to complete dryness and test its solubility in ether, petrol ether and benzene. Now add these solvents to some of the colloidal solution prepared in the last experiment, and note that the chlorophyll does not dissolve in any of these solvents. If, however, some salt solution, e.g., a little magnesium sulphate be added, the chlorophyll is precipitated from its colloidal state and is now soluble in ether and other solvents.

Experiment 18. To show that chlorophyll in the plant is probably in the colloidal condition.

Some nettle powder is carefully dried, e.g., by keeping it at 30°C. to 40°C. in an oven, and then further drying in a vacuum desiccater over sulphuric acid. Small quantities of this dry powder are put in test-tubes and different pure water-free substances such as acetone, ether, benzene and absolute alcohol are added. Note that these solvents are not coloured by the chlorophyll. It can be demonstrated that the extracted pigment is easily soluble in any of these substances.

Repeat the experiment with nettle powder moistened with a few drops of water, and note that the solvents are immediately coloured.

Experiment 19. Pure solvents are able to extract chlorophyll from fresh leaves.

Crush 10 grams of fresh leaves of nettle, horse-chestnut or elder in a mortar with some clean sand, and put the crushed material on a filter paper in a Buchner funnel. Add 20 c.c. pure acetone and suck it through by means of a water pump. Repeat this several times. The pure solvent is here able to extract the pigment.

It can also be observed that the leaf substance after extraction is brown, owing to the action of oxydases on the leaf. If therefore, the leaves are dipped first in boiling water, these oxydases are destroyed, and the leaf substance after extraction remains colourless.

Experiment 20. Treatment of fresh leaves with boiling water changes the condition of the chlorophyll.

Dry a quantity of leaves which have been put in boiling water and examine their solubility as in Experiment 18. Note that the chlorophyll in this powder is soluble in pure solvents.

It should be noted that colloidal chlorophyll is an electronegative suspensoid, and that it is a very excellent substance for demonstrating the properties of such colloids. It might even be worth while for this purpose to prepare a small quantity of pure chlorophyll (a + b) (see section D of this chapter).

In most suspensoids it is difficult to see when the precipitation has taken place, but here where any precipitated chlorophyll can immediately be extracted by ether, the method is open for quantitative work of high accuracy.

By the use of chlorophyll can be demonstrated such properties of colloids as the salt concentration required for precipitation, the effect on this of the valency of the precipitating ion, the stabilising effect of alkali and the reverse effect of acids, the action of protective colloids, etc.

Crude colloidal chlorophyll contains, of course, a good many accompanying substances which vary in composition and quantity, so comparable data are not obtainable by the use of such chlorophyll.

(To be continued).

NOTES ON THE COROLLA IN THE COMPOSITÆ.

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[With Thirty–Three Figures in the Text].

IN a previous contribution on the Compositæ (29) the writer explained that any phylogenetic scheme must take into account, among other data, " the form, development and colour of the corolla," and the present paper is an attempt to show that these characters in the corolla show a development which confirms the hypothesis that the course of evolution in the Compositæ has followed the lines indicated by the study of the pollen-presentation mechanism (29, p. 466).

So much has been done in the actual observation of the form, development and colour of the corolla, that there is little room for fundamental research on this part of the flower, except along the lines of casual morphology as indicated by Lang (19). There remains, therefore, the critical consideration of known facts in the light of the phylogenetic suggestions obtained from the study of the stamens and styles. I have been on the lookout for facts which would prove the changes suggested in the relations of the tribes (29) unsatisfactory or contrary to the true history of triba

differentiation as shown by the sum of the characters of the plant, but such facts, as will be shown, are not to be found among the characters of the corolla.

One might think that it was labouring the point to attach so much importance to the floral organs, but in addition to the arguments adduced previously as to their being the chief guide to affinities in this order, it is interesting to consider the geographical distribution which Bentham (3) took as his chief guide to the phylogeny of the tribes. Bentham maintained that the tribes of the Compositæ had acquired the essential characters now employed in classification before the dispersion of the order over the Pacific from its point of origin in the west of North America. Guppy (12) agrees and places the date of the origin of the tribes previous to the Tertiary submergence of the islands of West Polynesia. Except in the case of several more recently differentiated tribes this may be accepted and just as it reduces the importance of geographical distribution as a guide to the evolution of the tribes concerned, it emphasises the importance of the study of the floral characteristics.

Form of the Corolla.

There are three quite distinct types of corolla—(1) tubular and five-lobed (Fig. 1), (2) two-lipped with the posterior lip more or less reduced or absent (Figs. 2, 4-15), (3) ligulate and five-toothed (Fig. 3). The first type occurs in the disc florets and in the discoid capitula of most tribes. The second type occurs in the ray florets and in the discoid capitula of the Mutisieæ. The third type is confined to the Cichorieæ.

The phylogeny of the form and sex of the flower is the subject of an elaborate thesis by Uexküllung-Gyllenbad (32), and his observations confirm the hypothesis that the primitive type in the Compositæ is that of a hermaphrodite flower with a tubular, five-lobed corolla and that the truly ligulate corolla has been derived direct from this primitive type, while the numerous other forms are more or less modified bilabiate types. Apart from the systematic literature and the above thesis the references to the mature corolla form are somewhat fragmentary. There has been much statistical work on the variation in the number of ray florets, but beyond showing the possibilities of such variation this line of investigation has produced no results of phylogenetic value.

Considering the hypothesis that all florets other than those

with tubular and truly ligulate (*i.e.*, Cichoriaceous) corollas are modified bilabiate forms, we find many variations as confirmatory evidence. The ordinary ray floret is usually three-toothed with the posterior lip represented by the perfectly smooth surface at the top of the short tube by which the corolla is attached to the receptacle. Frequently, however, the short tube becomes more or less elongated and one or both of the suppressed lobes become developed to give a four- or five-lobed corolla with a deep indentation on the posterior side.

A typical case in *Helenium autumnale* was observed in the Cambridge University Botanic Gardens. The ray florets in some capitula were all markedly tubular, some being equally five-lobed and truly tubular (Fig. 4), while others were tubular with a deep indentation between the posterior lobes (Fig. 5). Still others showed four lobes with no trace of the fifth (Fig. 6), and others again, showed the fifth lobe more or less aborted (Figs. 7, 8). In a few florets the posterior lobes were large while the other three were united into a larger lobe with three teeth (Fig. 9). Thus in one capitulum could be seen most of the variations of the bilabiate ray floret. Similar abnormalities have been observed in *Calendula* spp. (Figs. 10, 11) and in other genera.

The four-lobed corolla is comparatively common among large ray florets and the vascular system is correspondingly enlarged but the vascular supply of the floret is the subject of a separate study and need not be considered here. It seems, however, to be more or less closely dependent on the width and size of the corolla. The five-lobed, pseudo-ligulate floret is rare and even when it occurs, the lobes are not usually uniformly developed nor do they resemble, except superficially, the truly ligulate floret of the Cichoriaceæ. All these variations are obviously partial reversions to the tubular, five-lobed type.

Another tendency shown by the three-lobed corolla is the development to a marked degree, especially in the Mutisieæ (Figs. 12-15) of the posterior lip. In *Calendula* and other genera showing the ordinary ray floret, the posterior lip may be represented by a small lobe lying at the base of the posterior indentation. This lobe may be larger and subulate (Fig. 10), or it may be divided (Fig. 11), orming a two-lobed lip of varying size. Distinctly bilabiate forms have been observed occasionally in most tribes (vide 3, 7 and 32 above).
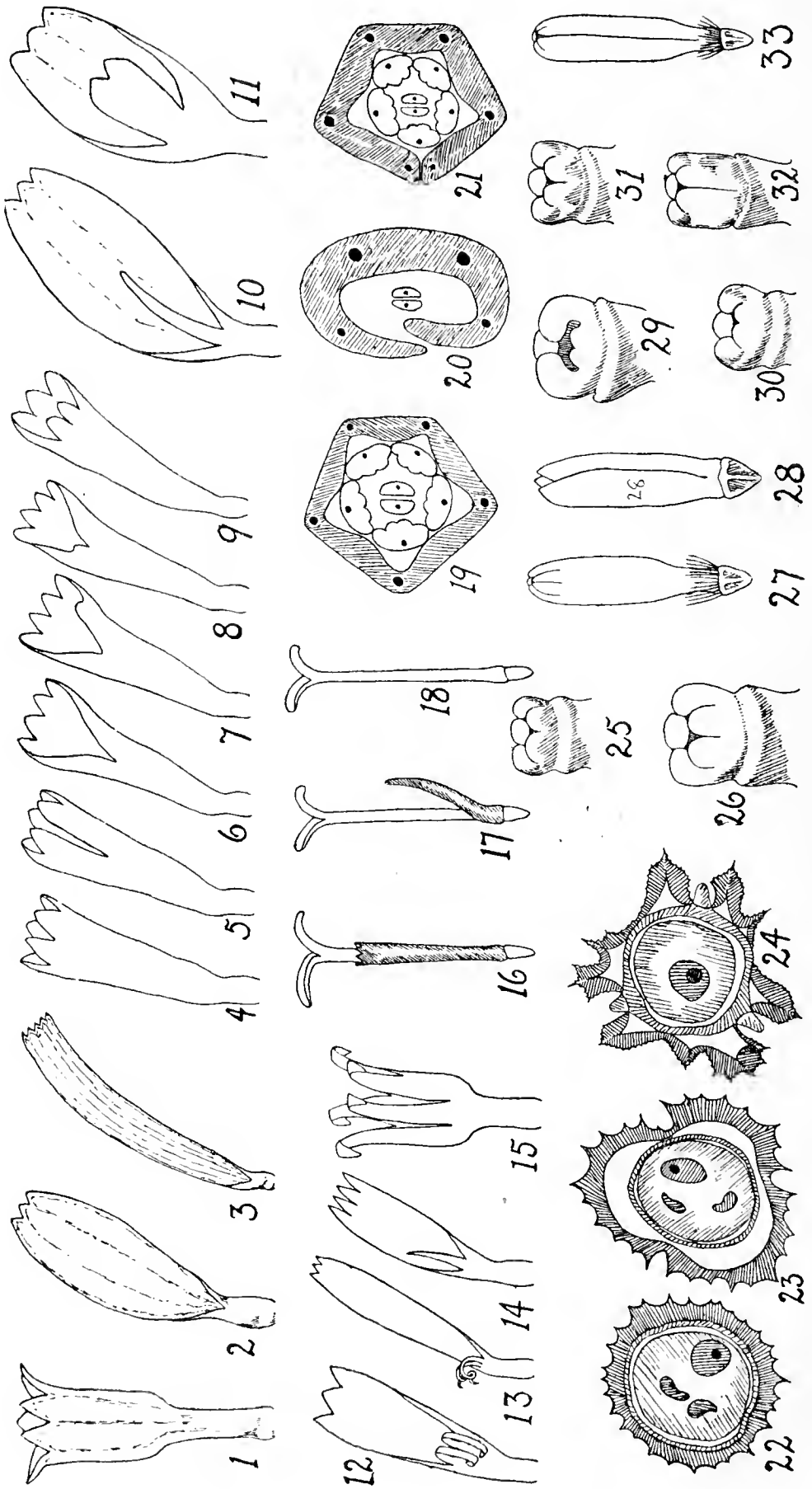
FIGS. 1-33. COROLLA IN COMPOSITÆ.
(For description see page 27).

In a comparatively few genera the corolla shows a tendency to *reductio ad nihilum*. *Baccharis, Conyza, Erigeron, Haastia, Heterothalamus, Psidia*, in the Astereæ; *Clibadium* and *Iva* in the Heliantheæ; *Gnaphalium* in the Inuleæ; *Doronicum* and *Petasites* in the Senecioneæ; and *Leria* in the Mutisieæ all show this tendency in some species. In *Haastia Sinclairi* (32; see Figs. 16-18) the female florets show various stages down to the entire elimination of the corolla. These cases, however, have little phylogenetic value and serve only to emphasise the tendency to reduction in corolla material, which is discussed later (p. 30).

The occurrence of a large proportion of the reduction forms in the Astereæ is of interest since it is suggested (29) that this group is the first of a reduction series leading through the Vernonieæ to the Eupatorieæ, and also in connection with the statement by Bentham (4) that the Astereæ and Inuleæ become discoid by the disappearance of the ray florets. The floret, in most cases where reduction is observed, is female, but the distribution of sex belongs more to the study of the composition of the capitulum, a subject which is dealt with in detail by Uexküllung-Gyllenbad,[1] and will be discussed in its phylogenetic aspect in a future contribution.

The Vernonieæ and the Eupatorieæ show similar tubular florets; the Astereæ show the typical tubular disc florets with bilabiate rays and with the above-mentioned tendencies to reduction in the corolla and the complete disappearance of the ray florets; the Heliantheæ, Helenieæ, Anthemideæ, Inuleæ, Calenduleæ and Arctotideæ have the tubular disc and bilabiate ray florets, which is the typical arrangement for the order in most genera, the Senecioneæ also show the typical arrangement, but a comparatively large proportion of the genera have discoid or disciform capitula; the Mutisieæ show

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[1] This author disposes of the idea that the disappearance of the anthers and the enlargement of the corolla in the bilabiate florets of the ray are correlated and concludes that such a method of compensation has no influence in determining the reduction in the androecium. As evidence he adduces numerous examples of (1) reduction in the stamens with no reduction in the corolla, (2) enlargement of the corolla with no reduction in the stamens, (3) reduction in the stamens with reduction also in the corolla, and (4) reduction in the stamens, with both enlargement and reduction of the corolla in the same species.

Figs. 1-3, typical corolla forms. Figs. 4-9, corolla forms in *Helenium autumnale*. Figs. 10-11, corolla forms in (10) *Calendula officinalis*, (11) *C. pluvialis*. Figs. 12-15, corolla forms in the Mutisieæ. Figs. 16-18, female florets in *Haastia Sinclairi*, (after Uexküllung-Gyllenbad). The corolla is shaded. Figs. 19-21, transverse sections of young florets :—(19) tubular; (20) bilabiate ; (21) ligulate. Figs. 22-24, sections of types of pollen grains:—(22) Tubifloræ; (23) *Senecio vulgaris*; (24) Cichorieæ. Figs. 25-33, floral development :—(25-27) tubular florets, *Senecio vulgaris* ; (28-30) ray florets, *Calendula officinalis* ; (31-33) ligulate florets, *Taraxacum officinale*.

several variations of the bilabiate and tubular corolla forms; the disc and ray arrangement occurs here also, but never in the Cynareæ. The tubular corolla in the Mutisieæ is frequently very deeply lobed and the posterior indentation may be deeper than the others as in *Helenium autumnale*. The bilabiate forms in this tribe usually have the anterior lip with three teeth or lobes and the posterior or upper lip takes on a variety of forms (Figs. 12-15). The Cynareæ have usually a regular, tubular corolla but the outer florets of the capitulum may be enlarged to form a ray; the corolla then becomes more or less irregular and the number of lobes increases to seven or eight in some species. These ray florets also show a tendency to become irregular or bilabiate. The Cichorieæ invariably have the truly ligulate corolla in all the florets of the capitulum. This corolla is always strap-shaped with five teeth. The florets towards the centre of the capitulum have the corolla closed to form a tube with the adjacent edges of the posterior petals tightly adpressed (Fig. 21). This is quite different from the æstivation of the bilabiate ray florets in which one side of the corolla overlaps the other (Fig. 20) before it unfolds to form the ray of the capitulum.

The Cichorieæ in this as in many other characters are distinct from all the other tribes, and the number of mutations which occur in the Compositæ gives a basis to the suggestion that the Ligulifloræ are monophyletic, derived comparatively recently from the Senecioneæ by mutation. Only in this way can one account for the pollen grains (2) as well as the corolla, stamens, style and even some nuclear details being of one peculiar type throughout the tribe. It is interesting to note also that *Senecio vulgaris* sometimes shows a somewhat similar type of pollen grain occurring in a small proportion among pollen of the Tubifloral type (Fig. 23). De Vries (33) and Trow (31) have shown that the ordinary ray floret is liable to appear or disappear quite frequently so that it is no matter for surprise if the ray has disappeared in the Eupatorieæ, Vernonieæ and Cynareæ. De Vries describes not a few mutations of various kinds in the Compositæ. Trow describes the origin by mutation of a fimbriate corolla in *Senecio vulgaris* and his explanation of the factors involved could well be applied in the case of the Cichorieæ.[1] Then if such a factor, probably a recessive, for true ' ligulateness ' does exist, it would furnish another explanation of those rare cases

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[1] The suggestion made by Trow that the absence of the fimbriate character is a function of the environment as well as the factor, f, is interesting in connection with a case recorded by Molliard (21).

of five-toothed ray florets. I am of opinion, however, that those exceptional forms are due to the irregular or abnormal development of the posterior lobes rather than to the distinctive process by which the truly ligulate corolla is developed. The genus *Hieracium* has a literature of its own, and although Ostenfeld (26) shows that polymorphism in this genus is correlated with apogamy, his results seem to point to the occurrence of mutations (as well as hybridisations) which would be preserved by the prevalent apogamy. Kajanus (16) also records a mutation. The occurrence of mutants in both the Senecioneæ and the Cichorieæ is especially interesting and the suggestion of the origin of the latter tribe by mutation is strengthened.

Development of the Corolla.

Floral development in the Compositæ is easily observed and has been the subject of numerous researches. Payer's figures (27) are not to be surpassed, and it is easy to follow the development of the three types of corolla from his illustrations of *Heliopsis scabra* ray and disc florets, *Centaurea jacea*, *Hieracium umbellatum* and *Cichorium Intybus*.

Other authors have merely extended the range of observations and included some abnormal forms. In the tubular florets the lobes are equal throughout all the stages of development, (Figs. 25-27). In the bilabiate florets the lobes become differentiated at an early stage (Figs. 29, 30); there may be a deep posterior indentation, and this, by the subsequent development of the usually abortive lobes, would give a pseudo-ligulate corolla, as in the Mutisieæ (e.g., *Ainsliæa*, *Catamixis*, *Pasaccardoa* and *Dinoseris*) and the rare cases in other tribes (e.g., *Stokesia* spp., *Anthemis* spp., *Helenium* spp., *Calendula* spp.). The deep indentations may occur on each side of the posterior lip and the development of this lip would give the bilabiate forms of the Mutisieæ and the abnormal, distinctly bilabiate forms in other tribes. The posterior lobes usually abort completely and there is practically always an over-lapping of the edges of the ' ligule' (Figs. 20, 28) which is not shown by Payer.

The ligulate floret arises in quite a different manner by the failure of the tissue connecting one posterior petal to the other to develop (Figs. 31-33). From a very early stage there is no doubt of the posterior split, and although the corolla tube may, at a later stage, elongate a little at the base it is always as different in appearance from the pseudo-ligulate rays as it is in development.

Exception has been taken to the teleological suggestion contained in the expression " economy of corolla material leads to the aggregation of the flowers into a capitulum " (29, p. 459) and the actual economy of corolla material effected in the capitulum has also been questioned. In the close crowding of the florets from the earliest stages it is obvious that aggregation has reached in the capitulum a stage where the food supply and space available for development of the young florets, exerts a distinct effect upon the size of the corolla. My opinion is that aggregation with its advantages in increased conspicuousness, etc., produced reduction in the size of the individual florets by the overcrowding which took place. This reduction would give further opportunity for the exercise of the tendeny to aggregation already present in the plant. Thus there would be a continuous reduction and aggregation mutually interacting until the limit of efficiency was reached. That limit is apparently reached in the disc and ray arrangement of the capitulum. The Dipsaceæ, Valerianaceæ and the Umbellifloræ are examples of the imperfect development of these two tendencies.

In the compound capitulum of *Echinops*, the dense corymbs, panicles or glomerules of capitula in many of the Eupatorieæ, Vernonieæ and a few genera in other tribes there is evidence of the continuance of this action and re-action beyond the limit of full efficiency.

Reduction in size would give marked economy of material and this, being useful, would tend to preserve the species showing the maximum reduction compatible with *full efficiency*. In the past, therefore, aggregation has led to economy, but at the present time it is economy which is the cause of the continued existence of the aggregation.[1]

In considering the actual economy of corolla material effected one must remember that before the aggregation into a capitulum took place, the ancestral Composite flower had developed into a tubular, *few-seeded*, high type flower, (cp. 34), visited chiefly by the higher insects, so that a comparison of the number of mature plants resulting from the seeds set in a capitulum must be made between the Compositæ and such cohorts as the Tubifloræ and the Umbellifloræ, where the flowers are few-seeded and aggregated only to a certain extent. Compare, for example, the capitulum of

[1] That the tendency to further aggregation is still present, but held in check, is shown by the various forms in which the capitula are grouped in the larger inflorescences and by anomalies such as the development of several concentric rows of secondary capitula in the bracts of the primary capitulum in *Inula glandulosa*, (25).

Helianthus annuus or *Taraxacum officinale* with the scape of *Digitalis purpurea* or *Verbascum Thapsus.* The economy then becomes obvious. Species in lower orders, such as the Ranunculaceæ and Caryophyllaceæ may develop more seeds with a smaller output of corolla material, but they have not the advantages of developing one or two seeds in each fruit which are possessed by the higher orders.

Colour of the Corolla.

Apart from descriptions in the systematic literature, the colour of the corolla in the Compositæ has not been the subject of research, except when species have been taken as examples in researches dealing with the relation of insects to flowers and a few scattered enquiries into isolated species. The distribution of colour in the various tribes, however, follows the lines of development already suggested to such an extent that it is worthy of consideration.

Practically all authorities are agreed that blue is the highest colour, and Avebury (1, p. 308) states his opinion " that all blue flowers have descended from ancestors in which the flowers were green————; and that they have passed through stages of white or yellow, and generally red, before becoming blue." Müller and Avebury disagree on the relative attractive power of white and yellow, but it seems probable that while moths are more attracted by yellow than by white, as Müller's results show, bees may be attracted more by white than by yellow, as Avebury's results prove. Müller, however, includes bees in his placing of yellow above white in the power of attraction. Willis and Burkill (35) confirm in general Avebury's scheme of colour values in regard to desirable insect visitors to flowers of the various colours.

An interesting experiment is recorded by Daniel (10) who propagated a pure white variety of *Chrysanthemum* by cuttings for about seventeen years and, among other evidence of degeneration, he found that the colour of the corolla became a more or less greenish yellow. Müller and Hildebrand (24) have pointed out that blue flowers frequently show atavism and revert to lower colours and that the reverse is rare. De Vries (33) gives many examples of this among the Compositæ.

It is not clear whether purple, which from the Mendelian work on pigmentation seems to be compounded of red and blue, is of a higher type than blue and, as the entomological results seem to indicate the contrary, the point requires experimental enquiry.

Considering the various tribes, we find that yellow from its prevalence and low colour value is presumably the primitive colour for the order. Yellow is the predominant colour in the disc florets in all tribes in which the disc and ray arrangement occurs, and the higher colours are usually present only in the ray florets.

In the Eupatorieæ and Vernonieæ no true yellow occurs, thus strengthening the hypothesis that these are end groups. Red, purple and blue are common in the Eupatorieæ, but blue seldom occurs in the Vernonieæ. The absence of blue when purple is common is of little importance, especially when blue is found, although not very frequently, in the Astereæ. Yellow, white and reds of various shades are typical of the Astereæ, Heliantheæ, Helenieæ and Anthemideæ. The Inuleæ are nearly always yellow, both in disc and ray florets, another point which justifies the removal of this tribe from the vicinity of the Astereæ towards the Senecioneæ. The Senecioneæ show violet and red in a few genera but the genus *Senecio*, which comprises over 1,300 species, has both disc and ray florets yellow, except in some of the more specialised sections, which have been separated from time to time as distinct genera, but which are included in the genus by Hoffmann (14). The Calenduleæ and Arctotideæ are usually yellow, both in ray and disc, but the ray florets may be white or purple. Only half-a-dozen genera in the Cichorieæ show blue flowers and the actual number of blue species form a small proportion of the tribe in which the predominant colour is yellow. Red and white flowers also occur occasionally in this group and the pure blue flowers are possibly an expression of the vigour of the recent and actively developing group which other data prove the Cichorieæ to be. The Mutisieæ and Cynareæ are usually red, purple or blue, yellow occurring more frequently in the former tribe and thus the placing of the Mutisieæ below the Cynareæ is supported even by the distribution of colour in the tribes. That the blue and purple of the Astereæ and Cynareæ is highly developed, similar to that in the Eupatorieæ and Vernonieæ, is indicated by the fact that such flowers are not known to give yellow varieties.

It will be seen that, although it is hardly possible to distinguish clearly the colour values of some of the central tribes, it is possible to decide that there is at least nothing in the distribution of colour in the order which is contrary to the previous suggestions of phylogeny, and that the colour of the corolla in these groups where the variation is not too wide, confirms the conclusions derived from other data.

Conclusion and Summary.

The characters of the corolla in the Eupatorieæ and Vernonieæ, although they do not confirm to any extent the inversion suggested in the position of these tribes, are quite in harmony with the change suggested by the study of the pollen-presentation mechanism. The corolla characters in whole or in part confirm the suggested lines of development for the Astereæ, Inuleæ, Cichorieæ, Mutisieæ and Cynareæ.

The interpretation of the ray floret as bilabiate and of the same type as the Mutisieæ is confirmed by the form in abnormal florets and by the floral development. The position of the Mutisieæ below the Cynareæ is justified still further by this breaking down of the distinction between the florets of that tribe and the ray florets in others; it is also proved to be natural by the scarcity of blue and predominance of yellow flowers in this group as compared with the Cynareæ. The development of an irritable pollen-presentation mechanism, as shown in the Arctotideæ, Mutisieæ and Cynareæ (13, 15, 17, 20, 28, 30) also supports the change, but that is the subject of a special enquiry.

It is suggested that the Cichorieæ arose from the Senecioneæ by mutation, and evidence in support of the hypothesis is adduced from the published accounts of mutations in the order. Describing a mutation in *Senecio vulgaris* in the ray florets of which "instead of being obscurely three-toothed at the tip, the ligules were often divided down to the basal tubular portion into three (sometimes two) long, narrow segments," Trow (31) states that "it is clear that the fimbriate character is inherited, and it may be assumed that a corresponding factor, F, suddenly dropped out of the constitution of the original plant." The hypothesis is that a factor similar to, but distinct from, this fimbriate factor acted to produce the Ligulifloræ. In this way the great similarity even in the most unimportant details throughout this sub-order can be explained, for if the group is monophyletic and of recent origin, it is obvious that it will not have had time to produce forms differing to the degree which obtains in the other tribes of the Compositæ. The fact that no intermediate types are found, although negative evidence and therefore not particularly reliable, also supports the view that the Cichorieæ arose by a sudden variation, which has not been repeated except, perhaps, in *Scolymus*.

There are several groups of data which are of phylogenetic importance and these will be considered later. The occurrence and

form of the pappus and the paleæ on the receptacle, for instance, indicate that some slight changes are required before a scheme can be formulated which will represent the development of the tribes as shown by the sum of the available data.

Quite apart from phylogenetic speculation, the Compositæ is a wonderful group and our British representatives well repay close study.   Material is abundant and has been observed by the writer on all possible occasions in the field, in herbaria and in botanic gardens, including the Jardin des Plantes at Rouen, where, as in most botanic gardens, the genus *Senecio* is well represented.

Acknowledgments are due to Professor M. C. Potter for access to books and periodicals.

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MARINE FUNGI IMPERFECTI.

By GEO. K. SUTHERLAND.

[WITH FIVE FIGURES IN THE TEXT].

JUST as in the case of the Ascomycetes the Marine Fungi Imperfecti have attracted much less attention than Phycomycetes, and so far no systematised attempt has been made to investigate either the extent of their occurrence or their life histories. Doubtless this neglect may be ascribed partly to the fact that the existence of marine forms has not been generally recognised, and partly to the disregard in which the Fungi Imperfecti as a whole are held, notwithstanding the economic importance of many. Consequently, of the very small number recorded, little beyond mere diagnostic characters is known.

The varied history of the first described species is worthy of note, inasmuch as it reflects, to a certain extent, the limited outlook as to the possibility of the existence of marine forms. This is the

monotypic genus *Blodgettia*,[1] recorded by Harvey, and placed by him among the Valoniaceæ under the name *Blodgettia confervoides.* Later botanists, while somewhat puzzled at the characters of the new alga which, if correctly described and diagnosed, justified the formation of a new order for itself, failed to grasp its true nature. Thus Farlow included it in his " List of Marine Algæ of the United States,"[2] with the reservation that the genus was worthy of further careful study. Bornet, who had also examined material, noted the incongruity of the conidia and explained these as separate parasitic algæ, at the same time suggesting that the mycelium was a form of *Cladophora* nearly related to *Cladophora prolifera.* It is to Wright that we owe the final determination of the parasite, to which his attention had been attracted while examining herbarium material of *Cladophora cæspitosa* from Bermuda. He was dissatisfied with the original description and in 1876 showed a specimen to the Dublin Microscopical Society. After obtaining fresh material from Farlow, he became convinced of its fungoid nature and placed it among the Dematiaceæ as *Blodgettia Borneti.*[1] This species is

[1] Trans. Irish Acad., Vol. 28, p. 25, 1881.

found in the filaments of *Cladophora cæspitosa* on the coasts of France and North America.

The first record of the occurrence in Britain of any marine Imperfects appears to belong to the early part of 1888, when Cooke and Massee gave a note on *Cladosporium algarum*[3] found by a correspondent on washed up fronds of *Laminaria flexicaulis.* A later examination of what they regarded as more mature material led them to transfer this form to the genus *Heterosporium.*[4] At the same time they described a species of *Phoma* also occurring on *Laminaria.* To both of these reference will be made in the following notes.

In 1894, Oudemans[5] described a species of *Septoria* occurring in the thallus of *Dictyota obtusangula* in the Celebes. For the following twenty years no further species seem to have been recorded.

During the past two years an extensive examination of marine algæ, conducted primarily with a view to investigating the extent of marine Pyrenomycetes, has revealed the presence of numerous Imperfects, and confirmed the opinion that these play an important

[1] Smithsonian Contributions to Knowledge, 1858.

[2] Proc. Amer. Ac. Arts & Sci., Vol. 10, 1875.

[3] Grevillea, Vol. 16, p. 80.

[4] Grevillea, Vol. 18, p. 74.

[5] Verslag. d. Kon. Akad. Wetensch., Amsterdam, Vol. 3, 1894, p. 54.

part in the disintegration of seashore organisms. Cultures bearing on their life histories and rôles were commenced in the laboratory, and the description of new species was held over pending the result of these and of wider observations on their occurrence. As these, not yet complete, must be interrupted for a time, it has been decided to issue a preliminary description of some of the new forms.

Since an examination of the named material at Kew, of numerous specimens from various points along the coast, and of laboratory cultures has led me to the conclusion that the form, described in 1888 as *Cladosporium algarum*, and later as *Heterosporium*, should be replaced in the former genus, a review of new evidence and a resumé of its position seem necessary, especially as this is one of the most abundant and widely distributed forms and consequently the more likely to find its way into collections. In its occurrence and habits it appears to be as cosmopolitan as the ubiquitous *Cladosporium herbarum*, abounding wherever rotting or dried fronds of *Laminaria* strew the upper beach. The conidia germinate rapidly, and the fronds, a few days after being cast up, become covered with tiny olive-coloured spots, which quickly extend their margins and run into one another, ultimately covering the whole with a thick felt, varying in colour from olive-green to black according to the humidity of the atmosphere and the growth more or less dependent on it. During dry weather growth is restricted, and the conidia are either blown away or fall into the thicket of conidiophores, which become darker in colour and cause the infected areas to stand out clearly against the usually bleached fronds. The conidiophores, at this stage, present the sharply truncated appearance of Fig. 1, 7. They possess, however, the power of renewing their growth on the recurrence of moist conditions, and produce either new conidia or pale coloured elongations of themselves as in Fig. 1, 5. The rapid and varied changes in atmospheric conditions prevent any great development of the latter, except during a continuation of moist weather, when the fronds become coated with a furry out-growth laden with dusty masses of olive conidia. Portions of infected thalli placed in moist stoppered vessels produce this growth in a few days.

That the fungus is not restricted to the region above high tide, is evident from the fact that fresh *Laminaria* fronds, collected from the tidal area, on isolation in the laboratory usually develop a luxuriant growth of *Cladosporium*. The conidia also germinate freely in salt water.

The mycelium developed in the deeper portions of the thallus may be hyaline, but towards the outer layers it becomes brown, bending sharply upwards into the dark coloured, erect, simple or slightly branched, stiff, fertile hyphæ already noted. These produce the conidia at their tips, and, when growing actively, elongate sympodially. The type of conidium is extremely varied (Fig. 1, 6.) These first formed are long and appear to act like sterigmata (Fig. 1, 4) from the tips of which arise either other sterigma-like forms or chains of smaller conidia. The longer connecting forms usually become 1–3–septate and vary in length from 20–40μ, with an average diameter of from 4–5μ. They germinate like the smaller ones, but not so readily, doubtless owing to a certain exhaustion of their contents and energy. During short periods of growth the conidiophores may produce the smaller continuous or uniseptate conidia directly. These vary in size from 5–15μ, × 5–6·5μ. The ones produced during moist conditions usually become darker coloured on exposure to drought, and all possess a thick resistant smooth wall. It is noticeable that a greater proportion of septate conidia are formed during dry conditions of growth.
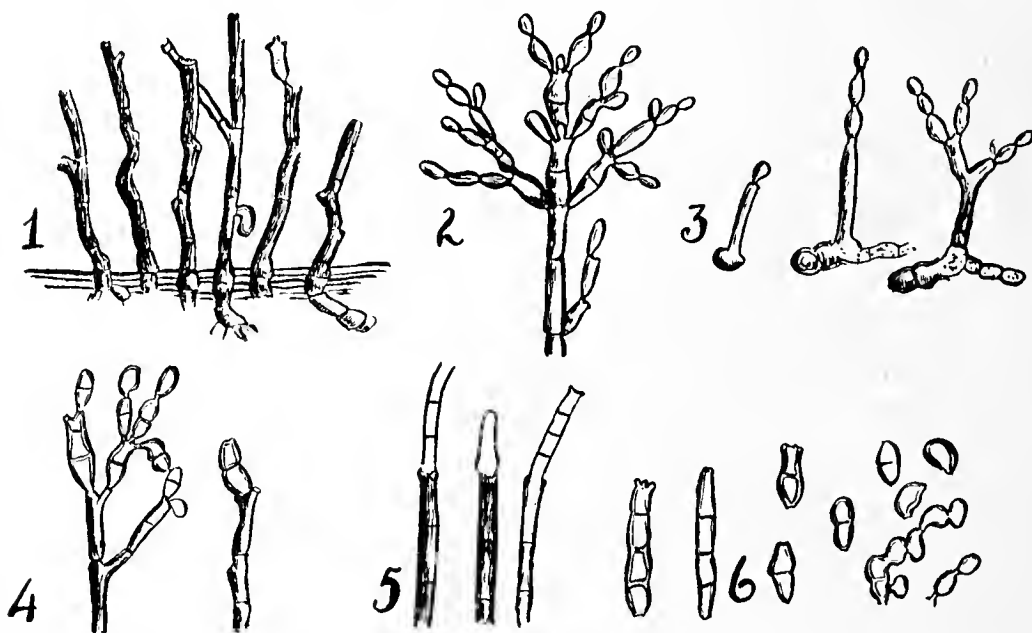
Fig. 1. *Cladosporium algarum*. *1*, conidiophores ; *2*, conidia from cultures ; *3*, germination of conidia in salt water ; *4*, attachment of conidia ; *5*, conidio-phores showing renewed growth ; *6*, types of conidia.

Culture experiments were first initiated to separate *Clado-sporium* from the other fungi accompanying it. In these, this species behaves very much after the manner of *Cladosporium herbarum* or *Cl. epiphyllum*, giving the same variety of fructifications. Fig. 1, *2*, represents a common type with regular sterigma-like bodies and

chains of ovoid or elliptical conidia connected by short necks. These are rarely septate. The fertile hyphæ form a luxuriant growth in most nutritive media. This is similar to what happens when infected fronds are kept in moist vessels. Conidia germinating in salt water develop a poor mycelium. Often they proceed at once to the formation of simple or slightly branched conidiophores bearing a limited number of conidia.

The whole habit of the fungus is characteristic of the genus *Cladosporium*, and so far no spinose conidia, such as described by Cooke and Massee, have been found in direct contact with the mycelium or its fertile branches, and continued growth in cultures and on sterilised fronds properly isolated has failed to produce these. In the natural state, by the seashore, however, the fungus is usually associated with other Imperfects, some of which have verrucose conidia. Two-celled or even four-celled conidia (not yet become muriform) of *Macrosporium* or of *Alternaria*, are often found scattered among the fertile branches of *Cladosporium* and consequently might quite well pass for *Heterosporium*. More numerous, however, are the conidia of a species of *Cercospora*, and the dimensions given by Cooke and Massee agree closely with the shorter and stouter dark forms of this fungus, but, although the granular contents give its conidia a somewhat rough appearance when glanced at casually, their walls are quite smooth. A consideration of the evidence would seem to justify the adoption of the former name.

DIPLODINA LAMINARIANA NOV. SP. (FIG. 2, *1–5*).

Mycelium hyaline; pycnidia grouped or scattered, globose or slightly flattened, at first immersed but finally erumpent with ostiole slightly developed, 110–160μ in diameter; pycnidiospores hyaline, at first continuous, becoming two-celled, elliptical or slightly curved, 8–12μ × 3–4·5μ.

Hab. Saprophytic on fronds of species of *Laminaria* along the coasts of Ayrshire, Dorset and Orkney—probably general.

This species is almost as abundant as *Cladosporium*, along with which it occurs normally. The mycelium is usually hyaline, but becomes faintly coloured towards the base of the pycnidia. These occur either grouped or scattered over the thallus as in Fig. 2, *1*. They appear first as small masses of coiled hyaline hyphæ placed well beneath the rind of the frond. As they develop and increase in size, the outer layer assumes a dark colour. Usually the

pycnidium is produced so near the surface that, when mature, the upper portion of the body becomes erumpent (Fig. 2, 2) and only the shortest of ostioles is present; when more deeply buried, a longer papilla-like mouth is necessary. In this case, usually the tip alone of the latter protrudes. The pycnidial wall (Fig. 2, 4) consists of several layers. To the outside there is an investing ring of dark thick-walled cells. This is lined by one or more layers of thin-walled hyaline parenchyma from which arise the short simple conidiophores. The minute conidia or pycnidiospores, at first continuous and elliptical, become slightly constricted and finally uniseptate (Fig. 2, 5). Frequently they assume a slight bend. The appearance of the septum may take place after the expulsion of
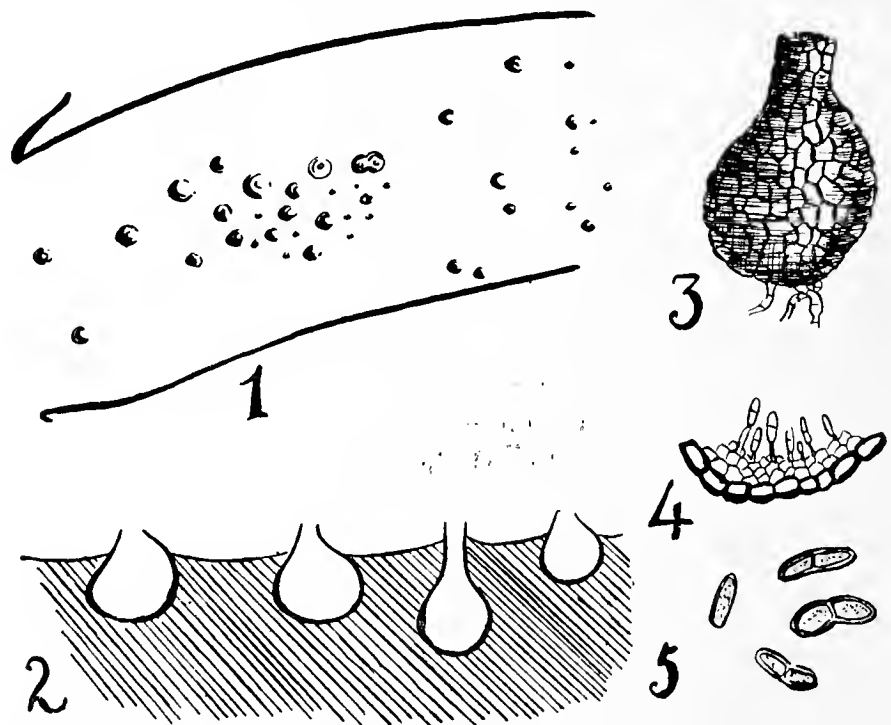
Fig. 2. *Diplodina laminariana.* *1*, thallus showing pycnidia; *2*, diagram showing types of pycnidia in section in thallus; *3*, pycnidium; *4*, pycnidial wall with conidiophores; *5*, pycnidiospores.

the conidium. The dimensions and description of this fungus show similarity to those given by Cooke and Massee for *Phoma Laminariæ*. As the spores of *Diplodina* are continuous almost until maturity, and may be expelled in this unicellular state, it is possible that the two are identical. A distinctly different *Phoma* has been met with on *Laminaria* fronds, but it is of much more restricted distribution. Its spores are smaller—being only about half the size—and consequently differ from *Phoma Laminariæ*, which, as noted above, corresponds more nearly with the unicellular state of *Diplodina*. Unfortunately a type specimen of the origina

Phoma has not been available, and consequently its connection with *Diplodina* and the smaller-spored *Phoma* could not be determined.

With reference to the naming of the species under consideration, a distinct disadvantage of the present artificial system, adopted with regard to pairs of genera like *Phyllosticta* and *Phoma*, or *Ascochyta* and *Diplodina*, may be noted. A generic distinction, based almost entirely on occurrence on the leaves in spots or on the stems, while applicable to fungi on higher plants, breaks down when it has to be applied to those occurring on Thallophyta. Even its application in the former has led to abuse and needless multiplication of species which can only be rectified by careful study of life histories and culture work. In the present instance, while the distinction laid down by Diedicke in the pycnidial walls of *Ascochyta* and *Diplodina* has been adopted in order to place this species in conformity with the existing classification, neither the generic value of this distinction, nor its universal application can be accepted as proved. The case for these pairs of genera is anything but satisfactory.

FUSIDIUM MARITIMUM nov. sp. (Fig. 3, *1–2*).

Mycelium hyaline, creeping, diffused ; conidiophores erect, simple or slightly branched with very long chains of conidia ; conidia hyaline, fusiform or cylindrical with pointed ends, $12\text{--}20\mu \times 3\text{--}5\mu$.

Hab. Saprophytic on *Laminaria* fronds and *Pelvetia* thalli. Orkney and Dorset,

This species of *Fusidium* occurs both on fronds collected above high tide mark and on decaying thalli periodically submerged. Freshly collected fronds from the tidal zone, isolated and grown in moist stoppered vessels in the laboratory, soon develop the hoary fructifications of this species. Its mycelium may penetrate the substratum or form loose webs over the surface layers. It is regular and hyaline, bearing numerous erect, simple or rarely branched, short, tapering conidiophores which merge into the long chains of conidia. These consist frequently of hundreds of units and reach a length of several millimetres. While the shorter chains are borne aloft, the longer ones are stretched and supported either on the conidiophores of *Cladosporium*, when present, or on the erect portions of one another. They form an irregular silvery lace work over the surface. The conidia are hyaline, fusiform or pointed cylindrical bodies. The points of contact are slightly flattened as in Fig. 3, 2.

MONOSPORIUM MARITIMUM nov. sp. (Fig. 3, *3–5*).

Mycelium creeping; fertile hyphæ, erect, hyaline with tree-like branching, densely interwoven to form a hemispherical mass; conidia hyaline or cream-coloured, borne singly on tapering branches, elliptical forms 14–20μ × 6μ, oval 12 × 8μ.

Hab. Saprophytic on decaying seaweed. Dorset.
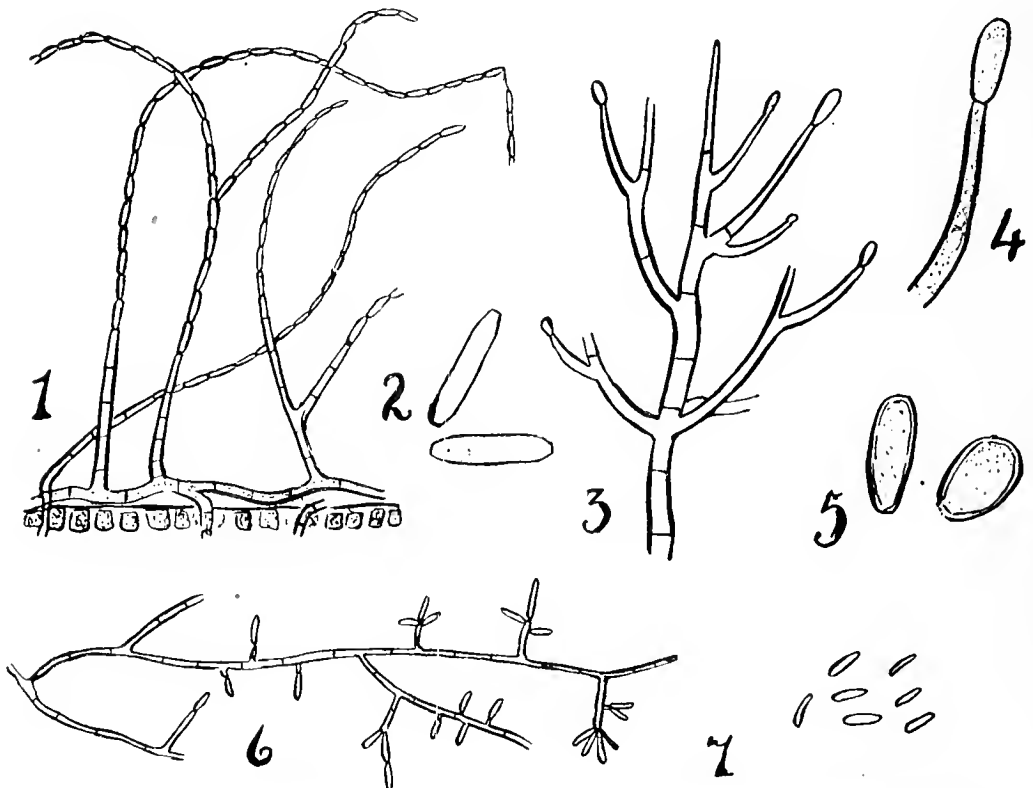
Fig. 3. *Fusidium maritimum* (1–2), *Monosporium maritimum* (3–5) and *Sporotrichum maritimum* (6–7) *1*, conidiophores and concatenate conidia of *Fusidium*; *2*, conidia; *3*, tip of branched conidiophore of *Monosporium*; *4*, branch with terminal conidium; *5*, types of conidia; *6*, mycelium and conidiophores of *Sporotrichum*; *7*, conidia.

The characteristic, irregular, pulvinate masses of this fungus are to be found wherever *Laminaria* fronds are decaying under fairly moist conditions. Like *Fusidium* it usually occurs along with *Cladosporium*. The mycelium is richly developed and forms a closely woven mass from which arise the erect much-branched fertile hyphæ (Fig. 3, *3*). The branching is varied, sometimes dichotomous, sometimes whorled. The conidiophores are densely packed together and taper upwards. At their tips are borne the single elliptical or oval conidia. These are smooth-walled, hyaline or cream-coloured. This genus is poorly represented in Britain.

SPOROTRICHUM MARITIMUM NOV. SP. (FIG. 3, 6–7).

Mycelium 1–1·5μ in diameter, procumbent, vaguely branched; conidia borne singly or in groups of two or three on short simple spinose conidiophores or sterigmata, continuous, elliptical, hyaline, 5 × 2μ.

Hab. *Laminaria* fronds. Dorset.

The creeping, delicate mycelium forms a loose web over the surface of the fronds. All over the hyphæ arise short, pointed, fertile branches bearing conidia (Fig. 3, 6). Frequently they arise on sterigma-like branches. This fungus may easily escape observation, as its mycelium ramifies along the surface at the base of the conidiophores of *Cladosporium* or mixed with the chains of *Fusidium*.

CERCOSPORA SALINA NOV. SP. (FIG. 4, 1–5).

Mycelium hyaline or slightly coloured; conidiophores simple or slightly branched with terminal or sub-terminal swellings from which arise the conidia; conidia varied, olive-green to brown in colour, short broad forms 30–45μ × 8–10μ and 3–5 septate, long types, 50–75μ × 6–9μ with 5–9 septa.

Hab. Saprophytic on various seaweeds along the coast of Aberdeen, Ayrshire, Dorset and Orkney.
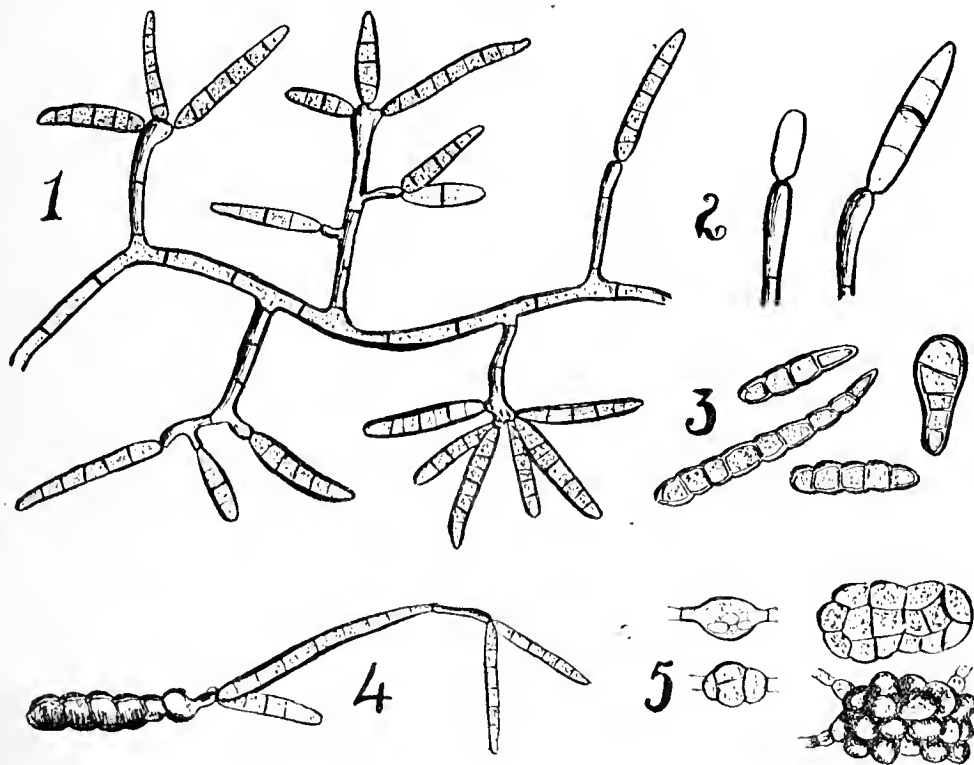
FIG. 4. *Cercospora salina.* 1, creeping mycelium with types of erect conidiophores; 2, developing conidia; 3, types of conidia; 4, germination of conidium showing immediate development of long, narrow type in salt water; 5, development of cell masses.

Cercospora plays very much the same part along the tidal zone that *Cladosporium* does above it. Like the latter it is widely distributed, and its conidia and mycelium may be found in almost any piece of decaying seaweed picked up from rock-pool or beach. Dried fronds of *Laminaria* showing *Cladosporium* usually also bear scattered conidia of this species. These are invariably of the short, stout, dark-coloured type and arise from a brown mycelium showing evidence of restricted growth. This would point to the adaptation of this saprophyte to thrive best in the zone either entirely covered with salt water or periodically submerged. Conidia and creeping superficial mycelium have been found on living plants. For example, they occur on blackened areas on actively growing specimens of *Furcellaria fastigiata*, but, owing to the presence of bacteria and also of a finer mycelium in the infected portions, it is uncertain which is the primary factor in attack.

The mycelium is developed in the sub-stratum, but creeping surface branches also abound. The hyphæ vary from 2–8μ in diameter and may be sparsely septate or consist of short, stout nodulose cells. This variability is even more pronounced when grown in cultures. While immersed portions are usually hyaline or faintly coloured, exposed hyphæ become light brown. The simple or branched conidiophores are erect or sub-erect, bearing conidia either singly at each tip or, by a later development of small swellings, aggregated in lax capitula of from three to five (Fig. 4, *1*). These arise as small buds and elongate rapidly, sometimes becoming uniseptate at an early stage (Fig. 4, *2*) and developing the other septa as they grow, sometimes attaining almost their full length before the appearance of any division. In the latter case the conidia are usually of the long narrow type. When the fungus is growing under dry conditions, as on the thalli strewn along the upper beach, the conidia are short, dark-coloured and slightly constricted. The same type is produced in cultures where the conidiophores are at all aerial, but, when there is abundance of moisture, the long, narrow multiseptate type is predominant (Fig. 4, *3* and *4*). The amount of moisture also affects the degree of colouration, both of the fertile branches and the conidia.

Cercospora salina responds to culture conditions and grows luxuriantly on agar extract of almost any seaweed. Naturally the conidia also germinate readily in salt water, but there the development of mycelium is very slight. If it fails to find a suitable sub-stratum it proceeds to form conidia of an extremely

long, attenuated type. These are nearly hyaline and borne at the end of short conidiophores tinged with light yellow. Frequently such conidia proceed directly to form other short fertile branches terminating in similar types (Fig. 4, *4*).

In many cases terminal or lateral swellings have been noticed on the mycelium. These increase in size and become divided by septa. In vigorously growing cultures, abundantly supplied with food material, some of these have been seen to form starting points for the development of fertile branches. They usually contain darker contents than the adjoining hyphæ. In a drop culture of salt water, to which a trace of lactic acid had been added, a stunted mycelium, devoid of conidia was produced. These swellings, however, appeared and, increasing in size, became divided by numerous septa to form hyaline cell masses which in their early stages, produced one or two hyphæ, but ultimately became darker, and, owing to the bulging of the cells, assumed the form of a small grape bunch. This development is shown in Fig. 4, *5*. These peculiar masses are still under observation. So far no attempt has been made to produce the conidia normally formed. It may be that they serve very much the same purpose as chlamydospores. The addition of lactic acid does not explain them, as similar drop cultures with the same quantity of lactic acid have produced normal conidia.

When grown on dry material above tide mark, the dark, stout thick-walled conidia might be regarded as belonging to *Helmintho-sporium*, but the type and habit of conidiophore, as well as the arrangement of conidia is distinctly that of *Cercospora*.

MACROSPORIUM LAMINARIANUM nov. sp. (Fig. 5, *1–4*).

Mycelium immersed in sub-stratum, with few creeping surface branches; fertile hyphæ erect, simple, flexuous, sometimes nodulose terminating in swollen tips; conidia large, oblong or broadly ovate, reddish-brown, verrucose, 35–70μ × 16–25μ.

Hab. Saprophytic on fronds of *Laminaria*, Dorset and Orkney.

Irregular spots both on dry and moist fronds are sparsely covered with this fungus, whose mycelium is very large, sometimes reaching a diameter of 15μ, hyaline when immersed, or reddish-brown on the surface. The fertile hyphæ are simple, rarely branched, more or less erect, smooth or nodulose, terminating in head-like swellings of a bright colour (Fig. 5, *1*). The thickening of the walls of the swelling and of the remainder of the apical cell is

interesting ; only that portion in contact with the conidium remains thin (Fig. 5, *3*). Continuation of growth may take place as in Fig. 5, *4*.

The conidia are borne singly and each possesses at the basal end a distinct dark-coloured scar of attachment or hilum. They vary from a short oval type, 35 × 25μ, to a long oblong form, 70 × 16μ (Fig. 5, *2*). At maturity their walls are thick, reddish-brown and verrucose. Altogether the conidia are easily distinguishable from the similarly muriform and verrucose ones of a species of *Alternaria*, along with which they frequently occur. Nor can they be confused with those of *Macrosporium Pelvetiæ*.

ALTERNARIA MARITIMA nov. sp. (Fig. 5, 5-7).

Mycelium diffused ; conidiophores erect, simple, rarely slightly branched, brown ; conidia in simple or branched chains ; pyriform with neck-like apex, dark brown, verrucose, 30-50μ × 12-18μ.

Hab. Saprophyte on *Laminaria* fronds, Ayrshire, Dorset, Orkney.
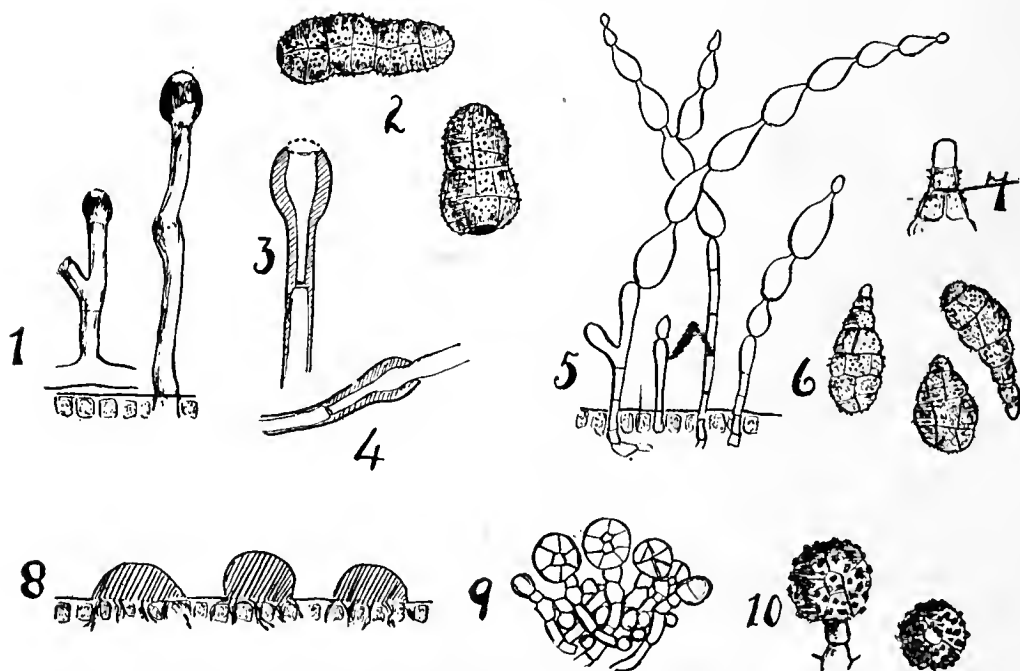
Fig. 5   *Macrosporium laminarianum* (1-4), *Alternaria maritima* (5-7) and *Epicoccum maritimum* (8-10), *1*, conidiophores of *Macrosporium* ; *2*, conidia with scars of attachment ; *3*, terminal cell of conidiophore, showing type of thickening ; *4*, renewed growth of conidiophore ; *5*, conidiophores of *Alternaria* with simple and branched chains ; *6*, types of conidia ; *7*, neck showing smooth cell ; *8*, diagrammatic section showing sporodochia of *Epicoccum* ; *9*, group of conidia ; *10*, conidia, one with pedicel, other showing point of attachment.

While examining bleached and dried fronds of *Laminaria*, collected in the Orkneys in 1914, muriform conidia of the characteristic *Macrosporium* type were noted. These were always

free upon the surface and, while mixed with the conidia of *Cladosporium*, for which the material had been collected, were invariably associated with a distinctive type of conidiophore. The difficulty of obtaining them attached in sections, added to their occurrence with other fungi, rendered cultures imperative. Agar extract of *Laminaria* proved most successful. The fungus was separated and fruited readily, forming the chains of conidia which distinguish the genus *Alternaria* from *Macrosporium*.

Similar conidia have since been found on the surface of freshly collected seaweed from the tidal zone, but as no mycelium could be found, there seems little doubt that this species is a saprophyte confined mainly to the zone above tide mark.

The mycelium, which varies from 2–8μ in diameter, is diffused and hyaline when immersed in the tissue ; aerial branches are brown. The same applies to mycelium grown in cultures. Erect conidiophores spring from the surface, ending in the slightly swollen tips shown in Fig. 5, *5*. On the fronds exposed to frequent periods of drought, these are short and simple, but, under the more even conditions experienced in cultures, they are usually longer and may be branched, forming a dense olive-green or brown felt over the surface. The conidia, grouped in chains, differ much, both in size and shape. At first olive-green, they become dark brown with the formation of a thick warty coat. The number of transverse and longitudinal septa varies considerably as well as the length of the neck-like apex, by which the conidia are attached to one another (Fig. 5, *6*). The cell or cells forming the latter are frequently smooth-walled (Fig. 5, *7*). This probably happens in the case where one conidium is well developed before the next in succession appears. Then the smooth neck cell is budded off before the formation of the next conidium.

The chains may be either simple or branched, in the latter case, the side chains arising either from neck cells or by the budding of any body cell on the side of a conidium. Usually they arise in succession and show successive stages of development, but frequently a number of almost equal small conidia are budded off quickly, and then increase in size. In this case the greatest development is not always found at the base of the chain (Fig. 5, *5*).

EPICOCCUM MARITIMUM NOV. SP. (Fig. 5, *8–10*).

Mycelium localised ; sporodochium hemispherical or pulvinate, aggregated on pink or reddish spots ; fertile branches short ; conidia globose, reddish-brown, roughly verrucose, 17–20μ in diameter.

Hab. Saprophytic on *Laminaria* fronds. Dorset and Orkney.

This species occurs on more or less regular patches of a pink colour on fronds cast up by the tide, being readily distinguished by the characteristic spots which stand out in marked contrast to the dark masses of *Cladosporium* on the same fronds. It has also developed on freshly collected material kept isolated in the laboratory, so that it seems capable of living also in the tidal zone but no trace, apart from scattered conidia, has been found on living fronds, so that it may be regarded as saprophytic.

The young hyaline or older pink mycelium is localised, and upon the irregular discoloured patches, which it forms, are borne the semi-globose or pulvinate, closely aggregated sporodochia (Fig. 5, *8*). The conidia possess short pedicels rarely exceeding 10μ (Fig. 5, *9*, *10*). They are globose structures, irregularly septate, covered with a rough verrucose, reddish-brown coat except where attached to the pedicel.

The variety of these species, representative of different subdivisions of Fungi Imperfecti, would point to the existence of a fairly extensive flora. But their main interest does not lie so much in their numbers as in their halophytic adaptations, in the rôle they play along the tidal zone, and in their connections, if any, with some of the Pyrenomycetes already known.

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THE VEGETATIVE ANATOMY OF *MOLINIA CÆRULEA*, THE PURPLE HEATH GRASS.

By Rev. T. A. Jefferies, F.L.S.

[With Nine Figures in the Text].

THE following account of the structure of *Molinia cærulea* results from a study of the grass carried out at the suggestion and under the guidance of Dr. T. W. Woodhead of the Huddersfield Technical College. The inquiry has proved most fruitful, especially from the ecological point of view, and an outline of the main results in that direction has already been published (2). The anatomy of the species, however, is only slightly less interesting than the ecology and shows important relations to it. Hence it has been deemed advisable to make the following details available as soon as possible.

The Purple Heath Grass (Fig. 1) is a perennial, growing in wet but well-drained situations in the fens and on the moors. It dominates miles of the Pennine uplands on siliceous soils, frequently to the exclusion over large areas of all other flowering plants. Where the drainage becomes blocked it yields place to such species as *Eriophorum vaginatum* and *Empetrum nigrum* ; where the water supply weakens the ground is held by *Nardus stricta*, *Calluna vulgaris*, etc. Typically *Molinia* forms circular tussocks from 8 to 20 cm. in diameter at the base, and raised to a similar height above the soil ; tussocks may be found, however, as much as 75 cm. across. The rhizomes are closely interlaced at, or a little above, the level of the soil, and below these we have the tangled mass of adventitious roots. The flat, narrow leaves generally stand up boldly and almost perpendicularly from 20 to 40 cm. above the rhizomes. Above the leaves, throughout the latter half of the year and often persisting through the next season, rise the inflorescences, their close-packed panicles looking like spikes, and upheld on tall
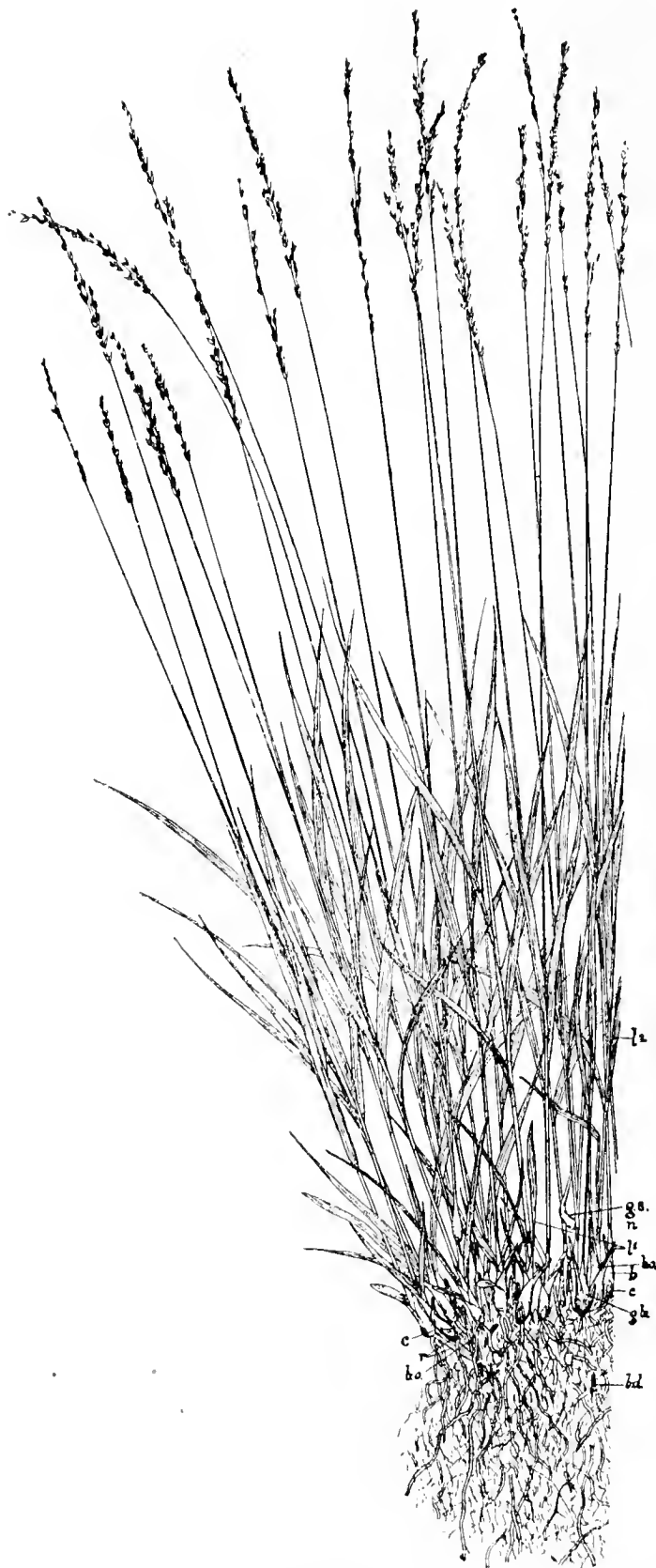
FIG. 1.   Half of a longitudinal section of a *Molinia* tussock.   Lettering :—
b., tuberous " basal " internode ; *b.d.*, decaying basal internode ; *b.o.*, old
basal internode with food reserve withdrawn, showing absciss layer scars ;
c., bud ; *g.b.*, galled base, basal internode not developed ; *g.s.*, galled and
distorted stem ; *l.¹*, first series of leaves ; *l.²*, second series of leaves ; *n.*, the
single " joint," nodes of second series of leaves ; *r.*, elongated rhizomes.

wiry haulms usually from 45 to 75 cm. long.   The flowers and frequently the leaf tips are deep purple, a feature reflected in the specific and common names of the plant.

I.   Root.

If the attempt be made to pull up a *Molinia* tussock it will almost certainly fail, because of the remarkable hold on the soil which the plant possesses through its enormous root system.   This forms an almost solid, tangled mass in the upper layer of the soil and penetrates to a great depth, spreading outwards slightly as it descends.   There are two types of root : cord roots and fibrous roots.   All the roots springing from the rhizomes are cord roots : these are about 1·5 to 2 mm. in diameter and descend from 15 to 45 cm. ; they retain their thickness throughout most of their length, tapering only very slightly until within 3 mm. of the tip, when they thin off rapidly and terminate in a blunt root-cap; they are so numerous that close to the rhizome they squeeze one another out of shape, and they are of great strength.   Cord roots do not usually give rise to branch cord roots : all their branches are fine, branched, fibrous roots.   These fibrous roots are given off sparingly all the way down from close to the soil surface to within two or three cm. of the cord root ends, but in the last two or three cm. they arise very freely.   The fibrous roots are of 0·3 to 0·8 mm. diameter and 5 to 13 cm. long, and they branch freely in all directions.
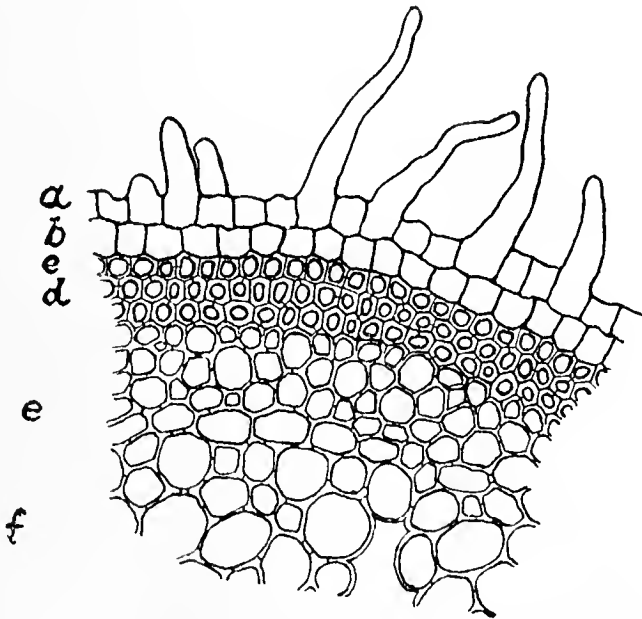
Fig. 2.   Cord root of *Molinia*: transverse section of piliferous layer, supporting layer, band of sclerenchyma, and outer zone of cortical parenchyma whose walls are not fully thickened ; mycorhiza not shown.   For details see Text.   × 100.

In transverse section the cord root shows on the outside (Fig. 2) a piliferous layer (*a*) of medium sized, thin walled, square cells with no cuticle and with a length in longitudinal section of four to eight times their diameter. Some of these cells bear root hairs which are abundant, from 100 to 350 microns long, and easily visible on the cord roots with a pocket lens ; at the base the hairs have a diameter nearly equal to that of the cell. Frequently the cells of this layer show the presence of mycorhiza, the mycelium being visible in the cells and on their surface, and occasionally penetrating to the cells of the second row. The piliferous layer is supported by a layer (*b*), occasionally two layers, of similar cells. Sections frequently show at certain points a breakdown of the superficial cells and mycorhiza in the cells of the second layer, which thus replace the damaged cells of the outer layer. In Fig. 2 the cell at *a* shows the beginning of this collapse, but the section is from a young root, as appears from the comparatively thin walled condition of the cortical parenchyma, hence the healthy state of the piliferous layer. Next we have a band of strengthening tissue (*c*, *d*) composed of several rows, generally three, of narrow sclerenchymatous fibres, having their walls pierced by a few fine pits and thickened at first with cellulose but afterwards becoming lignified. The outer layer (*c*) of this band of sclerenchyma is intermediate in structure : the cavities are slightly larger, and the walls thinner, than in the deeper layers, while the outer wall, being thickened on one side only, is considerably thinner. The fibres have a length of about 800μ, roughly three times the length of the neighbouring elements. Inside this strengthening zone we have the parenchymatous tissue of the cortex which occupies the greater part of the entire section and is divisible into three zones. The outer zone (*e*) is composed of cells irregularly arranged, of varying size and shape, the size tending to increase rapidly as we move inwards from the sclerenchyma. In the middle zone, the margin of which appears at *f*, the cells are practically circular and gradually assume an arrangement into radial rows. These rows, or groups of them, are separated by large air spaces, which provide for the aeration of the root in its wet habitat, are of schizogenic origin, and die out at the inner margin of this zone where the cells occupy the whole of the reduced circumference. The inner zone of the cortex shows a very perfect radial arrangement of the cells (see Fig. 3, *S*), the rows being closely applied one to another, and the individual cells becoming smaller and more rectangular as the endodermis is

approached.   The last of the extrastelar tissues, the endodermis, is
of remarkable strength and prominence (Fig. 3, *E*) : the cells in
transverse section are as deep as they are broad, and each is the
first of one of the radial rows of cortical cells; the outer wall is
thin and straight; the inner wall is almost semi-circular, often
thickened in the middle to three quarters of the depth of the entire
cell, lignified, and pierced by a series of fine pits which apparently
make provision for the transmission of solutions between the cortex
and the vascular tissues within ; the middle lamellæ are clearly
defined especially on the inner walls.   There are no cells in the
endodermis which can be picked out by their structure as special
passage cells : probably by virtue of the pits just described all the
endodermal cells are of that nature.
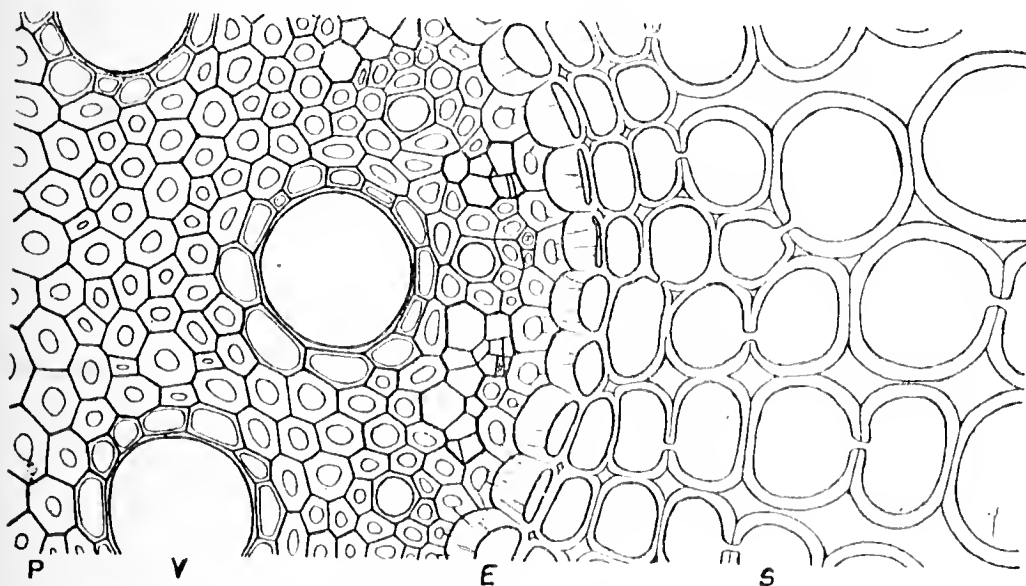
FIG. 3.   Cord root of *Molinia* :   transverse section   of endodermis and
neighbouring tissues.   Lettering :—E, endodermis showing pits connecting
with pericycle cells ; P, sclerenchymatous pith ; S, cortical storage tissue,
inner zone ; V, large vessel ; x, protoxylem ; p, protophloem.   × 300.

The stele (see Fig. 3) is enclosed in a pericycle of small, very
thick walled, pitted cells, forming a ring made irregular by the
convex surfaces of the endodermal cells to which they are applied.
Within the pericycle we have the polyarch ring, usual in mono-
cotyledons, of alternating phloem and xylem bundles, generally
numbering from twelve to twenty-four pairs and usually some
multiple of three.   The phloem groups are relatively small.   On
the outside of them and adjacent to the pericycle is the protophloem
(Fig. 3, *p*), the cells being smaller than the later developed elements
and frequently compressed.   The phloem consists of thin walled
sieve tubes and companion cells arranged in groups of very irregular

and varied outline. The groups of xylem have their protoxylem adjacent to the pericycle and large vessels to the inside. The protoxylem (x) is composed of narrow annular and spiral vessels. The xylem consists of tracheides, large vessels, and xylem parenchyma. Both annular and pitted tracheides are found, their ends tend to be blunter than usual, and they are very thick walled ; this tissue either in transverse or longitudinal section is easily confused with the sclerenchymatous ground tissue of the pith which in general appearance it closely resembles. The vessels (Fig. 3, V) are exceedingly large, their size being all the more striking because they are distributed singly, not more than one to a xylem group ; they are almost circular in outline, with abundant conspicuous pits, and each is surrounded by a layer of flat parenchymatous cells. Usually these vessels are developed only on the alternate xylem groups, the other groups of xylem remaining inconspicuous through the absence of the large vessel and the resemblance between the smaller wood elements and the ground tissue : but occasionally two neighbouring xylem groups converge on one vessel, forming a V section with a phloem group between the arms ; and sometimes two neighbouring groups have each a vessel and these two vessels become laterally apposed, when the circular outline of the single vessel is replaced by one roughly elliptical, including the two vessels, and these are separated by a flat partition of parenchyma, one cell thick, along the minor axis. These large vessels form a well marked zone around the pith. The xylem parenchyma, surrounding the vessels and scattered among the tracheides, has its walls slightly thickened, although in comparison with the other elements of the stele, the phloem excepted, it is thin walled ; in transverse section some of these cells around the large vessels are of considerable size. The whole of the central portion of the stele within the ring of large vessels is occupied by a sclerenchymatous pith (Fig. 3, P). This tissue is composed of exceedingly thick walled, long, sclerenchyma fibres, with fine pits and with ends rather bluntly pointed for such a tissue and sometimes flat ; towards the centre these elements increase in sectional area but there is no thinning of their walls : towards the margin the same tissue spreads out between the large vessels and occupies the regions between the vascular bundles ; the middle lamellæ are very prominent and give rise to strongly marked polygonal outlines as show in the figure. From the above description it will be realised that very thick lignified walls are a pronounced feature of the stele ; indeed it is chiefly to this

almost solid central cylinder, whose diameter is about one fourth
of the whole section, that the cord roots owe the remarkable
tensile strength which makes the plant so difficult to tear from
the soil.

Sections of the cord roots generally show the cortical cells
(Fig. 3, S) to have thick walls, prominent pits, and in the outer and
middle zones circular outlines, this rounding off of the angles being
associated with an increase in the number and size of the inter-
cellular spaces; the same cells are also usually crowded with small
starch grains. The wall thickening consists of cellulose, and both
this and the starch are shown by their subsequent history to be
forms of food reserve. The roots commonly function through three
seasons. Examination of a specimen towards the end of its third
summer, when the member was about two and a half years old,
showed the process of digestion already commenced : 15 cm. from
the rhizome the exterior was wrinkled and about one third of the
tissue in question, with its contents, had disappeared ; 2 cm. from
the rhizome digestion had begun but had made little progress. The
food reserve thus remains in the cortex of the root until the latter
ceases to function, when it is withdrawn. Young cord roots show
this cortical thickening, so that the reserve is deposited early.
During the active life of the root, the cortex with its abundant inter-
cellular spaces, probably functions as an aerating tissue. Roots
springing from shoots, where the development of the usual food
reserve in the stem has been interfered with by the galls referred
to later, are often unusually thick. From the old dead roots the
thickened tissue and starch grains have disappeared, leaving the
central cylinder surrounded by the endodermis and a loose detached
sheath, the sclerenchymatous band, with a few remnants of
incompletely digested cells (compare the tuberous internodes of the
leafy stem).

The structure of the fine roots shows in transverse section the
same general plan as that of the cord roots, but adapted to its
smaller dimensions. Again we find no cuticle, and a second row of
good sized, thin walled cells supports the piliferous layer. Root
hairs on these fine roots are rare and become common only on the
thicker and older portions : the usual root hair region is absent, and
the non-appearance of a cuticle suggests that the rootlets them-
selves function as absorbing organs. Thus *Molinia* shows a
reversal of the usual distribution of root hairs; they are absent
from the youngest rootlets, appear first as we move into the

mature region of the root system, and are abundant on the thick cord roots right up to the surface of the soil. Exceptions are sometimes found in the natural damp-air chambers, which occur frequently in tussocks growing up out of streams and runnels, and in which a thick fur of hairs is induced on all the roots traversing the chamber, whether thick or fine. The strengthening zone is generally represented in the fine roots by a slight thickening and lignification of the somewhat smaller cells of the third layer, especially on their outer and lateral walls : frequently there is no sign of a sclerenchymatous band. The cortical parenchyma comes next, from two to five layers deep according to the development of the member. The endodermis is of the same character as in the cord roots but the inner walls of the cells are not thickened to anything like the same extent : a well developed fine root showed fifteen cells in this ring. The central cylinder contains very small groups of bast, frequently five in number, and a relatively large amount of woody tissue : sometimes there is a single large vessel in the middle, and occasionally two close together, but frequently none at all. The stele of the fine roots is not so strongly developed, proportionately, as that of the cord roots. In longitudinal section one notices the abundant branching—two or three branches may appear at once in the field of a 4 mm. objective—which contrasts markedly with the scanty branching of the cord roots.

Before leaving the root, reference may be made to the frequent presence therein of chlorophyll. A tussock growing in a peat hag overhånging a stream had many of its roots exposed through the washing away of the peat, and some of its cord roots were green through the presence of chlorophyll, the chloroplasts being massed in the outer region of the cortex and chiefly on the exposed side. The same feature is common in the long cord roots near the sides of tussocks which have grown high up out of the soil, and which thereby expose their roots to light. It thus appears that the roots of *Molinia*, when accidentally exposed, can take on the work of carbon-assimilation, an illustration of the powers of adaptation which this species manifests in many directions.

II. Rhizome.

The rhizome of *Molinia* is reduced to a minimum : the shoots are packed together, and the rhizome is merely the necessary link for communication between them, and also between these shoots and the roots. The shoots when mature swell considerably just

above the rhizome and the slight spaces below are occupied by the cord roots, etc., the rhizome being everywhere crowded around and compressed by the buds, aerial shoots, and cord roots springing from it. The rhizome is a sympodium, each segment of which turns upwards into a leafy shoot and terminates as an inflorescence axis. Growth of the rhizome is continued by lateral buds which arise in the axils of scale leaves. The phyllotaxy throughout the species is $\frac{1}{2}$, but in the crowding that characterises the rhizome, it is generally obscured through displacement of members, with the result that very commonly buds are found on three sides. These buds may arise above the rhizome, below, or laterally, and they develop into short rhizomes and leafy stems which come off in all directions : there is nothing in *Molinia* like the straight ranks of shoots characteristic of *Nardus stricta*. The cord roots spring from the same nodes as the buds. Normally the rhizomes grow horizontally, slightly above the soil, but well covered by their crowded offshoots ; in some environments, however, they are uplifted on a mass of cord roots to a height of 20 cm. above the surrounding surface.
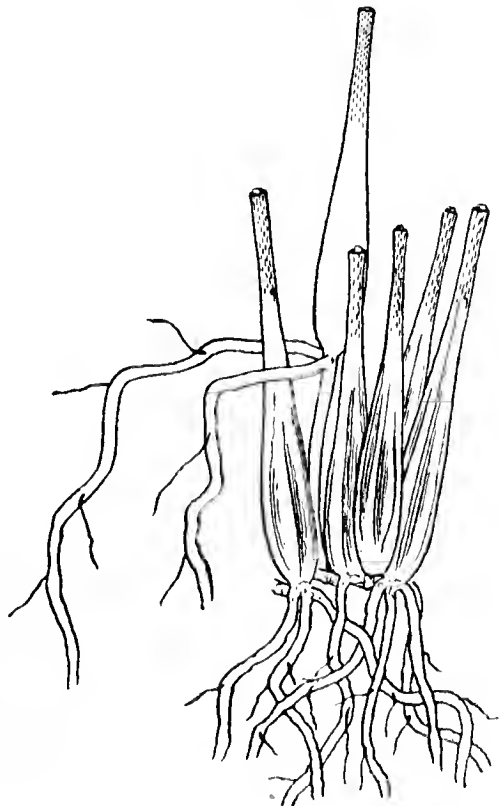
FIG. 4.   Elongation of rhizome and raising of root base.   The lower stems are old and have lost their food reserve.   Leaves removed.   Nat. size.

That the rhizome, despite its usually shortened form, retains the power to lengthen its internodes when necessary was shown by

the tussock whose roots were found to contain chlorophyll (see above, p. 56). The disappearance of the soil from the exposed side left the plant with more moisture in the humus collected in the body of the tussock itself than in the root region below, with the result that the new outer roots were found to be growing horizontally towards the centre of the plant and away from light and air (see Fig. 4). Several of the rhizomes had elongated an internode and grown upwards past the thickest part of the swellings on the shoots, so that the roots came off more than a cm., in one case over 2 cm. higher than the preceding ones and at a level where the shoots were sufficiently loosened to make way for the roots. In order to achieve this upward movement of the rhizomes, internodes were extended ten to twenty times their usual length, and the level at which the roots started was thereby permanently altered. Other cases recently investigated show that such elongation is by no means rare, but is to be regarded as the normal method of upbuilding when it is necessary for the tussock to ascend. It occurs where plants have been partly buried with ash after burning of the moors, and tussocks developing in a flush show the elongation repeated year after year, the roots coming off at successively higher levels, till the plant is lifted well out of the water (see Fig 1 *r, b.o.,* and *b.d.*)

Sections of the rhizome, as might be expected from what has been said of their macroscopic form, generally show a confused tangle of vascular bundles crossing the section in all directions. The structure is simplified, however, where elongation has taken place, and is found to consist of the following : the surface is protected by a small celled cutinised epidermis, stiffened by a band of sclerenchyma two or three cells deep ; parenchyma follows varying in depth from five to ten medium sized, irregularly arranged cells, not rows of cells as in the cortex of the cord roots ; the endodermis and pericycle are of small cells, within which lies a ring of vascular bundles whose xylem elements are abundant and thick walled ; the middle is occupied by a pith similar in appearance to the cortex. Usually the cells of the cortex and the pith are crowded with small starch grains, and this supply of reserve food is occasionally increased, as in the cord roots, by thickening of the cellulose walls of the starch containing cells.

III. Erect Aerial Stem.

The leafy stem of *Molinia* (see Fig. 1) is normally straight, thin except at the base, erect, glabrous and long, rising as already

mentioned, to a height of 45 to 75 cm.   It bears only two series of leaves: the lower given off immediately above the rhizome, the upper about 5 cm. higher up the stem (l^1, l^2); the leaves in each series spring from points so nearly at a level that they look like whorls.   Since the lower series is right at the beginning of the shoot the stem is said to have a single "joint" (n).   The stem divides naturally into three sections: first, the section between the two series of leaves, conveniently though inaccurately called the basal internode (b); second, the thin but very tough inflorescence stalk; and third, the axis of the inflorescence itself.

The basal internode, when mature, has a form closely resembling an Indian club (Fig. 4).   A healthy specimen about 5 cm. long is most typical and will serve best for purposes of description: it must be understood, however, that many are squeezed out of shape by overcrowding, and that all sizes are found from those less than 1 cm. high in young or dwarf plants to others approaching 10 cm. in tall, woodland forms.   The typical basal internode, then, is a swollen structure, rapidly expanding as it rises from the first node to a diameter of 5 mm., slowly tapering to 2 mm. or even less in the handle of the club, and then quickly expanding once more to form the slight ridge from which springs the second series of leaves.   The upper and thinner third of this internode is green; the lower swollen two-thirds is white.   The surface is smooth, shiny, and firm; the whole organ when pinched by the fingers is found to be quite hard.   One side is generally flattened by having been pressed in growth against the parent basal inter-node, which now stands, a withering hollow shell, close beside.   At the base will be found two, often three and sometimes only one, buds, ready for development at the coming of spring; and around these the decaying leaves of the first series, always dead by the time the basal internode is fully developed.

A transverse section across the thick part of the basal inter-node (Fig. 5, *A*) reveals the fact that we have here a well constructed and tightly packed organ for food storage.   The outer wall of the internode consists of a cutinised epidermis of small cells, strengthened by small celled, thick walled, mechanical tissue about three cells deep.   Within this lies the rest of the cortex composed of medium sized cells, about ten cells deep, having their walls thickened with cellulose and their cavities crowded with granules of food reserve.   This tissue possesses abundant schizogenic lacunæ of sizes varying up to that of the cells them-

selves, and frequent larger lacunæ, shown in the figures, where rupture of cells has taken place. The endodermis is not conspicuous, but the pericycle of small compact cells is clearly distinguished from it. Within the pericycle, and with it comprising an inner strengthening layer, we get a strong ring of schlerenchyma. Embedded in this mechanical tissue is a series of vascular bundles having abundant, thick walled, woody elements: the bundles are of
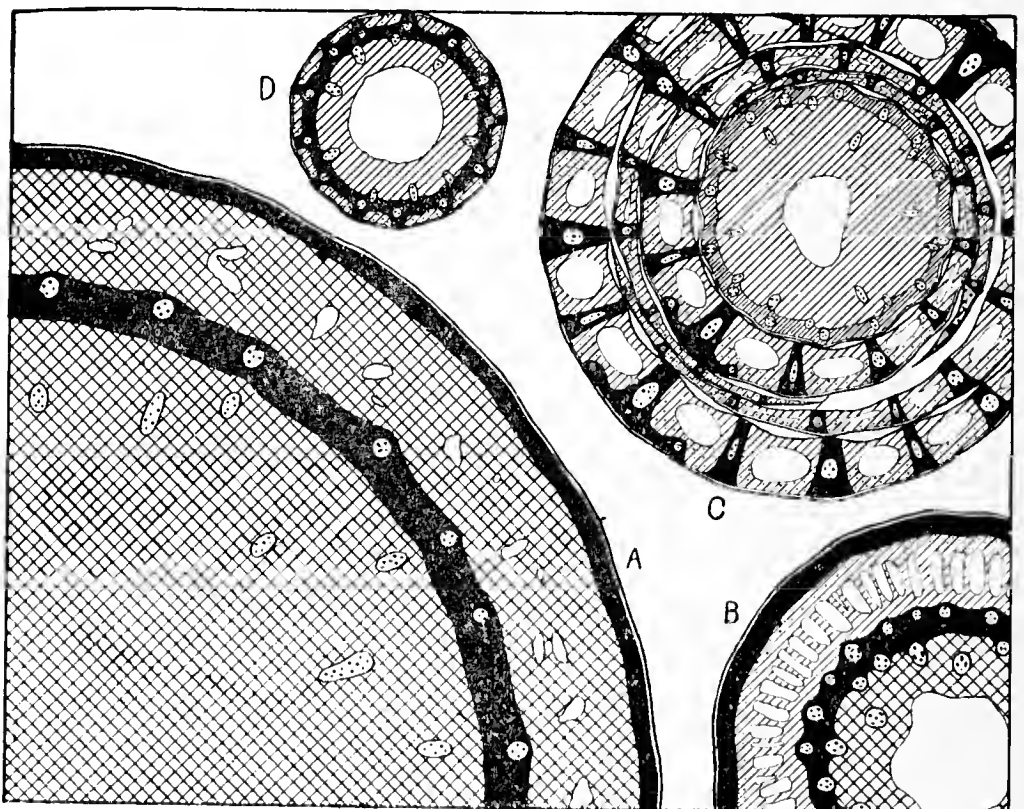
TISSUES: ■ Mechanical Strengthening; ⊠ Food Reserve; ▨ Soft Tissues; General, Assimilating, & Small Celled, ◉ Vascular Bundles. ▽ Lacunæ.

FIG. 5. Transverse sections of erect aerial stem, diagrammatic: A, through thick part of basal internode; B, through thin part of same; C, through portion enclosed by leaf sheaths; D, a little below inflorescence. × 25.

the usual monocotyledonous type, and many more are scattered inside this inner strengthening layer in an irregular double ring; there are none in the centre of the stem. All the rest of the large central cylinder, the spaces between the scattered bundles and the whole of the centre is occupied by ground tissue specially modified to act as storage tissue. The cells comprising this storage tissue (see Fig. 6) are largest towards the centre of the stem. Their walls are enormously thickened, the middle lamellæ being traceable in most cases through the middle of the walls, especially in the

neighbourhood of the vascular bundles.   In the angles between the cells exceedingly small intersticial spaces occur, and layering can occasionally be detected in the wall sections.   These walls are pierced by numerous pits (Fig. 6, *P*), which open gradually into the cell cavities, forming funnel shaped entrances.   Special tests by Gardiner's method demonstrated the fact of intercellular protoplasmic connection through these pits and the pit membranes.   On this account the basal internodes provide splendid examples of continuity of protoplasm, and they have the advantages of being compact, abundant, and capable of being kept a long time without pickling.   Tests with iodine solution, sulphuric acid, and double
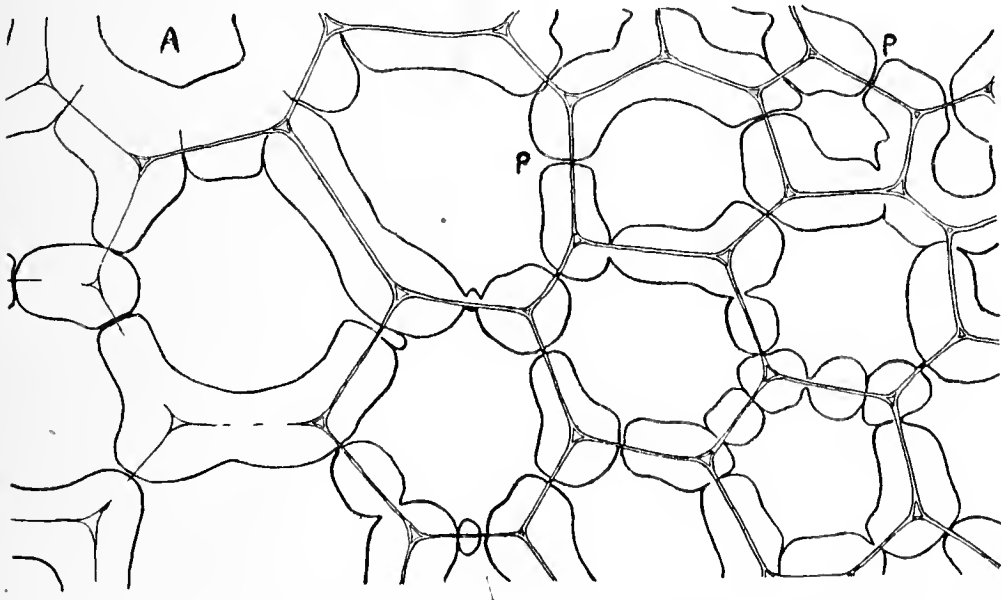
FIG. 6.   Storage tissue of basal internode, transverse section.   Left, near centre of stem ; right, approaching a vascular bundle ; at A, the cell wall has been cut obliquely, hence abnormal thickening ; continuity of protoplasm is indicated in some of the pits, as at P.   x 500.

staining with safranin and hæmatoxylin (Delafield's) showed the walls to consist of cellulose.   In addition to these thickened walls the cavities of the cells are packed with granules.   Tested with iodine the majority of these showed a clear starch reaction, but many remained suspiciously irresponsive to this test; they showed a decided light brown colour which suggested that some of the grains might be proteins, and further tests with Millon's reagent proved this to be the case.   In these swollen basal internodes we have therefore both starch and protein grains; no attempt was made to decide their proportions but the two kinds of grain are estimated to be present in the ratio three of starch to one of protein.   This is the only part of the plant in which protein granules have been observed : they appear to be absent from the food storage in rhizome and roots.

Successive sections show that the structure described, persists throughout the swollen part of the basal internode. When, however, the tapering becomes marked a transition sets in, and develops as we get higher up the internode until in the narrow green region (Fig. 5, *B*) we have the typical structure of a grass stem. The first change appears in the cortex where the cortical storage tissue is gradually broken up by a series of large lacunæ, until in the upper part we have an interrupted cortex consisting of radial strands of thin walled cells, generally in single rows but occasionally in groups, crossing a large air space. A corresponding change occurs in the centre where a space appears and gradually extends, while the wall thickening of the storage cells becomes less marked; but this does not begin as low down as the large lacunæ of the cortex. Finally we have the appearance of chlorophyll in the cortical cells adjacent to the subepidermal strengthening tissue, indicating photosynthetic activity in this region and explaining the greenness of this part of the stem.

The food stored in these basal internodes is not used during the summer fruiting season but remains throughout the winter; as soon, however, as the buds at their bases develop in the following spring they are found to be losing their contents: eventually nothing is left but the insoluble tissues forming the framework of the inter-node. These remains (Fig. 1, *b.o.*) consist of the outer wall of epidermis and sclerenchyma, the inner wall of pericycle, scler-enchyma, and vascular bundles, the scattered bundles, and a fringe of shrunken, dilapidated cell walls—all that remains of the dense storage tissue. In marked contrast with the functional swollen bases these empty shells collapse like straw between the fingers. The process of digestion by which these food reserves are used up is gradual, and on the same tussock we may find all stages of the process indicated by varying degrees of firmness, these differences being related to the varying stages of development reached by the subsidiary buds. For details of the actual break-down of the cells through digestion, see Woodhead (5).

Since these swollen bases have to endure throughout the winter when the leaves lie dead, it becomes important to protect them from dangers which might enter through decaying elements, and this is secured by means of absciss layers. Now absciss layers are usually associated with leaf-fall, a phenomenon which is some-what rare in herbaceous plants. Hence absciss layers are uncommmon in such types, and in grasses especially few have been described:

Ward, for instance, in his handbook on grasses (4) has no reference to
the subject.　In *Molinia*, however, absciss layers are well developed,
being found at the base of every leaf in both series.　By this
means the basal internode, which stands between the two series, is
protected both below and above from bacteria and other dangers
associated with decay.　Similar protection for the bulbs of *Scilla
non-scripta* has been described by Woodhead (6), where further
references to absciss layers in monocotyledonous plants will be
found.　These layers explain the fact that the dead leaves of *Molinia*,
when plucked, come away in handfuls without any effort, a feature
which is all the more striking when we remember the general
toughness of the plant, including the leaves; and it is true of
functional leaves late in the season as well as dead ones.　The
resulting leaf scars give a characteristic form to the tops of old
and decaying basal internodes (Fig. 1, *b.o.*, *b.d.*, and Fig. 4).
It should be added that the absciss layers of the upper series do
not extend across the inflorescence stalk, presumably because, if
they did, it would weaken a member which has to remain standing
throughout the winter, to give the fruits every opportunity of being
scattered.

A point closely related to the foregoing is that when the leaves
wither and fall the upper parts of the basal internodes retain their
greenness and continue to function until the buds put out their
leaves in the next season.　It thus appears that photosynthesis
goes on throughout the winter, and that *Molinia* despite its seasonal
foliage is to this extent an evergreen.　These active assimilating
organs are usually well protected from winds, etc. by the long dead
leaves, while plenty of light gets in to carry on the small amount of
assimilation possible in the one to two cm. of stem possessed of
chlorophyll.　Small as it may be, this feature is important and
suggests a comparison of *Molinia* with *Vaccinium Myrtillus*,
another heath plant which, while shedding its leaves, carries on the
work of photosynthesis by means of its green, winged stems.

We pass now beyond the " joint " to consider the structure of
the inflorescence stalk, whose length, thinness, and strength we
have already mentioned.　A short distance above the node, during
the summer, the transverse section is very simple (see Fig. 5, middle
of *C*): its outline is wavy or crisped ; a slight epidermis surrounds
a band of tissue, composed of small cells but not otherwise adapted
for strengthening purposes; within this ring the parenchymatous

ground tissue stretches across the whole space, the cells becoming larger as the middle of the stem is approached, a few breaking down altogether in the centre ; the vascular bundles are scattered in two irregular rings towards the margin, the outer ring lying in the band of small cells. At the other end of the stalk, just below the inflorescence, the section is considerably modified to secure greater rigidity (Fig. 5, *D*) : the vascular bundles are situated as before, but here a strong band of mechanical strengthening tissue appears, forming an unbroken cylinder deep enough to include the outer ring of bundles and the outer halves of the inner ring ; this cylinder is further stiffened by stereome girders passing to the epidermis from the bundles of the outer ring (compare the girdered bundles of the leaves), so that the cylinder has the section of an Ionic column ; a strong cutinised epidermis further increases its rigidity ; the spaces corresponding to the flutings of the column are occupied by small celled assimilating tissue, to which air is admitted through abundant stomata ; the centre is quite hollow for about half the diameter of the section. Compared with this region, that part which stands immediately above the "joint" is exceedingly weak : this seeming anomaly, however, is adundantly compensated by the sheaths of the upper series of leaves (Fig, 5, *C*), which are freely supplied with strong girdered bundles and completely enfold the weak part of the stem, usually three deep. Thus the apparent weakness is really an instance of the careful husbanding of resources, and of the disposal of strengthening tissues in a larger circumference where they will have greater efficiency as lateral supports. The strengthening zone appears in the stem itself as soon as we approach the top of the outer leaf-sheath, and reaches its full development before the topmost blade bends away from the stalk. Later in the season this mechanical tissue is developed in the weak part of the stem also so that, when the leaves decay, the stem can maintain its erect position without their support.

The main facts of the stem structure are summarised in Fig. 5, which gives a series of diagrammatic sections. They show especially the varying amounts and disposition of the mechanical tissues at different levels. A, is taken through the thickest part of the basal internode ; B, through its narrowest part, a little below the "joint" ; C cuts the stem where it is enclosed with the leaf-sheaths; and D illustrates the conditions obtaining generally between the leaf-sheaths and the inflorescence.

IV. Leaf.

Unlike its associates *Aira flexuosa* and *Nardus stricta*, whose leaves are setaceous through permanent inrolling, *Molinia* has a ribbon leaf. When wilting it rolls up considerably, and in dry seasons makes use of this power to reduce evaporation : but normally its leaf is flat. The sheath is split to the base ; the ligule is represented by a tuft of hairs, a useful character for identification ; the linear lanceolate blade is long, thin, smooth, tapering slightly to the ligule and running off above into a long fine tip ; though thin, the blades are exceptionally rigid : Ward (4) mentions that they are used locally for brooms ; there is a slight covering of hairs on the upper surface, especially towards the ligule ; the margin has a series of very fine teeth, which are outgrowths of the epidermal cells with walls containing a siliceous deposit.

Of this leaf, some account of which has been given by Lewton Brain (3), the under surface in transverse section is fairly even, while the upper is thrown into a series of low ridges. These ridges, of which there are eleven to fifteen on either side of the midrib, indicate the position of most of the vascular bundles, while the hollows are occupied by groups of motor cells. The vascular bundles are large and are made still more prominent by being girdered with stereome. According to the disposition of this stereome, which varies with the size of the bundles, the latter may be distinguished as of four types : midrib, main lateral bundles, secondary lateral bundles, and supporting bundles. The midrib is a large bundle with stereome passing to both surfaces and spread out on the under side into a broad sub-epidermal band, giving an inverted T section. The main lateral bundles occupy about every third ridge from the midrib to the margins ; their outline is practically circular and they fill up three-quarters of the thickness of the leaf under the ridge (see Fig. 7) ; broad flanges of strengthening tissue pass from these bundles to both epidermises, but do not spread out as in the case of the midrib. The secondary lateral bundles occupy the remaining ridges ; they have only about a third of the area in cross section of the main ones, and are of ovoid outline with the broad end of the egg towards the under surface ; these also are girdered above and below, but the mechanical tissue is but a narrow strip. The supporting bundles are a small pair on the flanks of and parallel to the midrib ; they have a band of stereome below them passing to the epidermis but none above ;

and they are peculiar also in that they stand beneath rows of motor
cells, thus occupying hollows, like the bundles in the leaves of
Glyceria fluitans and *Dactylis glomerata,* instead of the usual
position under the ridges ; in this position they strengthen the leaf
at the point where it is weakened by the development of air spaces.
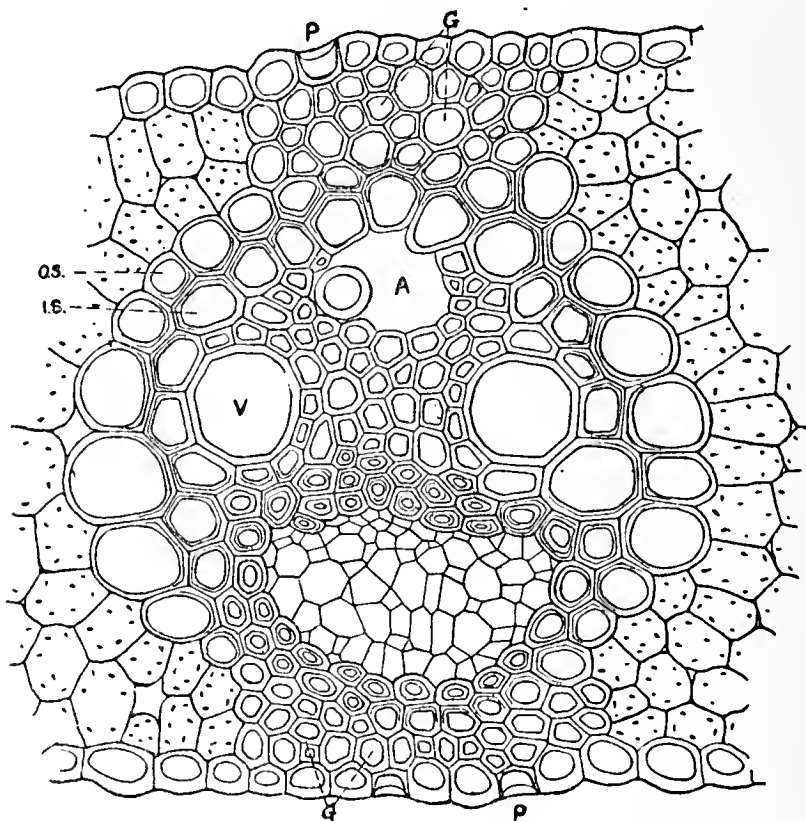
FIG. 7.   Transverse section of a main lateral bundle of the leaf.   Letter-
ing :—A., intercellular air-space ; G., stereome girder ; I.S., inner sheath ;
O.S., outer sheath ; P., plate-cell ; V., large lateral vessel.   × 400.

All the vascular bundles of the leaf have both an outer and
an inner sheath (Fig. 7) : the outer is composed of large cells, very
large in the middle of the section, with walls thickened especially
on the inner and lateral sides ; the inner sheath is thickened and
lignified throughout, and becomes continuous with the stereome
of the girders, into which the outer sheath also merges.   Pée-Laby
distinguished five orders of bundles in grass leaves, which Brain (3)
reduces to three, namely (1) bundles containing two large lateral
vessels and a prominent intercellular air space, (2) bundles with
large lateral vessels but no intercellular space, (3) bundles with
neither lateral vessels nor intercellular space.   In *Molinia* the
midrib and main lateral bundles are of the first order, the secondary
lateral bundles near the midrib of the second order, while the more
remote secondary lateral bundles and the supporting bundles are
of the third order.   In all the bundles the phloem is surrounded

by an exceedingly strong band of very thick walled elements (Fig. 7)
and the woody tissues, composed of annular tracheides and pitted
vessels, are both abundant and strongly thickened.   Each bundle
is accompanied by a pair of longitudinal rows of peculiar cells on
both surfaces of the leaf (*P*), marked off in transverse section by
having a darker colour and a concave instead of convex outer wall.
Examination of a piece of stripped epidermis shows these to be
provided with dumb-bell shaped plates (Fig. 9, *A*), probably siliceous
whence they may be called plate-cells, and the function of these
cells may be excretory.   It will be gathered from these details that
the *Molinia* leaf is exceedingly rich in wood elements and stereome
and this is the explanation of the rigidity of the leaves.   As Lewton
Brain remarks, " the leaf is peculiar in the great strength and
abundance of its mechanical tissue," a remark which would also
apply to the plant as a whole.
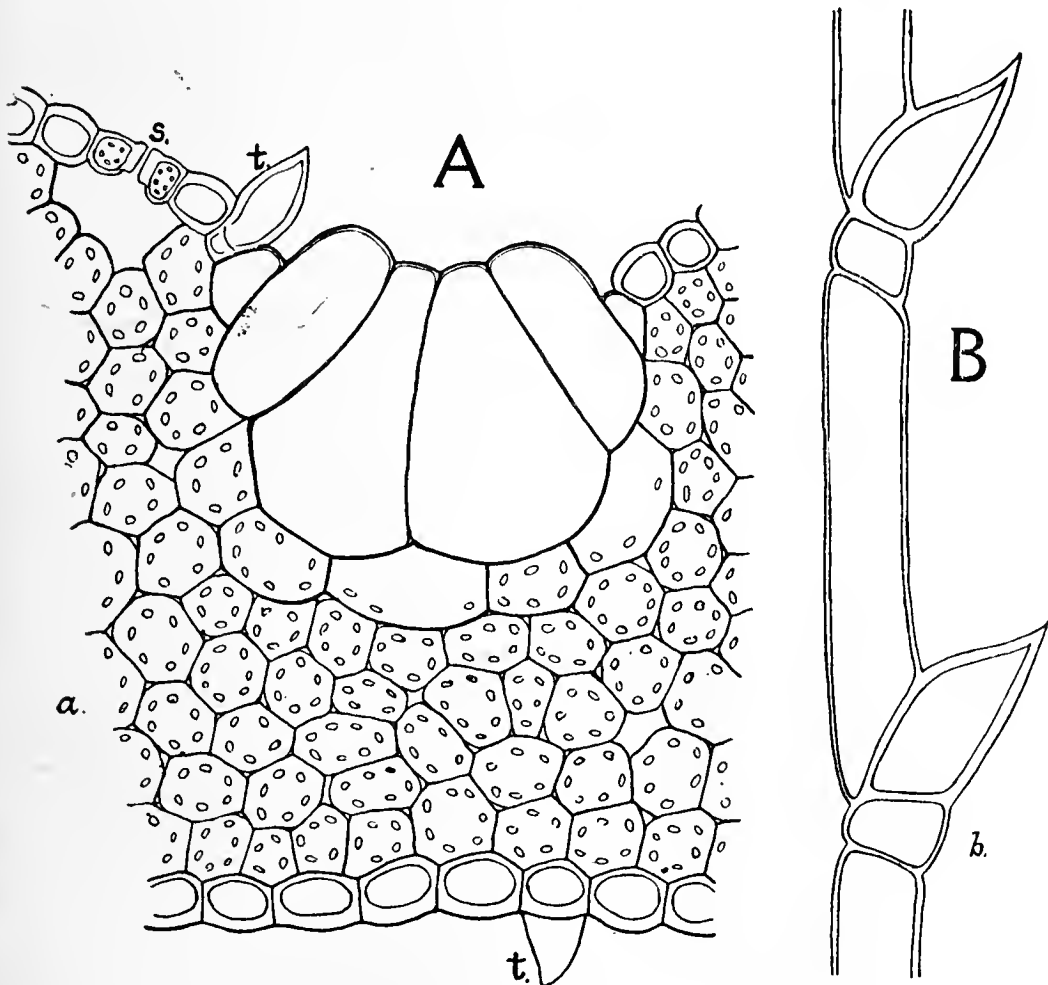
FIG. 8.   A.   Motor mechanism of leaf, transverse section.   B.   Teeth by
motor cells.   Lettering :—*a.*, assimilating tissue ; *b.*, " basal " cell ; *s.*, stoma ;
t., tooth.   A × 500, B × 800.

The motor mechanism (Fig. 8) is strongly developed and, after
the bundles, constitutes the most conspicuous feature in the

transverse section.· In the hollows between the ridges the motor cells form groups sharply distinguished by their huge size. Each group is composed of three or four large cells reaching half-way across the section with a small cell on either side. The outer walls are about half the thickness of those of the ordinary epidermal cells. The largest cells of the mesophyll adjoin the base of the motor mechanism, and frequently, especially near the midrib, appear to be losing some of their more solid contents and partaking of the nature of the motor cells. On either side of each group is a row of cells bearing teeth (*t.*), apparently having some definite relation to the motor cells: each tooth is an outgrowth of a small cell (see Fig. 8, *B*), which rests on another small "basal" cell below and presses against a long cell above, beyond which we have another tooth ; these teeth by exposing a surface which is large in proportion to the amount of their contents, probably increase the sensitiveness of the motor mechanism to changes in the atmosphere. Similar rows of teeth are found on the ridges, and others on the outer surface opposite the motor cells; in each case there are two parallel rows.
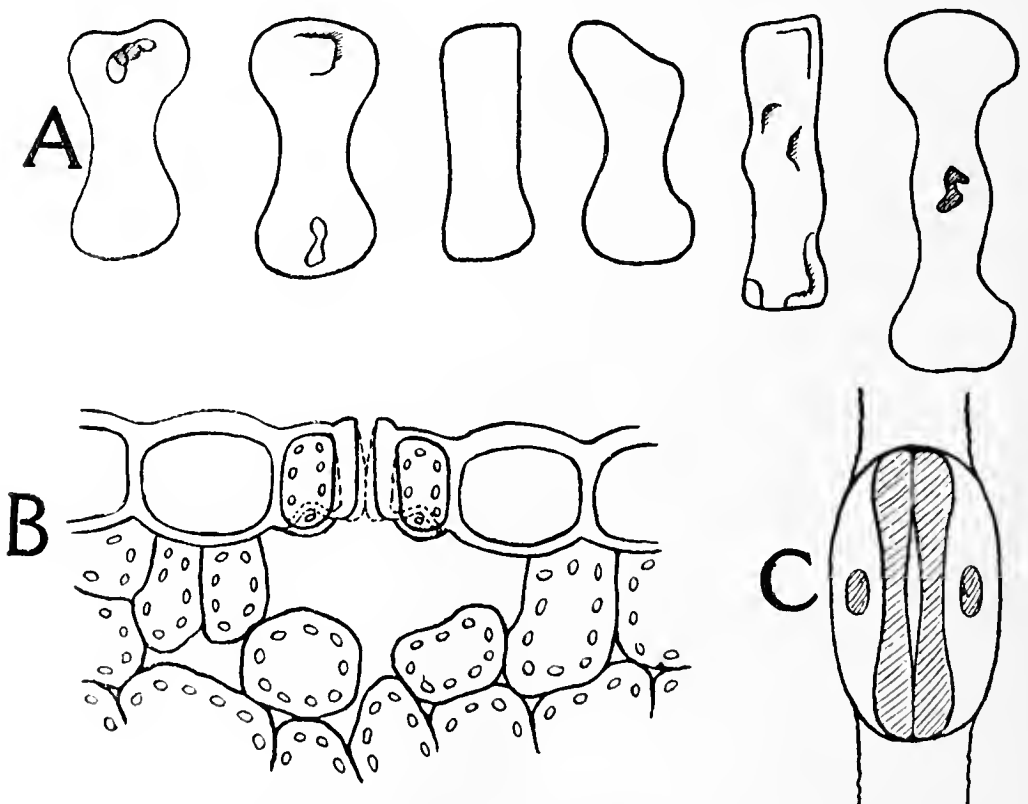
FIG. 9. A. Siliceous plates on epidermal "plate-cells" (P. in Fig. 7). B. Stoma in transverse section. C. Stoma in surface view. A × 1300 ; B and C × 1000.

Stomata are freely distributed on both surfaces of the leaf : on the upper surface they are distributed in the rows of cells next

or next but one or two, to the tooth-bearing cells by the motor mechanism (Fig. 8, *S*), so that when inrolling occurs they are quickly closed ; on the under or outer surface they are in rows practically opposite to those on the inner surface, and they are approximately equal in number. The structure of the stomata is shown in Fig. 9, *B*, *C*. The guard cells have slightly projecting lips, a thick wall by the air passage, and a thin internal wall whose distension or collapse opens or closes the aperture. As usual the guard cells contain chlorophyll.

Apart from specially modified elements, both sides of the leaf are covered with an epidermis of long but narrow, strongly cutinised cells. The mesophyll is composed of chlorophyll bearing cells (Fig. 8, *a*) which are small, polygonal and closely packed in transverse section but much more open in longitudinal, especially towards the inner surface ; in the middle of the mesophyll the cells are slightly larger and more loosely arranged. In the thickest part of this thin leaf, to right and left of the midrib, large air spaces or lacunæ arise by the breakdown of a number of the cells of the mesophyll. The wetness of the habitat usually favoured by *Molinia* involves a need of special facilities for aeration, which is met in this way, and the consequent weakening of the leaf is compensated by the large development of mechanical tissue. The small, supporting vascular bundles, previously described, are so placed as to give additional strength just where the leaf is weakened by the development of these lacunæ.

The leaf-sheath (Fig. 5, *C*) continues the structure of the leaf-blade in a modified form. The stereome of the girdered bundles tapers to the inside and expands towards the outer surface. The inner epidermis is not cutinised and there are no motor cells. A slight amount of chlorophyll tissue develops beneath the outer epidermis, but most of the mesophyll is wanting, and instead we have a large air space between every pair of bundles, except close to the margins where the sheath is too thin. The inner surface of the sheath is almost white, an appearance due to two or three layers of dead cells lying within the inner epidermis.

Frequently the bases of the leaf sheaths of the upper series of leaves show a prominent swelling. This is a gall produced by the larvæ of *Oligotrophus ventricolus*, one of the Cecidomyiidæ, a midge found during the progress of this study to be adundantly associated with *Molinia*, though only once before recorded in England, and which has been described by Grimshaw (1) from material supplied by the present writer. The galls are present on fully one fourth of the

aerial stems in the districts where this work has been carried on, and have since been found on examination to be common in several widely scattered areas. The development of the gall occasionally produces a curious looping of the haulm and some of the leaf sheaths surrounding it (Fig. 1, *g.s.*), but more commonly the swelling is concentric and does not deflect the stem (opposite *g.s.*). Sometimes the insect affects the stem before the basal internode has elongated, when the gall is produced at the base, instead of above the "joint," and looks very like the ordinary tuberous basal internode (*g.b.*). In this case, however, the inflorescence, its axis, and one or more leaves may be suppressed (above *g.b.*), and the basal internode does not elongate and swell to form a storage organ, the food reserve being deposited in the leaf sheaths around the nest of larvæ and in the subjacent cord roots instead of in the stem· From the presence of the larvæ between the stem and leaf-sheaths, whose blades had not opened when the check to stem development took place, it may perhaps be inferred that the eggs are deposited by the fly in the position in which the nests are found, an inference which is supported by the facts that young galls frequently show a puncture and that the female insect possesses an ovipositor. Apart from these early attacks, which are quite exceptional, no harm seems to be done to the plant. During the season the larvæ develop into pupæ and the galls increase in size to accommodate them. The mature insects emerge on the Pennines about the end of May.

V. Conclusion..

The inflorescence, flower, and fruit of *Molinia* have already been described by systematic and other writers, and my own investigations concerning them and the embryology and development are not yet complete. I have therefore limited this paper to the structure of the vegetative organs, the special points in which may now be summarised.

1. Among the *peculiar features* emphasis may be put upon (*a*) the unusual distribution of the root hairs, (*b*) the prominent endodermis of the cord roots, (*c*) the structure of the basal internodes and the nature of the food reserves they contain, (*d*) the absciss layers for the protection of the more lasting members, and (*e*) the vascular bundles of the leaves with their thick walls, double sheaths, and stereome girders.

2. Attention should be called also to such *general features* as (*a*) the wealth of mechanical strengthening tissue in all parts of the plant, (*b*) the remarkable development of xylem elements, (*c*) the

tendency to store up food reserves in various members—cord roots, rhizomes and basal internodes, to which might be added the seeds, and (*d*) the powers of adaptation which seem to lie latent in every part of the organism, e.g., chlorophyll in roots, rhizome elongation, and galled bases.

3. Finally the *biological value* of these structures may be stressed as relating them to the facts of distribution, etc. set out in the study of the plant's ecology (2). (*a*) The huge root system, the distribution of root hairs, the presence of mycorhiza, the abundant xylem, and the thin leaf plentifully supplied with stomata on both sides, indicate great powers of transpiration and a strong hold on the resources of the soil. (*b*) These in turn, when correlated with the wet habitat, flat leaf, and for a moorland plant, exceedingly large amount of assimilating tissue, suggest the ability to make food quickly and take full advantage of the short summers on the hills. (*c*) When to this we add the highly developed motor mechanism, the efficient and abundant storage tissue, and the evergreen character of the stems, we see how the disadvantage of delicate leaves is compensated. (*d*) Lastly the mechanical strength of the whole plant, together with its compact circular tussocks, gives it a power of resistance to wind and flood which go far to explain the success of *Molinia cærulea* as a coloniser and often a dominant species on our exposed uplands.

4. On the other hand, when studied in relation to its habitat on the Pennines, *certain weaknesses* appear. (*a*) The delicate nature of its ribbon leaf results in widespread withering of the leaf tips in the drying winds. (*b*) Its early development, by means of food reserves, exposes the young foliage to excessive cold. (*c*) Probably in consequence of the preceding, in cold seasons etiolation is so abundaut that wide stretches look yellow rather than green and in most years throughout the season " autumn " tints appear on all the leaves.

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THE VASCULAR ANATOMY OF THE TUBERS
OF *NEPHROLEPIS.*

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[With Three Figures in the Text].

Introductory.

ALTHOUGH from the point of view of their biological significance the tubers borne on the underground stolons of *Nephrolepis* have been the subject of frequent comment[1], their vascular anatomy does not appear to have received the attention it deserves. The investigations of Professor Heinricher, who studied the tubers under artificial conditions of culture, while dealing in a most interesting manner with the biological problems, leave the vascular anatomy out of consideration, and since 1889 no reference has been made to it. In that year Lachmann[2] made the observation that the single strand traversing the stolon breaks up at the base of the tuber into a number of smaller strands usually concentric or rarely bicollateral in structure, which form a network lying in the peripheral part of the tuber, parallel to its surface, and converge again towards the apex of the tuber, there uniting into a single strand. Lachmann, however, did not describe the changes which the strand undergoes in order to give rise to the reticulate system traversing the periphery of the tuber; and as these form an interesting parallel to the mode of elaboration of the dictyostele from the protostele in the ontogeny of many Ferns, they would seem to be worthy of description.

The material for this work consisted partly of tubers of *Nephrolepis cordifolia* from the Cambridge Botany School, and partly of fresh tubers of the same species obtained from the Cambridge Botanic Garden through the kindness of the Curator, Mr. R. Irwin Lynch, M.A., to whom I wish to express my hearty thanks.

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[1] Sperlich, A., Flora, 1906, p. 451 ; Sperlich, Flora, 1908, p. 341 ; Heinricher, E., Flora, 1907, p. 43.

[2] Lachmann, J.-P., "Contributions à l'histoire naturelle de la racine des fougères," Lyon, 1889, Thèse présentée à la faculté des sciences de Paris, pp. 155-6 ; Hofmeister, W., " Beiträge," etc., II. Abhandl. d. kgl. sächs. Ges. d. Wiss. V. Math.-Phys. Klasse III., Leipzig, 1857, p. 651.

Beyond the remarks contained in these two communications I am not aware of any reference hitherto made to the subject in question.

DESCRIPTIVE.

Several species of *Nephrolepis*[1] are now known to possess tubers, which are as a rule terminal swellings of short branches of the underground[2] stolons. The apex of the branch forms a minute mamelon at the distal end of the tuber. Only rarely, according to Professor Goebel's observations,[3] is the mamelon developed into a stolon while the tuber is still attached. The tuber is so completely protected by imbricating peltate scales that no part of its surface is left exposed. The fresh tubers examined by me contained enough sugar to give them a sweetish taste, and a copious brown precipitate with Fehling's solution confirmed the presence of a considerable quantity of a reducing substance. The outermost one or two layers of cells also contained a few starch-grains.

Fig. 1, A, illustrates the vascular relations between the stolon and the tuber. We shall follow the transition from the solid protostele of the stolon to the netlike stele of the tuber, as revealed by a series of transverse sections from the base upwards. But before describing these changes it will save some repetition to state that the manner in which the strands finally fuse up into the solid protostele at the apex is the reverse of the process of disintegration occurring at the base. In both these processes, however, individual variations are met with, which we shall later attempt to explain.

The strand of the branch-stolon penetrates the base of the tuber as a solid protostele for a few millimetres; its four or five protoxylems, hitherto more or less clearly seen,[4] may at this time become indistinct. The xylem then begins to dilate in a funnel-like manner and acquires a central mass of phloem which enlarges and is soon followed by pericyle, endodermis and ground-tissue in succession. The solid cylinder of xylem has been converted into a

[1] Heinricher, *loc. cit.*, p. 67. Tubers have also been recorded in *N. neglecta* by Lachmann. " Recherches sur la morphologie et l'anatomie des fougères," (Comptes rendus, CI, 1885, p. 605), and in *N. undulata* by Kunze (Bot. Ztg., 1849, p. 882) and by Hofmeister, (*loc. cit.*, p. 65) ; but these species may be synonymous with some of those mentioned by Heinricher.

[2] Under certain conditions, according to Sperlich (Flora, 1906, p. 454), tubers may be produced on the aerial stolons.

[3] Goebel, K., " Pflanzenbiologische Schilderungen I," 1889, p. 203. In one specimen at the Cambridge Botanic Garden, however, six of the tubers had produced stolons at their distal ends.

Attention may incidentally be drawn to the appearance of chlorophyll in the superficial layers of the young tubers as well as in some young underground stolons, *before* these organs have been exposed to light.

[4] Attention may here be drawn to a minor point, namely, that whereas in the aerial stolon there are usually four very well-marked protoxylem strands, in the underground stolons they are neither constant in number nor so well differentiated and regularly distributed.

hollow cylinder lined internally as well as externally by phloem, pericycle and endodermis, while the centre is occupied by a patch of ground-tissue which in this region is sclerenchymatous. This
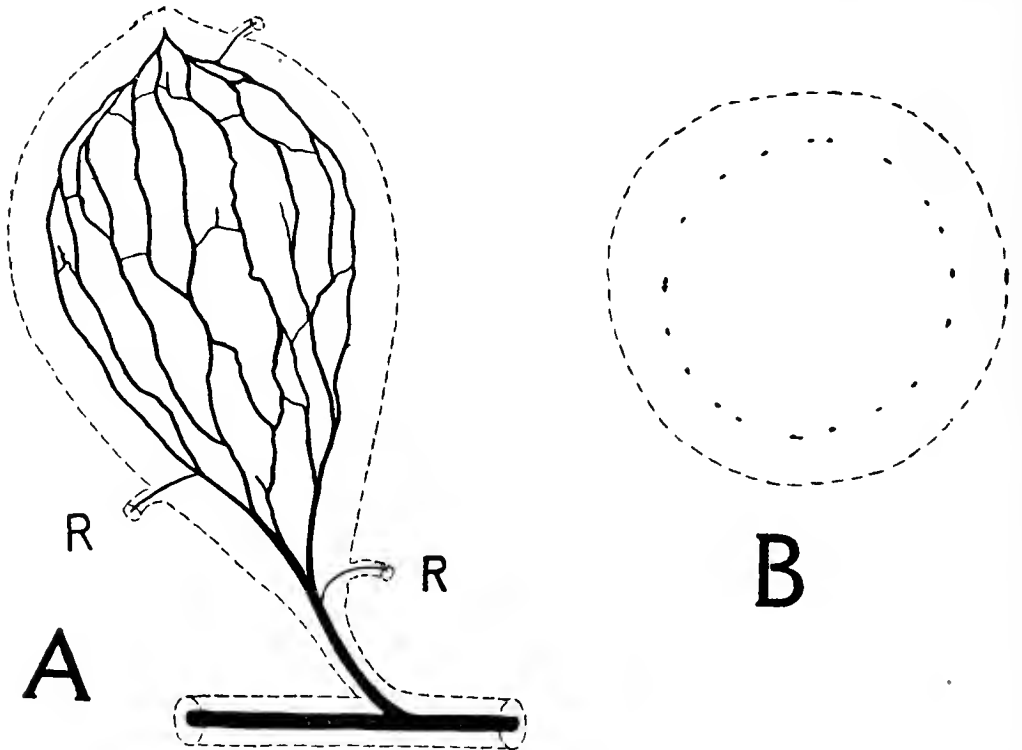
FIG. 1. *Nephrolepis cordifolia.* *A.*—Slightly diagrammatic representation of the vascular system of the tuber and its relation with that of the stolon. The broken line indicates the outermost limit of the cortex in both tuber and stolon. R, roots; their points of origin have no relation to the gaps in the reticulate stele of the tuber. *B.*—Transverse section of the tuber through its broadest part. *None of the gaps have any relation to leaf-traces,* whether rudimentary or other.

state of affairs does not, however, long persist, for, as the cross-section of the tuber increases, the stele also expands further and the xylem ring (Fig. 2, A) which has in places already become attenuated to only one or two layers of tracheides, becomes disintegrated into usually three or four arcs separated by gaps, with the result that the internal phloem becomes continuous with the external at the edges of the gaps. The arcs of xylem become further and further removed from the centre and from each other. Through the widening gaps first the internal pericycle, then the internal endodermis, and finally the central ground-tissue become continuous with the corresponding external tissues. The " pith ' has meanwhile lost its thick-walled character and can no longer be differentiated from the cortex. As we pass distally from the base of the tuber, the arcs of xylem divide repeatedly by constriction and give rise to a considerable number of strands which anastomose with each other, forming an irregular network lying parallel to the

surface of the tuber, a few millimetres inside it.   In a transverse
section of the tuber in its broadest part (Fig. 1, B) the vascular
system appears as a ring of about a dozen or more tangentially
flattened strands, each consisting of a plate of xylem completely
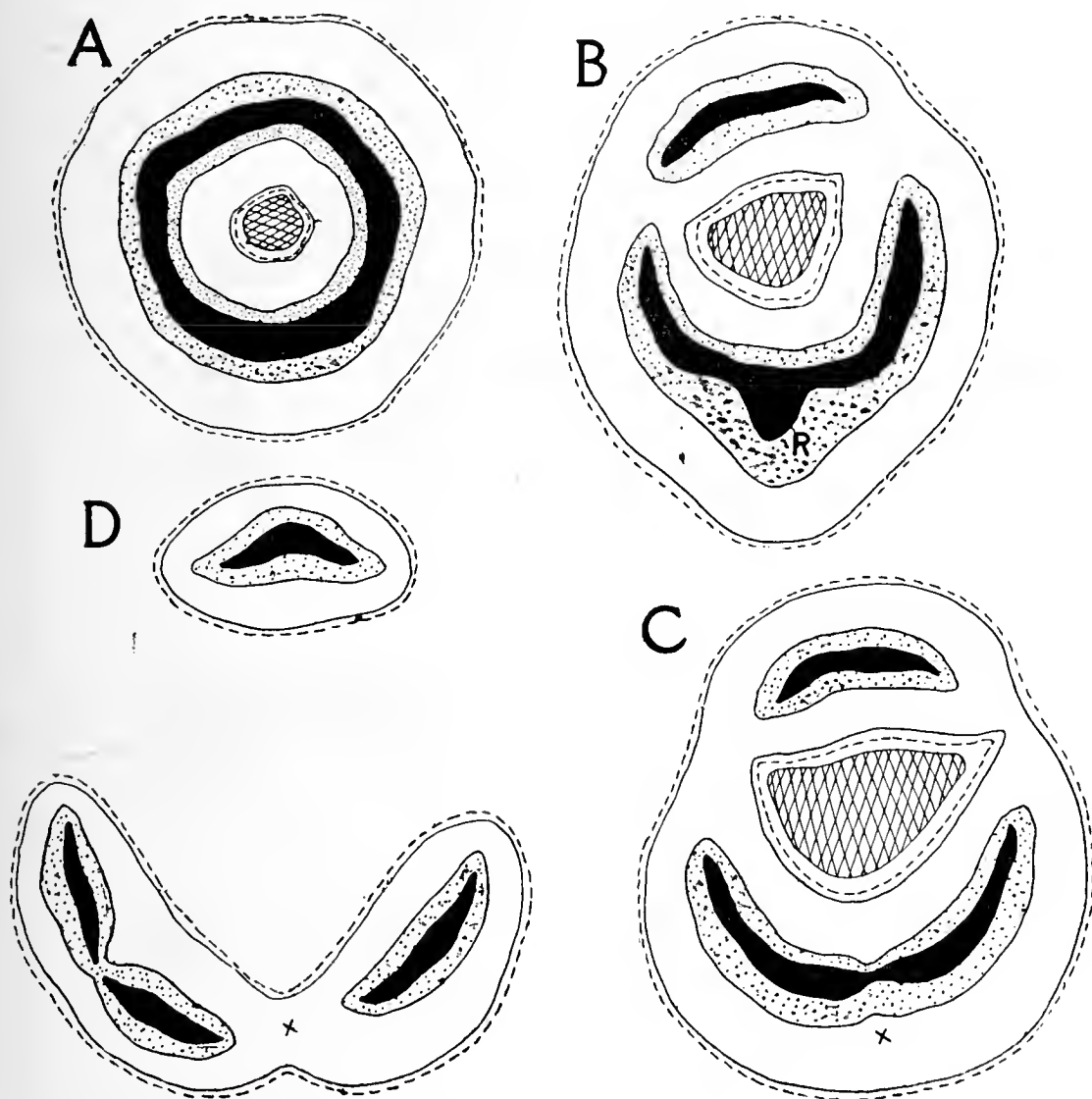
FIG. 2.   *Nephrolepis cordifolia.* Transverse sections of the *base* of the tuber,
the series running from below upwards.   *Xylem* solid black ; *phloem* dotted ;
pericycle left blank ; *endodermis* a broken line ; *sclerenchymatous pith* cross-hatched.

A has the appearance of a typical solenostele cut at a point where it is
uninterrupted by a leaf-gap. In *B* a root-stand is coming off at R, and gives
the impression that its exit is responsible for the gap (x) seen in *C* and *D*.   In
the two latter diagrams the detached root-stand is omitted.

surrounded by phloem, pericycle and endodermis.   The xylem-plate
is usually only one or two layers of tracheides thick, with the
smallest elements at its two extremities.   Distinctly spiral or
annular tracheides could not be made out clearly.   Bicollateral
strands such as Lachmann found in *N. exaltata* were not seen.

The course of elaboration sketched above is very often slightly modified, inasmuch as the internal phloem may effect a junction with the external *before* any internal pericycle has made its appearance. (Compare Fig. 3, which is from serial sections of the *apex* of a tuber). The xylem-ring is thus converted into a horse-shoe: the gap of the horse-shoe is at first merely bridged over by external phloem, which is in turn overlain by pericycle and endodermis, but
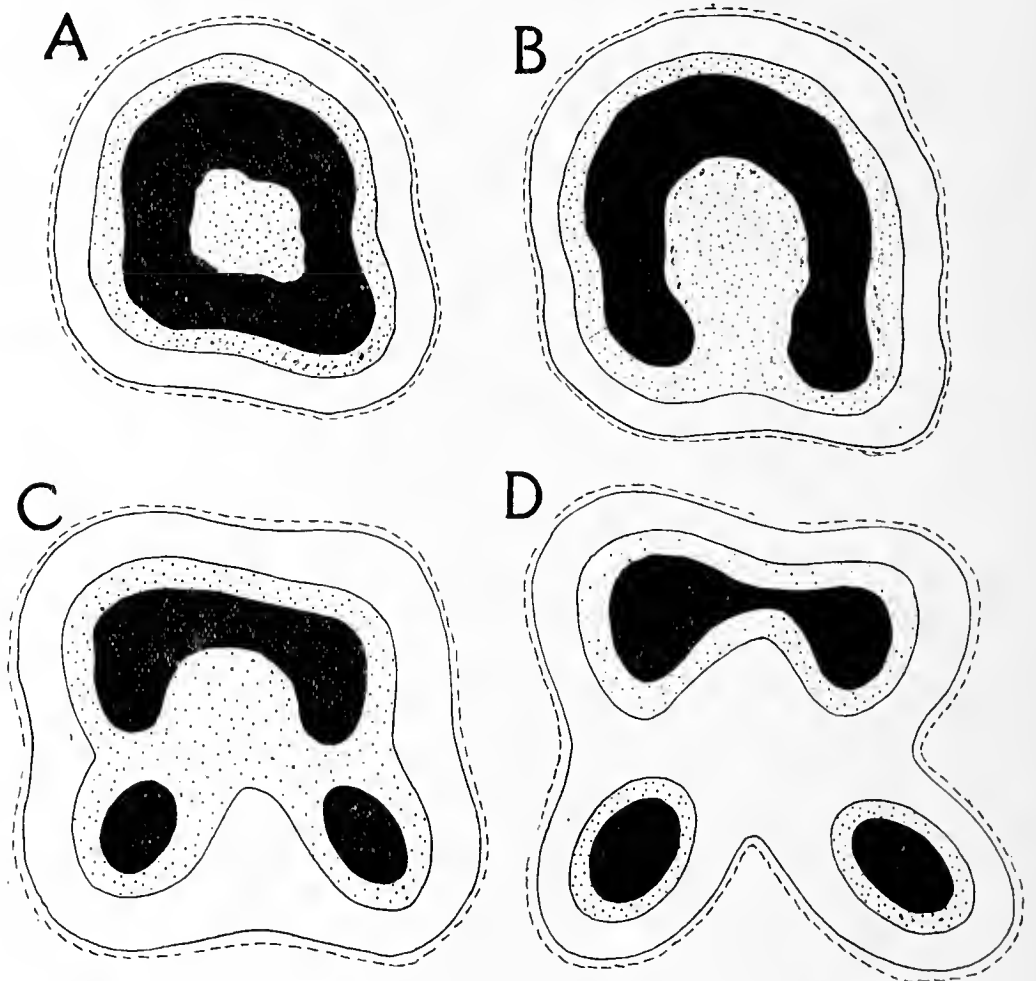
FIG. 3. *Nephrolepis cordifolia.* Transverse sections of the *apex* of the tuber, the series running from the apex downwards. The various tissues are indicated as in Fig. 2. Diagram *A* shows a stage resembling the " *Lindsaya-*type " of stele. For further explanation see Text.

it subsequently becomes invaded by the latter tissues in succession. Disintegration of the horse-shoe gives rise to three or four xylem-strands arranged in a ring. This process of disintegration may either begin before the pericycle has advanced far into the gap, so that we have the xylem-strands lying embedded in a single mass of phloem (Fig. 3, C) ; or it may be delayed till after the pericycle and endodermis have formed a complete internal lining to the xylem.

One cannot fail to notice the superficial but close similarity of some of the stages shown in Figs. 2 and 3 to those passed through

by many fern-rhizomes during their individual development from the protostelic to the dictyostelic condition.   Fig. 3, A, for example, is strongly suggestive of the "*Lindsaya*-type" of stele, while Fig. 2, A is exactly the appearance presented by a transverse section through the internode of a typical solenostelic fern.   Neglecting for a moment the mode of origin of the gaps, the appearance of a transverse section through the middle of the tuber does not materially differ from that of a dictyostelic rhizome.

Roots arise promiscuously on the tuber, but sometimes they are almost confined to the narrow basal end and to the apical mamelon.   As their strands are sometimes attached to the net-like stele at the base of a gap, sections through those regions (Fig. 2, B, C) give the impression that the gap is "caused" by the exit of the root-traces.   An examination of Fig. 1, A will, however, show that the point of origin of the root-strands does not follow any rule.

It seems probable that the relative duration of the different stages in the transition depends largely upon the shape of the tuber. If the increase of diameter from the stolon to the tuber is gradual (as shown in Fig. 1, A) the expansion of the vascular system is correspondingly slow, and the intervening tubular stage, with internal phloem, pericycle and endodermis, is of appreciable length. If, on the contrary, the tuber is sharply marked off from the stolon the different stages in the transition succeed each other rapidly and may even overlap, so that the xylem-ring may become disintegrated before it has acquired an internal pericycle, endodermis and ground-tissue (Fig. 3, C).   In the light of this suggestion some of the individual variations met with in the mode of transition may perhaps be satisfactorily explained.   This view appears to find support in the fact that in the pear-shaped tubers examined the transition was more rapid at the apex than at the base (that is, the narrower end).

Professor Heinricher (*loc. cit.*, p. 71) has found that the shape of the tuber seems to be characteristic for each species and suggests that it may be a character of taxonomic value.   Thus in *N. hirsutula* Presl *apud* Raciborski the tubers were pyriform, while in specimens of *N. tuberosa* (Bory, Willd.) Presl=*N. cordifolia* (L.) Presl they were found to be ellipsoid.   In view of the confused condition of the taxonomy of the genus such a distinction would be welcome, but it is difficult to say what importance can be assigned to this character, for variations in the shapes of tubers from the same plant are not rare.   In a few tubers from a specimen of the last-named species a complete transition from the pyriform to the ellipsoid

could be traced. Some of the tubers were flattened, having grown against the wall of the pot, while others were distorted by small pebbles in the soil. A few were spherical.

THEORETICAL.

In the foregoing account of the vascular anatomy of the tubers the main feature of interest is that the different stages through which the stele passes from the solid, cylindrical to the net-like condition are closely similar to those seen in the ontogeny of the solenostelic and dictyostelic Ferns, in which the influence of leaf-traces is justifiably regarded as the dominating factor in the evolution of the cauline stele (Boodle, Gwynne-Vaughan, Tansley)· My first impulse was to conceive of the tuber as possibly homo-logous with the "lateral plant" borne normally on the aerial stolon, which might have undergone swelling and a great reduction in the leaves, so that in its modified form it may serve as an underground organ for vegetative reproduction and water-storage. Certainly the position of the tuber on the stolon is exactly the same as that of a lateral plant, and the similarity in the stelar condition as traced from the base upwards encourages one in the hypothesis. The meshes in the network of strands might then be interpreted as leaf-gaps such as are known to exist in the Cactaceæ,[1] in spite of the extreme reduction of the leaves. The hypothesis is, however, not to be seriously entertained in view of the fact, already noticed by Heinricher (*loc. cit.*, p. 50), that the surface of the tuber shows no trace of leaves, even in a most rudimentary form.

Since then, none of the gaps can be looked upon as leaf-gaps, the stele in question is not entitled to be called even a perforated or dissected solenostele. Such a stele is not, to my knowledge, known to exist elsewhere among Ferns. It may be mentioned that in one underground stolon the strand was seen to break up into a few anastomosing ones arranged in a ring; these again fused up into a single strand at a point several millimetres from where the original strand divided. The portion of the stolon between these points showed neither any marked swelling nor any trace of leaves.

Structures which in external appearance (and apparently also in function) most closely approach *Nephrolepis* tubers are found in several species of *Equisetum* (*E. arvense*, *E. Telmateja*, *E. palustre*, *E. hiemale*) in which some of the internodes of the subterranean rhizome undergo swelling.[1] A point worth noticing is that in these

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[1] Ganong, W. F. "Beiträge zur Kenntnis der Morphologie und Biologie der Cacteen." Inaugural-Dissertation. Cited by Jeffrey, E. C., Trans. Canad. Inst., 1900, p. 35 of Reprint.

also the strands diverge widely from the base and converge again towards the apex (*loc. cit.* p. 41, and Plate I, fig. 3). Tubers similar to those of *Nephrolepis* have also been recorded by Ule[2] in a species of *Hymenophyllum*, but unfortunately nothing more is known about these.

Since the behaviour of the vascular strands in the tubers of *Nephrolepis* cannot be explained as being due to any influence from leaf-traces, we must look for an answer to the problem elsewhere. For a plausible suggestion, which would apply equally well to the case of *Equisetum*, I am indebted to Mr. R. H. Compton who believes that an increase in the diameter of an organ tends to cause a corresponding dilatation of its vascular system. As he has pointed out in another connexion,[3] " the impression given . . . . . is that the stele takes the opportunity afforded by the increased diameter to acquire a pith and expand." While dealing with the factors in the evolution of solenostely Mr. Tansley[4] has remarked that an increase in the diameter of the stele would be necessitated by any broadening in the span of the C-shaped leaf-trace, to the attachment of which on an originally solid stele he has ingeniously traced the root-cause of the departure towards solenostely. In the case of *Nephrolepis* and *Equisetum* the great increase in the diameter of the axis may exert on the stele an influence similar in effect to that of the enlarging leaf-trace on the cauline stele. For the proper supply of food and water to a storage organ of large diameter, it would be necessary that the vascular system should not have the form of a solid axial cylinder, but of an expanded hollow stele.

The further change, from the tubular to the net-like condition may be taken to be a natural result of the enormous dilatation of the stele, and the concomitant thinning of the tube in places to produce the " perforations." Contrary to what is the case in the normal fern rhizome, where the dilatation of the stele is, comparatively speaking, slight, we may confidently believe that the mere thinning of the tubular stele would suffice to produce gaps in it. In fact, a suggestion of such a mode of origin for these perforations is visible in their irregular shape, in the frequent occurrence, at their edges, of thin blindly-ending strands, and in the varying width of the

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[1] Duval-Jouve, " Histoire naturelle des *Equisetum* de France," 1864, p. 6 ff.; Luerssen in Rabenhorst's Kryptogamen-Flora, iii., 1889, figs. 203, 206, 209.

[2] Ule, E., Berichte d. d. bot. Ges., XV., 1897, p. (85).

[3] Compton, R. H., " An Investigation of Seedling Structure in the Leguminosæ," Journ. Linn. Soc. 1912, p. 97.

[4] Tansley, " Lectures on the Evolution of the Filicinean Vascular System," New Phytologist, 1907, p. 150.

ribbon-like strands composing the reticulate stele.

I wish to express my hearty thanks to Professor Seward for his kind interest in the work and also for revising the manuscript of this paper.

SUMMARY.

The strand of the branch-stolon penetrates the base of the tuber as a solid protostele for a few millimetres (Fig. 1, A), but rapidly expands in a funnel-like manner, acquiring, in succession, internal phloem, pericycle, endodermis and ground-tissue (Figs. 2 and 3). Sooner or later the funnel-like stele breaks up, while at the same time expanding enormously, into a hollow net-work of tangentially flattened ribbon-like strands (each concentric in structure), enclosing gaps of irregular shape and size. These strands converge again into a single protostelic strand. The latter as a rule ends in the apical mamelon, which contains the apical cell; but when the mamelon is developed into a stolon the strand is continued into the latter.

Root-strands arise promiscuously from this reticulate stele.

The process of fusion of strands at the apex is similar to the process of stelar disintegration at the base, the relative duration of the various stages in the transition depending upon the rapidity with which the tuber tapers towards its ends. This relation is inverse.

Attention is drawn to the superficial but close similarity of some of the stages in the transition to those passed through by many fern-rhizomes in their development from the protostelic to the dictyostelic condition. There being no sign even of reduced leaves on the tuber, the gaps cannot be traced to any influence from leaf-traces, which in normal fern-rhizomes have justifiably been held to be the dominating factor in the evolution of the cauline stele (Boodle, Gwynne-Vaughan, Tansley). It is suggested that the necessity of adequately supplying food to all parts of the tuber has "called forth" in the originally solid stele a dilatation great enough to transform it into a hollow net-work; this dilatation being similar to that "necessitated," according to Tansley, by the increase in the span of the C-shaped leaf-trace, to the attachment of which on an originally solid stele he traces the root-cause of the departure towards solenostely.

The reticulate stele of the tubers of *Nephrolepis* is unique because *all* the gaps in it are what have technically been called "perforations."

THE BOTANY SCHOOL, CAMBRIDGE,
March, 1916.

ALBERT STANLEY MARSH.[1]

O N January 6th, Captain A. S. Marsh of the 8th (Service) Battalion of the Somerset Light Infantry was shot through the heart by a sniper as he was passing a gap in the trench parapet near Armentières. Marsh was not 24 years old when he died and in him we have lost a botanist full of love for his subject and of promise for the future.

Marsh was the son of Mr. W. W. Marsh of Blacknell, Crewkerne, in Somerset, where he was born on February 1st, 1892. He entered Sexey's School at Bruton in 1903 with a Junior County Scholarship, and was a great success throughout his School career both in examinations and in the general life of the school. He gained two more county scholarships and also first-class honours in the Senior Oxford Local Examination, and took a conspicuous part in the school debating society and in other school activities. His headmaster writes that "his energy was amazing and he never appeared to find work a burden." In his third year, Marsh began to take a keen interest in natural history and started with great enthusiasm on the geology and botany of the district, making large collections of fossils and plants for school prizes. Among his close school friends were several boys who did well in science at the Universities and are now doing successful research.

Marsh also showed a marked talent for languages, both at school and later. For instance, he " got up " Greek for the Little-Go in a very short time (neither Greek nor Latin are included in the ordinary curriculum of the school), and later on he very quickly acquired a good working knowledge of German and French, spoken as well as written, in a way that impressed one as the way of a real linguist.

Marsh was considered a delicate boy when he first went to school, and was never an athlete, though his health rapidly improved, but he was a tireless walker, and always played a good game of fives, that favourite of so many students.

In December, 1908, while still under 17, Marsh entered for the scholarship examination in natural science at Trinity, Cambridge, and his work in botany was really wonderful for a boy of his age. At the time it was hard to be sure how far his high standard of knowledge was due to real scientific ability and how far to the excellent and careful teaching for which his school is well known.

[1] The writer has received valuable help from several of Marsh's friends at Cambridge as well as from the headmaster of his school.

But he was easily top of the candidates in botany, though by far the youngest of them, and he got an exhibition at Trinity, and the same year the Drapers' Company's " Soley " Scholarship. He came into residence at Trinity in October, 1909, and later on obtained a foundation scholarship there.

Of his undergraduate days it is difficult for one who was not his contemporary to write at all adequately. He was modest and reticent in demeanour, with a strong sense of humour and a pretty gift of irony, and he always gave one the impression of a great deal of personality beneath the quiet surface. One of his friends writes of " that sudden intense keenness and sparkling interest that used to bubble up when he was aroused about something and wanted to carry you with him. It was a great charm . . . ." Apart from the talent for languages, which has been already mentioned, he had distinctly literary tastes. Especially, as one of his close friends writes, was he attracted to the quaint or the bizarre. He contributed some excellent stuff to the humorous Cambridge Botany School " Tea-Phyt-ologist," an erratic production—it can hardly be called a periodical—of which three numbers appeared at irregular intervals. For his work he always showed a genuine love. After getting a first class in Part I of the Natural Sciences Tripos in 1912, he took Botany for Part II in 1913. During the last year or so before the final examination, perhaps he scattered his interest too much to be good for his botany and he rather neglected some parts of the subject, so that though he got a first-class he did not get it too easily. After his Tripos he was awarded the Frank Smart studentship in botany and migrated to Caius.

His favourite subjects in botany were ecology and taxonomy, but his interests were very wide and he definitely refused after his Tripos to confine himself to one line of research. During the long vacation of 1913 he carried out (with help from several others in the laborious work of surveying) the main part of an investigation of the vegetation of the salt marsh and sand dunes at Holme just north of Hunstanton in Norfolk. This work he continued at intervals till the summer of 1914, and the results were published in " The maritime ecology of Holme-next-the-Sea, Norfolk " (Journal of Ecology, June, 1915). For the lines on which it was conceived this is an admirable and admirably executed piece of work, bringing out very clearly certain of the edaphic relations of the salt marsh vegetation. In the long vacation of 1913, Marsh also carried out a small investigation on Cycad anatomy, " Notes on the anatomy of

Stangeria paradoxa " (New Phytologist, Jan., 1914). A large part of the winter of 1913-14 he devoted to investigating the anatomy of some xerophilous ferns and the results of this work were critically presented in " The anatomy of some xerophilous species of *Cheil. anthes* and *Pellœa* " (Annals of Botany, October, 1914).

Stimulated by the sudden appearance of *Azolla* in large quantities in a ditch by Jesus Close, he also at this time put together a summary of the curious sporadic occurrences of the two species of this plant in Western Europe—" The history of the occurrence of *Azolla* in the British Isles and in Europe generally " (Proceedings of the Cambridge Philosophical Society, February, 1914). All his papers are marked not only by sound critical ability, but by a certain distinction of style. One of his friends says " his precision in the use of language was a constant spur to a careless person like myself."

Perhaps Marsh's most promising work was his attack upon the conditions of competition between two closely allied species naturally inhabiting different types of soil, when grown in competition under controlled conditions on the two soils. The experiments he devised were already bringing good results when he left Cambridge to join the army.

In the spring and summer of 1914, Marsh was carrying on this work, finishing his Holme paper and collecting material for some research on the Ranales that he had in view. At midsummer several of us spent a fortnight or three weeks in Provence, for the study of the vegetation between Marseilles and the Maritime Alps. Marsh was of the party and revelled in his introduction to the vegetation of so distinct a climate and in his first glimpse of the high alpines. On his return to Cambridge, he demonstrated, as he had done the summer before, for Dr. Moss's field classes. For some time previously he had demonstrated in the elementary botany and elementary biology practical classes.

We were all rather dazed by the outbreak of war early in August and I remember Marsh reading Treitschke and trying hard to get the German standpoint. As un-militarist by nature as he could be, he evidently did some hard thinking away from Cambridge during September and when he came up in mid-October he at once joined the O.T.C. and put in every afternoon at the preliminary training. At the end of the month or early in November he applied for an infantry commission and in less than three weeks was given a commission as second lieutenant in the 8th battalion

of the Somerset Light Infantry. I well remember his excitement when he showed me the letter—he was like a girl with the invitation to her first ball. He joined his regiment within a week, and put in ten months of hard training before going to the front. I saw him only three or four times during that period; generally when he got leave for a day or two he came over to Cambridge and stayed at my house. In March he told me he was beginning to feel his feet, and indeed it was quite evident from his talk that he was getting a real grip of the work and of his men : in April he got his lieutenancy. His battalion went to France early in September and his first letter to me after that was about half full of the botany of the region where they were in billets. At Loos, his battalion was in support and was heavily shelled and sniped during the German counter attacks. The casualties were heavy, especially among the officers. As Marsh expressed it in a wonderfully vivid and very characteristic letter to another friend, " We were told that once we got the Germans on the run it would be all right, but they had the audacity to counter-attack ! . . . The high explosives dazed the men and the snipers slaughtered the officers." And then—after giving a (for him) quite exceptional glimpse of the after effect on his mind of the scenes he saw at Loos—he breaks off : " If you are fond of *Antirrhinum orontium*, this is the country for it." He promised to tell me all about Loos the first time he came home on leave, but that was never to be. Marsh's own company, " A," got off fairly lightly, and he escaped unscathed, but so heavy were the officer casualties that Marsh got his captaincy immediately, and commanded the battalion when it was soon afterwards inspected by the King. Then came the regular routine of alternating trenches and billets till January, when, just before he was to come home on leave, he was killed.

Marsh was, I believe, just beginning to find himself mentally when he joined the army, and it is impossible to say what he would have done if he had lived to return to botany, as he certainly would. I should not describe the work he actually did as " brilliant" though it was distinguished in style and of very excellent quality. He was very young—only 22 when he got his commission. His talents were certainly remarkable and his love for his subject most undoubted. I fancy his experience in the army was having a great effect on his character, which would have been evident when he returned to scientific work. I am sure he felt that here was a very serious job and though it might be, at any rate at first, an uncon-

genial job, it was up to him to make good in it. He certainly did make good. His brains and his underlying grit told, for all that he was a peace-loving student by nature and inclination. Though far from being "typically English" in mentality and tastes, he had some of the best English qualities—modesty, reticence, humour, pluck, and gaiety under trying conditions. One of his friends says that during the training, Marsh gave him the impression of acting from a sense of duty and of never being really keen on the work, though he did not confess anything of the kind. It may have been so: he did not give me that impression, but simply that of a man who put all of himself, as a man should, into the job he had taken up. His humour stood him in very good stead. "He was so cheerful—everything was always a joke" writes one of his brother officers. He evidently had a real hold on his fellows in the army. "I've never known a captain so much liked by his men" says one: "Nearly all the men spoke of him in their letters" written just after his death. And his servant wrote: "He was not only respected, but loved." He had the same hold on those who knew him well at Cambridge, and, quite apart from his scientific promise, his loss is very bitter to those who loved him.

A.G.T.

CARBON ASSIMILATION.

A Review of Recent Work on the Pigments of the Green Leaf and the Processes connected with them.

By Ingvar Jörgensen and Walter Stiles.

(Continued from p. 23).

F. Variations in the Quantity of the Leaf Pigments in Different Plants and under Different Conditions.

The part of Willstätter's work with which we have dealt so far concerns the characteristics of the pigments, their chemistry, and the methods for extracting them. Willstätter has further devised methods for the quantitative extraction and separation of the four pigments. Here, as in the aspects of the leaf pigments we have already considered, we are in the fortunate position of being able to neglect all earlier work on the subject, for it is now obvious that the methods employed by workers before Willstätter are imperfect and must give erroneous results. Thus, for instance, it is essential to separate the green and yellow pigments in order to obtain quantitative data as to the amount of chlorophyll present; and again other substances liable to be extracted with the pigments,

particularly small quantities of plant acids, will cause considerable alteration in the pigments. These considerations apply equally to the colorimetric and spectroscopic methods of estimation hitherto employed.[1]

Willstätter himself has so far used only colorimetric methods, for it is of no value to make measurements of a high degree of accuracy before possible errors in the extraction and separation of the pigments have been eliminated. When this has been done Willstätter suggests that quantitative spectroscopic analysis may prove very useful as a further refinement in the quantitative estimation of the pigments.

We shall give in some detail Willstätter's latest methods for the quantitative estimation of the pigments, more particularly as Willstätter in his latest publications (1915 b, 1915 c) applies them to plant-physiological work.

It is scarcely necessary to emphasise the extreme importance of obtaining reliable methods for the determination of the quantities of pigments in leaves. While Willstätter's earlier work in this respect, which is published in his book (1913), was mainly done in order to test the validity of his methods, yet he made also some estimations to determine whether there was any regularity in the variations in the quantity of the pigments in leaves, these estimations yielded figures very suggestive with regard to the physiological function of the pigments. Now Willstätter has definitely taken up the plant physiological aspect of this question, but it must not be forgotten that his work in this regard has so far been very limited and undertaken from a purely chemical point of view. It remains for the plant physiologist and ecologist to take up the methods and apply them in their various departments of research.

Of course the technique of these methods is not to be acquired without some trouble and practice, but their employment appears at present the only way to reliable results.

It is not our intention to give an historical survey of the considerations which led Willstätter to the methods he ultimately adopted. They were developed for the purpose of comparing the

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[1] For instance, in reference to a recent paper by Jacobson and Marchlewski (1912) where the authors claim to have shown that climatic conditions play an important part in regard to the production of one or other of the chlorophyll components, Willstätter points out that some of the errors committed by these workers were (1) only a fraction of the chlorophyll present was extracted, (2) an unknown portion of the extracted pigment was precipitated as phæophytin, and (3) only a portion of the phæophytin was isolated.

pigment-content of various extracts and preparations. Thus, if for instance, one should want to compare the amounts of chlorophyll (a + b) in two different crude chlorophyll extracts, the simplest thing to do is to separate the green from the yellow pigments by saponification with alkali, and then to compare colorimetrically the two chlorophyllin solutions so obtained. Further, by the saponification of a solution of known chlorophyll content, which can be used as a standard, it is possible to obtain an approximate value for the chlorophyll content of the two extracts.

If, however, one requires to find the ratio of the two chlorophyll components, their derivatives phytochlorin e and phytorhodin g must be separated from one another and separate comparisons made.

i. METHODS.

We shall describe first a simple method for the quantitative estimation of chlorophyll in a chlorophyll extract, and then the more exact method used for the estimation of the four pigments in fresh leaves.

(a) *Quantitative Estimation of Chlorophyll (a + b) in a Chlorophyll Extract.*

Ten c.c. of an acetone, or alcohol, extract is diluted to 100 c.c. with ether and 10 c.c. of this is poured into a separating funnel and diluted with a further 40 c.c. of ether.

An ether-diluted alcohol extract can be shaken at once with 4 to 5 c.c. methyl alcoholic solution of potash and the brown phase will appear. If the solution contains acetone, however, this must be completely removed from the solution before saponification, as chlorophyllins are destroyed by acetone. In this case a little methyl alcohol is added and the solution washed thoroughly with water.

After reappearance of the green colour, water is slowly added during gentle rotation of the separating funnel and the aqueous chlorophyllin solution is run into a 200 c.c. measuring flask. The ether solution of the yellow pigment is washed with a little more water to effect complete extraction of the chlorophyllins, and the watery layer added to that in the flask. The whole is made up with alcohol to 200 c.c.

The chlorophyllin solution is then estimated in a colorimeter by comparison with the standard solution.

(b) *Quantitative Estimation of Pigments in Fresh Leaves.*

1. *The Extraction of the Pigments.* The chief feature of the quantitative estimation of the pigments in fresh leaves is the primary treatment of them with watery acetone, and subsequent extraction with acetone containing only a low percentage of water.

The first treatment with weak acetone softens the leaves, removes plant acids, inhibits enzyme action, and at the same time removes no chlorophyll.

The details of the method are as follows:—In a mortar, 25 c.c. in diameter, are put 40 grams of fresh leaves with 50 c.c. 40% acetone ; the leaves are quickly mashed with 0·5 gram of quartz sand.   This serves the double purpose of facilitating disintegration of the leaf and of further diluting the leaf substances.

There are now added 100 c.c. 30% acetone, and the whole filtered on a Buchner funnel through a thin layer of talc, which keeps back the slimy protoplasmic matter.

After sucking the residue dry with the pump it is washed with 30% acetone until the filtrate runs off colourless.   The watery acetone is then sucked through.

The leaf substance is now treated with pure acetone and again sucked dry.   This is repeated until the acetone has removed all the pigment.   Complete extraction will require from 400 to 600 c.c. acetone ; towards the end of the extraction 5 to 10% of water is added to the solvent.

As the extract is obtained it is poured, in quantities of 100 to 200 c.c., into 200 to 250 c.c. of ether, and the acetone washed out with distilled water.

Finally the whole of the ether solution is dried with anhydrous sodium sulphate.   The extract is then divided into two equal parts, one of which is used for the determination of the green, the other for the determination of the yellow pigments.

2. *Separation of the Chlorophyll Components.*   The ether solution so obtained from 20 grams fresh leaves is converted into phæophytin by treatment with 0·5c.c. 2 N alcoholic hydrochloric acid in a boiling flask.

The ether is then evaporated off, first in a vacuum in the cold and then at 60⁰ for a short time under very low pressure.   The residue is dissolved in 1 to 2 c.c. of pyridine and heated on a steam bath. While still heated there is added to the pyridine solution 25 to 30 c.c. of boiling, concentrated potash solution in methyl alcohol.   This is

done so rapidly that the boiling of the pyridine solution is not interrupted. The brown phase appears and gives place rapidly to olive-green colour.

A vertical condenser is now fitted to the flask which is heated for two minutes on the steam bath. 5 c.c. water are added through the condenser and the boiling continued for another 1 to $1\frac{1}{2}$ minutes.

The flask is now cooled under the tap and its contents transferred to a 500 c.c. separating funnel with water and some ether. The liquid is then acidified with 20% hydrochloric acid, upon which the colour changes to a dull grey-green. 200 c.c. of ether are added and the funnel shaken strongly for some minutes. A little ammonia is added to the dull coloured, watery layer which is shaken up with small quantities of ether until the last is no longer coloured.

The mother liquor is made alkaline with ammonia on account of the flocks which separate out, and then acidified again in order that the ether may remove only a small quantity of the derivatives.

If the flocks are again washed with ammonia and little pigment thereby goes into solution, no phytorhodin has been destroyed in the saponification ; otherwise this has taken too long a time.

Before fractionation of the two chlorophyll derivatives the accompanying substances must be removed.

To achieve this the combined ether solutions are extracted 2 or 3 times with 30 c.c. of 12% hydrochloric acid and then with 10 to 15 c.c. of 20% acid till the acid layer finally separates in a colourless state. Further flocks form at the boundary layer, but they usually contain no green pigment. Besides the carotin, the ether also contains brown coloured substances.

The combined acid extracts are transferred to a 500 c.c. separating funnel with 200 c.c. of ether. They are neutralised with concentrated ammonia during gentle rotation until the watery layer is dull blue-violet in colour. The well-stoppered separating funnel is cooled under the tap and at the same time shaken first gently and then strongly. The watery layer is then usually pale blue and is run into a second separating funnel. By this method of neutralisation the last trace of the derivatives is brought into the ether.

The ethereal solution of derivatives is freed from methyl alcohol and pyridine by washing with 200 c.c. of water three times, with 1 to 2 c.c. of 3% hydrochloric acid added, as pure water would remove phytochlorin.

The phytochlorin is now separated by shaking 4 or 5 times with 3% hydrochloric acid, 400 c.c. being used altogether, and then several times with 5% acid until this is only feebly green. The extracts with the stronger acid require to be fractioned. This is effected by neutralising and extracting with 30 c.c. of ether, and then repeatedly extracting the ether with 3% hydrochloric acid until the volume of all the 3% hydrochloric acid extracts is brought up to 500 c.c. This constitutes the phytochlorin solution. The remaining liquid contains the phytorhodin which is extracted 4 or 5 times with 12% hydrochloric acid until the ether remains only slightly reddish.

3. *The Preparation of the Xanthophyll and Carotin Solutions.* The ether extract from 20 grams of fresh leaves is saponified with 2 c.c. of concentrated potash solution in methyl alcohol, the mixture being strongly shaken, first by hand and then for half-an-hour on a mechanical shaker. The liquid is then allowed to stand for some time and if it still fluoresces red it is skaken again and, if necessary, more potash is added. When the saponification is complete the solution is transferred to a small separating funnel and shaken with ether. A further 30 c.c. of ether is then added to the syrupy chlorophyllin salts. After shaking with water the upper ethereal layer contains the yellow pigments, the water the chlorophyllin salts. (Cf. Section E, experiment 6). After separation of the two layers, the watery layer may be again shaken with ether in order to determine whether the whole of the yellow pigments have been extracted.

The two yellow pigments are then separated according to the principle used in experiment 7. The ether solution is washed with water and methyl alcoholic potash to remove traces of chlorophyllins and small quantities of brown, acid, organic substances, and then twice more with distilled water. The ether is then evaporated at ordinary temperature under reduced pressure to a few c.c., and then transferred to 80 c.c. of petrol ether in a separating funnel.

From this the xanthophyll is extracted by repeated extractions with methyl alcohol as follows. (1) 100 c.c. 85%, (2) 100 c.c. 90%, (3) two extractions with 92%. If the last extraction is not colourless, further additions of 92% methyl alcohol are made.

In this way the xanthophyll in the methyl alcohol is separated from the carotin which remains in the petrol ether.

The xanthophyll is transferred to 130 c.c. of ether, by adding the latter to the methyl alcohol followed by slow addition of water and

subsequent separation of the watery methyl alcohol layer. The methyl alcohol is completely removed by further washings with water. The xanthophyll solution is then passed through a filter into a 100 c.c. measuring flask.

The solution is then cleared with a few drops of absolute alcohol and ether added up to the 100 c.c. mark.

The petrol ether solution of carotin is similarly washed, cleared and made up to 100 c.c.

4. *The Standard Solutions. The Chlorophyll Components.* The standard solution of the chlorophyll components are prepared by the saponification of a mixture of the methyl phæophorbides as follows :

0·0369 grams Methyl phæophorbide a (half hydrate)
$$(12 \times 10^{-5} \text{ gm.-mols. per litre}) ;$$

0·0124 grams Methyl phæophorbide b (water-free)
$$(4 \times 10^{-5} \text{ gm.-mols. per litre}).$$

The mixture is dissolved in 2 c.c. of pyridine and saponified with 35% methyl alcoholic potash in the manner described above, under (2), only as pure substances are used it is unnecessary to go through the treatment for purification with 12 and 20% acid. This gives about 500 c.c. phytochlorin e in ether saturated 3% acid, and of phytorhodin g in 12% acid.

These solutions can be kept for about a week, but after a longer time the colours change somewhat. In such a case the comparisons are better if the experimental solution is allowed to stand for a day.

The Yellow Pigments. The carotin solution is made up with petrol ether and the xanthophyll with ether, as follows :

0·0134 grams carotin in 500 c.c. petrol ether containing a little ether (5 × 10^{-5} gm.-mols. per litre).

0·0142 grams xanthophyll in 500 c.c. ether (5 × 10^{-5} gm.-mols. per litre).

The carotin solution can be kept in a well stoppered bottle in the dark for at least three weeks. The xanthophyll solution must be made fresh every day as it bleaches quickly, perhaps on account of impurities in the ether.

Instead of using solutions of the actual pigment it is possible to employ a solution of potassium dichromate as a standard solution. Willstätter gives the following thicknesses of aqueous potassium

dichromate solution and of the yellow pigments as corresponding to one another in colour intensity.

Carotin. 0·0286 grams per litre.				Potassium dichromate solution, 2 grams per litre.
100 mm.	...	...	... ...	101 mm.
50 mm.	...	...	... ...	41 mm.
25 mm.	...	...	... ...	19 mm.

Xanthophyll. 0·0284 grams per litre.				Potassium dichromate solution, 2 grams per litre.
100 mm.	...	...	... ...	72 mm.
50 mm.	...	...	... ...	27 mm.
25 mm.	...	...	... ...	14 mm.

ii. Results.

1. *The Total Content of Green and Yellow Pigments.*

As far as his observations go, Willstätter finds the chlorophyll content of leaves varies from 0·6% to 1·2% of the total dry weight, the greater number of leaves contain about 0·8% of chlorophyll of which three-quarters is chlorophyll a and one quarter chlorophyll b.   Much bigger variations were observed between leaves from the same plant than between the mean contents of leaves from different plants.   Shade leaves were found to be much richer in chlorophyll than sun leaves in proportion to the dry weight, but not in proportion to the leaf surface; for shade leaves, as is well known, are often very thin.

The total content of the yellow pigments (xanthophyll and carotin) was found to vary in different leaves from 0·1% to 0·2% of the dry weight, the xanthophyll contributing from 0·07% to 0·12%, the carotin from 0·03 to 0·08%.

Shade leaves were not found to contain a higher percentage of yellow pigments corresponding to their higher content of chlorophyll.

Leaves collected at different hours of the day were examined ; the time of day was found to be without influence on the chlorophyll content or the ratio of the pigments.   As this is of importance in regard to the function of the pigments in the processes of carbon assimilation we may quote here two tables taken from Willstätter's book to show how slight this variation is.

TABLE I.

Grams of Pigment in 1 kilo. dried leaves at different times of day.

Species.	Chlorophyll.		Yellow Pigments.	
	4 a.m.	5 p.m.	4 a.m.	5 p.m.
Sambucus nigra	8·49	8·30	1·48	1·57
Aesculus hippocastanum ...	9·58	8.75	2·07	1·91
Platanus acerifolia	6·82	6·21	1·06	1·35

TABLE II.

Ratio of Pigments in leaves at different times of day.

Species.	$\dfrac{\text{Chlorophyll a}}{\text{Chlorophyll b}}$		$\dfrac{\text{Carotin}}{\text{Xanthophyll}}$	
	4 a.m.	5. p.m.	4 a.m.	5 p.m.
Sambucus nigra	2·77	2·85	0·621	0·512
Aesculus hippocastanum ...	2·89	2·82	0·699	0·699
Platanus acerifolia	3·52	3·34	0·478	0·500

2. *Variations in the Proportions of the Two Chlorophyll Components.*

Willstätter found that the composition of chlorophyll present in different plants, and in sun and shade leaves of the same plant is approximately, though not exactly, constant.

The mean ratio of $\dfrac{\text{chlorophyll a}}{\text{chlorophyll b}}$ is 2·85, the greatest difference from the mean ·7 to ·8.

The variations appear to be produced by the conditions under which the leaves are growing. Thus it seems that some plants are ill suited for growth in the shade. Leaves of Sambucus for example, living in the shade, show abnormal chlorophyll relations, whereas real shade plants such as the Beech exhibit a normal chlorophyll content.

On the whole, shade leaves contain relatively less chlorophyll a than do normal leaves. For if shade leaves are excluded the average ratio of $\dfrac{\text{chlorophyll a}}{\text{chlorophyll b}}$ is 2·93 with extreme variations from this mean of ·5 to ·6.

Shade leaves give an average ratio of $\dfrac{\text{chlorophyll a}}{\text{chlorophyll b}}$ of 2·61 ± ·55, a number appreciably smaller than the mean for normal leaves.

The time of day is found to have no influence on the ratio of pigments.

3.   *Variations in the Proportions of the Two Yellow Pigments.*

The mean value of the ratio of carotin to xanthophyll was found by Willstätter to be 0·546 ± 0·15 to 0·2.

In the case of the yellow pigments, shade leaves show a wider divergence from the normal than they do in regard to chlorophyll. Thus normal leaves give an average ratio of $\frac{carotin}{xanthophyll}$ of 0·603 ± 0·1 corresponding to a molecular ratio of 1 : 1·5 to 2.   In shade leaves on the other hand, the average ratio of carotin to xanthophyll was as low as 0·421 ± 0·1.

4.   *Relation between quantities of Green and Yellow Pigments.*

The average molecular ratio of the total amount of green to the total amount of yellow pigment is 3·56, varying from 3·07 in the case of sun leaves to 4·68 in the case of shade leaves.

It will be observed that in the case of shade leaves the ratio of quantity of green to quantity of yellow pigments is raised.   An exception to this rule was found in the Plantain.   On the other hand in leaves well suited for growth in the shade values as high as 6 have been obtained for the ratio of green to yellow pigments.

It has been shewn above that in shade leaves the amount of chlorophyll a is raised in relation to that of chlorophyll b, while of the yellow pigments it is the xanthophyll which is relatively more abundant in these leaves: that is, of the green pigments the one poorer in oxygen is increased in amount, of the yellow pigments, the one richer in oxygen.

No simple relation could, however, be found between the ratios $\frac{chlorophyll\ a}{chlorophyll\ b}$ and $\frac{carotin}{xanthophyll.}$

G.   Final Remarks.

In the preceding pages we have endeavoured to give the outlines of the work of Willstätter and his co-workers, and we have emphasised the value of this work as one of the most brilliant researches in organic chemistry.   There has been in the past, and in spite of Willstätter's work there will probably be in the future, much loosely performed work on chlorophyll and involving the use of chlorophyll, so that it may be well to emphasise the criteria of purity of this substance that are now available.

These criteria are as follows :

1.   The ash should consist of pure magnesium oxide, and should weigh 4·5% of the weight of chlorophyll used.

2. The phytol content should be one-third of the molecule.

3. The chlorophyll must contain no yellow pigments. On saponification as described in experiment 6, the ether layer must remain colourless.

4. By saponification with alkali, the brown phase must appear (experiment 4) showing that the chlorophyll is not allomerised.

5. Phytochlorin e and phytorhodin g must be given as dissociation products (experiment 8).

6. In solution the chlorophyll must give the same spectrum as leaf extracts (showing there is no phæophytin present which would give absorption bands before the line E and between the lines E and F).

We have earlier referred to Étard's work in which the existence of a huge number of chlorophyll substances is asserted. There has now recently appeared a paper by Albert and Alexandre Mary (1915) in which the authors claim to have synthesised chlorophyll from nitrous oxide and aniline. It is indeed surprising that these workers, as the result of the synthesis of a substance with a green colour and a complex absorption spectrum, should put forward conclusions so completely at variance with Willstätter's work. But perhaps these authors have as much justification for their conclusions as Ewart (1915) who from the observation of a substance with a yellow colour and a simple absorption spectrum possessed by hundreds of substances, deduces the presence of xanthophyll in his preparations. It is perhaps significant that the " pure xanthophyll " extracted by Ewart should have properties different from Willstätter's.

The conclusions of Albert and Alexandre Mary and of Ewart have perhaps as sound a basis as that of Wager (1914), who is of opinion that *chlorophyll* is an auto-oxidisable substance which " in fact could replace pyrogallol in the quantitative estimation of the oxygen in the air." The simple phase test described in experiment 4 in section E of this chapter would have shown in this author's chlorophyll, the presence of the yellow pigments, which are of course autoxidisable (cf. experiment 6).

It may be well here to point out that Willstätter's researches only confirm the observations of the English physicist G. G. Stokes, whose work is mentioned by Willstätter with much respect. A few quotations from Stokes' work will show how near he came to the truth. In a paper published in the Proceedings of the Royal Society of London for 1864 he writes, " I find the chlorophyll of

land plants to be a mixture of four substances, two green and two yellow, all possessing highly distinctive optical properties. The green substances yield solutions exhibiting a strong red fluorescence, the yellow substances do not. The four substances are soluble in the same solvents and three of them are extremely easily decomposed by acids or even acid salts, such as bis-oxalate of potash, but by proper treatment each may be obtained in a state of very approximate isolation so far at least as coloured substances are concerned."

Although it is a matter of national pride that the discovery of the four leaf pigments should have been made by a British worker, yet on the other hand the almost complete neglect with which later investigators in this country have treated Stokes' work is certainly very discreditable. When the obsession for demonstrating the presence of formaldehyde in the leaf (started by Baeyer's hypothesis in 1870, and first 'experimentally' investigated by Pollacci in 1902), began in this country with the work of Usher and Priestley in 1906, these writers neglected the presence of *two* green pigments and completely left out of consideration the yellow pigments in their theory of carbon assimilation. How much of the recent inconsequent work on the same subject might have been avoided if all these later writers had been aware of, and taken notice of, the work of Stokes.

Again, the extraction and separation of the pigments without the aid of chemical action is due to Stokes. In a paper in the Journal of the Chemical Society for 1864, he says " For convenience and rapidity of manipulation, especially in the examination of very minute quantities, there is no method of separation equal to that of partition between solvents which separate after agitation. Bisulphide of carbon in conjunction with alcohol enabled the lecturer to disentangle the coloured substances which are mixed together in the green colouring matter of leaves."

The use of nettle leaves for extraction of chlorophyll was also recommended by Stokes in a paper published in Transactions of the Royal Society in 1852.

Considering that these observations were only side issues of Stokes' work, it is very remarkable that they should have been so correct. There can be no doubt that he did a great deal more work on chlorophyll than appears from his published work. He announced his intention of publishing work on chlorophyll, but it never appeared, and apparently nothing has so far been found among his papers referring in detail to these investigations.

(To be continued).
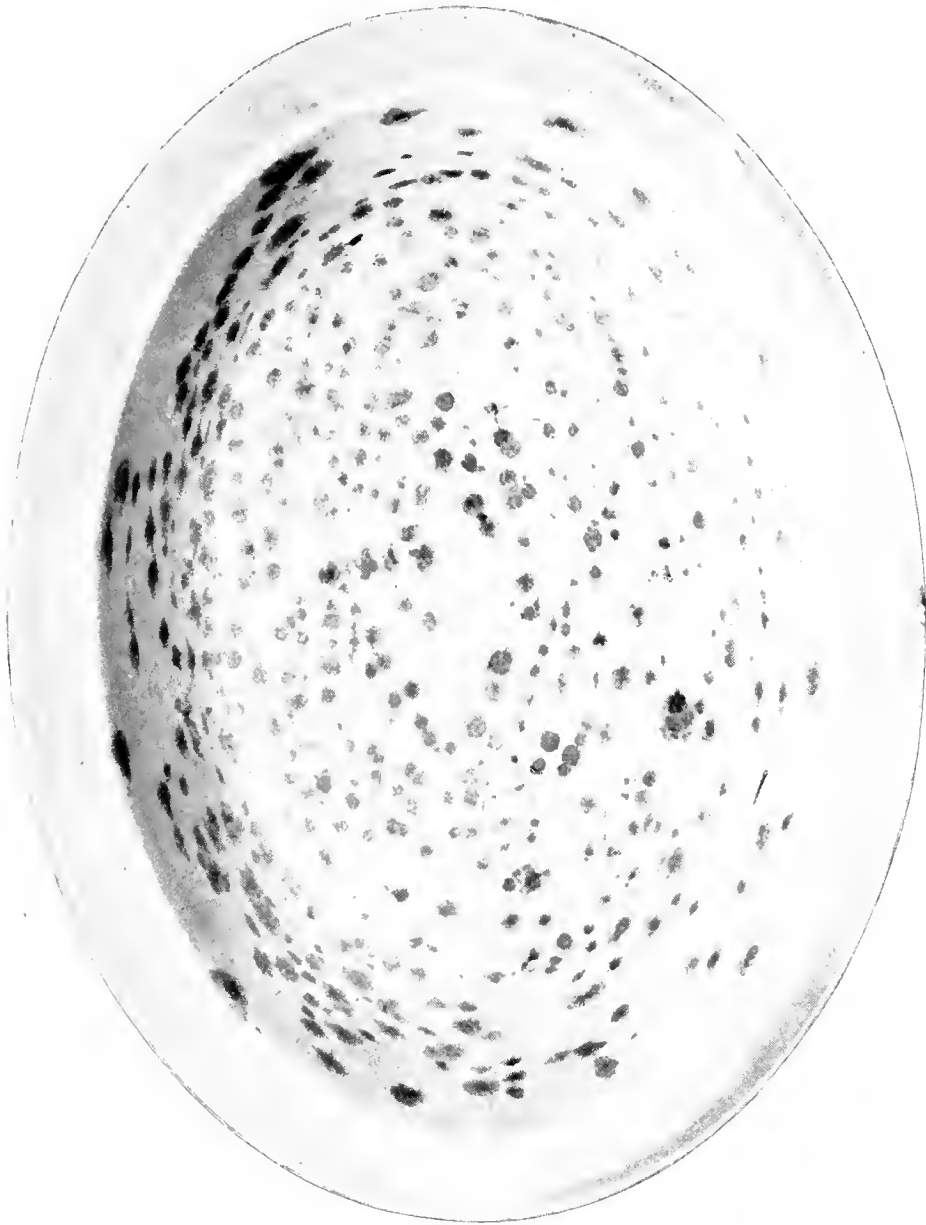
ACTON—A NEW PENETRATING ALGA.

ON A NEW PENETRATING ALGA.

By Elizabeth Acton, M.Sc.

[With Plate 1 and Two Figures in the Text].

THE alga here described appeared in an ordinary white pie-dish which had been standing for some years in the laboratory. The dish contained "*Cladophora* balls" collected a few years previously from Loch Kildona in South Uist.

Pale green patches suddenly began to form on the sides and floor of the dish. These increased gradually in size until the dish was nearly covered by them, but they have not increased in number since they first appeared. See Plate 1.

It was impossible to remove these patches by rubbing or scraping the surface of the dish, so that they were evidently growing underneath the glaze. In order to examine the alga, it was necessary to chip minute fragments of glaze from the surface of the dish and mount these on a slide. In this way it was possible to ascertain that a small filamentous green alga was growing between the glaze and porcelain of the dish, and by its growth, was separating the one from the other. The concentric zoning of the patches shewed that branches were extending radially parallel to the surface, but no branches were seen to penetrate either the porcelain or the glaze.

It will be obvious that fragments of a thallus obtained in this way were not sufficient for a complete investigation. No other dish in the laboratory shewed traces of this alga, and as the dish in question had contained only "*Cladophora* balls," these were examined to see if they contained a similar alga.

It was found that many of the dead cells of the *Cladophora* were covered by a small alga.[1] This was compared with the alga

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[1] *Cladophora (Aegagropila) holsatica* (the alga forming the balls) has very thick lamellose walls which persist for some time after the death of the cell in an apparently unchanged condition.

in the dish and was found to be identical with it. Text-fig. 1
shews portions of the thallus taken from the dish and from the
" *Cladophora* balls."

B   A
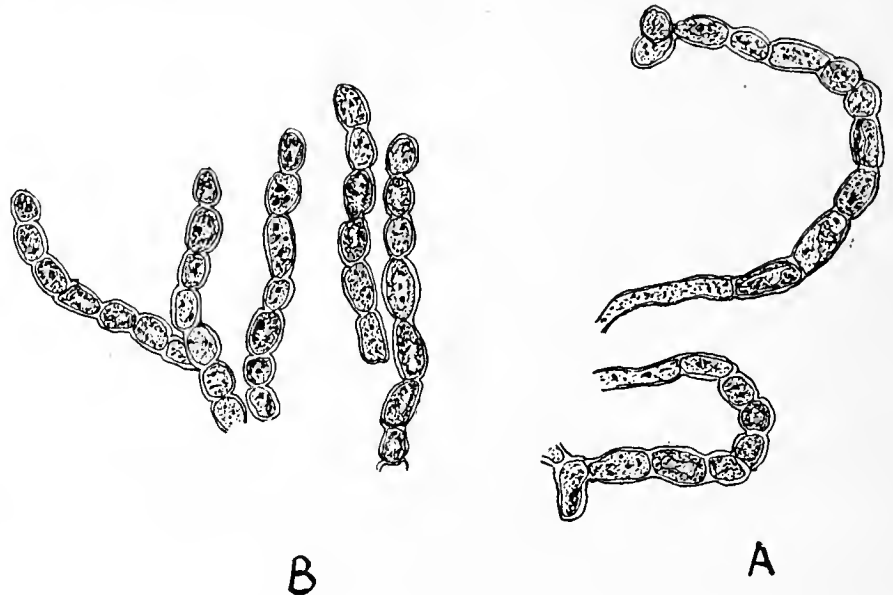
TEXT-FIG. 1. *A*, portion of thallus from pie-dish. *B*, portion of thallus
rom " *Cladophora* balls." × 370.

In the " *Cladophora* ball" the appearance of the alga seems to
vary according to the extent of decomposition of the old cell-wall.
A young thallus which has not completely covered the cell on
which it is growing usually shews numerous lateral branches given
off dichotomously and close together as in Text-fig. 2, D. As the
thallus grows the branches become closely crowded, the cells
become more regular and the original portions of the thallus often
die away. It then appears to consist of almost parallel filaments
of short, barrel-shaped cells running lengthways in the wall and
partially embedded in it. Owing to the death of the younger
portions of the thallus these filaments become disconnected. See
Text-fig. 1, B. Frequently the alga obtains entrance to the interior
of a cell and there develops irregularly as in Text-fig. 2, A.

As decomposition advances in the cell-wall, the latter breaks
up into pieces and many of the cells embedded in its surface die away.
Some of the cells which remain, however, put out branches which
penetrate deeply into the wall. The cells of these branches are
extremely long and thin and appear finally to lose their chlorophyll
(Text-fig. 2, B). It is possible that these penetrating branches act
as rhizoids which absorb nourishment from the decaying wall. The
surface cells connected with them are often large, with dense cell-
contents and probably separate eventually from the penetrating
branches and function as reproductive bodies. Text-fig. 2, C shews

a row of cells, some of which have begun to put out penetrating branches.
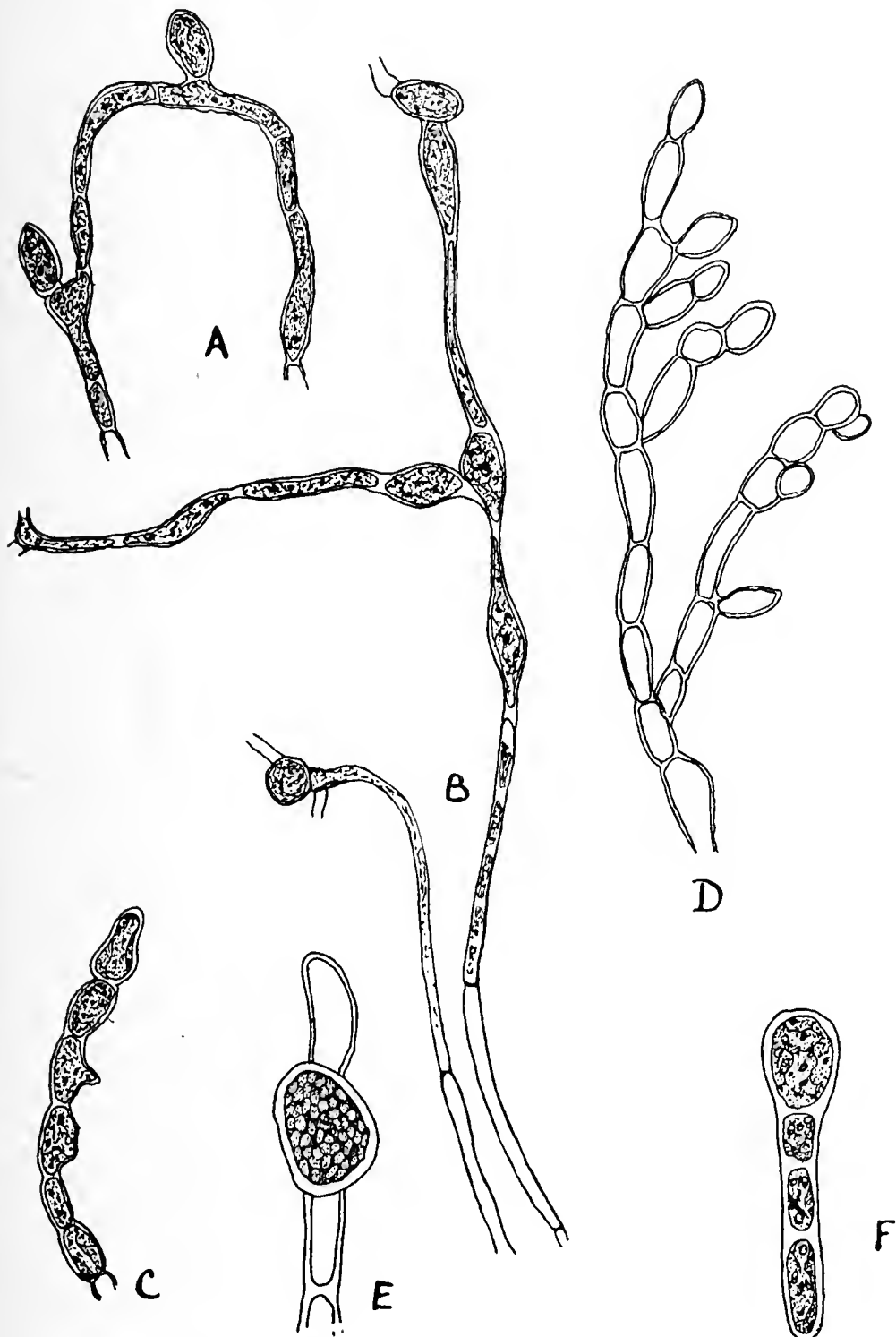
Text-fig. 2.   *A*, shews irregular branching of thallus ; *B*, portion of thallus shewing long, thin penetrating branches;   *C*, filament with cells beginning to put out penetrating branches ;   *D*, shews method of branching and shape of cells ;   *E*, resting spore crowded with starch grains ;   *F*, resting spore germinating.   A, B, C and D, × 540.   E and F, × 860.

The surface cells are from 8 to 10μ broad and 10 to 16μ long except when they are irregular in shape.   The penetrating cells may reach a length of 110μ and are only 2μ wide.

There is an irregular, lobed, parietal chloroplast and starch grains are nearly always present. They are often so abundant that they completely fill the cell (Text-fig. 2, E). The cells are multinucleate, 1 to 6 nuclei being present in a cell.

MULTIPLICATION.

Multiplication takes place by cells which become detached from the thallus. These may be either intercalary or terminal. Frequently the end cell appears to break away and germinate without a resting stage; or intercalary cells may round themselves off and develop a thick cell-wall as in Text-fig 2, E. Text-fig. 2, F shews a similar spore germinating.

No motile method of reproduction has been observed. Yet it is evident that such a state can exist, for the alga must have entered the cracks in the glaze of the pie-dish by means of minute motile spores. External conditions must have arisen which caused the formation of a large number of motile reproductive bodies, but no trace of these could be found.

SYSTEMATIC POSITION.

In discussing the systematic position of this alga, it will only be necessary to consider the chlorophyll-green penetrating algæ which have previously been described, for it is undoubtedly a member of the Chlorophyceæ.

Among these there are three genera—*Gomontia, Tellamia* and *Foreliella*—to which this alga bears some resemblance.

In 1889, Bornet and Flahault[1] founded the genus *Gomontia* to contain a shell-boring alga which they named *Gomontia polyrhiza.* The reproductive stage of this plant had already been described by Lagerheim[2] as a new species under the name *Codiolum polyrhizum.*

In 1895, Batters[3] described two shell-boring algæ which he placed in a new genus *Tellamia,* and in 1898, Chodat[4] founded a new genus *Foreliella* to contain his new species *Foreliella perforans* which penetrates the shell of the fresh-water mussel.

These three genera have many points in common, but *Tellamia* seems to be separated from the other two and from the alga under consideration by the form of its thallus.

[1] Bornet et Flahault. " Sur quelques plantes vivant dans le teste calcaire des mollusques." Bull. Soc. Bot. France, T. xxxvi, 1889.

[2] Lagerheim. " Ett Bidrag till Kanned om Slagtet Codiolum A. Br." Oefvers. af Kongl. Vet. Akad. Forhandl., 1885.

[3] Batters. " On some new British Marine Algæ." Ann. of Bot., 1895.

[4] Chodat. " Etudes de Biologie Lacustre." Bull. del'Herbier Boissier, June, 1898.

Two species *T. contorta* and *T. intricata* were described, both of which penetrate the periostracum of mollusc shells. The thallus is a flat expansion not bearing special penetrating branches. Batters himself places the genus near *Endoderma*. It is difficult to see why the genus *Tellamia* was formed, for there seems to be no reasonable objection to placing both *T. contorta* and *T. intricata* in the genus *Endoderma* which already existed.

The alga under consideration resembles very closely both *Foreliella perforans* and *Gomontia polyrhiza*. The question there-fore arises as to what characters separate these two genera. According to Chodat, who is responsible for this genus, *Foreliella* only differs from *Gomontia* in three points :—the chromatophore is different; the cell is uninucleate and the thallus is more markedly perforating. He does not state how the chromatophores differ, and as far as can be ascertained there is no important difference. The fact that the cells are multinucleate in *Gomontia* and uninucleate in *Foreliella* is not sufficient to separate the two genera, nor is the fact that the thallus is more markedly perforating in the one case than in the other. This latter fact is probably largely due to circumstance. The shell of *Anodon* is very soft compared with the shells perforated by *Gomontia* and therefore offers less resistance to the passage of the penetrating branches.

There is therefore no valid reason for separating *Foreliella* from *Gomontia*, and *Foreliella perforans* should be known as *Gomontia perforans*.

The new alga is evidently a species of *Gomontia*. The alga is perforating, the thallus consists of filaments radiating from a central point, and these branch dichotomously giving off in addition penetrating branches. The cells have a parietal, lobed chloroplast, 1–6 nuclei, and they are crowded with starch grains. All these facts are characteristic of *Gomontia*.

It differs slightly from the species *G. polyrhiza* in size and also in the shape of its cells. The terminal cells in *G. polyrhiza* are usually club-shaped, swollen at the end. In the new species they frequently taper towards the end.

Chodat[1] mentions a species *G. Manxiana*. I have not been able to find any description or any other mention of this species, so that it is impossible to discuss it here.

Collins[2] in 1897 described a fresh-water species *G. Holdenii*

[1] Chodat. "Algues vertes de la Suisse." p. 61.

[2] F. S. Collins. "Some perforating and other Algæ on fresh-water shells." Erythea, Vol. V, No. 9, 1897.

from the old shells of *Unio*. In this species the terminal cells taper at the end, but it is a very much larger species than the one under discussion, the cells sometimes reaching a diameter of 50μ.

The species from the "*Cladophora* balls" has therefore been characterised as follows.

GOMONTIA ÆGAGROPILÆ SP. NOV.

Thallo e filamentis a centro radiantibus, dichotomis atque ramulos tenues penetrantes emittentibus constante ; cellulis ramulorum apicalibus cylindricis vel attenuatis ; cellula unaquaque chromatophora lobata parietali prædita, nucleis 1–6, amyli granis plurimis.

Propagatio cellulis apicalibus vel intercalaribus a thallo segregatis, quæ aut statim germinant aut cystæ perdurantes crasse tunicatæ fiunt. Zoogonidia non visa.

Long. cell. 10–16μ (cell. penetr. usque ad 110μ); lat. cell. 8–10μ.

Hab. in parietibus emortuarum cellularum *Cladophoræ* (*Ægagropilæ*) *holsaticæ*, Loch Kildona, S. Uist, Hebridum Exteriorum.

The Botanical Laboratory,

The University, Birmingham.

DESCRIPTION OF PLATE I,

Illustrating Miss Acton's Paper on "A New Penetrating Alga."

Photograph of interior of pie-dish with thalli of *Gomontia Ægagropilæ* growing between the glaze and the earthenware. (Reduced to a little less than half-size).

THE UTILIZATION OF HERBARIUM MATERIAL.

By R. C. McLean,

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HOW many plants and families of plants remain imperfectly understood, for lack of adequate available material, can be known only to the professed systematist; but among such forms there are many with great promise of interest, and some at least, for instance the Balanopsidaceæ, Anonaceæ or the Lacistemaceæ, which may prove of considerable importance to the general scheme of the Angiosperm classification.

In almost all such cases the only material upon which scientific investigation is or has been possible is dry herbarium material, often of considerable age or indifferent condition of preservation.

Although it has long been customary to practise morphological analysis with such material, by simple soaking in water, either with or without heating, yet the results so obtained are seldom perfectly satisfactory, while the amount which can be learned of systematic affinity by macroscopic analysis of a flower, though valuable in itself, is confessedly incomplete, and has indeed failed, in many notable instances, as an index of affinity. There remain besides very many cases in which more or less dubiety exists, which a more intimate knowledge of the structures involved might well remove.

These considerations have led me to seek a practical method for the restoration of dried vegetable tissues as nearly as possible to their original state, in order that the microtome might be used in their examination.

There are several classic instances of the successful use of herbarium material in anatomical work. Among others, De Bary, Van Tieghem, Radlkofer and Solereder may be cited, while Tunmann (*1*) has illustrated how it may be employed for biochemical purposes, but apart from these fundamental works the method seems to have been neglected, especially since the elaboration of histological technique has produced more exacting requirements in regard to the quality of the material, and the growth of comparative morphology has rendered serial sections indispensable. Even for such purposes, herbarium material may be used; there are few structures too delicate for resuscitation, apart from actual protoplasm, especially if the specimens have been thoroughly and rapidly dried in the first instance. In this connection it may perhaps be allowable to draw the attention of collectors more

emphatically to the need of this care in preservation, since the possibilities of future study are strictly limited by the preliminary treatment. Where dry specimens are easily revived, much time and trouble may profitably be diverted from wet preservation and devoted to the improvement of the drying method.

Two methods which have been previously employed for resuscitation are quoted by Zimmermann (*2*), involving the use of Lactic Acid (*3*) or a mixture of this with Phenol. For some extremely delicate structures the first of these may be of some value, but a somewhat extended trial of it on herbarium specimens of Rhodophyceæ from Harvey's Australian collections has given very unequal results. The cell-walls of tissues which have been for a long time dry—a decade or upwards—appear to acquire a species of *rigor* which it is very difficult to break down. In other words the gel colloids of the walls lose part of their reversibility, and become to a certain extent incapable of reimbibition or at least of hydrosolution.

Where this condition is very pronounced Lactic Acid is powerless to rectify it. Such is markedly the case where there has been mechanical distortion during drying, or a very large percentage of shrinkage in volume.

The admixture of Phenol is a partial but not complete amelioration, and as the relatively slight solubility of this reagent makes it difficult to wash it out of a bulky organ, it has compensating disadvantages where subsequent dehydration is an object.

For the treatment of some algæ, or of objects accidentally dried for short periods, or of very minute objects, the above quoted methods may however be useful, and they lend themselves especially to the making of glycerine jelly mounts.

The method which I wish to describe is adapted to such cases as are not covered by the above methods, namely bulky, hard or resistant structures, or such as have undergone great change of form in drying. At the same time I would emphasise that it is equally applicable to the case of delicate organs, and will, if used with care, give results superior to those obtainable with Lactic Acid.

If the precipitation of the hydrogels which go to form the cell-wall has entered upon a state of quasi-permanence, no acid medium which does not at the same time cause solution will effect the desired change. Alkalis are more effective, and for the purpose in view I have had recourse to the old fashioned Potash.

Two distinct problems arise : (1) the restoration of the original form ; (2) the removal of air which has entered the cells, and is often very tenaciously retained.

The chief material studied was the stem of *Helianthus tuberosus*, which shows a wide range of histological differentiation, supplemented however by floral material from various families, some of which was as much as fifty years old.

Sections of three different thicknesses were laid on filter paper in a desiccator, where they stayed for a month, being for six days kept at a temperature of 100°C., in order to imitate the effect of prolonged air-drying as quickly as possible. After this treatment the sections, slightly curled, showed the brown colour of old herbarium specimens, and did not regain their form in water alone, which is characteristic of material long air-dry. These sections were then differentially treated in order to observe the effect of the factors involved in recovery, which may be catalogued as :—

    a.   Preliminary Treatment.
    b.   Size of Material.
    c.   Concentration of Medium.
    d.   Time.
    e.   Temperature.

To avoid needless detail the most successful treatment will be described shortly under each of the above headings.

a. *Preliminary Treatment.* Place the material directly into Absolute Alcohol, where it should remain for at least 24 hours. If the pieces are very delicate or very small, this is all that is required, but all pieces of appreciable thickness should be subjected to a reduced pressure of below 10 cms. of mercury during their immersion, to ensure proper penetration.

From the Absolute Alcohol bring the material down to distilled water through graded alcohols, as customary in cytological work. This ensures the complete replacement of the alcohol by water throughout the mass, and is very important in providing for the regular commencement of the swelling process.

Once in the water, the pieces may be left there indefinitely. The growth of moulds is prevented and recovery greatly hastened if they are placed in a hot chamber such as a paraffin oven during this period. In this way also much if not all of the brown dis-colouration may be extracted. All the changes from one medium to another must, in point of time, be proportioned to the bulk of the material, but roughly speaking the slower they are the better.

b. *Size of Material.* The thickest sections soak out best and material in bulk better than any sections. For this reason it is best to complete the recovery before proceeding to section, rather than trust to swelling out the latter after cutting.

c. *Concentration of Medium.* An aqueous solution of Potassium Hydrate of 4% to 8% strength. The former gives good results with Thallophytes, but a concentration not less than the latter is advisable for vascular material.

d. *Time.* Transfer the pieces from distilled water to Potash and leave them to soak for 6 to 9 days according to size and the degree of alteration in form which the material has undergone.

A decided improvement has been noticed if the soaking in Potash is allowed to proceed normally for about half the total time given, and thereafter the solution gradually concentrated down to about one-third of its original volume, during the remaining days. This may be advantageously performed under reduced pressure (see below).

e. *Temperature.* A moderate rise in temperature (up to 70°C.) during the preliminary soaking in water is all to the good and *shortens the time in Potash* to some extent. Prolongation of the water soaking also has this effect.

Heating *in* the Potash means ruination to cellulose tissues.

The action of Potash upon the tissues is not perfectly even. Thin walled parenchyma are perfectly restored. Epidermis, collenchyma, sclerenchyma, xylem and xylem parenchyma are equally good. Phloem and cambium are the least satisfactory, although if no great amount of sclerenchyma be present, so that drying does not produce destructive internal strains, these too may be completely restored.

The cognate problem of the removal of air is best resolved by the application of reduced pressure during the soaking in Potash, which may be associated with the gradual concentration of the medium advocated above. It is greatly aided, however, by perfecting the initial penetration with Absolute Alcohol in the manner above indicated.

The last process is neutralization. For this purpose mineral acids should be avoided. It is best accomplished by weak acetic acid (15–20% of Glacial) changed several times, which causes no noticeable solution of the membranes. Wash until the water, after standing with the object in it for several hours, appears neutral to litmus. If the material is very deeply coloured the acetic

acid may be shaken up first with ordinary bleaching powder, the excess of which is removed by filtration.

Dehydration, embedding and sectioning are performed normally. Staining presents slight difficulties. The solvent action of Potash upon the lignin substances affects the reactions which lignified walls should display. They do not, for example, give the familiar red colour with Phloroglucin.

I have not tested a range of stains from this point of view, because I found Fuchsin perfectly satisfactory. An aqueous solution (2%) may be employed in the usual way, the tissues being subsequently differentiated by acid alcohol; or a solution of Fuchsin decolourized by the addition of Sulphurous Acid may be used, which has a specific and direct affinity for lignified tissue. Stain in this solution for 10–15 mins. Wash in tap-water until excess of acid is removed. If more Fuchsin becomes oxidized during the washing out it may be removed by treating the sections with Absolute Alcohol. Counter stain with a saturated solution of Light Green in Clove Oil.

UNIVERSITY COLLEGE,
READING.
October, 16th, 1915.

LITERATURE.

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2. Zimmermann. Botanical Microtechnique. English translation by J. E. Humphrey. New York, 1893.

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DICRANOCHÆTE RENIFORMIS HIERON.,
A FRESHWATER ALGA NEW TO BRITAIN.

By W. J. Hodgetts.

[With One Figure in the Text].

*D*ICRANOCHÆTE, a genus of unicellular setigerous Green Algæ in which the setæ are branched in a characteristic dichotomous manner, was named and first described by Hieronymus[1] in 1887. The species described by him—*D. reniformis*, originally obtained from various localities in the Sudeten Mountains of Silesia—has since been recorded from several other Continental localities, but has hitherto not been found in Britain. The only other species of the genus so far known is *D. britannica* G. S. West,[2] found amongst submerged *Sphagnum*—on which it was probably epiphytic—in boggy pools on the slopes of Glyder Fach, N. Wales, and was figured and described by West in 1912.

The *D. reniformis* described below occurred as an epiphyte on the submerged stems and leaves of *Ranunculus aquatilis* and *Callitriche* sp. taken last April from a small pond at Harborne, near Birmingham. On these aquatic plants the alga was very abundant, always seeming to prefer the bright green younger parts where there was not much competition with other epiphytes; occasionally individuals were seen on filaments of *Œdogonium* which was also present in the pond. Hieronymus obtained his specimens on submerged mosses and liverworts (especially *Hypnum* spp., *Sphagnum* spp. and *Calypogeia Trichomanis*). The alga, when growing upon *Sphagnum* leaves, he observed, always showed a preference for the narrow chlorophyllous cells rather than the wider hyaline porose cells. Further, he never found the alga at a height of less than 500 metres; the Harborne locality, which is at a height of about 168 metres, shows, however, that at least occasionally it may flourish in lowland districts.

D. reniformis (Fig. 1, A, B) is unicellular, but sometimes the cells are united into short rows (as will be described below), the individual cells being circular, ellipsoidal, or more or less reniform in surface view, hemispherical-depressed, and attached by a broad flat base; in size the cells vary within wide limits, their longest

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[1] G. Hieronymus communicated his first account of *Dicranochæte reniformis* to the Botanical section of the Schlesische Gesellschaft on Nov. 10th, 1887; subsequently he published " Ueber *Dicranochæte reniformis* Hieron., eine neue Protococcacea des Süsswassers," Cohn's " Beiträge zur Biol. der Pflanzen," Bd. 5, Heft II, 1890, pp, 351-372, taf. XI, XII.

[2] G. S. West in Journ. of Bot., Vol. 50, 1912. p. 329.
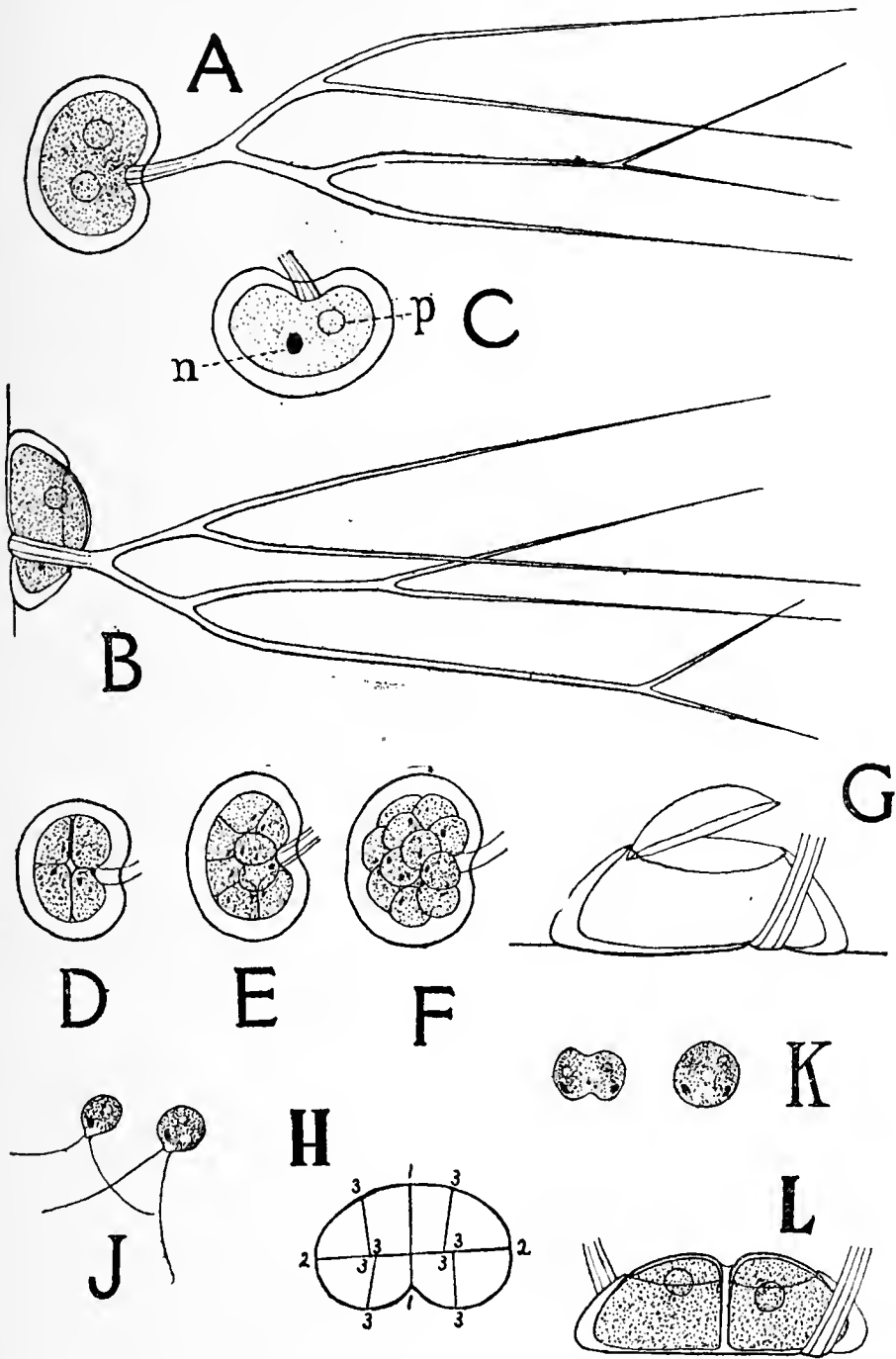
FIG. 1. *Dicranochæte reniformis* Hieron. A, vegetative individual from above, showing two pyrenoids ; the seta is flattened out in one plane. B, side view of adult vegetative individual. C, cell stained with Delafield's hæmatoxylin to show the nucleus *(n)* ; the pyrenoid *(p)* is unstained. D, E, F, zoogonidangia with almost mature zoogonidia, each of the latter showing a pigment-spot. G, dehisced zoogonidangium viewed laterally and showing the dorsal lid which has partly remained attached to the gelatinous sheath. H, diagram (after Hieronymus) showing the directions of the first three successive cleavages of the cell-contents prior to formation of zoogonidia. J, two zoogonidia, each showing an eye-spot and two cilia. K, two zygospores, each showing two conspicuous pigment-spots and several vacuoles. L, two individuals, viewed laterally, which have grown in contact with each other, a common gelatinous sheath being round both.

G × 1000, the others × 600.

diameter being 10-35μ, the height being 6-13μ. Characteristic of the species is the shallow notch or indentation on one side of the cell—which, however, is absent in very young individuals—the seta arising from the base of the cell, passing upwards through the notch, The dichotomously branched seta, however, is the most remarkable feature about the alga. It is often as much as 150μ long, and is composed of a hyaline gelatinous pectose substance, which gives no cellulose reaction and takes up stains only with difficulty. At the base, where it is broadest, a distinct lumen can frequently be seen with a high power, but it contains no visible contents and the long, finely attenuated branches are quite massive, as is sometimes the whole seta. Hieronymus mentions that occasionally the seta is completely unbranched, but such cases were never observed in the Harborne specimens. The number of branches in the fully developed seta varies considerably, Hieronymus figures as many as 14 in one case, but 4–8 was the usual number observed by me, although two very long simple branches are not infrequently found. The whole seta is very flexible and can withstand very rough treatment without being broken off.

The origin of the seta in the young individuals was worked out by Hieronymus, and his account, as far as could be observed, appears to be correct. The zoogonidia, by which the organism is propagated, first become attached by their hyaline anterior ends, but later settle on one side and then become "amœboid," *i.e.*, their outlines become somewhat wavy or irregular. The hyaline anterior end, after losing the two cilia, then grows out as a slender proto-plasmic thread which immediately secretes a tubular gelatinous wall round itself, the cell itself also taking on a membrane; the protoplasmic thread branches several times dichotomously and finally the branches, after attaining their adult size, cease growing, the delicate tubular branches becoming filled up with the gelatinous substance, while the protoplasmic contents gradually retreats back into the cell. The basal part of the seta, as stated above, sometimes retains a visible tubular structure and Hieronymus occasionally observed protoplasmic remains in the lumen by staining with iodine, eosin, etc. Hieronymus also observed occasional individuals— constituting his forma *pleiotricha*—with two or even three or four setæ arising from a corresponding number of indentations at various points round the cell, but so far such cases have not been observed in the present material. He explains these by assuming that the zoogonidia became attached in various irregular ways so

that the anterior end, from which the seta develops, was more or less overgrown by the rest of the cell, the seta thus growing out from some point of the flat basal surface, and at the same time forking near its base while growing between this surface and the substratum, the ultimate branches emerging at different points round the circumference of the cell. This explanation is supported by the fact that the individual setæ in these cases were usually simple or only forked once, at the same time being much shorter than the single seta of a normal individual. The same author also observed typical individuals which entirely lacked a seta, such cases being most frequent in the late summer generations of October. The hypothesis that the setæ serve as organs of protection against the attacks of various small aquatic animals, especially Infusoria, seems *a priori* quite reasonable.

We now come to the description of the cell-membrane in the adult individual. According to Hieronymus the very thin basal wall may sometimes give a cellulose reaction with chlor-zinc-iodine but prolonged treatment with this reagent failed to give it in the present instance. The dome-shaped part of the cell-wall is sharply marked off into two regions, the uppermost half being fairly thin and characterized by the deep red colour it readily takes on when stained with Congo-red, the rest of the wall remaining practically unstained ; this dorsal part is also coloured violet with chlor-zinc-iodine and undoubtedly consists of cellulose. Hieronymus states that in his specimens this part of the wall possessed minute cone-shaped, pointed, cellulose protuberances or tubercles, and these are very conspicuous in his figures, sometimes being arranged in two tolerably regular concentric circles, forming a sort of crown to the cell. In the Harborne material, however, these tubercles could not be found, the rounded dorsal part of the wall being always quite smooth even when examined under the highest powers ; the absence of these protuber-ances is the most conspicuous difference between the English specimens and those figured by Hieronymus. The difference is hardly specific, and indeed Hieronymus states that they were not always present ; perhaps the smooth form ought to be looked upon as a variety—which may be termed var. *lævis*—differing from the type only in this respect.[1] In individuals stained with Congo-red it can easily be seen that the red-stained dorsal part of the wall is seated on the protoplast like a sort of cap, and is not continuous

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[1] *D. reniformis* var. *lævis* :—superiore parte tuberculis orbata ; cætera ut in typo.

with the thin basal wall, which, according to Hieronymus, is also of cellulose, the peripheral region of the cell being surrounded by a thick sheath of gelatinous pectic substance. This gelatinous sheath (Gallertscheide) is practically unstained with Congo-red but very readily takes up such stains as methylene blue, methyl green, safranin and fuchsin, its composition being apparently the same as that of the seta. Especially in the older individuals it may be seen that this sheath overlaps to a certain extent the edges of the cellulose "cap" (cf. Fig. 1, B). The very young plant which arises from a zoogonidium, at first possesses a uniformly thin membrane which does not take on a distinct colour with either Congo-red or chlor-zinc-iodine. In a slightly older individual the convex part of the wall, as sometimes also the flat basal part, may be stained with Congo-red—less distinctly with chlor-zinc-iodine. About this stage a peripheral ring of gelatinous substance is observed, and, according to Hieronymus,[1] this is secreted by the protoplast through a circular split which makes its appearance in the originally continuous cellulose wall, separating the upper dome-shaped portion from the flat base. The gelatinous sheath is thus not derived from the original cellulose wall but is directly secreted by the protoplast. The process is not very easy to follow owing to the difficulty in finding individuals in the right stages of development, the early stages being passed through very rapidly. The sheath grows considerably in thickness and also in a vertical direction, thus pushing the cellulose "cap" further from the base of the cell, the edges of the sheath, as already stated, later overlapping the lower part of the "cap." When stained with safranin or fuchsin the gelatinous sheath shows a distinct radiating fibrillar structure such as is shown by the sheaths of many Conjugatæ.

The bright green chloroplast is single, with the form of an inverted watch-glass, and lies against the upper surface of the cell, where it is most favourably placed for receiving the light. A single conspicuous pyrenoid is usually present in the chloroplast, but the larger individuals have frequently 2 or 3 (rarely 4) pyrenoids. Hieronymus seems to have mistaken small granules of some other nature for pyrenoids, for, speaking of the latter, he says,[2] "bei mancher Individuen werden dieselben sehr zahlreich sodass man bisweilen 50 und mehr davon zählen kann!" He also states that there is no amylaceous envelope (Stärkehülle) to the pyrenoid; but

[1] *loc. cit.,* p. 357.
[2] *loc. cit.,* p. 359.

in fresh material stained with iodine the pyrenoid always gave a very distinct starch reaction so that a starch-sheath appears to be present. In one case when some of the material had stood for some weeks in water many of the cells were found to have lost their pyrenoid and were filled with minute angular starch-grains, but such starch-grains were never observed in the fresh material, although according to Hieronymus, scattered starch-grains are frequently present in old cells. For an elaborate description of the pyrenoid and its reactions to various stains, reference should be made to the above-quoted paper. The cell cavity below the chloroplast is filled with cytoplasm and a single nucleus, which may be demonstrated in alcohol material by staining with Delafield's hæmatoxylin, is present, usually lying close against the flat basal wall (Fig. 1, C).

As already stated, the organism is propagated by zoogonidia, every individual sooner or later becoming a zoogonidangium. The number of swarm-spores produced in each cell varies from 4 to about 30 (? 32), the most frequent numbers being 4, 8 and 16; in the larger individuals the number does not appear to be always a multiple of four; in one case 30 (? 32) were counted by me, this being the largest number observed. Hieronymus states that the contents of a cell about to form zoogonidia, divides by successive divisions, of which the directions of the first three are shown diagrammatically in Fig. 1, H, the later divisions being quite irregular. This appears to be true of the smaller cells which form 4 or 8 zoogonidia (see Fig. 1, D, E), but in the case of larger individuals, numerous cleavages were seen to appear more or less simultaneously at the periphery of the protoplast, gradually extend inwards, meeting internal cleavages and finally dividing the contents into a variable number (not more than 32) of polygonal masses, which do not all lie in the same plane. Hieronymus noted the presence of 2, 4 or 8 nuclei in cells, the chloroplasts of which showed no evidence of division, so that the cleavages of the chloroplast do not appear to proceed along with the division of the nucleus. A few hours before the zoogonidangium dehisces, the swarm-spores round themselves off and each develops a small bright red pigment-spot (see Fig. 1, D, E, F). The zoogonidia are enclosed in a hyaline gelatinous vesicle, a similar but less conspicuous investment being round each individual swarm-spore; owing to the swelling of this vesicle pressure is exerted on the dorsal cellulose "cap" and eventually this is slowly pushed on one side, the vesicle with its contents emerging gradually until it finally lies free in the surrounding

water. The "cap" frequently remains adherent to the gelatinous sheath of the cell at one spot, empty sheaths with the caps attached in this way being quite commonly observed (see Fig. 1, G); frequently, however, the "cap" is pushed right out of the sheath. The vesicle surrounding the swarm-spores gradually dissolves, the latter escaping one by one; they were never seen to swarm in the vesicle. The pyrenoid disappears prior to the formation of zoogonidia and is apparently formed *de novo* in the young plant, since pyrenoids could not be detected in the zoogonidia, although, according to Hieronymus[1] one or several "pyrenoids" are formed in each zoogonidium before liberation from the mother-cell. The liberation of the zoogonidia was always observed to take place in the early morning, and was quite easily seen by mounting *Callitriche* leaves, etc., which were covered with the alga, in water and keeping the slide in a damp chamber to prevent evaporation; many individuals were usually seen to have dehisced and the zoogonidia more or less active in the surrounding water when the preparation was examined early the next morning. Formation of swarm-spores took place freely in April, but during the warm weather which set in about the middle of May their formation was only rarely observed. Apparently spring is a time of active propagation with the alga.

The zoogonidia (Fig. 1, J) are rounded or pear-shaped, uninucleate, 6·25–8·5μ in diameter, biciliate, the cilia being inserted at the somewhat pointed hyaline anterior end where, placed laterally, is the small red pigment-spot. The chloroplast is more or less parietal and contains small starch-grains, but no pyrenoid was observed; one or more vacuoles are often present. The zoogonidia are actively motile only for a few minutes, soon becoming quiescent, settling down and forming new individuals as already described. On one occasion numerous swarm-spores, apparently derived from the *Dicranochæte* were observed to have *four* cilia, but as there was some doubt as to whether they actually did belong to the alga or not, their fate was not observed. However, at another time, in a preparation which contained large numbers of quiescent zoogonidia, undoubtedly of *Dicranochæte*, many of these were seen to be larger than usual (their longest diameter being about 11·3μ) and to possess *two* very conspicuous red pigment-spots (see Fig. 1, K). These had lost their cilia, but were certainly derived by fusion of two of the swarm-spores as they were exactly twice the size of the ordinary

[1] *loc. cit.*, p. 366.

zoogonidium, many of which, in the quiescent state and lacking cilia but with the usual single red pigment-spot, were present in the same preparation. The fusion cells (zygospores) possessed a very delicate membrane and a vacuolate chlorophyllous contents; in some of them, two distinctly separate masses of chlorophyll were present, each mass containing one pigment-spot. Unfortunately, formalin had been run under the cover-glass to restrain the movements of certain active swarm-spores, so that the development of these fusion cells could not be followed, and I was never successful in observing them again. There seems no doubt, however, that the swarm-spores of *Dicranochæte* may occasionally function as gametes and fuse in pairs, the quadriciliate swarm-spores which were observed being possibly formed by fusion of two of the biciliate ones.

No vegetative division appears to take place in this alga, but it was observed that young individuals which happen to be close together or in contact grow with the contiguous parts of their gelatinous sheaths flattened against each other and fused together (Fig. 1, L). Sometimes short filaments of 3 or 4 cells are produced in this way, presenting the false appearance of having been produced by vegetative division. Such cells are firmly united to each other and remain so when the filament is forcibly torn away from the substratum.

According to Hieronymus many of the cells appear to pass through the winter in the vegetative condition. He also noticed certain cells which had formed a thick wall round themselves inside the usual membrane, constituting a sort of aplanospore, in which condition they probably perennate.

The only other species of *Dicranochæte* recorded, as already stated, is *D. britannica* G. S. West, from N. Wales. This alga was free in a deposit obtained by squeezing submerged *Sphagnum*, but, according to West, there is every reason for supposing that when living it was epiphytic upon this moss. *D. britannica* differs from *D. reniformis* in its cells being more or less globose and in having a thick lamellose wall, with no indentation, the seta being dorsal or sub-dorsal. The method of reproduction of *D. britannica* is unknown but from the mode of occurrence of the alga it seems to be brought about by zoogonidia.

Dicranochæte seems to occupy a somewhat isolated position in the Chlorophyceæ, branched setæ being present in no other genus of Green Algæ, although small branches are sometimes given

off from the bristles of *Glæochæte*,[1] a genus of the Cyanophycæ which has been placed by some authors in the Chlorophyceæ, but which is certainly not allied to *Dicranochæte*, Blackman and Tansley[2] place *Dicranochæte* in the family Chlorococcaceæ of the Protococcoideæ, the characteristic feature of this family—in which they include, besides *Dicranochæte*, the genera *Chlorococcum*, *Sykidion*, *Characium* and *Halosphæra*—being the method of reproduction which is effected solely by zoogonidia, vegetative division occurring only in the palmelloid stages. With other setigerous green algæ *Dicranochæte* seems to show little or no direct affinity owing to the absence of vegetative division in the latter, there being no evidence that this character is other than primitive. The absence of setæ in such a genus as *Characium* does not preclude the possibility of an affinity between it and *Dicranochæte* as these structures in algæ have certainly been evolved along several widely separated lines of evolution.

In conclusion I wish to express my indebtedness to Mr. W. B. Grove for helping with the literature.

[1] See G. S. West's " British Freshwater Algæ," 1904, p. 345.

[2] " Classification of the Green Algæ," New Phytologist, Vol. I, 1902, p. 92.

CARBON ASSIMILATION.

A Review of Recent Work on the Pigments of the Green Leaf and the Processes connected with them.

By Ingvar Jörgensen and Walter Stiles.

(*Continued from* p. 96).

It is well to remember that Willstätter's work has not exhausted chemical investigation on the subject of leaf pigments. There is a great deal yet which is not clear, and much which is very *hypothetical*, as for instance, the relation between the two green pigments, the reactions occurring in the changes from chlorophyll to chlorophyllin salts, the oxidations and reductions of chlorophyll derivatives, and above all, that which is of the greatest interest to us, the photo-chemistry of chlorophyll. On this last subject we have so far had no publication from Willstätter, although it is evident from his papers that he has been working at it and has realised that the phenomena of carbon assimilation such as we know them in living plants cannot be imitated by experiments with the four pigments " in vitro." Knowledge of the photo-chemistry of chlorophyll will probably help us to estimate the true significance of many of the observations which have already been made on chlorophyll outside the plant.

We have so far mainly dealt with Willstätter's work in organic chemistry, and in a later chapter we shall discuss Willstätter's plant physiological work; before concluding this chapter it must, however, be mentioned that Willstätter's physico-chemical work, that on the state of aggregation of chlorophyll, for example, does not appear so brilliant and convincing as his work in organic chemistry. Although the extension of our knowledge of the colloidal state of chlorophyll must be regarded as a great advance, yet Willstätter's arguments and experiments on this point are not very complete, and he seems intentionally to avoid any detailed discussion of the question. The reason for this may be found in the fact that before the subject is properly attacked, an investigation of the colourless substances which accompany the pigments in the chloroplasts, as thorough and as detailed as that of the pigments themselves, is necessary. It is to be hoped that Willstätter or some other equally capable organic chemist will direct his attention to this subject which so much needs investigation.

Willstätter's work is one of those monumental pieces of research which are of permanent value. In the following chapters we shall deal with another piece of work which will always retain its value—the work of F. F. Blackman on the intake of carbon dioxide by the leaf.

CHAPTER III.

The Path of Gaseous Exchange.

The passage of carbon dioxide from the outside medium into the leaf in the case of submerged water plants almost certainly takes place by diffusion in aqueous solution through the outer walls of the epidermal cells in the same way that substances will diffuse from cell to cell within the plant.

In the case of lower plants like the mosses the path must be the same, as the surface layer of the leaf is uniform throughout. In the higher land plants, on the other hand, there are two paths by which gases might diffuse into and out from the leaf. There might be diffusion through the cuticle of the epidermal cells as in submerged water plants or mosses, or the diffusion of gases might be principally through the small perforations, stomata, which occur in varying abundance over one or both surfaces of the leaves of higher plants, but which comprise only a fraction of the total area of the leaf. It is a possible alternative that both cuticle and stomata may be utilised for diffusion, in which case it becomes of interest to determine the relative importance of the cuticle and stomata in gaseous diffusion into the leaf.

The work on the paths of gaseous exchange before the researches of F. F. Blackman, like the work on chorophyll before Willstätter's, is all open to the criticism that the experimental methods used were imperfect. It is therefore not to be wondered at that a mass of contradictory results was obtained, and that none of the views of earlier workers had been established. It will be sufficient for us to refer here to the observations of Garreau (1850), Merget (1877-8), Wiesner (1879), Boehm (1889) and Wiesner and Molisch (1889), who have urged that the stomata are the path of gaseous exchange, while Boussingault (1868) and Barthélemy (1868) have advocated the contrary view, that the intake of carbon dioxide takes place through the cuticle. Mangin (1888) took up an intermediate position that diffusion through the cuticle is insufficient to account for the whole of the gaseous exchange. During assimilation he concluded that practically all the gaseous exchange takes place through the stomata as the pressure of carbon dioxide in the external air is insufficient to cause much diffusion through the cuticle.

It is unnecessary for us to go into a detailed description of the results and conclusions of these workers nor into a criticism of

their results. It is enough to say that none of them have furnished indisputable evidence on the matter, and their work becomes chiefly of historical interest after the clearing up of the problem by F. F. Blackman in whose papers on the subject (1895 a, 1895 b) a summary and criticism of earlier work is to be found.

The essence of Blackman's work, is the measurement of the quantity of carbon dioxide passing in and out of the two surfaces of living leaves on which the distribution of the stomata is known. For this work it was necessary to devise a special apparatus by which could be measured the small quantities of carbon dioxide with which one has to deal in such experiments. This apparatus is described in the first of Blackman's papers (1895 a). By its means a current of air either free from carbon dioxide or containing any desired concentration of this gas, is passed over the surface of a leaf in a closed chamber and the intake or evolution of carbon dioxide by the leaf measured. This is effected by estimation of the carbon dioxide in the gas leaving the leaf-chamber by passing this through standard baryta solution which is subsequently titrated against standard hydrochloric solution.

For details of the apparatus we must refer to the description in the paper cited above. It is especially noteworthy that although the apparatus is complicated yet the manipulation is exceedingly simple, consisting only in the turning of taps. The different parts of the apparatus are in duplicate so that two different surfaces of a leaf or two different parts of a plant, can be examined under exactly similar conditions at the same time.

Special mention should be made of the plant chamber by means of which the two surfaces of a leaf can be examined simultaneously. This chamber consists of two circular rims of brass, 5 millimetres deep and 36 millimetres in diameter, to one face of each of which is hermetically cemented a plate of thin glass. Through the brass rim are drilled at opposite ends of a diameter two small holes, into each of which a copper tube of 1 millimetre bore is soldered. These form the channels by which the gas enters and leaves the chamber. For convenience of handling one tube is curved half way round the rim of the half chamber so that it lies parallel with the other. The leaf to be examined is slipped between the two half-chambers and hermetically sealed to them by means of wax, and the leaf is then ready for experimentation. For leaves of different forms, plant chambers of different shapes may be used.

Blackman experimented with various kinds of leaves, including

those where the stomata were limited to the lower surface only and those where the stomata were present on both surfaces. Usually the carbon dioxide evolved from the leaf into a current of air free from that gas was the quantity measured, but results were also obtained for the intake of carbon dioxide which show the path traversed by the gas is the same whether it is travelling into or out of the leaf.

In the following table we have summarised the results obtained by Blackman for the ratios of the amounts of carbon dioxide given out from the two surfaces of the leaves of various plants.

TABLE III.

Plant.	Peculiarity.	Stomatic ratio. Upper surface / Lower surface	CO_2 respired. Upper surface / Lower surface
Nerium oleander	Very thick cuticle	$\frac{0}{100}$	$\frac{3}{100}, \frac{6}{100}$
Prunus laurocerasus	„ , „	$\frac{0}{100}$	$\frac{0}{100}, \frac{4}{100}$
Hedera helix	„ „ „	$\frac{0}{100}$	$\frac{4}{100}$
Platanus occidentalis	Thin cuticle	$\frac{0}{100}$	$\frac{3}{100}$
Ampelopsis hederacea	„ „	$\frac{0}{100}$	$\frac{3}{100}$
Polygonum sacchalinense	„ „	$\frac{0}{100}$	$\frac{6}{100}$
Alisma Plantago	Aquatic plant. More stomata on upper surface	$\frac{135}{100}$	$\frac{135}{100}, \frac{120}{100}$ $\frac{115}{100}, \frac{113}{100}$
Iris germanica	Isobilateral leaf	$\frac{100}{100}$	$\frac{105}{100}, \frac{110}{100}$
Populus nigra	Stomata on both surfaces, fewer on upper	$\frac{100}{575}$	$\frac{100}{375}$
Helianthus tuberosus	„	$\frac{100}{240}$	$\frac{100}{273}$
Tropœolum majus	„	$\frac{100}{200}$	$\frac{100}{265}$

The numbers in the foregoing table show how constantly the path of carbon dioxide from the leaf follows the distribution of the stomata. Similar results were obtained in the case of carbon dioxide absorbed in assimilation. Thus, in the cases of *Ampelopsis hederacea*, *Platanus occidentalis* and *Polygonum sacchalinense*, where all the stomata occur on the under surfaces of the leaves all the carbon dioxide was found to enter by the lower surface. None

was taken up by the astomatic upper surface. In the case of *Alisma Plantago*, which has stomata on both sides of the leaves, there was found in every experiment performed a constant tendency for the absorption of carbon dioxide to be greater by the upper surface where the stomata are more frequent. Confirmatory results were obtained with *Tropæolum majus* and *Acer platanoides*.

From these experiments the conclusion is drawn that the intake and evolution of carbon dioxide takes place through the stomata. The only alternative explanation is that in the case of leaves with the stomata confined to the lower surface the cuticle on the lower surface is fifty to a hundred times more permeable to carbon dioxide than the lower surface. It seems impossible to suppose this the case, especially as leaves with thin cuticles gave results exactly similar to those obtained with leaves possessing very thick cuticles.

Although accepting Blackman's results in regard to the exhalation of carbon dioxide in respiration, Brown and Escombe (1905 a) are of opinion that his method is not so well adapted to investigations of the intake of carbon dioxide in assimilation, chiefly because the amounts of carbon dioxide dealt with seldom exceeded 0·1 c.c. with a possible experimental error of one-tenth of that amount. Brown and Escombe therefore performed some experiments similar to Blackman's under conditions which admitted the measurement of carbon dioxide taken in by the two sides of a leaf on which the distribution of stomata was known.

The following tables taken from Brown and Escombe's paper exhibit their results.

TABLE IV.

Respiration from the two Surfaces of various Leaves.

Plant.	Time in hours.	Leaf area in sq. cms.	CO_2 evolved in c.cs. $\dfrac{Upper}{Lower}$	Ratio of CO_2 evolved. $\dfrac{Upper}{Lower}$	Ratio of stomatic distribution $\dfrac{Upper}{Lower}$
Canna indica	4·75	28·27	$\dfrac{8·41}{20·76}$	$\dfrac{100}{246}$	$\dfrac{100}{246}$
,, ,,	5·0	28·27	$\dfrac{5·55}{17·90}$	$\dfrac{100}{322}$	,,
,, ,,	4·23	28·27	$\dfrac{3·04}{6·40}$	$\dfrac{100}{210}$	,,
Rumex alpinus	5·5	59·44	$\dfrac{1·03}{3·60}$	$\dfrac{100}{286}$	$\dfrac{100}{269}$

TABLE V.

Assimilation by the two Surfaces of various Leaves
illuminated on Upper Surface.

Plant.	Time in hours.	Leaf area in sq. cms.	CO_2 assimilated in c.cs. $\dfrac{Upper}{Lower}$	Ratio of CO_2 assimilated $\dfrac{Upper}{Lower}$	Ratio of stomatic distribution $\dfrac{Upper}{Lower}$
Colchicum speciosum	5·75	59·44	$\dfrac{4\cdot34}{3\cdot26}$	$\dfrac{100}{72}$	$\dfrac{100}{119}$
Senecio macrophyllus	4·75	28·27	$\dfrac{3\cdot90}{3\cdot60}$	$\dfrac{100}{92}$	$\dfrac{100}{126}$
,, ,,	4·25	28·27	$\dfrac{5\cdot80}{4\cdot20}$	$\dfrac{100}{72}$	$\dfrac{100}{126}$
Rumex alpinus	5·0	59·44	$\dfrac{5\cdot70}{8\cdot90}$	$\dfrac{100}{144}$	$\dfrac{100}{269}$
,, ,,	5·5	59·44	$\dfrac{7\cdot50}{9\cdot81}$	$\dfrac{100}{130}$	$\dfrac{100}{269}$
Nuphar advenum	2·0	76·97	$\dfrac{2\cdot20}{0\cdot00}$	$\dfrac{100}{0}$	$\dfrac{100}{0}$
Catalpa bignonioides	1·85	79·03	$\dfrac{0\cdot00}{4\cdot91}$	$\dfrac{0}{100}$	$\dfrac{0}{100}$
,, ,,	2·3	79·03	$\dfrac{0\cdot00}{8\cdot96}$	$\dfrac{0}{100}$	$\dfrac{0}{100}$

Brown and Escombe's results confirm Blackman's in regard to leaves with the stomata confined to one surface. With leaves bearing stomata on both surfaces, when illuminated on the upper surface there is always less intake of carbon dioxide by the lower surface than might be expected from the relative distribution of the stomata over the two surfaces, whereas the ratio of carbon dioxide respired from the two surfaces follows very closely the ratio of stomatal distribution.

Brown and Escombe explain this result in the following way. During respiration, if there is a steady evolution of carbon dioxide, the rate at which this will escape from the leaf will be independent of the degree of opening of the stomata, for should the stomatal aperture decrease, the partial pressure of carbon dioxide inside the leaf will correspondingly increase and the rise in 'diffusion potential' will counterbalance the effect of diminished stomatal aperture.

In the case of assimilation, on the other hand, the 'diffusion potential' will remain constant, for the partial pressure of the carbon dioxide diffusing inwards varies constantly from 0·0003 atmosphere outside the leaf to zero where there is complete absorption of the carbon dioxide. Hence, if the stomatal opening

varies, the rate of intake of carbon dioxide must also vary and the results obtained may be due to the greater degree of opening of the stomata on the illuminated side. Brown and Escombe also think that as more energy is absorbed by the chloroplasts of the palisade parenchyma, carbon dioxide will be more rapidly utilised in that part of the leaf and consequently the diffusion gradient will be steeper in the intercellular spaces of the palisade into which the stomata of the upper surface open, which will also favour a more rapid intake of carbon dioxide by the stomata of the upper surface.

Further evidence as to the path of carbon dioxide into and out of the leaf has been obtained by investigating the gaseous exchange when the stomata are artificially blocked. It had previously been asserted by Boussingault that the path of carbon dioxide intake was through the cuticle. This conclusion was based on an experiment in which leaves of Nerium were painted over with lard. In one the astomatic upper surface was so covered, in the other the lower surface, with the result that the leaf with its stomata blocked assimilated more. Blackman shows that this result is due to the use outside the leaf of too high a concentration of carbon dioxide (more than 30%).

The results of Blackman's own experiments with leaves of *Nerium oleander* are given in the subjoined table and show clearly the effect of increasing the carbon dioxide concentration.

Table VI.

Mean percentage of CO_2 present in each experiment.	CO_2 in c.c. decomposed per unit area.		Ratio of amount of CO_2 decomposed per unit area.	
	Normal leaf.	Vaselined leaf.	Normal leaf.	Stomata blocked.
6	0·07	0·01	1	0·14
6·3	0·055	0·01	1	0·20
7·5	0·046	0·017	1	0·21
14	0·18	0·04	1	0·37
55	0·049	0·067	1	1·3
50	0·043	0·069	1	1·5
97	0·033	0·060	1	1·8

Some further experiments made with *Nerium oleander* show that more carbon dioxide passes through the vaselined under surface than through the unvaselined cuticle of the upper surface, so that coating the leaf with vaseline does not render it impervious to the passage of carbon dioxide.

Injecting the leaf with water has a similar influence on altering the ratio of CO_2-intake by the two surfaces as coating the lower surface with vaseline. By such injection the intercellular spaces are filled with water and diffusion of carbon dioxide can then only take place in solution; hence the stomatal surface no longer possesses such an advantage over the upper surface in regard to the passage of carbon dioxide through it. The following table shows the relative amounts of carbon dioxide respired from the two surfaces of leaves under various conditions.

TABLE VII.

Condition of Leaf.	Relative output of CO_2.	
	Upper surface.	Lower surface.
Normal leaf	1	39
Injected ,,	1	10
Vaselined under surface	1	3

Blackman thus comes to the conclusion that the epidermis with its cuticle is slightly permeable to carbon dioxide, but that under normal conditions, by far the greater part of gaseous exchange takes place through the stomata. Under artificial conditions, such as waterlogging the intercellular spaces or blocking the stomata, the passage of carbon dioxide through the cuticle, though not actually greater, may become of relatively more importance.

An extended series of experiments bearing on the same matter of the path of carbon dioxide into the leaf has been made by Stahl (1894). His method consisted in artificially blocking the stomata on parts of the leaf and showing that after exposure to light, starch formation is limited to the regions of the leaf where the stomata were unblocked. Some similar experiments were made independently by Blackman who confirms Stahl's observations.

The experiments of Blackman, Stahl and Brown and Escombe appear to show conclusively that the path of diffusion of carbon dioxide into the leaf is mainly or entirely through the stomata. But there are three facts which rendered difficult the acceptance of this evidence alone. These facts are (1) the large amount of carbon dioxide absorbed by a leaf during active assimilation; (2) the low partial pressure of carbon dioxide in the atmosphere—the carbon dioxide only amounts to about 3 parts per 10,000 of the atmosphere ; and (3) the very small fraction of the leaf surface occupied by the stomata.

Brown and Escombe have shown that the leaf of *Catalpa bignonioides* can absorb from ordinary air 0·07 c.c. of carbon dioxide measured at N.T.P. per sq. cm. of leaf surface per hour. The area of the stomatal openings is only 0·09% of the total leaf surface. Hence diffusion through them must take place at the rate of 7·77 c.c. per sq. cm. per hour.

Now experiments made by Brown and Escombe showed that a normal solution of sodium hydroxide exposed to moderately still air containing about 3 parts of carbon dioxide per 10,000 absorbs this gas at ordinary temperatures at the rate of about 0·120 c.c. per sq. cm. of absorbing surface per hour, and this is only increased to a maximum value of 0·177 c.c. per sq. cm. per hour when the rate at which the air is passed over the absorbing solution is increased.

Hence, if the diffusion of carbon dioxide into the leaf takes place entirely through the stomata, this absorption of carbon dioxide must take place about 50 times as fast as it would by a solution of normal sodium hydroxide of which the exposed surface had the same area as the stomata.

Brown and Escombe were thus led to investigate the rate of diffusion of gases through small apertures in a septum. Their method of procedure was as follows : 200 c.c. of normal sodium hydroxide were placed in a flat-bottomed flask which was left open in comparatively still air containing the normal amount of carbon dioxide. The surface of the liquid was about 10 cm. in diameter. A very steady and uniform absorption then took place at the rate of about 0·25 c.c. carbon dioxide per hour.

In order to obtain a suitably perforate septum between the absorbing liquid and the other air, the neck of the flask was passed through the bottom of a small glass cup to which it was cemented. The annular space of the cup was then filled with mercury. A flat-bottomed nickel crucible was inverted over the mouth of the flask so that the edges dipped into the cup of mercury, and in this way a perfect mercury seal was obtained. A hole of the desired size was made in the bottom of the nickel crucible.

A number of such pieces of apparatus with variously perforated septa were prepared at the same time, and after displacing the air in them with air freed from carbon dioxide they were exposed to the atmosphere under the same conditions. As a result of these experiments, Brown and Escombe came to the conclusion that with small apertures the rates of diffusion are proportional, not to the areas, but to the diameters of the opening. The following table

summarises their results with regard to carbon dioxide. Similar results were obtained with water vapour.

TABLE VIII.

Diffusion of carbon dioxide through apertures of various sizes.

Diameter of aperture.	CO_2 diffused per hour.	CO_2 diffused per sq. cm. per hour.	Ratio of areas of apertures.	Ratio of diameters of apertures.	Ratio of CO_2 diffused in unit time.
22·7	·2380	·0588	1·00	1·00	1·00
12·06	·09280	·0812	·28	·53	·39
12·06	·10180	·0891	·28	·53	·42
6·03	·06252	·2186	·07	·26	·26
5·86	·05558	·2074	·066	·25	·23
3·23	·03988	·4855	·023	·14	·16
3·22	·03971	·4852	·020	·14	·16
2·12	·02608	·8253	·008	·093	·10
2·00	·02397	·7629	·007	·088	·10

In order to explain this result, Brown and Escombe consider first the case of a disc capable of absorbing carbon dioxide and freely exposed to the air. If the latter is perfectly still, convergent streams of carbon dioxide will creep through the air towards the disc to replace that absorbed, and a steady gradient of density will be established, and if surfaces are drawn passing through all the points of the same carbon dioxide density, these surfaces will form ' shells ' surrounding the disc. If the disc is a perfect absorbent of carbon dioxide, these shells will vary in density from zero at the absorbing surface to a maximum density which is that of carbon dioxide in air. This will theoretically be at an infinite distance from the disc but is practically reached at a point 5 or 6 diameters from the disc. Now Stefan has examined mathematically the exact converse of this case, namely, evaporation from a circular surface of liquid. Stefan obtained the following formula for the amounts of evaporation from such a surface :—

$$M = 4ka \frac{P-p''}{P-p'}$$

Where M is the mass of liquid evaporated in a given time, k the coefficient of the diffusion of the vapour, a the radius of the disc of liquid, P the pressure of the atmosphere and p' and p'' the pressure of the vapour at the surface and at an infinite distance from it respectively.

The formula given by Larmor for the absorption of carbon dioxide by a perfectly absorbing disc, assuming the formation of

such shells of equal density is essentially the same.   It is:—

$$Q = 2k\rho D,$$

where Q is the quantity absorbed in any time,

> k the coefficient of diffusion of carbon dioxide in air,
>
> ρ the density of atmospheric carbon dioxide,
>
> D the diameter of the disc.

Brown and Escombe explain their results in regard to the rate of diffusion through perforate septa as due to the same cause, namely, that when a gas is diffusing through such a perforate septum, shells of equal density are formed outside the perforation just as in the case of the absorbent disc, and the same 'diameter law' will hold.

The accompanying diagrams show the various systems of shells.   Fig. 1 is the case of the shells over a perfectly absorbent disc.   The density of the diffusing gas varies from ρ at a remote distance from the surface to zero at the surface itself.   In Fig. 2 are represented the shells produced on the inner side of a perforated diaphragm opening into a large space in which the gas is rapidly absorbed and where the density of the gas at the perforation is kept at a maximum by a constant current of air.   The density of the gas here varies from ρ at the diaphragm to zero at the surface. In Fig. 3 is represented the case of a perforated septum like the
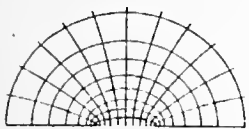
Fig. 1.
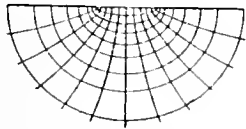
Fig. 2.
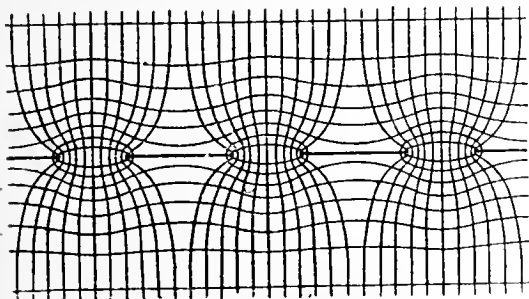
Fig. 4.
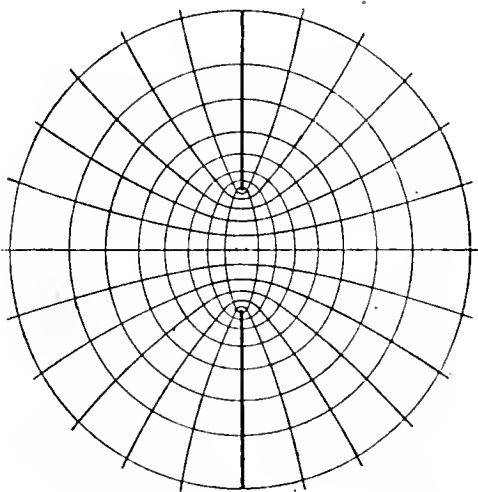
Fig. 3.

FIGS. 1—4.

FIGS. 1 AND 2.   Diffusion "shells" formed outside and inside a perforation in a septum.

FIG. 3.   Diffusion "shells" outside and inside a perforation in a septum in perfectly still air.

FIG. 4.   Lines of flow through a multiperforate septum.

case shown in Fig. 2, but in which there is perfectly still air outside, so that shells are produced outside as well as in.   Here the density of the gas will vary from ρ at a remote distance outside to $\rho_{\prime}$ at the perforation to zero at the absorbing surface.

In the case of the leaf in still air, we are dealing with an approximation to this last case, but as in actual fact there will always be more or less movement of air outside the leaf there will be a corresponding approach to the conditions indicated in Fig. 2.

If the partial pressure of carbon dioxide in ordinary air is called P, then in the last case, from Larmor's formula, we have the quantity passing through any shell outside $= 2k(P-P_{\prime})D$, where D is the diameter of the perforation.   Similarly, the quantity passing through any shell inside $= 2kP_{\prime}D$.

Now when a constant flow is established, these two quantities must be the same so that

$$2k(P-P_{\prime})D = 2kP_{\prime}D$$
$$\text{whence } P-P_{\prime} = P_{\prime}$$
$$\text{or } P = 2P_{\prime}$$

That is, in still air the pressure of carbon dioxide at the perforation will only be one half of the pressure of the gas there when this is kept constantly renewed.   Consequently the diffusion gradients will only be half as steep in the former case and the rate of absorption will correspondingly be reduced to half.

Similarly, in the case of the leaf, the rate of passage of carbon dioxide will be increased in the same way when the air outside the leaf is constantly renewed, provided that the cells surrounding the space into which the stomata open are perfect absorbers of the gas.   Also, other things being equal, the velocity of flow through the stomata will be proportional, not to the areas of the stomata, but to their diameters.

Since the surface of the leaf is perforated, not by one, but by many stomata, the researches of Brown and Escombe on diffusion through multiperforate septa become of great interest in relation to the intake of carbon dioxide.   The multiperforate septa consisted of sheets of celluloid of thickness 0·08 to 0·1 mm. in which a series of holes at definite distances from one another were punched.   The septa were fixed to the open ends of glass tubes containing sodium hydroxide.   The following table gives some of Brown and Escombe's results.   In each case the area of cross sections of the tubes was measured and the diffusion through the perforate septum compared with that down an open tube.

Table IX.

Diameter of each hole 0·380 mm.
Length of tube 1·0 cm.

Distance of holes apart in diameters.	Number of holes per sq. cm. of septum.	Percentage area of holes on unit area of septum.	$\dfrac{\text{Septum diffusion}}{\text{Open tube diffusion}} \times 100.$
2·63	100·00	11·34	56·1
5·26	25·00	2·82	51·7
7·8	11·11	1·25	40·6
10·52	6·25	·70	31·4
13·1	4·00	·45	20·9
15·7	2·77	·31	14·0

It will be observed from these numbers that the obstruction offered to the diffusion of gases by a multiperforate septum is considerably less than the actual obstruction of area. Thus when the area of perforation was less than 3% of the whole area of the septum, the actual diffusion through the perforations was 51·7% of the diffusion taking place through an open tube of the same area of cross section. That is, the diffusion through the septum is nearly 15 times as great as it would be if it were simply proportional to the area of the cross section. As the distance between the holes is increased, the efficiency of the area of the perforations increases until the holes are about 10 diameters apart. In this case and in cases where the distance apart of the holes is increased, the diffusion through the perforations is about 40 times as much as it would be if it were proportional to the area of cross section of the tube.

The accompanying figure (Fig. 4) illustrates what Brown and Escombe imagine to be the lines of equal density and the lines of flow of gas through such a multiperforate septum. The lines of flow of gas diffusing towards the septum will be approximately parallel at some distance from the septum, but as they pass through the perforations they converge, the velocity of flow increasing at the same time owing to the production of ellipsoidal density shells round the opening. After passing through the opening, the lines of flow diverge and as lines of flow from adjacent perforations cannot cross each other (for otherwise there would be shells of different density crossing each other, which is impossible) they must bend round and become once more parallel, the velocity of flow at the same time diminishing. From a consideration of Fig. 4, it is easy to understand how it is that the perforations under favourable conditions of distribution are so efficient for diffusion through them.

Brown and Escombe's results lead them to the general conclusion that "the interference of the density shells of small

holes set at 10 diameters or more apart is small, each hole beyond this limit acting almost independently according to the diameter law.'

Now in the leaf of *Helianthus annuus*, for example, the stomata on the under surface are actually about 8 diameters apart. The stomata themselves open into cavities in which shells of diffusion may form. The under surface of such a leaf is therefore a multiperforate septum in which the perforations are so far apart that practically each single opening can exercise its full efficiency as regards diffusion through it, without interference from its neighbours. We may, therefore, expect the diameter law to hold, and the rate of diffusion of carbon dioxide through the stomata to be proportional to the linear dimensions of the stomata.

Assuming the stomata to be circular in shape instead of elliptical as they actually are, Brown and Escombe have worked out the quantity of carbon dioxide capable of diffusing into the leaf under various conditions. Under the most favourable circum-stances, when the stomata are wide open and the carbon dioxide in the air outside the leaf is in constant motion so as to maintain the greatest possible pressure of carbon dioxide there, we have the following data :—

> Diameter of stoma, 0·00107 cm.
>
> Length of tube, 0·0014 cm.
>
> Number of stomata per sq. cm., 33,000.
>
> Area of cross section of stoma, $9·08 \times 10^{-7}$ sq. cm.

Under these circumstances the theoretical value for the quantity of carbon dioxide absorbed by the leaf is 2·578 c.c. per sq. cm. per hour.

If, on the other hand, the air outside the leaf is perfectly still the maximum quantity of carbon dioxide entering the leaf is 2·095 c.c. per sq. cm. per hour.

These values are far higher than the observed quantities of carbon dioxide taken in by the leaf. Thus Thoday (1910) found a leaf of *Helianthus annuus* was capable of increasing in dry weight by about ·17 milligrams per hour per sq. cm. which corresponds to an intake of carbon dioxide of only about 0·14 c.c. of carbon dioxide per hour measured at normal temperature and pressure.

Hence, the stomata, in spite of the relatively small area of the whole leaf surface they occupy, could yet allow the diffusion through them of many times as much carbon dioxide as actually passes through. There is then, every reason to regard the results of Blackman and Brown and Escombe as affording definite proof that the path of intake of carbon dioxide into the assimilating aerial leaf of higher plants is mainly through the stomata.

Chapter IV.

The Factors Influencing the Intake of Carbon Dioxide.

A. General Remarks.

As we have already said in our introductory chapter, carbon assimilation is a complex of processes which probably obey quite different laws. Thus we know that one or more of these processes must be photo-chemical since light is required for carbon assimilation. Consequently one would hardly expect to express the relation between the amount of carbon dioxide used and the various factors which influence the intake of carbon dioxide in a simple way.

It is the great merit of F. F. Blackman that many years ago he called attention to the complexity of the processes of carbon assimilation, and showed that it was impossible to construct such a curve as a temperature-assimilation curve without regard to the possible effects of other factors. The result of Blackman's analysis of the intake of carbon dioxide under various conditions is expressed in his principle of limiting factors, and summed up in his work ' Optima and Limiting Factors' in Annals of Botany for 1905, a paper with which every student of plant physiology should be well acquainted, as the considerations contained therein are so fundamental for all biological processes.

Before F. F. Blackman's publications, investigators dealing with the influence of a factor on any physiological process, spoke of the factor having minimum and maximum values, below and above which the process does not take place and an optimum value at which the process proceeds at its greatest rate. In carbon assimilation there was alleged to be an optimum value of temperature at which assimilation is greatest. Similarly, there was supposed to be an optimum carbon dioxide supply and an optimum illumination for carbon assimilation. The optimum values obtained by different authors did not show any concordance, and Blackman pointed out that the method of experimentation in which, for instance, the influence of carbon dioxide and light are neglected when the effect of temperature is considered, is utterly illogical and cannot be expected to give results of any clear value.

Blackman states the principle of limiting factors as follows: " When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the ' slowest ' factor."

Thus the rate of carbon assimilation in the leaf may depend on five obvious factors :—

1. Carbon dioxide supply.
2. Water supply.
3. Intensity of illumination.
4. The quantity of chlorophyll.
5. Temperature.

Any one of these might act as a limiting factor in assimilation.

We may quote with advantage an illustration of the operation of limiting factors given by Blackman. A leaf is supposed to have so much light falling on it as would give energy sufficient to decompose 5 c.c. of carbon dioxide per hour. If, now, the leaf is subjected to such a pressure of carbon dioxide that 1 c.c. of carbon dioxide is assimilated by the leaf per hour, there is sufficient energy provided to enable the whole of this carbon dioxide to be assimilated. When the pressure is raised to double the amount, so that 2 c.c. diffuses into the leaf per hour, the energy is sufficient to bring about the assimilation of the whole of the carbon dioxide, and so on until the pressure has been increased to five times its original value. But if the carbon dioxide supply is further increased, no further increase in carbon assimilation will take place as the energy is only supplied at a rate sufficient to allow 5 c.c. of carbon dioxide to be assimilated in an hour. Whatever the value of carbon dioxide supply above this value, the amount of assimilation will always be the same, *i.e.*, the maximum possible for the value of light intensity. The curve connecting assimilation and carbon dioxide supply will therefore be of the form ABC (Fig. 5). On the other hand, if the light intensity be now increased to double its value, it will be sufficient to allow 10 c.c. of carbon dioxide to be assimilated in an hour, and increases in carbon dioxide supply, will result in a steadily increasing carbon assimilation with increasing carbon dioxide supply, until this latter gives an assimilation of 10 c.c. an hour, when illumination will again put a limit on assimilation, and a curve of the form ADE will be obtained. With still stronger light, the curve AFG would be produced. Thus it is impossible to investigate the relation between carbon dioxide supplied and the amount of assimilation without considering the factor of light. Similarly, other factors must be taken into account and care must be taken that a factor other than the one under consideration is not acting as a limiting factor.

The principle of limiting factors is, of course, of general application where a process depends on a number of factors. It is, indeed, rather an elaboration of the ' Law of the Minimum ' which had been applied to agricultural problems by Liebig as far back as 1843.
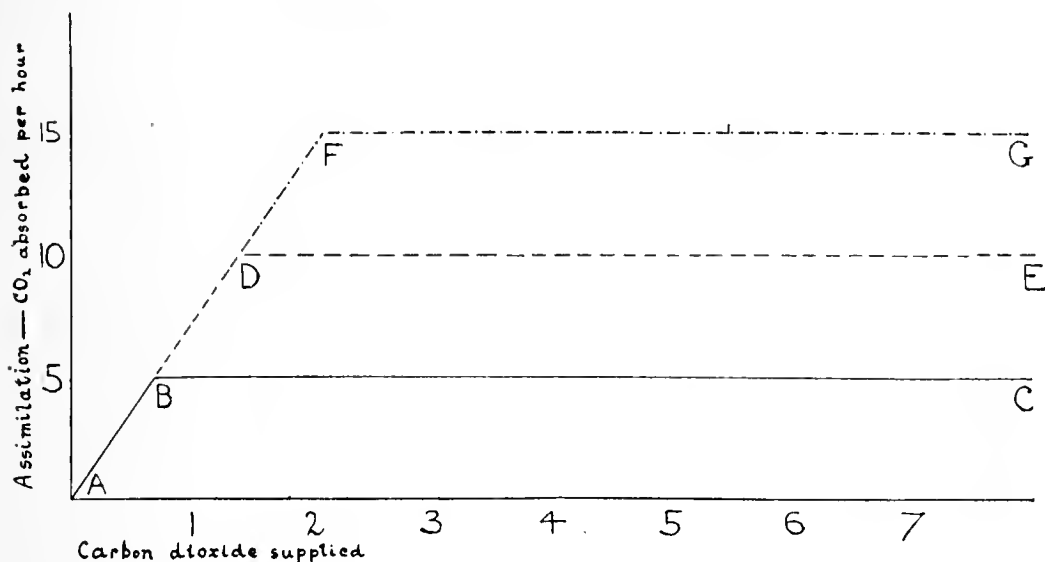
FIG. 5. Scheme to illustrate the action of a limiting factor. (After F. F. Blackman).

The question of the optimum value of a factor requires some consideration.

As regards temperature we have already referred to the van't Hoff rule that for every rise of 10°C the rate of a chemical reaction is doubled or trebled. If this law were followed throughout it is clear that there could be no optimum temperature for assimilation, which would increase more and more rapidly with increasing temperature. As a matter of fact for several plant processes the van't Hoff rule is followed between say 5°C and 29°C, but at higher temperatures it is quite clear that the rate of metabolic change in the organism slows down and the rule does not express the relation between temperature and the process.

To explain this slowing down at high temperatures Blackman introduces a ' time factor.' Thus at 25°C and lower temperatures the initial assimilation rate is maintained unchanged for a consider- able time, but at higher temperatures, 30°C and over, although the leaf after exposure to light commences to assimilate at a rate given by the van't Hoff rule, this initial rate of assimilation cannot be maintained but falls off regularly, and the higher the temperature the more rapid the falling off. If then the assimilation at various

temperatures is measured over a considerable time it is certain that a temperature will be found at which assimilation is at a maximum. This apparent optimum temperature will however vary according to the time elapsing between the commencement of assimilation at that temperature and the actual measurement of assimilation owing to the rapid falling off in assimilation due to the time factor.

We shall later discuss the time factor in more detail, but in this place we would comment on the use of such expressions as ' time factor.' It is of course desirable that the same terminology should be used wherever possible in physiology as is employed in pure chemistry and physics. But while analysis of physiological processes has not proceeded far this is not always possible, and it is to the credit of F. F. Blackman that he has introduced the terms ' limiting factor,' ' time factor,' which permit discussion of physiological processes without involving premature assumptions as to their nature.

The work of F. F. Blackman and his pupils has been largely concerned with the influence of the various factors temperature, light and carbon dioxide supply on the rate of carbon assimilation. Before the publication of his ' Experimental Researches on Vegetable Assimilation and Respiration ' there had indeed been much work on the influence of these factors on assimilation, but as none of these previous workers had recognised the principle of limiting factors it seems unnecessary for us to discuss their results here. Again the search for an optimum value of the various factors has not helped to elucidate the problems.

We shall first deal with the work of Blackman and his pupils on the relation between assimilation and the chief environmental factors, carbon dioxide supply, light intensity and temperature. Blackman expresses this relation as being such that " the magnitude of this function in every combination of these factors is determined by one or other of them acting as a limiting factor. The identification of the particular limiting factor in any definite case is carried out by applying experimentally the following general principle. When the magnitude of a function is limited by one of a set of possible factors, increase of that factor, and of that one alone, will be found to bring about an increase of the magnitude of the function."

We give here (Fig. 6) the curves obtained by Blackman and Smith (1911 b) showing the inter-relationship between carbon assimilation and the three external factors in the case of *Elodea.*
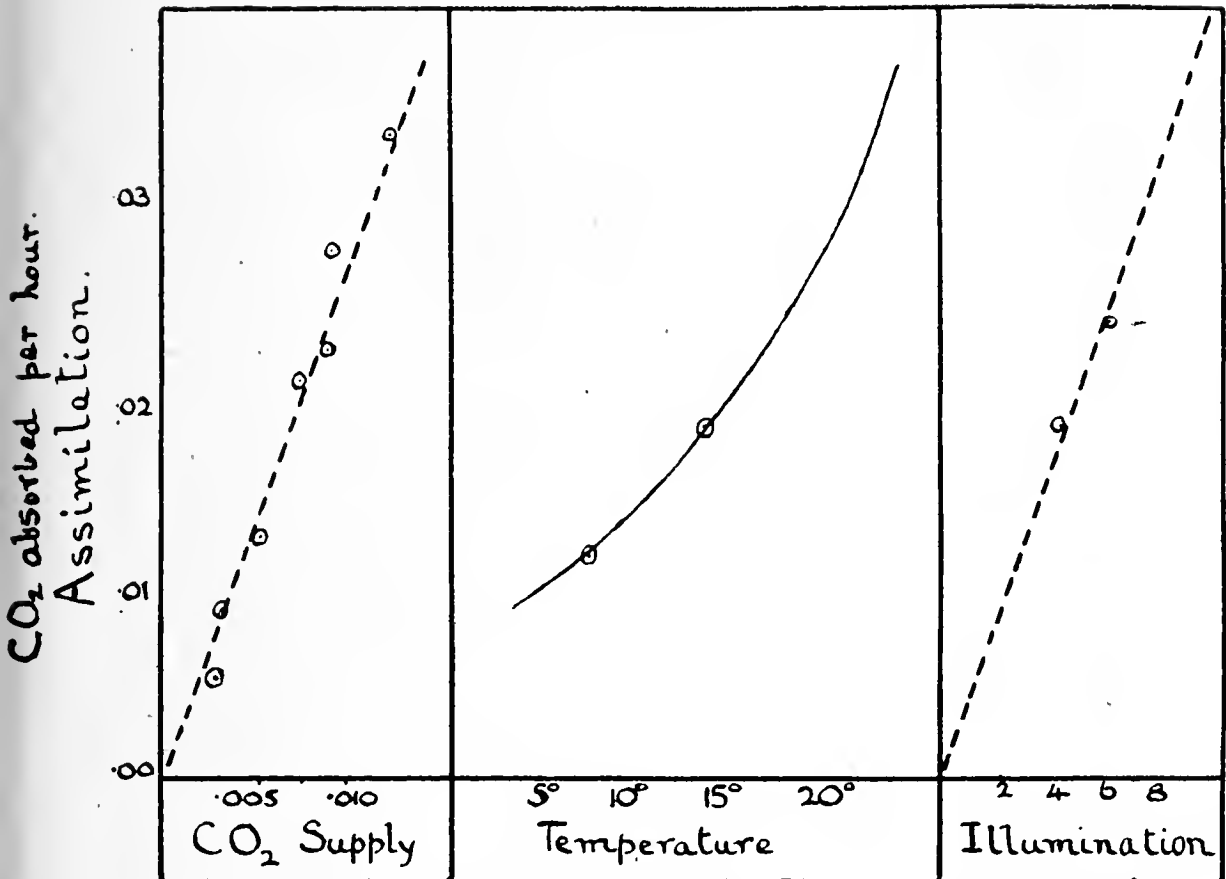
Fig. 6. Inter-relation of environmental factors and assimilation in *Elodea*. (After Blackman and Smith).

The first curve shows the relation between assimilation and carbon dioxide supply when this latter is the limiting factor; the second curve shows the amount of assimilation at different temperatures when temperature is the limiting factor, and the third the relation between assimilation and illumination when the intensity of light is limiting. From these three curves we can obtain the minimum of carbon dioxide supply, temperature and light intensity which are required for any quantity of assimilation.

To be continued.

LABORATORY NOTE.

THE POLLEN OF *ECHEVERIA RETUSA* LINDL. AS LABORATORY MATERIAL.

IN the course of experiments with the pollen of various plants oi the purpose of demonstrating the formation of pollen-tubes tc elementary classes, I have recently made use of that of *Echeveria retusa* Lindl.

The pollen of this plant gives such exceptionally good and reliable results that it seems desirable to place it on record as useful laboratory material.

Pollen grains germinate readily in hanging-drop preparations of 15% cane sugar and form tubes with unusual rapidity. For example, the average rate of growth of the tubes at a temperature of 21°C. is 540μ per hour, so that preparations suitable for class-demonstration purposes can be obtained without difficulty within an hour of transferring the pollen to sugar solution.

To ensure satisfactory results, it is advisable to use sterile slides, etc., when making the drops and to transfer pollen from recently opened anthers.

Pollen-tubes so obtained provide useful material for making direct measurements of the rate of growth of such structures and shew cytological features similar to those described by Strasburger (1) for the pollen tubes of species of *Allium*.

The cytoplasm exhibits active streaming movements, and, as the tubes increase in length they form remarkable plug-like transverse septa.

These plugs (Propfen) originate as ring-like thickenings of the wall, increase rapidly in thickness and quickly form a conspicuous pad or plug blocking the cavity of the tube.

The substance of which the plug is composed is highly refractive and gives the reactions characteristic of callose., e.g., it stains blue with a watery solution of aniline blue and gives a rose-red colour with corallin-soda.

The pollen of several species of *Sedum* and of species of *Crassula* also germinates rapidly in sugar solutions of fairly high concentration.

Echeveria retusa is a native of Mexico and like the majority of Crassulaceous species is a leaf-succulent with rosette habit.

Rapid germination of the pollen and a preference for solutions of relatively high osmotic pressure may well be biological features in these plants associated with the xerophytic conditions under which they occur in nature.

It would be of interest to know whether the pollen of other groups of succulents, e.g., Cactaceæ, exhibits similar tendencies.

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LITERATURE.

1. Strasburger, E. Das botanische Praktikum, 1902, p. 537.

ON THE REMARKABLE RETENTION OF VITALITY OF MOSS PROTONEMA.

By B. MURIEL BRISTOL, M.Sc.

[WITH THREE FIGURES IN THE TEXT].

IN October, 1915, a collection was made of about fifty samples of soil taken from different parts of the country, with the object of ascertaining by means of cultures what algæ are present in different soils in the form of resting-spores. Among the soils collected were eleven most interesting samples very kindly provided by Dr. T. Goodey. These were taken from specimens upon which he had already worked in connection with the protozoa of the soil,[1] and had originally been obtained in 1912 from the sample house at the Rothamsted Experimental Station, Harpenden. At this station, samples of soil have been taken periodically since 1843 from the different experimental plots, and have been stored in bottles, the corks of which have been sealed with leaden capsules to prevent the loss of the contained water by evaporation and to insure against the possibility of infection by dust. The soils appear to have been partially dried by exposure to the air, and then passed through a sieve having ¼-inch meshes, before being bottled.

All of the samples used in the present work were taken from the top 9 inches of soil; seven were taken from the Broadbalk plot in the years 1846, 1856, 1865, 1868, 1869, 1881 and 1893 respectively, one from Agdell in 1867, one from Barnfield in 1870, one from Hoosfield in 1868, and one from Geescroft in 1865.

In setting up cultures of the soils great care was taken to prevent infection from outside, by a preliminary sterilisation of all vessels and instruments to be used, and of the culture medium into

[1] T. Goodey, M.Sc., " Note on the Remarkable Retention of Vitality by Protozoa from Old Stored Soils." Annals of Applied Biology, Vol. I, Nos. 3 and 4, 1915.

which the soils were to be introduced.[1] The culture solution was placed in the sterilised vessel to a depth of about half-an-inch, and about 2 or 3 c.c. of the soil to be examined were then introduced into the solution by means of a sterilised spatula. The vessel, closed with its lid or cotton-wool plug, was then placed under a glass case to keep the dust from accumulating on its surface.

The culture medium used was a mineral salt solution consisting of :—

Potassium dihydrogen phosphate ($KH_2 PO_4$)	1·0 gm.
Sodium nitrate (Na NO_3)	1·0 gm.
Magnesium sulphate (Mg SO_4)	·3 gm.
Calcium chloride (Ca Cl_2)	·1 gm.
Sodium chloride (Na Cl)	·1 gm.
Ferric chloride (Fe Cl_3)	·01 gm.
Distilled water	1000 c.c.

Owing to unfavourable conditions of climate, *viz.*, short days, absence of sunlight and low temperatures, growth in the cultures was very slow, and it was not possible at the time of writing to make a complete and systematic examination of them. A few blue-green algæ had begun to appear, but the most interesting fact was the growth of moss protonema from certain of the soils obtained from the Rothamsted Experimental Station. The protonema first appeared in the form of a few fine green threads growing from a particle of soil ; the threads rapidly branched until they became easily visible to the naked eye as tufts of varying sizes. The soils in which the protonema was observed were Barnfield 1870, Hoosfield 1868 and Agdell 1867, so that it has been produced after prolonged drought, after 46, 48 and 49 years respectively ; none was found in any of the Broadbalk soils nor in Geescroft 1865.

Examined under the microscope, the protonema is seen to have the structure characteristic of any filamentous moss protonema. The cross walls are oblique throughout the length of the filament, and the outside walls are very thin ; the end cells are slightly tapering and rounded at the apex. Each cell of the filament contains a parietal layer of cytoplasm in which are embedded numerous small chloroplasts. Near the ends of the filaments the chloroplasts are disc-shaped, relatively small and fairly widely separated (Fig. 1, A),

[1] All the vessels, including small glass boxes and small conical flasks or wide-necked bottles fitted with plugs of cotton wool for stoppers, were heated three times in a dry steriliser to 120°-130°C, and kept at that temperature for about an hour ; the culture medium was heated three times in a steam steriliser for at least two hours on each occasion.

but in the older parts of the filament they become very much crowded together and somewhat irregular in shape, and are elongated in the direction of the long axis of the cell (Fig. 1, B).
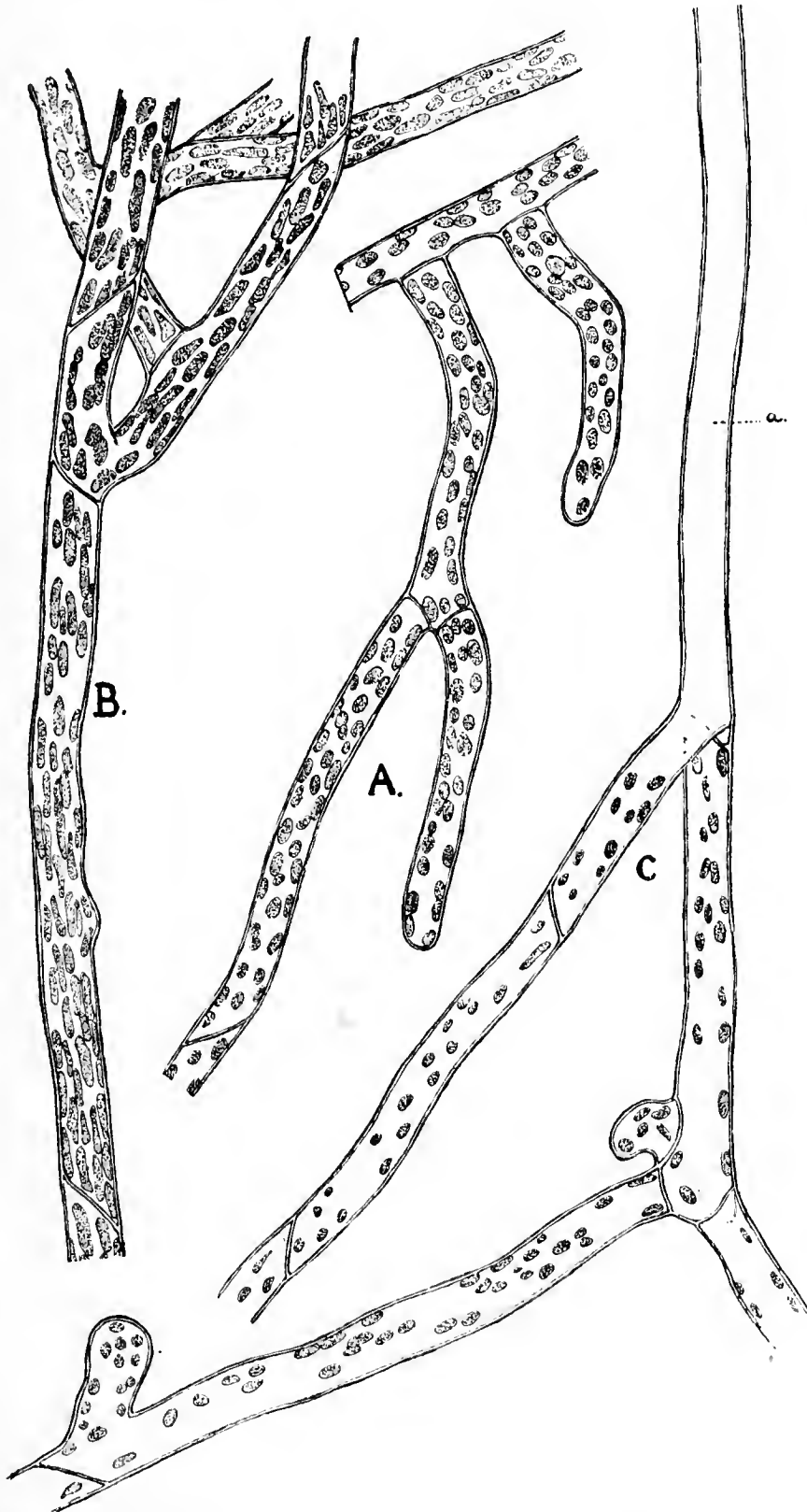
FIG. 1. Moss protonema produced in culture of Barnfield soil 1870. A, apical cells; B, middle cells; C, basal cells of filament. *a*, colourless attaching cell. × 428 diameters.

If a small tuft of protonema is transferred with its attached soil to a glass slide, and the soil is then very carefully broken up and removed by means of needles, it is possible to isolate the resting-cells from which the green protonema is produced. These resting-cells are not spores, but evidently constitute a resting filament of a moss protonema which was growing when the soil was
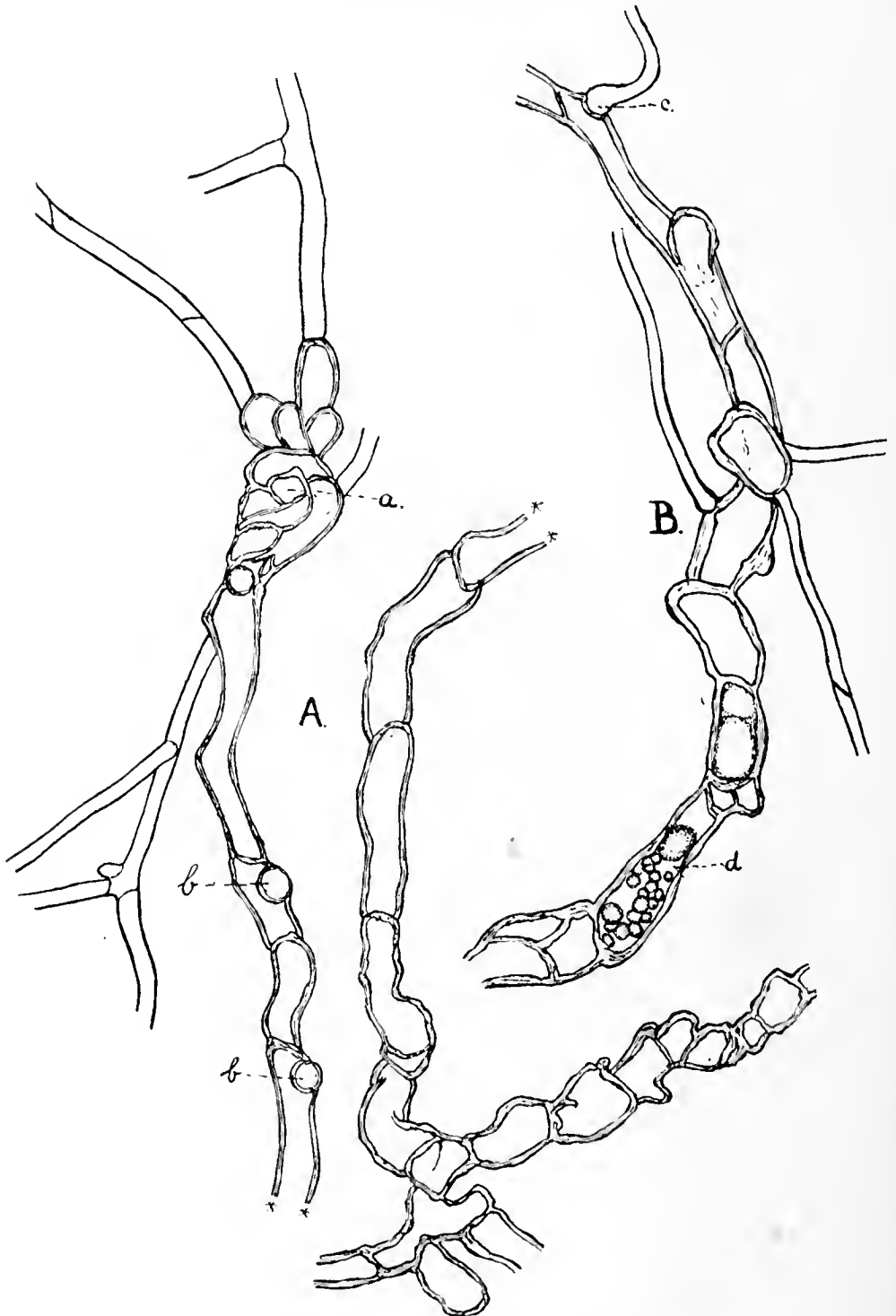
Fig. 2.   Distorted resting filaments showing attached vegetative filaments.
a, cluster of dwarf branches ; *b*, attachment of lateral branch ; *c*, swollen base of vegetative filament ; *d*, cell filled with oil globules.   × 130 diameters.

dried nearly fifty years ago, and which has remained dormant ever since. The cells of the filament possess all the characters of true resting cells; they have become very much enlarged, and an intense thickening of the walls has produced considerable distortion, not only in the shape of individual cells but also in the growth of the whole filament (Fig. 2). In some cases a number of dwarf branches have been produced, which by being crowded closely together have given the filament the appearance shown at *a* in this figure.

The walls of the cells are extremely thick and lamellose and have assumed a deep brown colour. The thickenings are somewhat irregular, often with the production of projections both on the inside and on the outside of the wall; the ends of the cells are frequently enlarged to form a head with densely striated walls (Fig, 3, B). Circular markings can also be seen on the lateral walls of some of the cells showing where side branches have become detached from the main filament (Fig. 2, A, *b*).

Two kinds of cells can be distinguished from one another by their contents. The great majority have the structure shown in Fig. 3, B, where the walls are enormously thickened and a lining layer of cytoplasm can easily be detected under the microscope by reason of a number of minute globules of oil which it contains. In these cells a nucleus lying close to the cell-wall can frequently be seen without staining. A few other cells are rendered most conspicuous by being completely filled with large globules of a greenish-yellow oil, which gradually turns to a deep brown colour, and finally black, on treatment with a 1% solution of osmic acid. The walls of these cells are often somewhat less thickened than those of the first kind (Fig. 3, A).

In ordinary green protonema-filaments reserve food is stored in the form of starch; the existence of a fatty oil in the cells of the resting filaments is unique, and is interesting as being correlated with their extreme retention of vitality.

When suitable conditions for growth are supplied, young filaments are produced as outgrowths from the walls of the resting cells, and the latter gradually lose their oily contents. At first the young filaments are colourless, but as cross-walls are formed and the filament increases in length small scattered disc-shaped chloroplasts appear. The cell arising directly from the resting filament is slightly swollen at the base (Fig. 2, B, *c*) and appears in all cases to remain permanently colourless, and its wall gradually assumes a light brown colour (Fig. 1, C, *a*). It is not until three or four cross-walls have been produced in the young filament that the cells

develop the very numerous chloroplasts characteristic of the lower cells of a well-grown protonema.
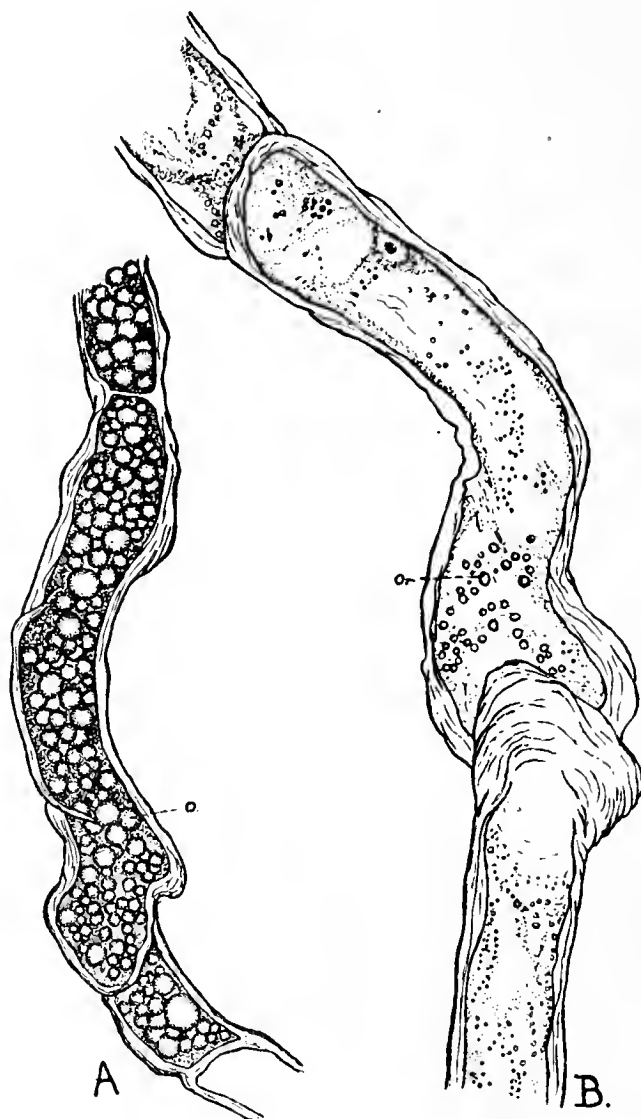
FIG. 3. Cells from resting filament. A, cell filled with globules of oil; B, cell with enormously thickened walls and a few small oil globules. *a*, oil globules. × 428 diameters.

A subsequent examination of some of the dry soil from which the cultures had been made, and also of fresh samples obtained from the Rothamsted Laboratory for the purpose, which had been soaked out with water for a few hours, revealed the presence of the resting protonema in the soil itself, and proved without the slightest possibility of doubt that the presence of moss protonema in the cultures was not the result of a foreign infection. The cells of the resting filaments observed in the dry soils were all completely filled with oil, having the appearance of the cell at *d* in Fig. 2, B; this indicates that the two kinds of cells described above only represent different stages of development, and that probably all the cells were originally completely filled with oil.

No previous record has been made of a resting moss-protonema of this nature. Goebel[1] has described certain resting buds, developed from the protonema, which on being provided with suitable conditions for growth give rise directly to a leafy stem; but this appears to be a condition quite different from that described above.

The only other resting state in mosses which has been recorded is still more different, for here buds produced on a leafy stem become arrested in their development, lose their chlorophyll and assume a dark brown colour. These changes appear to be produced as the result of unfavourable conditions of growth, and under proper conditions the buds develop either directly or by the formation of a secondary protonema into perfect plants.[2]

Moss spores contain chlorophyll and are usually very short-lived, though in a few cases spores have been known to retain their vitality for months. Hence the power to produce a resting protonema-filament which is able to resume growth, even after half a century, is a great asset to the plant in preventing its extinction through adverse climatic conditions.

The Barnfield soil when first removed from its sealed bottle in 1912 was found to contain 10% of water[3]; but during the three years which elapsed before these cultures were set up a gradual drying of the soil had taken place, through the cotton-wool plug with which the bottle was closed, until the soil contained only 3·3% of water. These percentages of water are too low for a protonema to exist in a vegetative condition, and it is certain that the resting filaments have been lying dormant for nearly half a century in the condition in which they are now found in the soils. The resting filaments have thus retained their vitality during a period of forty-two years in a soil containing only 10% of water, and a further period of three years in which the soil was gradually drying until it contained only 3·3% of water.

In conclusion, I desire to express my thanks to Professor G. S. West for suggesting this very interesting subject for study, and to Dr. E. J. Russell of the Laboratory, Harpenden, for his help in providing me with fresh samples of soil from the original bottles.

[1] Goebel. "Das Prothallium von Lycopodium inundatum." Bot. Zeit., 1887, p. 161.

[2] Campbell. "Mosses and Ferns," 1905, p. 190.

[3] I am indebted to Dr. T. Goodey for this figure.

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CARBON ASSIMILATION.

A Review of Recent Work on the Pigments of the
Green Leaf and the Processes connected with them.

By Ingvar Jörgensen and Walter Stiles.

(Continued from p. 135).

B. Temperature.

From the researches of van't Hoff it is well known that the
relation between temperature and the reaction velocity of a good
many chemical reactions can be expressed in a simple way, revealing
the fact that in many cases the reaction rate at moderate temper-
atures is increased 2 or 3 times for a rise of 10°C.   On the other
hand, animal physiologists have shown that although a smooth
temperature-metabolism curve can be constructed which gives the
relation between temperature and respiration, yet this curve does
not obey the van't Hoff rule.   Similar curves have been obtained
for plant respiration by Kuijper (1910) and for one aspect of plant
growth (Leitch, 1916).   Such a curve is shown in Fig. 7 and its
relation to true van't Hoff curves with different coefficients (Q_{10})
exhibited.   Some consider such a curve as made up of portions of
several van't Hoff curves having different constants (Pütter, 1914).
Krogh (1916) points out that it is not very probable from *a priori*
considerations that the van't Hoff rule should be followed, as we
have to do, not with a simple chemical reaction, but with a complex
series of reactions possibly taking place in a heterogeneous system.
And even if the difference between the heterogeneous system and
a system is solution could be neglected, yet the shape of the
curve would still be affected if a limiting factor were operative.
Thus oxygen pressure in the tissues might be a limiting factor.

Owing to Blackman's recognition throughout his work of the
effect of limiting factors, our knowledge of the relation between
temperature and carbon assimilation is much clearer.   It is recognised
by Blackman that in investigating the influence of temperature on
carbon assimilation, no other factor must be limiting the rate of the
process, as in such a case, the amount of the carbon assimilation is
simply dependent upon the value of the limiting factor and is not
related to the temperature.

The influence of temperature on carbon assimilation is described
in two papers, one by Miss Matthaei (1904) and a second by
Blackman and Matthaei (1905).   In these papers will be found a

general account of the apparatus and method used. Isolated leaves of Cherry Laurel (*Prunus laurocerasus* var. *rotundifolia*) were used in most experiments, while for some, leaves of *Helianthus tuberosus* were employed. The leaves were carefully selected and after
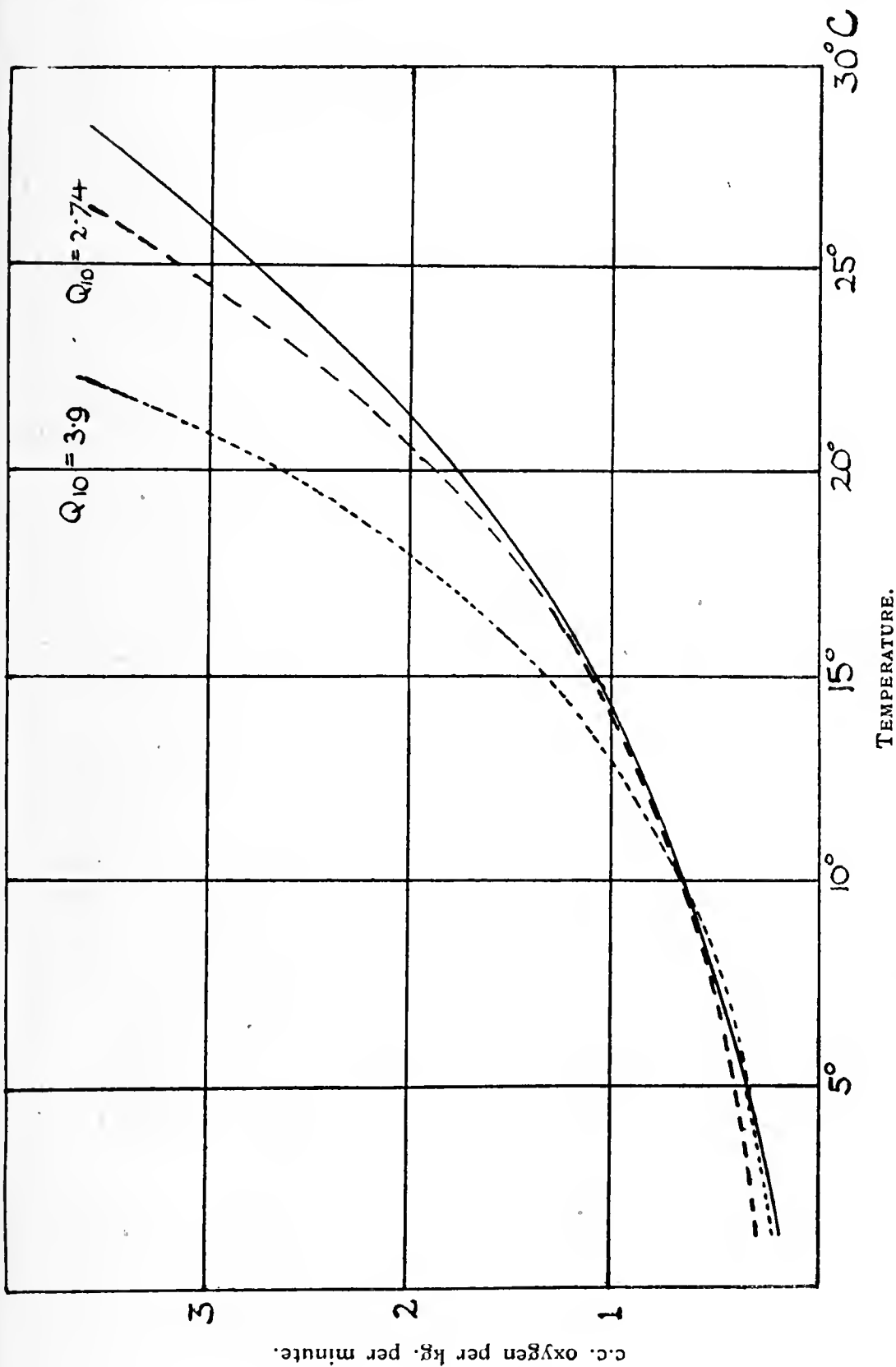
FIG. 7. Temperature metabolism curve of a fish compared with van't Hoff curves. (After Krogh).

picking were kept for 24 hours before experimentation at fairly constant temperature, with their stalks in water in covered beakers exposed to diffuse light. It was found that the previous history of the leaf, especially as regards nutrition and temperature changes, is very important in determining the amount of assimilation. The significance of this we shall refer to later.

For each experiment a fresh leaf was employed and this was kept with the cut end of its stalk in water in order to keep down loss of water from the leaf through transpiration. The leaf was contained in a chamber through which air containing a known quantity of carbon dioxide was passed at a known rate. Analysis of the outflowing gas gave the necessary data for determining the intake of carbon dioxide. As a source of light, incandescent gas or Keith high pressure gas was used. For the work described in the second paper, sunlight alone was used.

It is assumed that assimilation and respiration take place simultaneously in the leaf, and for this purpose the respiration at each temperature was obtained by measuring the output of carbon dioxide in the dark. On adding this to the value of the 'apparent assimilation,' the 'true assimilation' is obtained.

At higher temperatures the respiration is more difficult to estimate, for oscillations occur much too big to be accounted for by experimental errors. Also, during an assimilation experiment, the respiration is constantly changing on account of the assimilation. An approximation to its value was therefore obtained by measuring it in the dark before and after an assimilation experiment and taking the mean value.

Another complication has to be taken into account when high intensities of light are used. It was recognised by Brown and Escombe (1905) that light falling on a leaf would bring about a rise in temperature of the leaf, and they endeavoured to calculate this from a knowledge of other conditions of the leaf. Blackman and Matthaei (1905) show that the values obtained by Brown and Escombe depend on the values of six other quantities which are not all known, and hence the value obtained by calculation is not very likely to be correct.

Blackman and Matthaei therefore made direct measurements of the internal temperature of the leaf by means of small thermo-couples of copper and constantan. One junction was embedded in the midrib of the leaf, and the other kept in a water bath. The internal temperature of the leaf was measured by bringing this water

bath to such a temperature that the E.M.F. of the combination of two thermocouples was zero. The two couples are then at the same temperature and the temperature of the water bath containing one junction is consequently the internal temperature of the leaf.

In this way it was shown that the values obtained by Brown and Escombe for the rise in temperature of the illuminated leaf were much too small. The following table giving some of the values obtained by Blackman and Matthaei shows how much higher the internal temperature of the leaf may be above that of its surroundings when subjected to intense illumination.

TABLE X.

Effect of Light in Raising Internal Temperature of Leaves.

Source of Light.	Relative Intensity of Light.	Temperature of bath containing Leaf Chamber.	Internal Temperature of Leaf.
Keith high pressure gas burners.	13	11°C	15°C
,,	26	11°C	23·7°C
,,	45	13·5°C	30·5°C
Brilliant sunlight in July.	—	18·6°C	22·4°–30·7° C

In all experiments with high light intensities the internal temperature of the leaf was therefore measured.

In all experiments 800 c.c. of air containing from 0·8% to 2·8% of carbon dioxide were passed over the leaf per hour. As this was never used up it was supposed that carbon dioxide was not a limiting factor. The experiment was allowed to run for $1\frac{1}{2}$ to 2 hours before measurements were made, in order to render the conditions constant. The amount of carbon dioxide absorbed during consecutive hourly or two hourly periods was then measured. This gives the value of the 'apparent assimilation' to which is added the value found for the respiration in order to obtain the 'true assimilation.'

Assimilation at Low and Medium Temperatures.

As unit intensity of light was used the light from a single incandescent gas burner when the front of the mantle was 130 cms. from the leaf. With this illumination, assimilation could be detected at as low a temperature as —6°C. With increasing temperature the assimilation rapidly increased up to 3°C, above which, increase

of temperature had no effect on the rate of assimilation. This remained the same right up to 33°C. The accompanying curve (Fig. 8) shows the result obtained with unit intensity of light. It will be observed that it resembles the curves shown in Fig. 5 illustrating the action of a limiting factor. It is evident that this would be the result if the intensity of light were acting as a limiting factor over that part of the curve above 3°C.
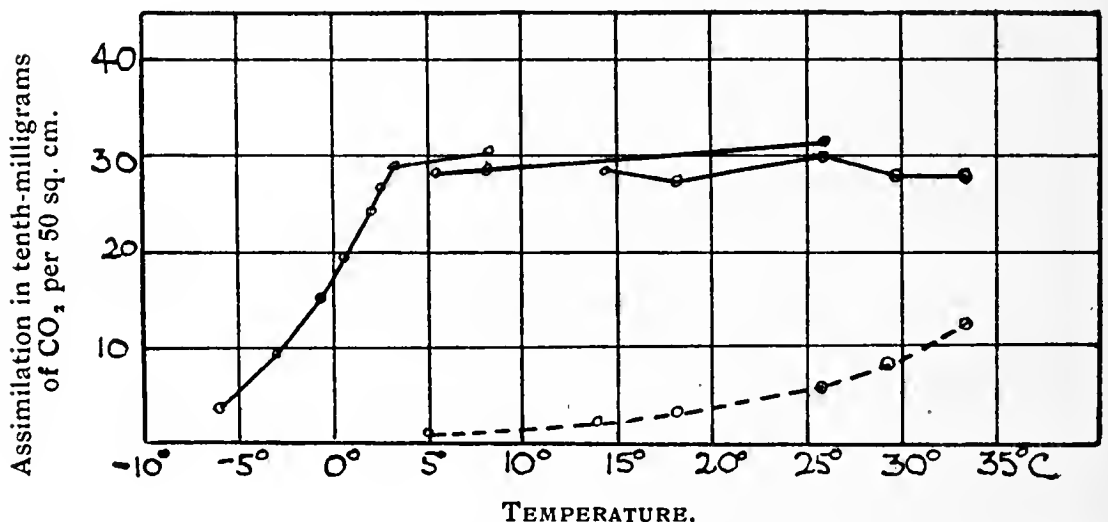
Fig. 8.   Curve illustrating effect of temperature on assimilation of Cherry Laurel with unit intensity of light.   The broken curve indicates the respiration. (After Matthaei).

Consequently, if the light intensity is doubled, one would expect tne first part of the curve to be much longer, and increase in temperature to produce a corresponding increase in assimilation until this has reached a value twice as great as that given by the horizontal part of the curve when unit intensity of light is employed. Similarly, if the light intensity is still further increased a yet higher temperature has to be employed before the limiting action of light will become evident.

The curves shown in Fig. 9 illustrate this clearly. They show graphically the results obtained by Miss Matthaei for the relation between temperature and assimilation when 1, 2 and 4 units of light intensity were employed. They indicate that provided light is not a limiting factor and the carbon dioxide supply kept constant and in excess, the higher the temperature, the greater the assimilation. Increasing the temperature will, however, produce no change in the rate of assimilation if the light intensity is below a certain value and so acting as a limiting factor.

Assimilation at High Temperatures.

It is thus possible to construct a curve showing the relation between temperature and assimilation when neither light nor carbon dioxide supply is a limiting factor. Above 25°C, however, a fresh complication arises. Below 25°C the amount of assimilation remains constant hour after hour, but above this temperature the rate of assimilation decreases with time. The initial rate of assimilation cannot be maintained. In all the experiments the rate of assimilation during the first $1\frac{1}{2}$ hours was not measured; measurements were then made of the assimilation taking place in successive hours. Finally the leaf was darkened and the respiration measured. The following tables show typical series of results for the assimilation of the leaf at a low temperature and at a high temperature during successive hours.

TABLE XI.

Assimilation of Leaf of Cherry Laurel at 8·8°C.

Area of Leaf 44·6 sq. cms.

Light Intensity.	Time.	Apparent Assimilation.	Real Assimilation per 50 sq. cms. per hour.
Unit	12.30–2.0 p.m.	Preliminary	Preliminary
,,	2.0–4.0 ,,	·00375	·0023
,,	4.0–6.0 ,,	·0037	·00225
Twofold	8.0–9.40 ,,	Preliminary	Preliminary
,,	9.40–11.40 ,,	·00665	·0039
,,	11.40–1.40 a.m.	·0066	·0039
,,	1.40–3.40 ,,	·00645	·0038
,,	3.40–5.40 ,,	·0065	·00385
,,	5.40–7.40 ,,	·0065	·00385

TABLE XII.

Assimilation of Leaf of Cherry Laurel at 37·5°C.

Area of Leaf, 36·0 sq. cms.

Respiration, 0·0019 grams per hour.

Light Intensity.	Time.	Apparent Assimilation.	Real Assimilation per 50 sq. cms. per hour.
45	10.30 a.m.–12.0 noon	Preliminary	Preliminary
,,	12.0 noon–1.0 p.m.	·0154	·0237
,,	1.0–2.0 ,,	·0106	·0176
,,	2.0–3.0 ,,	·00795	·0139
,,	3.0–4.0 ,,	·0059	·0109

These two tables show very clearly the different relation between assimilation and time at temperatures below and above 25°C. Whereas the assimilation proceeds at a constant rate at 8·8°C as long as the experiment is continued, at 37·5°C there is a rapid falling off in the rate of assimilation throughout the experiment. A time factor comes into play. The facts observed in Miss Matthaei's experiments indicate the three following laws in regard to the time factor (Blackman, 1905).
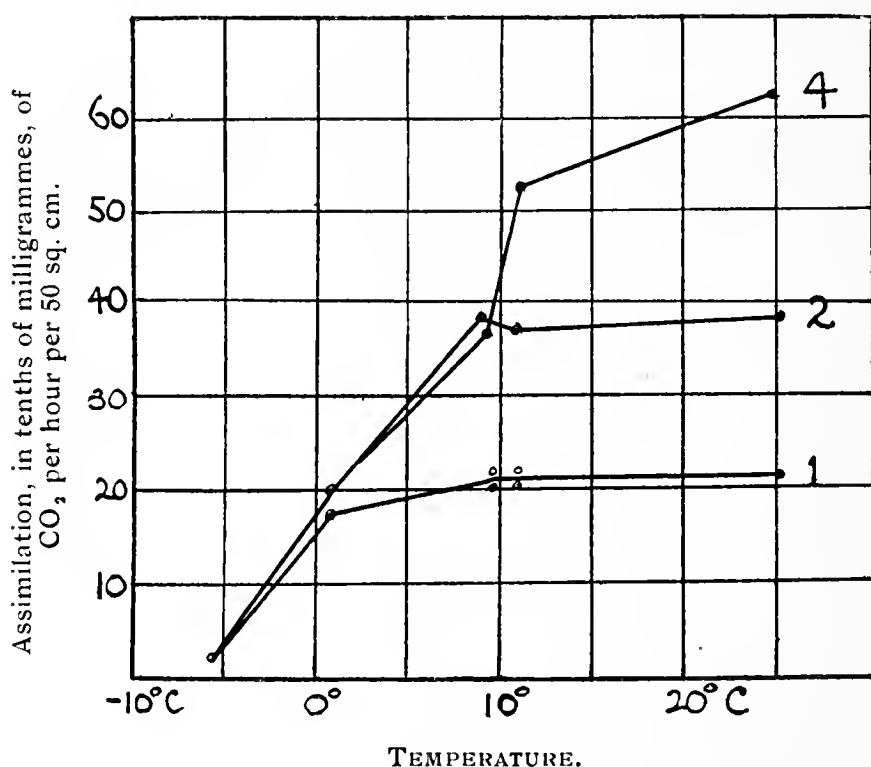
FIG. 9. Curve illustrating the effect of temperature on assimilation of Cherry Laurel under the influence of light of different intensities.   1, unit intensity of light ; 2, twofold intensity ; 4, fourfold intensity.   (After Matthaei).

1. At high temperatures the initial rate of assimilation cannot be maintained, but falls off regularly.

2. The higher the temperature the more rapid is the falling off.

3. The falling off at any given temperature is fastest at first and subsequently becomes less rapid.

It thus becomes impossible to measure the highest possible assimilation at any temperature, but Blackman estimates this initial value of the assimilation by two methods. Firstly, below 25°C no time factor is involved and the assimilation numbers obtained therefore give a correct value of the initial values of the assimilation. The curve obtained from these numbers is a van't Hoff curve in which the temperature coefficient for a rise of 10°C is 2·1.   By

assuming that the van't Hoff rule is followed above 25°C we can obtain by calculation the initial values for assimilation at higher temperatures.

Secondly the initial assimilation at a high temperature may be determined from the values actually measured after various times. At any temperature the curve between time and assimilation is plotted and the curve continued back to a point where the value of the time is zero: the value corresponding to this is the initial assimilation.

Blackman's diagram illustrating these methods is shown in Fig. 10. The broken line is the temperature assimilation curve
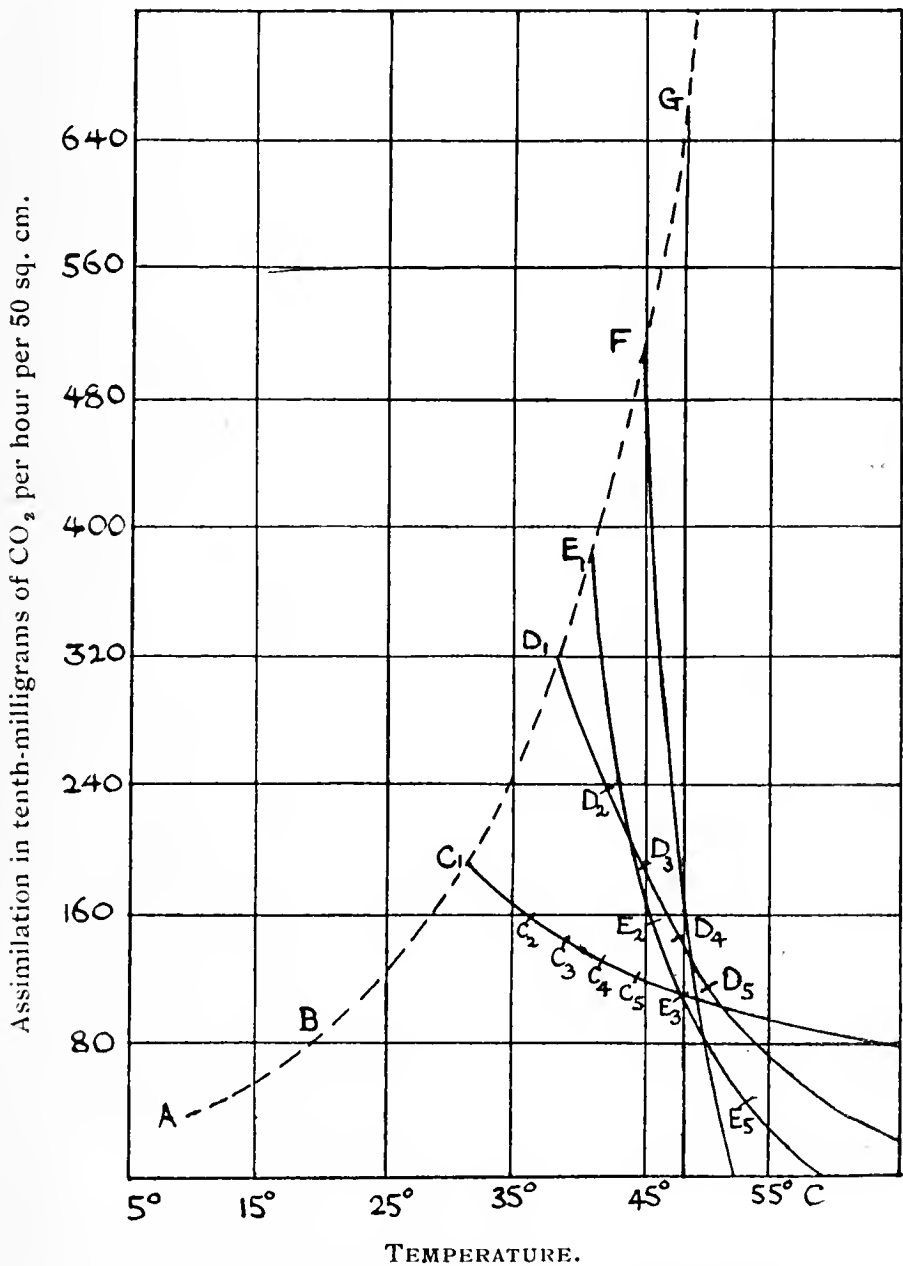
FIG. 10. Curve showing initial assimilation maxima at different temperatures. For further explanation see text. (After Blackman).

obtained by continuing the van't Hoff curve obtained for temperatures below 25°C. As to the second method estimations of the assimilation were made during four successive hours at 30·5°C, 37·5°C and at 40·5°C. The values for assimilation so obtained are plotted against time on the same diagram (the abscissæ now having a time significance) in the curves C_2—C_5, D_2—D_5 and E_2—E_5 respectively, the points C_2, C_3, etc. giving the values of assimilation obtained in the experiments. These curves are continued backwards to a point representing zero time, which gives the initial assimilation. The curves are so arranged in the diagram that the position representing zero time in each case is that also representing the temperature of the determinations, so that if the initial values of assimilation are given by the van't Hoff rule, they will fall on the curve drawn on that assumption. This is shown to be the case, so that as Blackman says, there is satisfactory evidence for a preliminary acceptance of the theory that the initial values of assimilation at high temperatures follow the van't Hoff as well as at low temperatures, although above 25°C the existence of the ' time factor ' prevents the direct measurement of this maximum possible assimilation. The suggested form of the assimilation time curves at still higher temperatures are shown at F and G (Fig. 10). At G the temperature is supposed to be reached at which the assimilation falls at once to zero.

Experiments conducted by Blackman and Matthaei (1905) in which natural illumination only was employed show that different leaves may have different temperature coefficients for assimilation. Thus whereas Cherry Laurel has a temperature coefficient of about 2·1, that of *Helianthus tuberosus* was found to be about 2·5. The results are summarised in the accompanying diagram (Fig. 11).

A further point brought out in these researches is that the assimilation rate is affected by the season of the year, thus leaves are more active in February than in April, and from January to March than from October to mid December. These facts are at present unexplained, but they have no effect on the temperature coefficient which is independent of seasonal variation.

Owing to the more rapid falling off of the assimilation with time the higher the temperature, the temperature at which greatest assimilation is observed will depend upon the time which elapses between the commencement of the experiment and the measurement of the assimilation. Thus in the case of Miss Matthaei's measurements the highest value of the assimilation for the first hour after the experiment had run its preliminary 1½ hours is given at 37·5°C,

while for the fourth hour it is given at 30·5"C. Thus the optimum value obtained will depend upon the time that has elapsed between the commencement of the experiment and the measurement of the
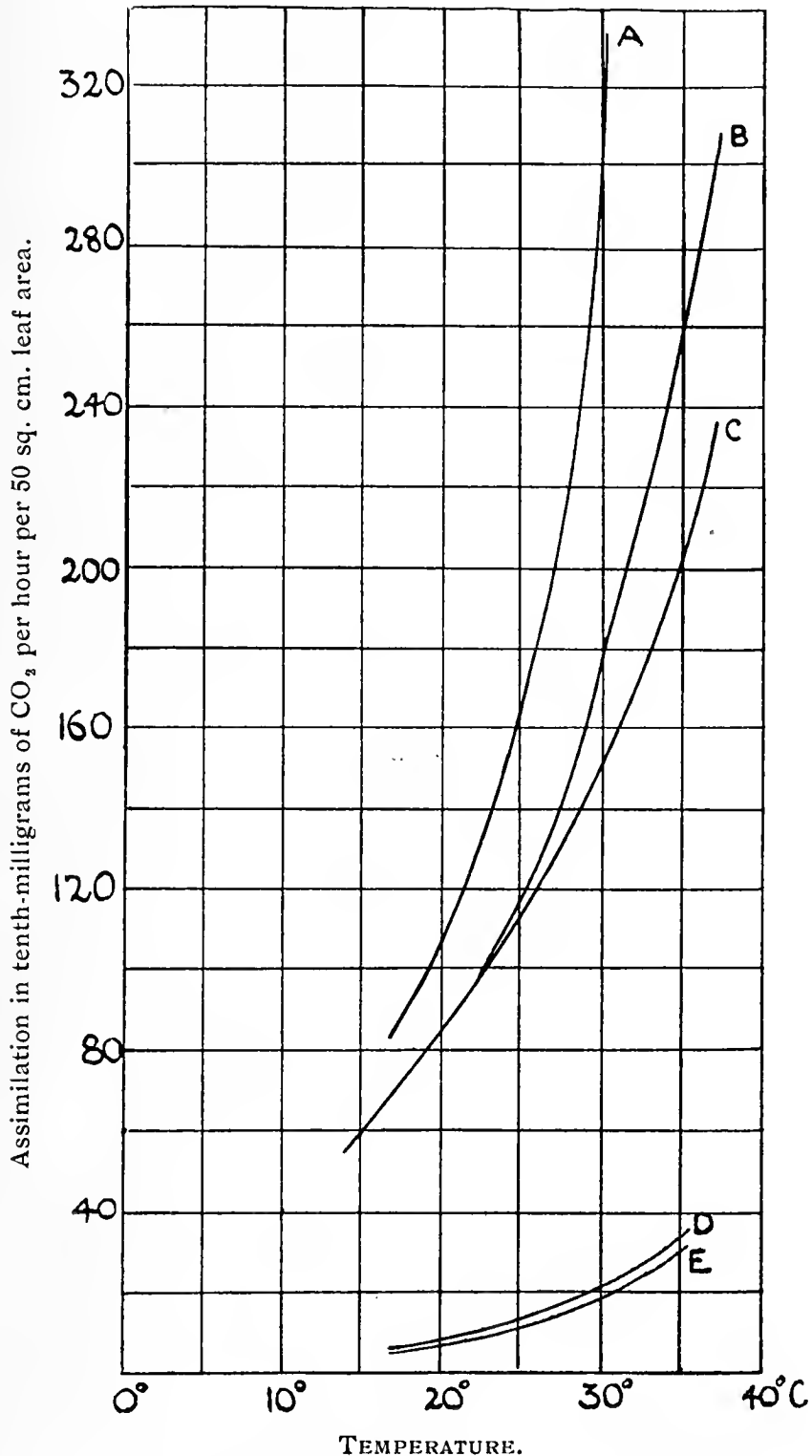
FIG. 11. Curves of assimilation and respiration of *Helianthus* and Cherry Laurel at different temperatures. A, curve of initial assimilation maxima for *Helianthus* ; B, the same for Cherry Laurel ; C, curve for assimilation of Cherry Laurel two hours after the initial moment of heating to the particular temperature ; D and E, temperature-respiration curves for *Helianthus* and Cherry Laurel respectively. (After Blackman and Matthaei).

experiment.   Obviously it would also depend on the previous history of the leaf as regards temperature.

In regard to the existence of the time factor Blackman points out that the rate of hydrolytic action of enzymes always shows a marked optimum temperature effect, and he cites Kjeldahl (1879) who showed that malt diastase hydrolysed increasing quantities of starch up to about 63°C after which the action fell off quickly, becoming nothing at 86°C, this being due to the destruction of the enzyme by heat.   The apparent production of an optimum is thus due to two opposed processes, the hydrolytic action of the enzyme and the destruction of the enzyme by heat.

This characteristic of enzyme actions was later elaborated by Tammann (1892, 1895) and by Duclaux (1899) and it has come to be called Tammann's principle.   Fig. 12, taken from Duclaux, illustrates clearly how the optimum is produced. The curve AB shows the relation between temperature and the enzyme action if the enzyme activity remains unimpaired, the curve CD represents the relation between temperature and quantity of enzyme, and the curve AOE represents the actual curve between temperature and the enzyme action.

Recently some continental writers (Kanitz 1915, Rahn 1916) have taken the trouble to point out the application of Tammann's principle to Blackman and Matthaei's results.   Not only did Blackman himself point out the similarity of his results with those in the case of enzyme actions, but he also recognised that the matter was probably more complex. Thus he says " Physico-chemical finality is not to be attained in this matter, but special research might at least show how far the recorded optima for assimilation and respiration are real metabolic truths and how far they are illusions of experimentation."

With the further criticism of Rahn we need not deal, as it attempts to explain why Blackman did not get a result which as a matter of fact he actually obtained.

Kanitz also criticises Blackman on account of the manner in which the curve of initial assimilation values at high temperatures is obtained.   He points out that the number taken by Blackman for the temperature coefficient of assimilation in the case of Cherry Laurel was quite arbitrary ; that it might have been 2·4 equally with 2·1.   However, an examination of Miss Matthaei's figures are sufficient to show that the choice of 2·1 as the temperature coefficient between 5°C and 25°C was fully justified.   Below 5°C the temperature

coefficient increases with decreasing temperature. This may be due to some other factor coming into play and it is a general phenomenon in life processes (cf. *e.g.*, Krogh's temperature-metabolism curve in Fig. 7). Again in the case of many chemical reactions the temperature coefficient gradually decreases with rise of temperature, and this may perhaps be the case in carbon assimilation. In any case Blackman was more justified in assuming as an approximate temperature-coefficient at temperatures above 25°C the value obtained from 5°C to 25°C than the value between —6°C and 5°C. It must be kept in mind that the theoretical curve of initial assimilation maxima is necessarily an approximation.
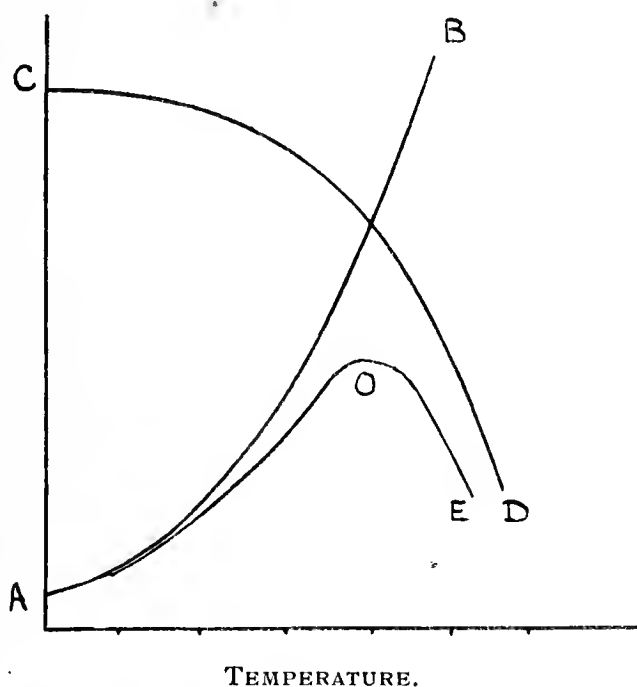
TEMPERATURE.

FIG. 12. Curves showing the relation between temperature and rate of enzyme action. (After Duclaux).

Kanitz further criticises the method of obtaining the initial assimilation values obtained by carrying back the time-assimilation curves to zero time. He asserts that the numbers so obtained give the amount of assimilation which actually takes place during the first hour of the experiment, whereas the number actually required is the assimilation which would take place in an hour if the initial rate of assimilation remained constant throughout that hour. This criticism also is due to imperfect consideration of Blackman's curves. The first measurements of assimilation were made by Miss Matthaei from 1½ hours to 2½ hours after the experiment was started. The number so obtained can be taken as approximately representing the rate of assimilation 2 hours after the commencement

of the experiment. Blackman actually continues his curves back for 2 hours, not $1\frac{1}{2}$ hours, and the value so obtained should therefore represent approximately the initial rate of assimilation. Kanitz's criticism is thus founded on a misconception.

The further criticism of Blackman's work offered by Kanitz is based on the assumption that Blackman's construction (Fig. 10) and the application of the time law for enzyme action are mutually exclusive. This is evidently not so. Blackman himself realised the resemblance between his results and those obtained for enzyme actions, but preferred to express his results in a non-committal form to making assumptions which were unproved by experiment.

With the criticisms of Blackman's construction offered by some other continental writers we need not deal, as they amount to no more than the expressions of personal opinion.

Kanitz points out how from a consideration of Duclaux's curve (Fig. 12) it is obvious that the position of the optimum is no fixed point, but must depend on the quantity of enzyme present, which will also depend on the previous history of the system: on its previous temperature, on the velocity with which it is brought to the optimal temperature, etc. It is interesting in this connection to recall that Miss Matthaei found it necessary, in order to obtain uniform results, to take particular care that all leaves used in her experiments were subjected to the same treatment for the 24 hours between their removal from the tree and the commencement of the experiment.

It may also be mentioned here that some investigators regard the optimum temperature as the highest temperature which can be maintained continuously without a depression of the function resulting, and recently Miss Leitch (1916) has adopted this idea for the case of growth. It is clear, however, that if it is really Tammann's principle or something strictly analogous to it that is involved in 'time factors' in carbon assimilation and growth, the position of the optimum, as Kanitz points out, is not a definite fixed point, but depends upon other factors which will only remain constant if the plants are subjected to the same previous history.

C. Light.

The effect of light on the intake of carbon dioxide has been indicated in the previous section of this chapter. Reference was there made to Miss Matthaei's experiments in which it was shown that intensity of light may limit the intake of carbon dioxide, in

which case increase of temperature produces no effect on the rate of assimilation. The results have already been shown graphically in Fig. 9.

Blackman and Matthaei (1905) have also made extensive series of observations on assimilation under different conditions of natural illumination which show strikingly the influence of light as a limiting factor. The general arrangement of the experiments was similar to that employed previously and referred to in the previous section of this chapter. The leaves experimented upon were contained in a leaf chamber as before through which a current of carbon dioxide was passed such that the supply of carbon dioxide never limited the intake of the gas. The experiment summarised in the following table may be regarded as typical.

TABLE XIII.

Assimilation by a leaf of Helianthus tuberosus under Natural Illumination.

Area of Leaf 70·1 sq. cms.

800 c.c. of air containing 2·5% CO_2 passed over the leaf per hour.

Date : July 30th, 1904.

Temperature 18·0° to 18·3°C.

Assimilation at this temperature when light is not limiting is ·0093 grms. per 50 sq. cms. per hour.

Time P.M.	Illumination.	Temp. of Bath.	CO_2 in grams absorbed by leaf.	Real assimilation in grams CO_2 per 50 sq. ems. per hour.
12.30–1.30	—	—	Preliminary	Preliminary
1.30–2.30	Heavy laden clouds	18·2	0·0011	0·0015
2.30–3.30	Violent thunderstorm at first ; then slowly clearing up	18·3	0·0032	0·0030
3.30–4.30	Brighter ; no rain	18·3	0·0073	0·0059
4.30–5.30	Sun at first, then clouded over ; storm driving up	18·3	0·0050	0·0043
5.30–6.30	Overcast, steady rain ; 6·10, heavy storm	18·0	0·0007	0·0010

It will be observed that the intake of carbon dioxide is in none of these measurements near the value given when light is not limiting, and in each case the assimilation must be a measure of the light only. The assimilation shows marked variations parallel with the light conditions.

Besides a large number of similar determinations by Blackman and Matthaei made at various temperatures with light the limiting factor when natural illumination was employed a few confirmatory measurements were made with the water plant *Elodea* by Blackman and Smith (1911 b); the results have already been shown graphically in this chapter (see Fig. 6). These writers have also shown that Pantanelli's results (1903) rightly interpreted, indicate that assimilation is directly proportional to the intensity of light used until either carbon dioxide supply or temperature becomes a limiting factor. The earlier experiments of Reinke (1883) also support the conclusion.

From the results of his experiments Blackman concludes that where temperature and carbon dioxide supply are in excess the rate of assimilation is proportional to the intensity of illumination. There is thus for every temperature a minimum value of the light intensity which is sufficient to allow the maximum assimilation rate to take place at that temperature always presuming no other factor is limiting. By using perforated screens in front of the leaf to cut off part of the sunlight, Blackman and Matthaei were able to show what proportion of sunlight was required to give the maximum possible assimilation at 29·5°C. Thus it was shown that in bright sunlight during the middle of the day in August the maximum assimilation possible at 29·5°C in the case of Cherry Laurel was given by 0·36 of full sunlight and in the case of *Helianthus* by 0·69 of full sunlight. It would be expected that *Helianthus* was therefore capable of a much higher rate of assimilation than Cherry Laurel at the same temperature, and this is indeed the case (cf. Fig. 11).

Indeed Blackman and Matthaei show that when light is the limiting factor equal areas of different plants equally illuminated produce the same amount of assimilation. Blackman and Smith (1911 b) have shown that the same law holds with water plants (cf. Fig. 13).

With the bearing of these results on the general question of energy in regard to assimilation we propose to deal later.

D. Carbon Dioxide Supply.

The influence of carbon dioxide supply upon assimilation has been investigated by Blackman and Smith in the case of submerged water plants. *Elodea* was chiefly employed for this purpose, but experiments were also made with a water-moss *Fontinalis*, and a few isolated observations were also made with *Ceratophyllum* and *Potamogeton*.

In order to examine the intake of carbon dioxide by submerged water plants, a stream of water containing carbon dioxide dissolved in it was passed over the leaf. By estimations of the carbon dioxide that had passed over the leaf in a definite period the rate of intake of the gas could be measured. The procedure employed with land plants was somewhat modified. A description of the method is given in the eighth paper in Blackman's series of researches (Blackman and Smith, 1911 a).

With small-leaved plants such as *Fontinalis* and *Elodea*, sprigs of the plant were used instead of single leaves as in experiments with Cherry Laurel or *Helianthus*. In most of the experiments medium temperatures and medium illumination were employed. The value taken for the carbon dioxide supply is the mean value of the carbon dioxide concentrations of the liquid before and after passage through the chamber. The 'real' assimilation was calculated as in the experiments with land plants already described.

The results obtained with both *Fontinalis* and *Elodea* are shown in the accompanying curves (Fig. 13). In the weaker

Carbon dioxide supply in grams of CO_2 per 100 c.c. water.
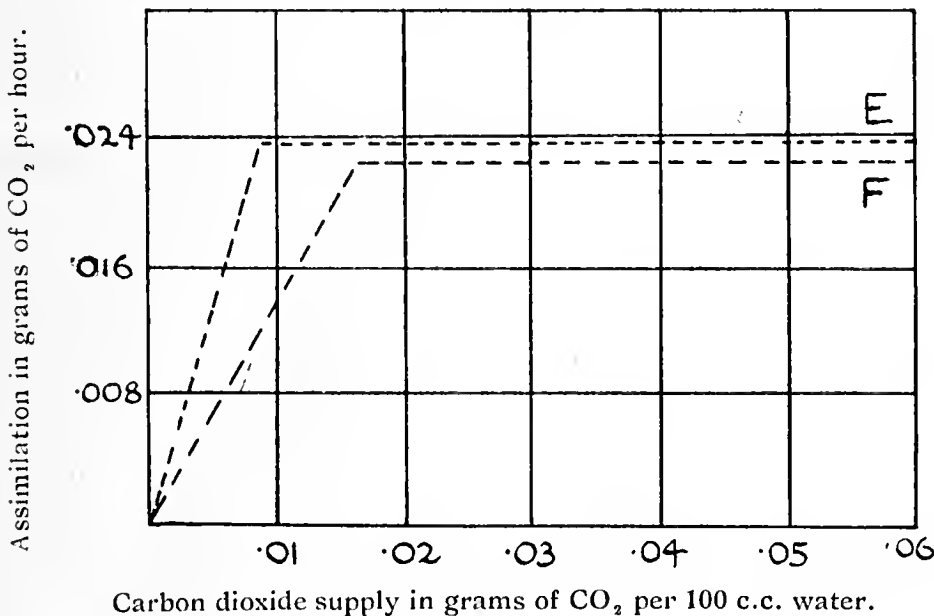
Fig. 13. Curves illustrating the influence of the magnitude of carbon dioxide supply on assimilation. E, *Elodea* ; F, *Fontinalis* (after Blackman and Smith).

solutions of carbon dioxide, the assimilation increases directly with increase of carbon dioxide supply. Neither light nor temperature are limiting. In each case, however, a point is reached where increase in carbon dioxide supply is no longer accompanied by a corresponding increase in assimilation. The latter remains constant however much the carbon dioxide supply is increased. Here, either temperature or illumination is limiting the rate of assimilation.

In order to obtain a greater assimilation, either the light or the temperature would have to be increased. In this particular case it can be shown by increasing either light or temperature that it is light which is the limiting factor. Some earlier experiments of Treboux (1903) and Pantanelli (1903) also show clearly the proportionality between carbon dioxide supply and assimilation until light becomes a limiting factor.

Another noteworthy point is that where carbon dioxide supply is the limiting factor, *Fontinalis* assimilates only about half as much carbon dioxide as *Elodea* for any particular concentration of carbon dioxide. It suggests that the difference is due to there being less obstacle to the diffusion of carbon dioxide up to the chloroplasts in *Elodea* than in *Fontinalis*. Moreover, as values obtained with *Ceratophyllum* and *Potamogeton* are of the same order as those obtained with *Elodea*, it appears to be a class distinction between Bryophytes and Phanerogams.

It is to be observed that no depression of assimilation occurs even with such a high concentration of carbon dioxide as 0·0536%. This is 33·92% of saturation, and constitutes an environment as rich in carbon dioxide as is an atmosphere containing 30% of the gas. With higher concentrations, however, the 'real' assimilation becomes much depressed. This is not to be regarded as evidence that there is 'a primary optimal amount' of carbon dioxide for assimilation. Blackman regards the depression of assimilation in high concentrations of carbon dioxide as due to a narcotic effect of the strong carbon dioxide upon protoplasm, which has been previously shown by many workers (cf. Chapin, 1902). The depression has no direct relation to carbon assimilation. Blackman thus concludes that " in the curve expressing, in any given light, the relation of assimilation to the whole range of CO_2-concentrations from zero to saturation, we may separate off the falling end-part of the curve as an effect of narcotic poisoning. The third and last phase thus contrasts with the first two phases, which are specific assimilation effects, the first rising in a straight line where the CO_2 is limiting and the assimilation proportional to it, and the second a horizontal line where the assimilation is limited by the light (or the temperature) and is independent of increase of the CO_2-supply."

To be continued.

RECENT DEVELOPMENTS IN THE STUDY OF ENDOTROPHIC MYCORHIZA.

BY M. CHEVELY RAYNER. D.SC.

SINCE the middle of the 19th century it has been recognized by botanists that invasion of the roots of a vascular plant by fungal hyphæ may take place regularly without implying parasitism of the kind which is ordinarily responsible for the symptoms of disease. During the latter part of the century many cases of regular association between the roots of one of the higher plants and the vegetative stage of a fungus were described, and the view was clearly formulated by Pfeffer and others that such a condition involved a ' community of interest ' on the part of the two organisms.

The existence of such mutual relations had already been recognized in the lichens. In this group de Bary had described lichen species which show intimate relations between the algal and fungal constituents as regards nutrition, indicated by the formation of fungal haustoria which penetrate the algal cells. Such species are connected by transition forms with the commoner types in which the ' balance of power ' is more evenly maintained either by increased resistance on the part of the algal cell or decreased demands on the part of the fungus. De Bary described the state of affairs among the lichens as a condition of *symbiosis* and the use of the term was soon extended to other cases of more or less evident mutualism in both plant and animal kingdoms.

The regular association of mycelium with the roots of vascular plants noted by Kamienski (1881) and others was recognized as a case of this kind and the name *mycorhiza* used to describe it by Frank in 1887 has since passed into common botanical usage. The researches of Frank, his recognition of two types of mycorhiza which he named *ectotrophic mycorhiza* and *endotrophic mycorhiza* respectively and the conception of symbiosis which he founded have become an integral part of the literature dealing with

the physiology of plants and need not here be further elaborated. They mark the first stage on a path of enquiry since traversed by many investigators.

The object of this review is to consider certain recent researches on endotrophic mycorhiza, to note their bearing on current conceptions of the physiology of the relationship, and to take stock more especially of their significance in the region of experimental ecology. The subject has hitherto received inadequate treatment in botanical text-books. Observations have accumulated and have been recorded as isolated details in the life-histories of certain plants rather than as expressions of a fundamental biological phenomenon common to plants and animals. It is felt that the advance in knowledge resulting from a study of recent researches into the physiology of the subject must materially alter this conception and will focus the attention of botanists on the physiological similarity of the phenomena underlying these remarkable associations with those already revealed by a study of infectious disease in the realm of animal pathology. Full historical reviews of the earlier literature on mycorhiza with very complete bibliographies have been published in the papers here reviewed; and these render it unnecessary to do more than briefly indicate the general history of research on the subject from the time of Frank up to the beginning of the present century.

The value and importance of Frank's work can be judged by the position still assigned to it in botanical literature and by appreciation of the fact that his general interpretation of the physiology of the association has been a starting-point for many subsequent researches.

Frank clearly recognized the possibility of specialized relations between plant and fungus in certain groups. He drew attention to the necessity for comparison of *infected* with *non-infected* plants in order to determine the exact nature of the relations in any given case, and he pointed out that material was not then available for the purpose of such comparisons.

It is significant of the difficulties that beset such researches that this region of the mycorhiza problem remained so long unexplored, and that the exact relations between plant and fungus in two of the families mentioned by Frank as likely to prove 'special cases,' namely, Orchidaceæ and Ericaceæ, have only recently been elucidated. The demonstration of such specialized relations in these two groups and the recognition of their true nature are an important contribution to our understanding of endotrophic myco-

rhiza. The narration of them is a fascinating chapter in the history of biological research, and the facts themselves have an immediate and practical bearing on the problems of soil ecology.

From 1887 onwards, researches on mycorhiza fall naturally into two groups : *viz.*, those dealing with the ectotrophic and endo-trophic forms respectively. Despite the accumulation of a large number of facts bearing on the physiology of the relationship it is not yet clear whether, in the case of the ectotrophic forms, we are dealing with an association between plant and fungus differing only in degree from that in endotrophic forms, or whether the two types became differentiated at an early stage in their evolutionary history and differ from one another, not only in degree, but also in the essential character of the infection.

Stimulated by the researches of Frank, records of many obser-vations on endotrophic mycorhiza were published during the last decade of the 19th century. For a detailed list of these reference may be made to the papers by Gallaud, Burgeff and Rayner named at the end of this review. Among them may be mentioned those of Groom on the saprophytic Monocotyledons and *Thismia aseroë* (1894, 1895) ; Thomas on *Corallorhiza* (1893) ; Janse on the endo-phytes of many Javanese plants (1896); Chodat and Lendner on *Listera cordata* (1898) ; Nobbe and Hiltner on *Podocarpus* (1899) ; Magnus, who published an exhaustive study of the mycorhiza of *Neottia nidus-avis* (1900), and Stahl (1900) whose treatise " Der Sinn der Mykorrhizenbildung " is in part a record of the author's experimental investigations and in part a comprehensive account of the interpretation of the physiology of the relationship which he based upon them. The views of Stahl on the subject of mycorhiza have been widely quoted in botanical text-books. Certain of his experimental observations and conclusions are, however, at variance with those recorded in one of the more recent papers here reviewed. A brief discussion on the matter under dispute will be found towards the end of the present paper (p. 173).

These earlier researches showed that the relation between plant and fungus—more obviously in the case of plants like the saprophytic orchids and *Thismia*—are intimate and complex, with apparently a balance of profit on the side of the higher plant. There is frequently a definite succession of phases in the life-history of the fungus, the predominance of one or other of these phases being expressed by a differentiation of the invaded tissues of the root into morpho-logical regions.

In the first phase the mycelium vegetates actively in the living

cells of the root, forming a system continuous with hyphæ ramifying in the soil around the roots. Invasion of the root-cells is tolerated by the plant, which yields up a certain amount of nutritive material to the intruding hyphæ. Some degree of parasitism on the part of the fungus in this phase may doubtless be inferred in the case of green plants like *Listera* and the majority of orchids, in which there is evidence that the root-cells yield a supply of carbohydrate to the mycelium. In the case of non-chlorophyllous plants, e.g., *Neottia*, which are equally dependent with the fungus upon organic carbon compounds in the soil, it is not clear that the fungal partner can benefit to any extent at the expense of the plant.

The second phase is initiated by increased resistance on the part of the root-cell. The protoplast assumes the rôle of phagocyte, attacks the hyphæ which have invaded and vegetated in it, and ultimately destroys and digests them.

So much is clear from the researches named above and from those of later workers, who inferred a ' symbiosis ' analogous to that which exists between the leguminous plant and its tubercle organism. The exact nature of this symbiosis has given rise to much speculation and can be more profitably discussed in the light of recent researches.

It has usually been assumed that ability of the fungus to utilize organic carbon and nitrogen compounds is an important factor in the nutrition of the plant, especially in the case of non-chlorophyllous saprophytes like *Neottia* and *Monotropa*. Up to the beginning of the present century, however, there was no experimental evidence of the degree of interdependence of plant and fungus, nor was anything known of the life-histories or systematic positions of the fungi concerned. There are cases of endotrophic mycorhiza, moreover, in which the root-cells are invaded without such changes in the cell-structure as allow deductions to be safely drawn as to exchange of metabolic material. It is clear that interpretations of the physiology of the association based on cytological observations of plants in which such changes do occur cannot validly be extended to cover all the cases described.

Speculations as to the evolutionary history of such relationships were also unprofitable until more knowledge was available as to the behaviour of *infected* as compared with *uninfected* plants; the time and source of infection; and the growth of the endophytic fungus on different nutrient media outside the plant. Only when such evidence became available could a theory of symbiosis be formulated which would include all the observed cases and link

them up in any kind of evolutionary sequence or make clear the bionomics of mycorhiza in vascular plants.

The necessity for such evidence was first clearly recognized by Noël Bernard (1—12) whose work marks the beginning of a new stage in the history of research on mycorhiza—another landmark in the field of investigation opened up by Frank and his fellow-workers nearly twenty years earlier.

When Bernard undertook his researches into the bionomics of the relationship between plant and fungus in the orchids, it must be realized that no information was available as to time or mode of infection of the orchid seedling, nor was anything known of the degree of dependence—if any—of the two organisms upon one another, or of the systematic position of the fungi concerned.

On the other hand great difficulty was experienced by orchid-growers in germinating the seeds of certain species,—a source of much loss and inconvenience in this branch of horticulture. Bernard very sagaciously assumed that this practical difficulty might have to do with a critical stage in infection by the appropriate fungus and directed his researches with the view of proving this hypothesis experimentally.

He thus summarized the chief objects of his investigation.

1. To germinate seeds of the orchids under aseptic conditions on sterilized media suitable for their culture.

2. To isolate the root-fungi, grow them in pure culture and identify them with certainty.

3. To compare—for each orchid species—the behaviour of aseptic cultures with those of seeds infected by the endophyte.

By using seeds removed aseptically from sterilized capsules and sown under aseptic conditions, Bernard demonstrated the impossibility of raising uninfected orchid seedlings and showed incidentally that infection takes place subsequent to seed-sowing. He then proceeded to isolate and cultivate the root-fungi from various species of orchid, and was successful where all previous workers had failed.   He tested the identity of the fungi isolated in this way by inoculation of sterile seeds, induced successful germination of the most refractory species by this means, and so demonstrated conclusively the obligate nature of the association.

In later papers he described the details of infection in various orchids and the germination thereby effected, together with many interesting observations showing the delicacy of the physiological adjustment.   For example, early experiments with *Cattleya* and *Cypripedium* showed that the fungus from *Cattleya* was effective in

inducing germination of seeds of *Cypripedium* and *vice versa*. With species of *Phalænopsis*, *Vanda* and *Odontoglossum*, in which the seeds were notoriously difficult to germinate, the relation is more specialized, and each plant species has its specific endophyte which alone is effective in causing germination. This specificity was shown experimentally by sowing seeds of *Phalænopsis* and infecting some cultures with their own endophyte and others with fungi obtained from the roots of species of *Cattleya* and *Odontoglossum*. Thus, using the endophyte from *Cattleya*, the seed was killed, no germination took place and there was no digestion of the fungus by the cells of the embryo. With that from *Odontoglossum*, infection of the seed occurred, germination was at first normal but was followed by digestion of the fungus in the cells of the embryo and subsequent arrest of growth. Infected by the fungus found in the parent plant, germination proceeded in the usual way and gave a well-developed plant. Again, a high degree of physiological adaptation was shown by experimental cultures which indicated a critical stage in the development of the seedling—earlier or later according to the species. In some orchids practically no germination takes place without infection ; in others, presumably relatively primitive, e.g., *Bletilla hyacinthina*, development goes so far as the production of a tubercle with several leaves : it never reached the stage of root-formation in any species investigated by Bernard. Of great biological interest also is the experimental demonstration that the orchid plant sets a limit to the spread of its fungus partner, shown not only by digestion of the mycelium in certain cells of the root, but also by complete exclusion of hyphæ from the chlorophyllous tissues of the shoot.

Bernard was struck, as all biologists must be, by the parallelism between certain aspects of ' symbiosis ' in the orchids and the resistance to disease described as ' immunity ' in animal pathology, and the machinery of phagocytosis which is one of the means by which such immunity is secured. His subsequent researches were based upon this point of view. It is much to be regretted that they have been brought to an end by the untimely death of the author.

In 1909, Burgeff (13) published a monograph on the root-fungi of the orchids in which he reviewed the work of Bernard, confirmed his experimental results and gave an account of his own original researches on the mycorhizal fungi of many orchid species. Bernard had referred these fungi to the genus *Rhizoctonia*, after provisionally placing them in the genus *Oospora* when first isolated. Burgeff proposes the new generic name *Orcheomyces* for the group

without making any definite suggestion as to their systematic affinities.

The experimental evidence obtained by Bernard and Burgeff is contradictory with regard to the practicability of maintaining the fungus in pure cultures without impairment of its power of effecting germination. For example, Bernard found that a root-fungus isolated from *Cattleya* was scarcely capable of inducing germination after 17 months in pure culture outside the plant. Burgeff, working with another orchid, found the powers of the fungus unimpaired after 26 months cultivation on artificial media. This author also expresses the opinion that in certain cases the fungus isolated from endemic species can be used for inducing germination of the seeds of tropical species—an observation which bears out the earlier observations of Bernard and is of practical interest to orchid-growers.

Such inconsistencies suggest that the degree of dependence varies in different orchids. The more specialized the relation between the two partners, the more easily is the adjustment thrown ' out of gear ' by cultivating the fungus outside the plant.

Until 1915, the case of the orchids remained unique as being the only one in which the endophyte had been isolated and in which an obligate relation between the two symbionts had been experimentally demonstrated, but in that year Rayner (14) showed that a similar condition of obligate symbiosis existed in Ericaceæ. It was proved experimentally that seedlings of *Calluna vulgaris*, raised under aseptic conditions from sterilized seeds do not develop beyond the seed-leaf stage and are incapable of forming roots. The infecting fungus was isolated, grown in pure culture and proved to have the morphological characters of the genus *Phoma*, a member of the Hyphomycetes. Sterile seedlings growing in suitable media under aseptic conditions and inoculated from such pure cultures formed a vigorous root-system, and developed into normal plants.

The case of *Calluna* is probably characteristic of ericaceous plants in general. The relations between fungus and plant are as specialized and remarkable as in the orchids, although in many respects different, and are of considerable interest ecologically in view of the edaphic peculiarities of ericaceous plants.

The occurrence of endotrophic mycorhiza in the roots of Ericaceæ had long been known, and the more obvious facts described. Such cytological observations as had been made, however, gave no clue to the nutritive relations, nor was anything known experimentally of the physiology of the relation in this family of plants. The facts brought to light are striking and in some respects

unique among Flowering plants. The root-fungus of *Calluna* not only forms mycorhiza but extends throughout the plant in an extremely attenuated condition into the tissues of the shoot and leaves. In turn the ovary of the flower becomes infected and hyphæ grow across from the wall to the seed-coats of the young seeds within the ovary chambers. When these are ripened and shed they carry with them their fungal partner in the form of delicate hyphæ on the surface of the seed-coat. Conditions responsible for the awakening to growth of the plant embryo at germination favour a corresponding activity in the mycelium, which infects the young seedling and determines in this way its further development.

Doubtless the low germination capacity and irregular germination observable in ericaceous seeds may be attributed—as in the orchids—to failure of the fungus to ' make good ' at a critical stage, perhaps sometimes to death of the seed-coat mycelium from desiccation.

As compared with the orchids, the problem of infection in Ericaceæ has been solved in a different way. Infection is more certain, but the plant takes the increased risk inseparable from the presence of a facultative parasite in the assimilating tissues. In this case also there is *immunity*, determined, as in the orchids, by the power of the plant-cell to hold the invader in check.

The extensive researches of Gallaud (15) on mycorhiza are worthy of notice. They were undertaken to investigate cases in which the relations between plant and fungus are simpler and presumably more primitive than in the orchids. The author describes in detail numerous cases of mycorhiza selected from different groups of plants, many of which were previously unrecorded. He discusses certain morphological structures in the endophyte and their functions, and gives a general critical survey of the state of knowledge at the time when the papers were written.

Gallaud's original observations may be summarized as follows:—

The habit of forming endophytic mycorhiza is common and widely distributed in both Monocotyledons and Dicotyledons and occurs in plants belonging to widely separated families.

Infection of the roots is constant for many species, inconstant for others and shows morphological characters which permit of classification into groups.

None of the fungi concerned was successfully isolated by Gallaud. When growing as endophytes spores are not formed and their systematic position therefore remains doubtful.

From comparison of plants of the same species, e.g., *Arum maculatum*, Gallaud could find no difference between infected and uninfected plants attributable to infection. He held, moreover, that the communication between the internal and external systems of mycelium is too scanty to support Stahl's view as to the rôle of the fungus in absorption.

No definite relation of cause and effect was established between infection and the production of root hairs.

The physiology of the relationship is discussed at some length and the facts interpreted as follows. It is believed that mycelium living in the soil is attracted chemically by the roots and becomes closely applied to them. Hyphæ penetrate either directly or at special cells and there is evidence that penetration is resisted by the plant-cell. This mycelium is invariably intracellular in the outer cortex. In the inner cortex near the vascular cylinder, it forms well-developed haustorial organs (*arbuscules*). Abundance of food-material so obtained leads to the formation of reserve organs at the ends of young hyphæ (*vesicles*), which can serve for immediate growth, or function ultimately as reproductive organs after the death of the plant. There is evidence of resistance to invasion on the part of the plant-cells, passively by the deposition of membrane over invading hyphæ, and actively, after invasion, by digestion of the intracellular suckers (*arbuscules*) transforming them to structureless organs (*sporangioles*). These organs (*arbuscules* and *sporangioles*) are regarded as representing two stages in the development of the same structure and as the physiological homologues of the "pilzwirthzellen" and "verdauungszellen" of the orchids.

Active digestion of the mycelium is confined to certain cells and is characterized by definite cytological changes affecting the nucleus and other cell-organs. After digestion the cytoplasm and nucleus again become normal. There is good evidence of absorption of starch by the fungus, but the plant ultimately recovers the greater part of the absorbed material, albeit possibly in an altered form.

Gallaud was led to believe that in the types of mycorhiza he describes, as compared with the much more advanced orchid-type, there is no true symbiosis, ("symbiose harmonique") between plant and fungus. The latter he regards as a special kind of 'internal saprophyte' of the roots.

In view of the facts described, this use of the term *saprophyte* is misleading, and it would seem more intelligible to describe the invaders as *internal parasites* of a special kind whose further

development is strictly localized and controlled by the digestive power of the root-cells. It may surely be said that in these plants, as in the animal body, *immunity*—in the sense of an absence of pathogenic symptoms following upon the presence of a foreign organism in the tissues—is determined by the activities of certain specialized cells or phagocytes.

The details of nutrition in the non-chlorophyllous orchids are still so uncertainly known, that the case of *Gastrodia*, recently described by Kusano (16), is of special interest.

The curious saprophytic orchid, *Gastrodia elata*, is a native of Japan, where it is frequently found growing in woods, below *Quercus serrata* and *Quercus glandulifera*. The whole vegetative body consists of a colourless, rootless tuber, 10–17 centimetres long at maturity, associated with which are invariably a number of small daughter tubers, arising as offsets from the parent. It is noteworthy that the latter are relatively very abundant as compared with tubers of flowering size. The rhizomatous portion of the tuber bears scale leaves and the whole structure is invested with a corky covering like a potato. The immense inflorescence appears at the end of May and is produced only by tubers of full size. The plant is destitute of chlorophyll, has no root-system, and the corky covering of the tuber must render it a singularly inefficient absorbing organ.

Before the work of Kusano, *Gastrodia* was assumed to be a humus-saprophyte with mycorhiza,—in spite of the absence of roots! Nothing was known definitely of the mode of nutrition. This author has now demonstrated a very remarkable case of symbiosis between the *Gastrodia* tuber and the rhizomorphs of *Armilleria mellea* (*Rhizomorpha subterranea*), a common fungus on dead and living roots of *Quercus* in the neighbourhood. Kusano regards the rhizomorphs as usually saprophytic on *Quercus*, but they are recorded as troublesome parasites of the potato-tuber on farms near the woods.

The history of the association described by Kusano is briefly as follows. The great majority of the small tubers are entirely free from fungal infection; investigation at various stages of development yielded no evidence of an association with any fungus-mycelium, and gave no clue as to the mode of nutrition of the plant. Such tubers never flower. Flowering tubers, on the other hand, are invariably infected. The rhizomorphs of the fungus ramify on the surface and at certain points penetrate the flesh of the tuber by special haustorial branches. Below the surface, these haustoria give off mycelial branches which are responsible for an elaborate and highly-differentiated infection of the outer tissues of the tuber.

The mode of penetration of the tuber and the effect on the plant-cell is quite similar to that produced by the haustoria of parasitic flowering plants such as *Cuscuta* or *Orobanche.* Infection is limited in a radial direction and spreads tangentially only around the point of infection. In this infected region three zones may be distinguished. In the outer zone there is evidence of resistance on the part of the cell, but the general appearance of these cells suggests a ' balanced mutualism.' In the zone below, the fungus forms ' clumps' as in typical orchid mycorhiza; the nucleus and cytoplasm degenerate and are ultimately absorbed by the fungus. There is no evidence here of a beneficial symbiosis; the action of the fungus is entirely destructive, although the hyphæ themselves ultimately disappear and apparently undergo auto-digestion.

In the innermost zone of cells ' the balance of power' is readjusted, the cell cytoplasm increases in amount and the mycelium undergoes digestion by the plant. Kusano describes this inner region as " the chief metabolic centre " for the plant; food-materials, derived from the fungus, accumulate in the cells, serving ultimately for the development of the inflorescence and for the nutrition of the daughter-tubers. The rhizomorphs attached to the tuber are continuous with those in the soil, not uncommonly with branches bearing the typical *Armilleria* fructifications. The antagonism between the two components of this curious " mycorhiza " is shown by the reciprocally destructive actions described above and also by the interesting fact that occasionally the resistance of the plant is inadequate and the fungus parasitizes the tuber in precisely the same way as it does in the case of potato.

The more striking features of the association may be summarized as follows :—

1. Young tubers are uninfected and continue to grow only so long as they are attached to the parent-tuber. Failing infection by rhizomorphs of *Armilleria mellea,* they never reach the flowering stage. The majority of these daughter-tubers and their offsets undergo a gradual diminution in size from lack of food-material, and die without flowering.

2. Infection takes place from a branch of the rhizomorph, which infects the tuber locally. The infecting strand is continuous with the mycelium outside, and is the only means by which the tuber can take up food-material from the soil.

3. The plant shows a remarkable degree of resistance to invasion by the fungus, the rhizomorph of which is parasitic in its mode of entry and in its behaviour in certain zones of the infected

tissue. In another region there is accumulation of food-material brought in by the fungus and the mycelium is ultimately digested. The production of flowers and the nutrition of the offsets is entirely dependent upon this and the plant must be regarded as completely parasitic on the fungus during part of its life-history.

4. The fungus is entirely unmodified by the association and produces normal fructifications, a condition unique among fungi forming endotrophic mycorhiza.

5. The use of the term 'mycorhiza' must be extended if it is to cover a case of this kind, in which an association is formed between a fungus mycelium and the *shoot*-tissues of a flowering plant.

Kusano suggests that in *Gastrodia* there is represented the first step in the formation of a special mode of parasitic life of the fungus under special circumstances, which gives rise ultimately to a reciprocal exchange of food-material of benefit to the host. He considers that in the evolution of mycorhiza, the *Gastrodia* arrangement is probably a primitive stage. His conclusions in this respect are, however, open to criticism in view of the extreme specialization and reduction of the plant. They are indeed difficult to maintain after his demonstration that the rôle of 'host' is played by the fungus and not by the tuber.

Nothing is at present known of the behaviour of the seeds of this remarkable orchid at germination. In view of the dependance of the majority of orchid seeds upon infection at a critical stage of germination and of the observed facts of vegetative infection of the *Gastrodia* tuber the results of experimental seed cultures will be awaited with particular interest.

Much has been, of necessity, omitted from this review of recent work, and much yet remains to be learned from experimental research. The view of Frank—that special relations might be expected to exist in certain families of plants—has proved to be well-founded. Since these relations, though equally complex, are strikingly different in the two groups for which they have been demonstrated, they have probably been independently evolved and present different physiological problems. Demonstration of an obligate relation between plant and fungus in the orchids and in Ericaceæ introduces a new conception of the physiological relation and it is evident that the work of the past fifteen years has made it possible to define the term symbiosis, as used by Frank, with greater precision. Those cases, for which more specialized relations have been demonstrated experimentally, may be regarded

as expressions of the diverse ways by which a compromise has been attained by different plants when confronted with similar problems.

The work of Stahl has already been mentioned (p. 163). Some comment is required on the want of agreement between the conclusions reached by this author with regard to the behaviour and significance of the endophyte in Ericaceæ and those of Rayner in the paper on *Calluna* reviewed here. Stahl's conclusions as to the rôle of the fungus in mycorhiza were based largely on comparative cultures of mycorhiza plants grown in untreated soil and in soil which had been 'sterilized' by heat or ether vapour. Having described experiments in which seeds of *Vaccinium*, sown in May in 'sterilized' soil, germinated without difficulty and produced well-rooted seedlings, which, in October of the same year were "völlig pilzfrei," he thus summarizes his conclusions regarding Ericaceæ :

"Während manche obligaten Mycorrhizenpflanzen, wie wir früher gesehen haben, der Anzucht aus Samen und der Kultur grosse Schwierigkeiten bereiten, lassen sich die Ericaceen auch ohne Gegenwart von Wurzelpilz unschwer kultivieren und ihre Samen gehen, zwar oft langsam, aber in grossem Procentsatz und sicher ohne Mitwirkung symbiotischer Pilze auf " (*loc. cit.*).

Since seedlings six months old were "*pilzfrei*," the seeds from which they emerged must also have been free from the fungus. This involves recognition of a mode of infection in *Vaccinium* very different from that which takes place in *Calluna* and—since well-rooted seedlings were grown without infection of the roots—the absence of an obligate relation determining the formation of roots. The observations of Rayner on *Calluna* record an invariable infection of the seedling from the testa of the seed with subsequent development of root. Failing such infection, roots are not formed and the seedlings perish. This author also records ovarial infection for species of *Vaccinium*. It is true that experimental proof has not yet been offered that the course of events is similar in *Vaccinium* or that root-formation is dependent upon infection at a critical stage in the development of the seedling. The occurrence of ovarial infection in the genus, however, points to a similar habit in this respect, and the presence of hyphæ in the ovary presumably involves infection of the seed-coats as the seeds mature.

This recent work on Ericaceæ certainly casts doubt on the experimental results claimed by Stahl and renders it necessary to demonstrate the practicability of raising 'fungus-free' seedlings of *Vaccinium* under rigidly aseptic conditions. The increased growth

of plants in the partially sterilized soil which he used is easily interpreted in the light of recent work on soil sterilization.

It is still only possible to speculate on the evolutionary history of the mycorhiza habit in plants. The facts, as they are known at present, fit into such a scheme as the following. The roots of vascular plants grow ordinarily in the soil—a medium teeming with micro-organisms. There is evidence that bacteria, lower algal forms, and fungi tend to aggregate about these roots,—owing, possibly, to the excretion of minute quantities of organic material. This chemotactic influence may operate more effectively in some cases than in others and determine the entry of mycelium into the roots and, hence, the occurrence of plants possessing endotrophic mycorhiza. Whatever the ultimate cause, it is certain that internal infection of the root-cells by fungal hyphæ is a common and widespread phenomenon among flowering plants. In most of these cases the condition is limited to the formation of mycorhiza, and the mycelium is rigidly excluded from the chlorophyllous tissues.

In the simpler cases described by Gallaud, there is evidence of exchange of nutritive material with a balance of profit ultimately on the side of the plant. The fungus invades the root as a parasite, but the resistance of the root-cells to invasion is such that the parasitic habit is held in check and the mycelium grows for a time as a kind of internal saprophyte in the root-tissues. Ultimately, by digestion of the invading mycelium (mycolysis), the plant recovers the material of which it has been robbed and may profit otherwise to a variable extent depending on the metabolic activities of the fungus. It is possible, for instance, that in some cases the endophyte can utilize atmospheric nitrogen, whereas in others, *e.g.*, the orchids, this power seems to be absent (see Burgeff, *loc. cit.*). It is not very clear that the individual mycelium can profit in the long run, but it is sheltered temporarily from competition and from desiccation, and parts survive which are set free by the eventual decay of the roots. In certain plants, *e.g.*, the orchids and Ericaceæ, such relations have become more specialized and more intimate, and have resulted in the condition of obligate symbiosis described—obligate for the plant, but not for the fungal partner.

The problem of ensuring infection has been solved very differently in the two groups, and each has its attendant risks and advantages. The evolutionary history must have been long and complex in both, and cases representing side-lines of development might be expected to occur. The case of *Gastrodia* among the orchids may be of such a kind and others will doubtless be brought

to light. There is, for instance, that of *Monotropa* among Ericales. This plant has been investigated by more than one worker. The cytological facts are well-known, but the physiological nature of the association between plant and fungus still awaits experimental investigation.

Such a scheme as that outlined above is consistent with the view held by Bernard and by the writer of the present paper, namely:—that the phenomenon of mycorhiza in plants is only an expression of the warfare waged continually by all organisms against parasitic invasion of their tissues. The flowering plant possessing mycorhiza has done more than hold the invader in check; it has turned the intrusion to its own advantage.

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UNIVERSITY COLLEGE, READING.

CARBON ASSIMILATION.

A Review of Recent Work on the Pigments of the Green Leaf and the Processes connected with them.

By Ingvar Jörgensen and Walter Stiles.

(Continued from p. 160).

E. Chlorophyll Content.

So far we have dealt with environmental factors. It has been seen from Blackman's analysis that the laws governing the intake of CO_2 in relation to these factors cannot yet be expressed in simple physical and chemical terms, but the experimental facts so far obtained can be conveniently expressed in terms of the action of limiting factors.

Nor would it seem any more probable that an enquiry into the relation between an internal factor, *e.g.*, chlorophyll, and the intake of carbon dioxide should yield results of any greater physico-chemical definiteness. In this section we shall deal in some detail with investigations made with a view of determining the relation between assimilation and chlorophyll content, and more particularly with the recent work of Willstätter and Stoll (1915). At present only preliminary accounts of the extensive work of these investigators are available; consequently it is difficult to form a correct judgment of the value of the work and the validity of the arguments put forward in support of the hypotheses advanced.

There is a strong contrast between Willstätter's and Blackman s expression and generalisation of experimental results. While Blackman carefully avoids premature conclusions and tries to find non-committal expressions which will embody all his experimental results, Willstätter advances a simple definite hypothesis and attempts to obtain experimental data which will support his theory.

In this section we shall only give Willstätter's experimental data, in a later chapter we shall deal with the various theories of carbon assimilation which he has advanced.

The main result of his work is a demonstration of the complexity of the processes of carbon assimilation; an opinion which has often been expressed by earlier workers. For instance Pfeffer (1897) says that the chloroplasts "are only capable of assimilatory activity when all the component parts co-operate in an appropriate manner, and that the final result is produced not by a single reaction but by the agency of a complicated and self-regulatory mechanism."

Ewart (1896, 1897) and Pantanelli (1903) have published data which also tend to show that chlorophyll is not the only internal factor in the processes of carbon assimilation.

Willstätter, however, expresses this opinion in a far more dogmatic way, postulating that the chloroplast is the seat of a photochemical reaction and that the product formed in this reaction is subjected to an enzymatic action which takes place at the boundary between chloroplast and plasma. In this latter process oxygen is supposed to be evolved. His contention is that the efficiency of the assimilatory process depends not only on the amount of chlorophyll but also on the amount of enzyme, and in his investigation he has examined extreme cases where either chlorophyll or enzyme are in excess.

The very great importance of Willstätter's work lies in the fact that for the first time quantitative estimations of the pigments have been made; in an earlier chapter we have stated in some detail the methods employed by Willstätter and also pointed out how unreliable were the estimations of all of the earlier workers. The principle used in the analysis is the saponification of the leaf extract with alkali and the subsequent abstraction of the yellow pigment with ether. The chlorophyllin solution is then compared colorimetrically with standard solutions. Thus the disturbing influence of the yellow pigments is avoided; however, it must be pointed out that the information obtained only holds for the chromogen complex; as regards the phytol part of the chlorophyll molecule which is split off in the saponification we do not get any information.

Willstätter's experience from earlier investigations where the pigments were estimated in leaves collected at various times of the day and at various seasons led him to the conclusions that the amount of pigment is not altered during the processes of assimilation; this view is confirmed here, for Willstätter finds no appreciable difference in the amount of pigments as the result of assimilation.

Of course it has been assumed before Willstätter's time that the assimilation varies with the amount of chlorophyll, but it had not been possible definitely to estimate the chlorophyll content or to differentiate between the part played by the chlorophyll and the part played by the plasma.

Thus, for instance, Weber (1879) found that equal areas of the leaves of different plants under the same conditions had different assimilatory powers. Haberlandt (1882, and see 1914) explained Weber's results by determining the number of chloroplasts per unit area in the plants used by Weber and showing that there is a parallelism between the assimilatory activity and the number of chloroplasts. His results are exhibited in the following table.

TABLE XIV.

Relation between Assimilatory Activity and Number of Chloroplasts.

Species.	Assimilatory Activity per unit area.	Number of Chloroplasts per unit area.
Tropoeolum majus	100	100
Phaseolus multiflorus	72	64
Ricinus communis	118·5	120
Helianthus annuus	124·5	122

But of course there is no evidence or even probability that all chloroplasts contain the same amount of chlorophyll, so that this attempt to correlate assimilatory activity with quantity of chlorophyll is extremely crude.

While we have no criticisms to offer in regard to Willstätter's chemical analysis of the pigments, it appears from his preliminary account that the experimental arrangements in his assimilation experiments may be open to considerable criticism. As, however, he promises a detailed paper in which " many remarkable details in the experimental arrangement" are to be described, it seems desirable to defer such criticism to a later period.

The main principle of his method of experimentation is the same as that used by earlier workers, *e.g.*, Kreusler (1885–1890) and Blackman. The noteworthy features of Willstätter's method are :

(1) The carbon dioxide is determined by weight in an absorption apparatus.

(2) *The high intensity of illumination.* He uses a ½ watt Osram lamp of 3000 candle power at 15–25 cm. distance from the leaf chamber (corresponding to a light intensity of 48,000 to 130,000 lux).

(3) *The rapid stream of carbon dioxide* (4·5 litres per hour).

(4) *The method of temperature measurement.* The temperature of the gas in the leaf chamber is measured (presumably by a mercury thermometer) ; it is obvious, in view of Blackman's experiments, that this is indeed very unsatisfactory, particularly when such high light intensities are employed.

The experimental conditions used by Willstätter are such " *that the assimilation of a normal well assimilating leaf cannot be increased by increasing the carbon-dioxide concentration or the light intensity.*" The temperature, which is kept constant, generally 25°C, is " favourable to assimilation."

Expressing this in terms of Blackman's principle it must mean that neither light nor carbon dioxide is a limiting factor. The amount of carbon dioxide assimilated can then only depend on internal factors and the temperature.

The ratio between the quantity of chlorophyll and the carbon dioxide assimilated in a certain time is termed by Willstätter the assimilation number (assimilation number =

$$\frac{\text{amount of } CO_2 \text{ assimilated in one hour.}}{\text{chlorophyll content.}})$$

Approximately constant values of the assimilation number would indicate that the assimilation depended only on the amount of chlorophyll, if variable values are obtained it means that other factors come into play.

It is to be regretted that only the tables which *illustrate* the conclusions are given by Willstätter; none of the preliminary work necessary for the justification of the conclusions is quoted.

Normal Leaves.

In Table XV we give the results obtained by Willstätter for normal leaves.

TABLE XV.

Assimilation Numbers of Normal Leaves.

Concentration of CO_2, 5%.

Rate of Gas Current, 4·5 litres per hour.

Species.	Temp.	Light Int. in Lux.	Wt. of Leaves gm.	Dry Weight gm.	Leaf Surface sq. cm.	Chlorophyll content mg.	CO_2 ass. in 1 hr. gm.	Ass. No.
Rubus Eubatus ...	25°	48000	5·0	1·80	356	16·2	0·094	5·8
Syringa vulgaris ...	25°	48000	12·0	3·45	371	14·1	0·091	6·5
Sambucus nigra ...	25°	48000	8·0	2·20	343	17·8	0·117	6·6
Ulmus	25°	48000	8·0	2·35	421	13·0	0·089	6·9
Prunus Laurocerasus	30°	75000	10·0	3·40	—	12·2	0·098	8·1
Primula	30°	75000	10·0	0·90	—	11·4	0·105	9·1
Hydragea opuloides	30°	75000	10·0	1·20	—	9·2	0·060	6·5
Pelargonium zonale	30°	75000	10·0	0·96	—	12·5	0·093	7·4

From the numbers given in this table there would seem to be a rough parallelism between the amount of chlorophyll and assimilation. But in all the cases given the leaves were in the same stage of development and all were rich in chlorophyll. On the other hand leaves from the same plant, but in different stages of development, exhibit much wider variations in the assimilation number, as the following table shows.

TABLE XVI.

Assimilation Numbers of Leaves from the same Plant,
but in different Stages of Development.

25°   5% CO_2 48000 lux.

Species.	Age of the Leaf.	Weight of Leaves gm.	Dry Weight gm.	Leaf Surface cm².	Chloro-phyll mg.	CO_2 ass. in 1 hr. gm.	Ass. No.
Acer pseudo-platanus	4–6 leaf at the top of the branch 23rd June	6·0	2·0	358	5	0·059	11·8
	leaves from the base of the branch same day	6·0	2·15	469	24	0·124	5·2
Tilia	young light green leaves 25th June	8·0	2·05	421	5·2	0·074	14·2
	lower dark green leaves 26th June	8·0	2·55	530	22·5	0·148	6·6
Taxus baccata	young branch 27th June	20	5·65	—	27·6	0·131	4·7
	last year's branch 28th June	20	7·05	—	47·5	0·102	2·1

It will be observed from this table that the chlorophyll content increases with the age of the leaves ; so does the assimilatory power, but not in the same degree. Consequently the assimilation number decreases.

Further data in regard to the variation of the assimilation number with the state of development of the leaves are given in the following table.

Table XVII shows clearly that after a time, although the chlorophyll content increases, yet the assimilation number diminishes. From this fact Willstätter concludes that the chloro-phyll is present in excess, while some other internal factor (enzyme) is limiting the rate of assimilation.

Autumn Leaves.

In autumn leaves the conditions are very complex. The general rule is that with decreasing chlorophyll content the assimilation decreases as is shown in Table XVIII. Leaves rich or poor in chlorophyll give the same assimilation number.

TABLE XVII.

*Assimilation Numbers of Leaves of the Same Species
at different times in Spring.*

25° 5% CO_2. 48,000 lux.

Date.	Species.	Weight of Leaves gm.	Dry Weight gm.	Leaf Surface cm².	Chlorophyll mg.	CO_2 per hr. gm.	Ass. No.
29th April ...	Aesculus	8	1·68	211	8·1	0·090	11·1
7th May ...	Hippo-	8	1·65	374	12·1	0·146	12·1
3rd June ...	castanum	8	2·35	386	19·8	0·127	6·4
4th May ...	Tilia	6·0	1·31	344	5·0	0·053	10·6
12th May ...	cordata	6·0	1·29	463	6·9	0·110	16·0
5th June ...		6·0	2·11	421	17·3	0·123	7·1

TABLE XVIII.

Assimilation Numbers of Autumn Leaves.

Date.	Species.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm².	Chlorophyll content mg.	CO_2 ass. per. hr. gm.	Ass. No.
14th July ...	Sambucus nigra	8·0	2·05	359	18·8 [a]	0·116	6·2
14th October	Sambucus nigra (leaves easily detached)	8·0	1·64	356	8·2	0·049	6·0
2nd November	Populus pyramidalis (dark green leaves)	8·0	2·55	376	15·2	0·152	10·0
2nd November	Populus pyramidalis (yellow green leaves)	8·0	2·35	363	3·9	0·031	7·9

In many cases, as will be seen from the next table, the assimilation number increases at the beginning of autumn and diminishes later.

TABLE XIX.

*Assimilation Numbers of the Same Species at
different times in Autumn.*

Date.	Appearance of Leaf.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm².	Chlorophyll content mg.	CO_2 ass. per hr.	Ass. No.
30th July ...	deep green thin	4·0	1·55	271	19·7	0·080	4·1
17th September	green	4·0	1·55	336	12·5	—	6·6
5th October ...	green with yellow spots	4·0	1·45	344	7·8	0·064	8·5
19th October ...	almost yellow	4·0	1·35	337	2·1	0·010	4·8

Some leaves which remain green until they fall off keep their assimilatory power, so that in spite of the lateness of the season and high chlorophyll content, the assimilation numbers are still high. Yet in other cases where the chlorophyll content of the leaves in autumn is still high the assimilation numbers are remark-ably low, while there is a considerable difference between the assimilation numbers of young and old leaves from the same plant. These results are given in Tables XX and XXI.

TABLE XX.

Autumn Leaves with High Chlorophyll Content and Normal Assimilation Numbers.

Date.	Species.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm².	Chloro-phyll content mg.	CO_2 ass. per hr. gm.	Ass. No.
26th October ...	Cydonia japonica var. Moerlosii (falling leaf)	10·0	4·50	301	16·3	0·119	7.3
27th October ...	Clerodendron trichotomum (fallen leaf)	6·2	1·28	211	9·3	0·115	12·3
14th October ...	Lonicera tatarica (fallen leaf)	4·0	1·20	299	3·3	0·020	6·1

TABLE XXI.

Autumn Leaves with Same Chlorophyll Content but different Assimilation Numbers.

Date.	Species.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm².	Chloro-phyll content mg.	CO_2 ass. per hr. gm.	Ass. No.
11th November	Ampelopsis tricuspida Veitchii (fresh, green)	7·0	1·50	—	6·8	0·014	2·0
16th October ...	Ampelopsis quinquefolia (older, green)	5·0	1·05	251	6·4	0·006	0·9
17th October ...	Ampelopsis quinquefolia (younger leaves from apex of shoot)	5·0	1·15	325	6·4	0·050	7·9

The low assimilation number after exposure to low temperatures is rapidly increased on exposure to higher temperatures. Thus in the case of leaves of *Ampelopsis Veitchii* gathered at 4°C the assimilation number gradually increased from less than 0·8 to 2·6 after 1½ hours at 25°C, and increased up to about 4 when it remained constant.

Leaves poor in chlorophyll (Yellow Varieties).

Leaves poor in chlorophyll, *e.g.*, yellow varieties with a chlorophyll content of from 15% to 3%, or even less, of that of the leaves of the normal green varieties, exhibit a marked deviation in the proportionality between chlorophyll and assimilation. The numbers given in Table XXII for the yellow varieties are not maximal values of the assimilation, for it was found impossible to use the maximum light intensity without injuring the leaves. Under certain conditions it was found that at 15°C instead of 25°C the absolute amount of carbon dioxide absorbed by equal surfaces of yellow and green varieties can in the former reach a value as high or even higher than that taken in by the leaf of the normal variety.

TABLE XXII.

Assimilation Numbers of Leaves of Green and Yellow Varieties of Elm.

Ulmus, 5% CO_2, 3000 candle power at 35 cm. distance (24,000 lux).

Variety.	Temp.	Weight of Leaf gm.	Dry Weight gm.	Chloro-phyll mg.	Leaf Surface cm².	CO_2 ass. per hr. gm.	CO_2 per sq. metre gm.	Ass. No.
Chlorophyll poor ...	25°	8·0	2·0	0·95	321	0·075	2·3	79
do.	15°	the same leaf	2·0	0·95	321	0·056	1·7	59
Chlorophyll rich ...	25°	8·0	2·35	13·0	421	0·089	2·1	6·9
do.	15°	8·0	2·35	13·0	421	0·059	1·4	4·5

Willstätter points out that from this table it may be observed that the assimilation of chlorophyll-poor and chlorophyll-rich leaves is very similar at lower temperatures, but that the quantity of assimilation per unit quantity of chlorophyll is much greater in the yellow varieties.

Willstätter seems to indicate that the conditions in the yellow varieties are of great importance in judging the factors which influence the assimilation. As temperature variations do not influence the assimilation in such leaves, while decrease in light decreases the assimilation, he concludes that in this case the enzymatic system is more developed than the chlorophyll system, which thus controls the rate of assimilation. The reaction of the chlorophyll system being photochemical it may be assumed to have a temperature coefficient not far from unity, while the enzymatic process has a temperature coefficient of a magnitude of 2 to 3. In the normal leaf the chlorophyll system is more developed and the enzymatic process limits the rate of assimilation. Consequently

here variations in light intensity are without influence on the assimilation, while temperature variations influence the rate of assimilation considerably. However, no experimental results are given which justify these conclusions, and however interesting they may be they can at present only be accepted as postulates.

Etiolated Leaves.

The complexity of the processes involved in carbon assimilation is also made clear by examination of the behaviour of etiolated leaves.

On this subject observations were made by Miss Irving (1910) at Blackman's suggestion. Blackman assumed that in the case of etiolated leaves "the whole assimilatory apparatus might be efficiently developed except the green pigment, and as this increased by degrees, so the power of photosynthesis would increase. Thus the amount of chlorophyll present would then be the limiting factor for assimilation, and interesting data might be looked for relating the amount of pigment present to the amount of photosynthesis that could be effected."

This expectation was not confirmed by the preliminary experiments carried out by Miss Irving, who found not only that etiolated shoots possessed no power of assimilation, but that shoots that had developed a considerable green colour did not possess the power.

In Miss Irving's experimental arrangement the plants were supplied with their own respiratory carbon dioxide, and the light to which they were exposed was the feeble light from a north window.

Willstätter, using a much stronger light intensity (48,000 lux) and 5% carbon dioxide, obtained results which justified Blackman's expectations that chlorophyll is really a limiting factor. There is a possibility that in Miss Irving's experiments light was a limiting factor.

TABLE XXIII.

Assimilation Numbers of Etiolated Leaves becoming Green.
Phaseolus vulgaris.

Temperature 25°C, 5% carbon dioxide. Light intensity, 48,000 lux.

Light.	Appearance.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm^2.	Chlorophyll content mg.	CO_2 ass. in 1 hour gm.	Ass. No.
Not previously illuminated	Pure yellow	5·0	—	—	$<0·1$	0·007	>70
11th June, after 6 hrs. illumination	Greenish yellow	4·4	—	—	0·3	0·040	133
29th May, after 2 days' illumination	Yellow-green	5·0	0·70	216	4·0	0·096	24
31st May, after 4 days' illumination	Grass green	5·0	0·74	260	7·8	0·104	13·3

It will be observed that the assimilation numbers are very high, indicating that it is the chlorophyll which is limiting assimilation.

Chlorotic Leaves.

Willstätter has also examined chlorotic leaves and finds, in spite of the low chlorophyll content, comparatively low assimilation numbers showing that the chlorophyll is only partially utilised. Of course one would not expect if one of the essential elements (iron) were wanting, that the assimilatory apparatus should be properly developed. Willstätter considers that the fact that in chlorotic leaves even a small amount of assimilation takes place, makes the assumption of B. Moore (1914), that iron plays an important part in the assimilatory process, even more improbable than ever.

Although it is premature to attempt to summarise the results of Willstätter's plant physiological work, it seems reasonable to conclude that under certain circumstances when no other factor is limiting, the amount of chlorophyll determines the intake of carbon dioxide by the leaf. Further it is clearly brought out by Willstätter's experiments with leaves in different states of development, yellow varieties, etiolated leaves, etc., that besides chlorophyll other internal factors are operative. This idea is not novel, as it has been expressed for instance by Pfeffer and Blackman, but Willstätter, for the first time, determines the relation between quantity of chlorophyll and assimilatory activity. The novelty which Willstätter claims for his researches lies in the exposition of carbon assimilation as consisting of two different processes, one photochemical and one enzymatic, the complete experimental proof of which Willstätter has not yet brought forward. For plant physiologists there should be nothing new in this view, as from Blackman's experiments it was seen that carbon assimilation had a temperature coefficient between 2 and 2·5 and consequently the photochemical reaction must be coupled with chemical reactions. But it would indeed be noteworthy if the famous German chemist should succeed in convincing plant physiologists that in what Blackman terms the " katalytic honeycomb of the cell " only two processes are concerned in assimilation.

Willstätter's theories of carbon assimilation, both those expressed in his book and in his more recent papers, we shall refer to later.

<center>CHAPTER V.</center>

<center>*The Products of Carbon Assimilation.*</center>

<center>A. GENERAL REMARKS.</center>

In this chapter we propose to deal with the production of substances in the leaf as a result of the assimilatory processes. We shall confine our attention here to a consideration of the substances known to be produced; later we shall deal with the theories of carbon assimilation advanced to explain the production of these substances.

The substances which are known to be produced as a result of carbon assimilation are oxygen and carbohydrates, and the evolution of oxygen by the green plant in sunlight was one of the earliest known facts of plant physiology.

Although a few workers have made investigations on the laws governing the evolution of oxygen in sunlight, notably de Saussure (1804), Bonnier and Mangin (1886) and Maquenne and Demoussy (1913), yet the subject is one which has probably received less attention than any other aspect of carbon assimilation, and of recent years seems scarcely to have attracted that attention which it deserves.

The formation of carbohydrates in the leaf as a result of assimilation was not recognised until the classical researches of Sachs (1862) established this fundamental fact. Since then the production of carbohydrates in the leaf has been the subject of almost continual research, notably by English workers, yet it cannot be claimed that even now our knowledge of the organic products of carbon assimilation is very definite.

Although the production of oxygen and of carbohydrates in the leaf are merely two different aspects of the same process or set of processes, yet the two have always been investigated quite independently of one another. It is therefore convenient to discuss these two questions separately, and we shall therefore in the following section of this chapter review our present knowledge regarding the evolution of oxygen from the assimilating leaf, before passing on to consider the various questions raised in connection with the formation of organic material.

<center>B. THE EVOLUTION OF OXYGEN.</center>

That green parts of plants under the influence of light evolve oxygen, was established by Priestley, Senebier and Ingenhouss towards the end of the eighteenth century, but it was de Saussure

(1804) who first attempted to obtain quantitative data as to the relation between the amount of oxygen evolved and the carbon-dioxide absorbed by the assimilating plant.

Saussure found that the volume of oxygen evolved in a given time by the plant was less than the volume of carbon dioxide absorbed, and he came to the conclusion that part of the oxygen of the carbon dioxide was used in assimilation. It is strange that Saussure should be so often quoted as the discoverer of the fact that the volume of oxygen given out by the assimilating plant is equal to the carbon dioxide absorbed. Thus even Sachs (1882 or see 1887) says "As an essential point, it is at the same time to be insisted upon here that the volume of oxygen evolved is equal to the volume of the carbon dioxide taken in, as de Saussure and, later and more exactly, Boussingault have already established." It is therefore worth while to quote Saussure's conclusion in his own words, " Il résulte de toutes ces expériences, que les plantes, en décomposant le gaz acide carbonique, s'assimilent une partie du gaz oxygène qui y est contenu."

As Saussure's work is perhaps not always easily accessible, it may be worth while to give his actual results here.

Saussure placed a suitable number of plants in a large vessel containing an artificial atmosphere comprising about 21% oxygen and the rest nitrogen, to which was then added carbon dioxide. The plants were then exposed to sunlight on a number of successive days (6 to 18) and at the end of the period the gas in the vessel was analysed.

The following numbers were obtained with 7 plants of periwinkle (Vinca minor).

	Before.		After.
Nitrogen ...	4199 c.c.	...	4338 c.c.
Oxygen ...	1116 „	...	1408 „
Carbon dioxide	431 „	...	0 „
	5746		5746

Oxygen evolved	292 c.c.
Carbon dioxide absorbed ...	431 „
Oxygen absorbed by plant ...	139 „

In the following table are summarised all Saussure's experiments on this subject.

TABLE XXIV.

Oxygen evolved in Assimilation (de Saussure).

Species.	Oxygen evolved.	CO_2 absorbed.	Oxygen absorbed.
Vinca minor	292 c.c.	431 c.c.	139 c.c.
Mentha aquatica ...	224 ,,	309 ,,	86 ,,
Lythrum salicaria ...	121 ,,	149 ,,	27 ,,
Pinus genevensis ...	246 ,,	306 ,,	60 ,,
Cactus opuntia	126 ,,	184 ,,	57 ,,

Such experiments as these, of course, take no account of the respiration of the plants which is certainly going on in the intervals between the illuminated periods and is generally assumed to continue concurrently with assimilation during the illuminated periods as well. The same criticism is to be levelled against the experiments of Boussingault (1864) and others, who obtained a ratio of oxygen evolved to carbon dioxide taken in, of approximately unity, and to those of Schloessing (1892, 1893), who obtained numbers for the $\frac{oxygen}{CO_2}$ ratio considerably greater than unity (1·05 to 1·33). Similar numbers were also obtained for lichens by Jumelle (1892), and for mosses by Jönsson (1894).

It was Bonnier and Mangin (1886) who attempted to separate the gaseous exchanges due to assimilation and respiration. For this purpose they employed four different methods.

1. By successive exposure of the same green tissue to darkness and light in a closed vessel and measurement of the change in content of oxygen and carbon dioxide of the vessel in which the tissue is enclosed during each period, it is possible to obtain data for the gaseous exchange due to assimilation alone.

Thus if in any time
  c' is the carbon dioxide evolved in the dark and
  o' ,, ,, oxygen absorbed in the dark
  The respiratory coefficient $\frac{CO_2}{O_2} = \frac{c'}{o'} = r.$

Similarly if in the same time
  o is the oxygen evolved in the light and
  c ,, ,, carbon dioxide absorbed in the light

Then the total oxygen produced by the assimilatory
process in the given time is o + o'

and the total carbon dioxide absorbed in assimilation
is c + c'

And the true assimilatory coefficient is

$$\frac{o + o'}{c + c'} = \frac{O}{C} = a.$$

2. The second method employed by Bonnier and Mangin is
based on Bernard's observation (1878) that by the use of chloroform
the assimilation may be suppressed and respiration alone takes
place. By comparison of the gaseous exchanges taking place in
two similar quantities of leaves exposed to light under the same
conditions, but in which one was anæsthetised with ether, and the
other not, the gaseous exchange due to assimilation may be
estimated.

3. Bonnier and Mangin's third method is based on the
suppression of assimilation by removal of all carbon dioxide from the
neighbourhood of the leaves. Two similar vessels contain equal
weights of similar leafy tissue; one of the vessels contains
concentrated barium hydroxide solution, the other an equal volume
of pure water. In the former, not only is the carbon dioxide
of the atmosphere removed and assimilation prevented, but the
carbon dioxide evolved in respiration is absorbed by the baryta. So
that, as in the second method, the difference between the oxygen
content and carbon dioxide content of the two vessels at the end
of the experiment, gives the true values for oxygen and carbon
dioxide evolved and absorbed respectively in assimilation.

4. The fourth method depends on the measurement of the
gaseous exchanges in branches of the same plant which are
unequally green. Thus a yellow branch of *Euonymus japonicus* on
exposure to light evolved 2·89 units of carbon dioxide and absorbed
2·11 of oxygen, while in the same time a green branch evolved 2·27
of oxygen and absorbed 0·54 of carbon dioxide.

The four different methods gave concordant results. The
results obtained by Bonnier and Mangin for a number of species at
different times of the year are shown in Table XXV. It will be
observed that the true assimilatory coefficient is always greater than
unity, whereas the respiratory coefficient is below unity. The
consequence of this is that the apparent assimilatory coefficient,
which neglects the respiration, is always lower than the real

assimilatory coefficient. Thus Bonnier and Mangin explain the fact that Boussingault obtained a ratio of $\dfrac{O_2}{CO_2}$ of about unity.

TABLE XXV.

Assimilatory Coefficients for Different Leaves (Bonnier and Mangin).

Species.	Month.	Real Assimilatory Coefficient $\dfrac{O_2}{CO_2}$	Respiratory Coefficient $\dfrac{CO_2}{O_2}$	Apparent Assimilatory Coefficient.
Tobacco	November	1·12	0·73	1·00
Ivy	,,	1·09	0·86	1·00
,, ...	,,	1·08	0·80	1·01
Bramble	,,	1·06	0·84	0·91
Ivy	December	1·06	0·84	0·88
Butcher's Broom	,,	1·08	0·78	0·92
Broom	March	1·16	0·87	1·09
Pinus sylvestris	,,	1·17	0·80	0·88
,, ,,	,.	1·12	0·85	1·04
Chestnut	June	1·06	0·83	0·99
Lilac	,,	1·06	0·96	1·05
,, ...	,,	1·05	0·93	1·02
Holly	February	1·24	0·75	1·13
Chestnut	April	1.16	0·82	0·91
Broom	February	1·16	0·85	0·92

In an extended series of observations on the respiration and assimilation of succulents, Aubert (1892) has obtained similar values for the assimilatory coefficient of ordinary plants, but much larger values for succulents. He concludes that the $\dfrac{O_2}{CO_2}$ exchange due to assimilation is greater than unity for all plants. For ordinary plants the ratio is not very far removed from unity, but for succulents it may be much larger. For ordinary plants the ratio varied from 1·05 to 1·23, numbers which agree closely with Bonnier and Mangin's observations. Some of the values obtained by him for succulents are given in the accompanying table. The values quoted for *Sedum Telephium* and *Opuntia tomentosa* show that at different times the ratio for the same plant may vary greatly.

Having regard to the peculiar metabolism of succulents, however, the relation of these assimilatory coefficients to the assimilatory process is doubtful.

Table XXVI.

Assimilatory Coefficient for Succulents (Aubert).

Species.	Date	Temperature.	Assimilatory Coefficients.
Aloe spinosa	23 July	24°C	2·45
Crassula arborescens ...	2 ,,	32°C	3·57
Mammilaria Newmanniana ...	,,	,,	3·51
Opuntia tomentosa	,,	,,	4·68
,, ,, ...	23 ,,	24°C	7·59
Sedum carneum	2 ,,	32°C	1·55
,, reflexum	,,	,,	1·40
,, Telephium	,,	,,	1·24
,, ,, ...	23 ,,	24°C	1·34

Recently Maquenne and Demoussy (1913) have called in question Bonnier and Mangin's results. As with all other workers on this subject, these investigators used a closed vessel as plant chamber connected to a reservoir containing 8 or 10 parts of carbon dioxide to 100 of air, from which the leaf chamber was filled after evacuation.

The leaf chamber was exposed to light and after a convenient time the gas in the chamber was analysed.

The respiratory and assimilatory coefficients of a large number of species were measured; the results are given in the following table.

Table XXVII.

Respiratory and Assimilatory Coefficients (Maquenne & Demoussy).

Species.	Respiratory Coefficient.	Apparent Assimilatory Coefficient.
Ailanthus	1·08	1·02
Aspidistra	0·97	1·00
Aucuba	1·11	1·10
Begonia	1·11	1·03
Cherry Laurel	1·03	0·97
Chrysanthemum	1·02	1·01
Dahlia...	1·07	1·07
Haricot	1·11	1·12
,, ...	1·07	1·07
Ivy	1·08	1·00
Lilac	1·07	1·03
Lily	1·07	1·00
Mahonia (autumn)	0·95	0·99

TABLE XXVII—*continued.*

Species.						Respiratory Coefficient.	Apparent Assimilatory Coeffieient.
Maize ...	...	...	...	...	...	1·07	1·05
Oleander	...	...	...	...	...	1.05	1·01
Pea ...	...	...	...	...	...	1·07	1·04
Pear ...	...	...	...	...	...	1·10	1·08
Poppy	...	...	...	...	...	1·09	1·09
Privet ...	...	...	...	...	...	1·03	1·02
Rhubarb	...	...	...	...	...	1·02	1.00
Ricinus	...	...	...	...	...	1·03	1·03
Rose ...	...	...	...	...	...	1·02	1·00
Spindle-tree ...	...	...	...	...	...	1·08	1·02
Sorrel ...	...	...	...	...	...	1·04	1·04
Tobacco	...	...	...	...	...	1·03	1·04
Turnip...	...	...	...	...	...	1·11	1·06
Vine ...	...	...	...	...	...	1·01	0·99
Wheat...	...	...	...	...	...	1·03	1·02
Wild Grape ...	...	...	...	...	...	1·00	1·01

From their results Maquenne and Demoussy conclude that the value of the apparent assimilatory coefficient lies between that of the respiratory coefficient and unity, especially as the leaves were probably at a higher temperature during the assimilatory period than in the dark, and as the respiratory coefficient rises with temperature, higher respiratory coefficients probably correspond with the assimilatory coefficients given.

They therefore conclude that the real assimilatory coefficient approximates to unity.

For if c is the volume of oxygen evolved in assimilation alone, and if d is the volume of carbon dioxide absorbed in assimilation alone,

And if a is the volume of carbon dioxide evolved in respiration, and if b is the volume of oxygen absorbed in respiration,

$$\frac{a}{b} = m, \text{ the respiratory coefficient,}$$

and $\frac{c-b}{d-a}$ = the apparent assimilatory coefficient.

We see that $\frac{c-b}{d-a}$ is between 1 and m.

When, as in the general case m $>$ 1

then also $\frac{c-b}{d-a} > 1$

whence $1 - \dfrac{c}{d} < \dfrac{b}{d} (m-1)$

and when $m < 1$

and $\dfrac{c-b}{d-a} < 1$

we have $1 - \dfrac{c}{d} > \dfrac{b}{d}(m-1)$

Now $1 - \dfrac{c}{d}$ is the difference between the real assimilatory coefficient and unity and $\dfrac{b}{d} (m-1)$ is actually not greater than $0 \cdot 01$ in the first case, nor less than $-0 \cdot 01$ in the second. Hence the real assimilatory coefficient differs from unity by a quantity less than $0 \cdot 01$.

From the results we have collected together in this section it becomes quite clear that the relation between the oxygen evolved in assimilation and the carbon dioxide taken in, is by no means definitely determined. Yet this is a question of great importance in regard to the problem of carbon assimilation, for in the determination of the nature of a reaction or series of reactions, it is of first importance to know the quantitative relation between the initial substances and the products of the reactions.

(To be continued).

THE TRANSLOCATION OF LATEX AND THE
MULTIPLE RAZOR.

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[With Six Figures in the Text].

THE present position of our knowledge of the function of latex is indicated by Haberlandt (3), who, after devoting several pages to the work already done, says, "The preceding discussion sufficiently emphasises the need for further experimental research upon this subject." There is an increasing amount of evidence for the view that laticiferous tissue forms a distributive system for elaborated food-materials, proteids, carbodydrates, etc., and the only important objection is made by Kniep (4), who quotes experiments by himself and Treub (10), as showing that, if *Euphorbia* plants are kept in the dark for several weeks, the starch grains in the latex do not disappear to any appreciable extent. Schimper (7) also interpreted his results as disproving the translocation of latex. Mangham (5) emphasises the negative evidence of previous researches on the translocation of *carbohydrates* by laticiferous tubes and vessels, but it is largely a question of the interpretation of results and of an over-emphasis of the behaviour of one constituent (the starch grains) of the latex. The presence of proteolytic enzymes in the latex of *Ficus* (2) certainly does not confirm the view that latex has no nutritive value.

Schwendener (8) and Faivre (1) have given somewhat in-conclusive experiments in support of the translocation of latex, and as this is almost a necessary concomitant of the nutritive value, the writer has devised an instrument with the aid of which it is hoped to solve the problem.

The latex in the laticiferous vessels is usually, if not always, under a certain amount of pressure, with the result that when the system is disturbed by a leaf being cut off for examination, the condition of the latex in the leaf itself and also in the stem is no longer the same. The pressure causes the latex to exude, chiefly on the cut surface next the stem. It is necessary, therefore, if the condition of the latex in *different parts of the leaf* is to be studied, to have some means of retaining the latex of the leaf in the same condition as it is before any cutting has been done. A number of workers on translocation have felt the want of an instrument for

cutting a leaf or stem *simultaneously* into a given number of parts. The instrument described below has been successful in some preliminary experiments.

This " Multiple Razor " is composed essentially of a number of wooden blocks threaded on two bolts, some of the blocks having just the two holes necessary for the bolts and others being sawn half-way down and fitted with small screws which, when tightened, grip a safety razor blade inserted in the cut. The razor blocks are fitted with the blades and the ' blind ' blocks may be inserted between them on the bolts. When the razor blocks alone are used there is half an inch between the blades, and this can be increased by half-inches up to any required distance. The holes for the bolts should be bored a little larger than the diameter of the bolts so that each block moves a little. When all the required blocks have been threaded in their proper positions on the bolts they are tightened up with butterfly screws and a final adjustment of each razor block must be made to bring all the razor edges level with each other. Then by means of the butterfly screws they can be screwed tightly so that no movement of the individual blocks is possible.

It has been found convenient to use the single-edged blades supplied with the Christy Safety Razor. Plane or lime wood forms satisfactory material for the wooden blocks. The instrument could be varied to suit any particular requirements, but the following details may be of use as showing a medium size ' multiple razor ' for general use. The letters refer to Figs. 1—3.

Number of blocks required :— Razor-blocks, 16.

<div style="text-align:center">Blind blocks, 20.</div>

Dimensions of wooden block :— 3″ x 2″ x $\frac{1}{2}$″.

Saw-cut :— exactly half-way between B and C, $1\frac{1}{4}$″ to $1\frac{1}{2}$″ deep.

Positions of holes, H and K :— centre of H, 1″ from top (AB).

<div style="text-align:center">„   K, $2\frac{1}{4}$″   „   „</div>

Positions of small screws, F and G :— $\frac{1}{4}$″ from top (AB), 1″ apart.

<div style="text-align:center">These are $\frac{1}{2}$″ steel screws.</div>

Bolts, P and Q :— length, 12″; thickness, $\frac{1}{4}$″.

<div style="text-align:center">Number of threads per inch, 21.</div>

Butterfly screws, R and S :— width, $1\frac{1}{4}$″; thickness, $\frac{3}{16}$″.

Washers for above :— width, $\frac{5}{8}$″; thickness, $\frac{1}{16}$″ to $\frac{1}{8}$″.

The back of the razor blade is $1\frac{9}{16}$″ long and projects beyond the ends, forming a convenient hold ; the edge is $1\frac{7}{16}$″ long. The blade is rather a strong one, $\frac{1}{64}$″ thick. The depth of the blade, $\frac{19}{32}$″,
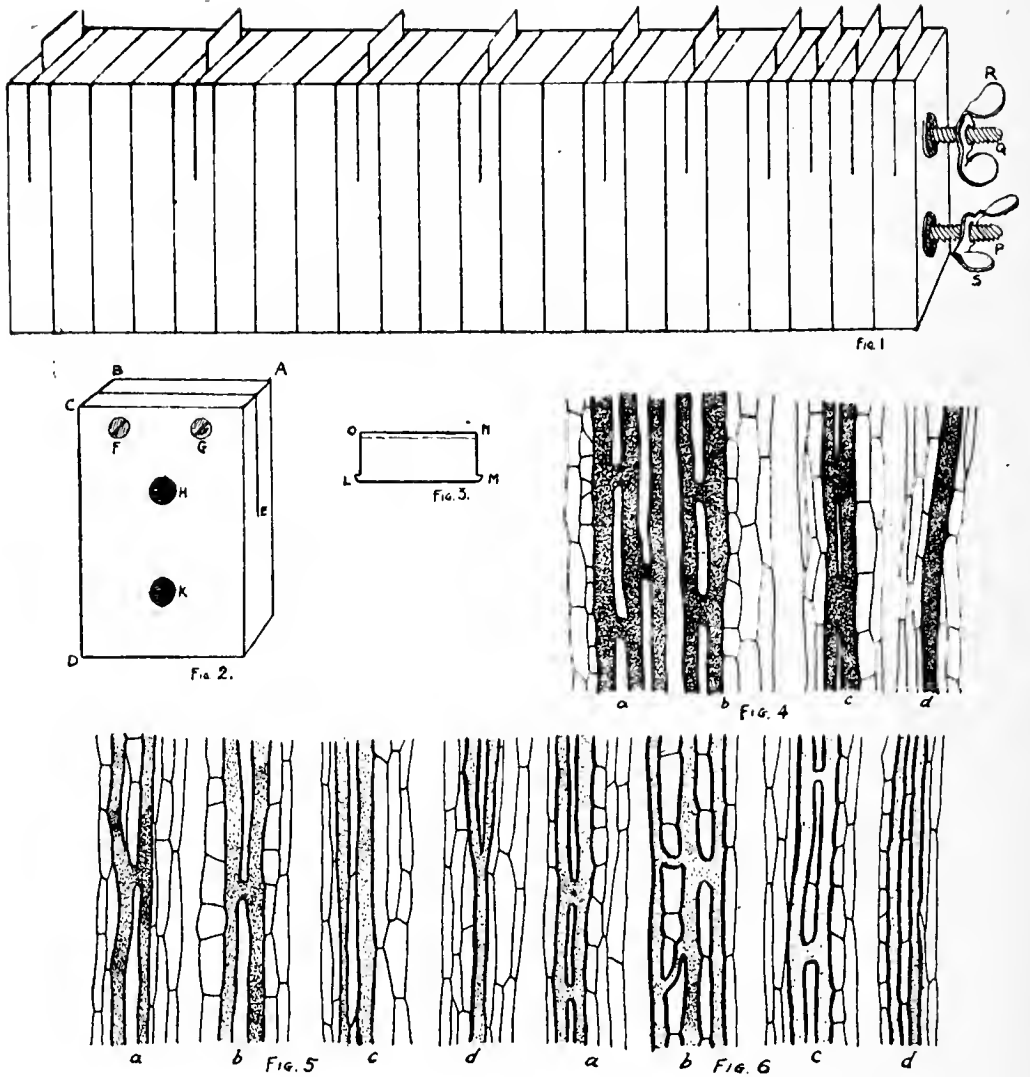
FIGS. 1–6.

DESCRIPTION OF FIGURES.

Figs. 1–3.   Scale 1 cm. to 1 inch.

Fig. 1.   The complete 'Multiple Razor,' showing the blades $\frac{1}{2}''$, $1''$, $1\frac{1}{2}''$ and $2''$ apart ; for details see text.

Fig. 2.   A razor-block, for details see text.

Fig. 3.   A razor-blade,   ,,   ,,

Figs. 4–6.   Longitudinal sections, × 104, of laticiferous vessels in midrib of leaf of *Lactuca virosa*, L.

> (a) from basal portion.
>
> (b) from penultimate basal portion.
>
> (c) from penultimate apical portion.
>
> (d) from apical portion.

Fig. 4.   Normal leaf.

Fig. 5.   Leaf after 48 hours in darkness.

Fig. 6.   Leaf after 4 days in darkness.

limits the thickness of tissue which can be cut as, when mounted, it extends only $\frac{3}{4}''$ beyond the top of the block.

Being easily and inexpensively made the instrument should prove of some use in botanical laboratories wherever translocation experiments are carried out either in teaching or in research.

Two species of *Lactuca* have been examined for latex translocation. Faivre (1) used seedlings grown in darkness and Kniep (4) criticises the relevancy of his results because the laticiferous vessels may grow in darkness while there is no increase in the latex, which therefore becomes diluted with a watery fluid. This objection was avoided by growing the plants in the open in flower-pots until at least six well-developed leaves were present. *Lactuca virosa*, L. was examined first. The potted plant was taken from the garden; one leaf was arranged carefully and without injury along a block of wood and 'guillotined' with the multiple razor, which cut the leaf into four parts, each half an inch long, the severance of the parts taking place at exactly the same time as the dividing of the leaf from the plant. The latex in each part should, therefore, have been in the same condition as it was in the uncut leaf; at least, there should have been the same amount of latex in each portion as there was before any cutting took place.

A series of thick longitudinal sections of the midrib then showed the latex to be dense and abundant in each part of the leaf. No gradation in density could be traced in the different parts of the leaf (Fig. 4, a–d).

The plant was then placed in darkness for 48 hours and a leaf similar to the first one was treated in the same way. The longitudinal sections here showed a marked difference; not only was the latex less dense and less abundant even in the basal part of the leaf, *i.e.*, the part next the stem, but the portions showed a successive decrease in the density of the latex from base to apex (Fig. 5, a–d).

The experiment was carried further and in four days a third leaf was found to have very little latex, so little indeed, that it was difficult to distinguish whether there was any gradation (Fig. 6, a–d). *Lactuca scariola*, L. was also examined and yielded similar results.

This gradation within the leaf has been found in the sugars of the sieve-tubes by Mangham (5) in his work on translocation of carbohydrates and emphasises in the matter of translocation the affinity between latex and assimilable reserves already pointed out

by Faivre (1) in the composition, origin, and behaviour under varied external conditions.[1]

Transitions from secretory sacs to laticiferous vessels are known in the Compositæ and the development of this problem may assist, as certain other physiological phenomena do, in the elucidation of the relationships within the order itself and also of those of the order with the Campanulaceæ (9, b and f). Considerable progress has been made with this phylogenetic problem (9 and unpublished work exhibited at the British Association at Newcastle, 1916),[2] and the results seem to show that there has been an evolution in physiology, more or less parallel to that shown by the morphology of the groups within the Compositæ. As it is obviously necessary to know the physiological function of the anatomical structures before we can make any deductions of phylogenetic value from laticiferous tissue it is intended to extend the present work as much as possible within the Campanulaceæ and Compositæ.

[1] It should be noted that although latex seems to be almost certainly of nutritive value in the Compositæ it may not have the same function or show the same behaviour in other orders. Cf. Parkin (6), on the water-storage function of latex in tropical plants.

[2] Some of the preparations discussed above were on exhibition at the Newcastle meeting of the British Association, Section K.

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THE NATURAL HISTORY OF A SIBERIAN COAL :

An Abstract by Professor Zalessky (Petrograd)

of his Original Paper.

Histoire naturelle d'un Charbon (Russian and French), by M. D. Zalessky. Mém. Comité géol., nouvelle sér., livr. 139. Petrograd. (4to, pp. 74, pls. XIII). Price 4 R. (8s.).

THIS work represents the detailed description of a coal and the fossil organisms discovered in it. Some pieces of this coal were picked up by the geologists A. A. Sniatkov and V. C. Pancratov on the bank of the river Tam (Siberia), where it is entered by the tributary Spuskovoj, and the seam of this coal, hitherto not yet found, should be subordinate to rocks of the upper Kemmern series of a common section of the palæozoic deposits of the Kuznetsk basin. The readers of the Russian Geological Messenger are acquainted with this kind of coal, named Fornite, from a previous report by M. D. Zalessky and A. A. Sniatkov in this review, No. 4, vol. I, 1915. The first chapter is an introduction into investigation and before entering the subject, the formation of coal is touched on in general, the interesting new fact is cited of a discovery of calcareous concretions in the Brusnitzin Coal-seam of Kolchugino coal-mine, in the Kuznetsk basin. These portions of the mother substance of the coal are constituted of a mass of decayed leaves of *Mesopitys Ichihatcheffi* among which a great many scarcely deformed, decorticated branches of the same plant of various thickness are so well-preserved, as to lead the author to believe that the coal-seam of such a constitution may have resulted from accumulation of vegetable remains *in situ*. The mass of leaves he compares with forest-litter, discriminating, however, the circumstance that this litter was formed in a swampy forest, and the decay of the leaves and branches took place under water and not in the open air. The drift of vegetable remains is admitted by him only within swamp precincts. Making reference to sapropel coals he touches on another interesting fact, namely the accumulation of the green Alga *Botryococcus Braunii*, in a great mass, on the shore as well as on the bottom of the Ala-Kul, a bay of the lake Balkhash, giving the clue to the solution of the origin of Bogheads and Torbanites constituted, in the opinion of some authors, from the accumulation of fossil Algæ.

The second chapter deals with the nature of the new coal, to which the author gives the name *sapromyxite*, instead of the former

name ; it represents an accumulation of slimed remains of the strap-like thalli of sea-weeds compared by the author with *Himanthalia lorea*, the brown Alga of the family Fucaceæ living in the streak of the tide in the Atlantic ocean. The Algal nature of the fossil was proved by the investigation of the exterior of the lamina into which the coal has split on weathering, as well as by the microscopical examination of these plates where the female conceptacles with oogoniæ included in them, possessing each an oosphere, were discovered ; in one case there was also a portion of a slimed, isolated antheridium. This fossil Alga allied to *Himanthalia lorea* is called by the author *Himanthaliopsis Sniatkovi.* A characteristic feature is the black colour of the coal in bulk ; it is, however, brownish red when cut into thin plates, fully recalling the brown colour of the Phacophyceæ in transmitted light, a fact that makes the author suppose that the colour of the coal depends upon phycophacin, the dyeing matter of the brown Alga, though it is not verified. A microscopical examination of the slimy and time-hardened thalli of this brown Alga, partly converted into resin (the partial solution of the coal obtained by the solvents of resin supports this conclusion), revealed within their layers two forms of fungi. One form reminds one of the present-day mucor moulds, and the other of the radiating mushroom fungi. There are found, besides, crowded accumulations of globular bodies $0·6\mu$–$7·5\mu$ in size, passing sometimes into sausage-like or other quite fantastic forms in which the author sees yeast similar to the present day, which live in the mucilage that trickles down the stems of the trees. Besides, these yeast-like organisms there occur in the slimy mass of Algæ accumulations of more minute coccus-like bodies in the form of thin lamellæ, flows and clusters. The author has called these coccus-like bodies *Micrococcus myxophilus*, presupposing that these accumulations represent a colony of bacteria : he points out, however, that these coccus-like bodies do not noticeably differ from the minute forms of the mentioned yeast-like organisms, and he makes allowance that the coccus-like bodies may be spores of yeast. Besides the moulds, the yeast and the cocci in the sapromyxite, were found a radiolarian from the group of *Radiolaria Acantharia* (the first representative of this group discovered in the fossil state), a flagellate, a ciliate, two rotatoria and a beetle of the Staphylinoidea group. On the surface of the thalli of *Himanthaliopsis Sniatkovi* occur pseudo-branching threads composed of a series of cells having the size of 38μ–62μ and the width of 8μ–$12·5\mu$ with distinctly marked

sheath permeated as it seems by peroxide of iron. The author compares these threads with those of *Cladothrix dichotoma* and names them *Cladothricinium Pancratovi*.

In chapter III all organisms mentioned have a detailed description. To moulds referable to mucor-moulds, the author has given the name *Mucorvelium palæomycoides*, to the radiating mould *Actinomycoidium floccidum* and to the yeast organisms *Mycogemma saccharomycoides*. The radiolarian is called *Acanthosphæra parvula*, the flagellate is described under the name *Disoma flagellata*. One of the rotatoria is referred to Rattulidæ and described under the name *Palæorattulus elegans*, and the other belonging to the *Brachio-nidæ* is named *Thoracozoon brachionoides* (in the work *Doracozoon brachionoides* is a misprint). The beetle of the Staphylinoidea group is named *Microcantharis minutus*. In chapter IV the author expounds the formation of the new coal by accumulation of fucoid sea-weeds (drifted by the tide and waves of the sea) on the sea-shore where they were converted into mucilage and covered by moulds, but not on the bottom of the sea as Moor had once supposed for all coals. In conclusion the author points out the important significance of this coal for the solution of geological problems, because the recognition of sea-weeds in *Himanthaliopsis Sniatkovi* makes it probable that the sediments of the coal-bearing series of Kuznetsk basin were deposited in a sea gulf but not in a continental lake as was formerly supposed. A tricoloured autotype in water colours from nature by Miss E. D. Kovalsky and phototype plates from micro-photographs made by the author accompany the work.

GEOFFREY BOLES DONALDSON.[1]

CAPTAIN Geoffrey Boles Donaldson is the third young Cambridge botanist killed in action. He was the only child of the late Mr. Donaldson of Londonderry and of Mrs. Donaldson, late of Lower Quinton, Stratford-on-Avon. He was educated at Oundle School and came up to Caius College in October, 1912, with an open scholarship and also a leaving scholarship from his school. Donaldson was very keen on botany and did very well, taking a first class in Part I of the Natural Sciences Tripos in June, 1914. He joined the party from the Botany School which visited the South of France in June and July of that year, and took the most enthusiastic interest in the vegetation, making very full and interesting notes of everything we saw. I remember one gloriously fine evening at a remote village—Roquesteron—having a long talk with him about ecology and the most promising lines of work. He was splendidly full of vitality and keenness. How little any of us dreamed that of the five young men of that party three would be dead in little more than two years—two of them killed in action on the soil of the country they were then visiting for the first time. To his frank, attractive personality Donaldson added not only unlimited energy but very marked modesty and consideration for others. He gave the impression of great solidity of physique, mind and character.

On the outbreak of war he was one of the first to join the O.T.C. and went to the first Cambridge training camp at Royston during August and September. In October he was gazetted second lieutenant in the 7th (Territorial) battalion of the Royal Warwickshire Regiment. He got his lieutenancy in December and during 1915 was for some time instructor in musketry to the 2/7th battalion of his regiment. When he got long enough leave he used to write to me and ask about the botany of the places he was going to stay in with his mother. After a visit of this kind to the Norfolk Broads, he wrote me a long letter discussing and asking questions about the interpretation of the vegetation, which showed that he was still taking a keen interest in botany.

Donaldson was gazetted to a captaincy in March, 1916 and went to the western front in May with his battalion. On July 21st, the battalion was ordered to attack and capture part of the German

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[1] The information in this notice is derived partly from the *Times* notice of Donaldson's death, and largely from letters, copies of which his mother has been kind enough to send to the writer.

line. Donaldson's company was in the first line and he led his men into action in a most gallant manner. He succeeded in piercing the German first and second lines and was instrumental in killing and making prisoners a large number of the enemy. Unfortunately the battalion on his left failed to get across " no man's land " and Donaldson's company was cut off and prevented from returning. He was killed by a bomb thrown at him by one of the enemy. In that particular attack the battalion was the only one of the division which succeeded in penetrating the enemy's line and it received the praise of the higher command. The second in command of the battalion writes that this was entirely due to the cool and well-timed leading of Donaldson and another captain and to the splendid discipline of the men.

His colonel writes that " he was always a most reliable and painstaking officer. No work was too much for him and I shall always feel what he did during our training greatly helped to bring my battalion to a high state of efficiency." His company sergeant-major, who was struck down just before him, writes " Since Captain Donaldson was called upon to take command of C company, his methods, treatment of his men, and unconcern for danger have been the admiration of all his N.C.O's and you may think of him as conducting the grim business of war as calmly as his studies at home." One of his subalterns writes " He made a splendid company commander : he was always cheerful and everyone liked him and worked well for him." A private in another company told his wife that he often wished he was in Donaldson's company, as the men said he was so splendid and all loved him.

Donaldson, when he joined the army, had not of course had time even to begin to show what he could do in botany, to which he intended to devote himself—in fact he had only just taken the first part of the Tripos. But with his ability, solidity and keenness —qualities he afterwards showed as a soldier—there is no reasonable doubt that he would have done very well. We Cambridge botanists are paying a very heavy toll of our highly endowed young men, but we have good reason to be proud of the stuff of which they are made.

A.G.T.

CARBON ASSIMILATION.

A REVIEW OF RECENT WORK ON THE PIGMENTS OF THE
GREEN LEAF AND THE PROCESSES CONNECTED WITH THEM.

BY INGVAR JÖRGENSEN AND WALTER STILES.

(Continued from p. 193).

C. THE CARBOHYDRATES OF THE LEAF.

The presence of starch in the leaf was recognised by von Mohl as long ago as 1837, but it was Sachs (1862, 1864) who identified this starch as a product of assimilation by showing that it appeared in the chloroplasts after exposure to light and disappeared in the dark, and he also showed that chlorophyll was necessary. Sachs' conclusion that starch produced in the chloroplast is the first visible product of assimilation is well known. The method of detection of starch by decolorisation of leaves by alcohol and subsequent treatment with an alcoholic solution of iodine is still the current method of detection of starch in the plant.

It was later recognised that many leaves never elaborate starch and A. Meyer (1885) classified plants into classes according to the quantity of starch their leaves contain. Leaves forming little or no starch were known to yield extracts which reduced cupric solutions and which were optically active, and it was therefore concluded that such leaves contained reducing sugars. In addition the non-reducing disaccharide cane sugar was actually extracted in a crystalline form from leaves of Vine by Kayser (1883).

Brown and Morris (1893) justly pointed out that there was no proof that the cupric reducing substances in the leaf were sugars, and they therefore tested for different sugars. They state that the

only sugars they found were sucrose, glucose, fructose and maltose. Pentoses were tested for, but not found. It is to be regretted that Brown and Morris do not state definitely what tests they applied for the various sugars, as their results have been accepted without ques- ion by most later workers. The presence of cane sugar seems quite definitely established, as leaf extracts after treatment with invertase increase in reducing power and change in optical activity, and this change is not very different from that which would result if the increase in reducing power were due to the inversion of cane sugar into glucose and fructose. They were also able to show the presence of maltose by obtaining maltose phenyl-osazone from leaf extracts. Similarly glucose phenyl-osazone is produced, but no evidence is given as to why it is concluded that d-glucose and d-fructose are the only hexoses present, beyond the fact that they could obtain no other phenyl-osazones. It should be noted that d-mannose gives the same osazone as d-glucose and d-fructose, while the l forms of these three hexoses which are always stated to be absent from the leaf, give a phenyl-osazone of the same crystalline form and the same melting point as the d forms of the sugars (see *e.g.*, Tollens, 1914). More recent work, however, has never succeeded in revealing the presence of any hexoses other than d-fructose and d-glucose, though on the other hand it must be admitted that no definite evidence has so far been brought forward in favour of the absence of all other hexoses. For example, Parkin (1911) was unable to obtain any osazone from extracts of snowdrop leaves other than glucose phenyl-osazone, and hence concludes that galactose and mannose are both absent. This argument is satisfactory for galactose, but it does not hold for mannose, as that sugar gives the same osazone as glucose and fructose.

Moreover, the recent work of Davis, Daish and Sawyer (1916) has cast grave doubt on the presence of maltose in leaves, which they find only present in estimable quantity as a result of enzyme action in leaves not instantaneously killed. Contrary too, to Brown and Morris, these last workers conclude that free pentoses are present in leaves.

They base this conclusion (Davis and Sawyer, 1914) on the fact that leaf extracts contain substances soluble in 80% alcohol which are not precipitated by basic lead acetate, which are unfermentable by ordinary yeasts and which exercise a cupric- reducing power after other sugars have been fermented away. There are of course many sugars which would fulfil these conditions,

as the only hexose sugars fermented by ordinary yeasts are *d*-glucose, *d*-mannose, *d*-fructose and less easily *d*-galactose, while numerous disaccharides with cupric-reducing power are not fermented by yeasts, as well as the pentoses, while there is always the possibility of the presence of substances with cupric-reducing properties other than sugars.

The further evidence of the presence of pentoses is derived from the fact that these purified plant extracts on subjection to distillation with hydrochloric acid according to the Kröber-Tollens process (see Tollens, 1914) yield a weight of phloroglucide which would be given by practically the same amount of pentose calculated as a mixture of *l*-arabinose and *l*-xylose. It must be admitted that the concordance is not very striking and Kluyver (1914) has pointed out that the presence of hexoses and disaccharides in such a solution is a source of error, as on distillation with hydrochloric acid these also give small quantities of furfural-like compounds which yield an insoluble phloroglucide, so that the method could not give any very accurate value for small quantities of pentose in presence of large quantities of other sugars. Davis and Sawyer admit the truth of this criticism, but point out that the error actually introduced in this way is small (about 18%).

Nevertheless the evidence that the *only* sugars present in the leaf are sucrose, *d*-glucose, *d*-fructose and pentoses does not carry complete conviction.

Besides sugars and starch Davis, Daish and Sawyer have estimated the complex derivatives of the pentoses, the pentosans, by distillation of the leaf-matter insoluble in alcohol by the Kröber-Tollens method.

The table overleaf may therefore be regarded as summing up our knowledge in regard to the presence of carbohydrates in the leaf.

The absence of *d*-mannose, which is closely related to *d*-glucose and *d*-fructose in chemical constitution and in its behaviour as regards fermentation by yeasts appears to have been generally accepted without the production of any sound evidence in support of the opinion. It is also generally assumed that the *l* forms of the hexoses are completely absent from the leaf. Thus E. F. Armstrong (1913) says : " In spite of frequent search it has never been possible to detect *l*-glucose or *l*-fructose in the leaves of plants, and the work of Brown and Morris leaves hardly any doubt that hexoses of the *d*-series and their polysaccharides are the only products of

assimilation." However, it is not clear how Brown and Morris's work leads to this conclusion. The hexoses of the *l*-series are not fermented by yeasts and there is no evidence in Brown and Morris's paper that these workers tested for hexoses of the *l*-series after the *d*-hexoses had been fermented away. Davis and Sawyer, on the other hand, show that if this is done there still remain cupric-reducing and optically active substances which they conclude are pentoses, and they seem inclined to regard them as a mixture of *l*-arabinose and *l*-xylose, and they support this contention with determinations of pentoses by the Kröber-Tollens method in the way we have already described, but without showing any great concord-

TABLE XXVIII.

Carbohydrates of the Leaf.

Group.	Compound.	Evidence of Presence.
Polysaccharides[1]	Starch ...	Test with Iodine Solution (Sachs). Hydrolysis with Diastase (Brown and Morris) or with Taka-Diastase (Davis, Daish and Sawyer).
	Pentosans ...	Leaf matter insoluble in alcohol yields furfural on distillation with concentrated hydrochloric acid (Davis, Daish and Sawyer).
	Dextrin ...	Leaf matter insoluble in alcohol but soluble in water which possesses optical activity and reducing properties after treatment with taka-diastase occurs sometimes in Potato (Davis and Sawyer).
Disaccharides...	Sucrose ...	Extracted by Kayser from Vine leaves. Inverted with invertase and weak acids (Brown and Morris).
	? Maltose ...	Production of maltose phenyl-osazone from leaf extracts (Brown and Morris). Microchemical test by production of osazone. Presence denied by Davis, Daish and Sawyer.
Hexoses ...	*d*-Glucose *d*-Fructose	Cupric reducing power of plant extracts. Production of glucose phenyl-osazone from plant extracts (Brown and Morris, Parkin).
Pentoses ...	? *l*-Arabinose ? *l*-Xylose	Purified alcoholic extracts of leaves yield furfural on distillation with concentrated hydrochloric acid (Davis and Sawyer). Presence denied by Brown and Morris.

[1] Exclusive of cellulose and pectin substances.

ance between the values obtained by this method of estimation and that of the reducing power.

The problem of determining the different sugars in the leaf is one of extraordinary difficulty owing to the large number of members of the group and the similarity of their properties. At present we cannot regard as settled even the question of what sugars are definitely absent from the leaf. This question is nevertheless of much importance in the quantitative estimation of sugars in the leaf and as the results of such analyses are likely to be used in connection with theories of assimilation, the exact identity of the leaf sugars may be of fundamental importance in obtaining an understanding of the assimilatory process.

It seems to us, therefore, that before forming a final judgment as regards the carbohydrates of the leaf and before accepting in all their details the results of quantitative analyses already made, there is required a thorough investigation that will settle which carbohydrates are present and which are not, as definitely as Willstätter has settled the question of the leaf pigments.

In the following sections of this chapter we summarise the analytical methods employed for quantitative carbohydrate analysis of the leaf and the results obtained by their means, but it should be understood that some of these results may have to be modified when fuller knowledge is obtained of this important but extremely difficult subject.

D. Quantitative Estimation of the Carbohydrates of the Leaf.

The quantitative estimation of the carbohydrates of the leaf was first seriously undertaken by Brown and Morris for *Tropæolum*; their results are given in their well-known paper in the Journal of the Chemical Society for 1893. Since then the most noteworthy contributions to the subject are those of Parkin (1911) on the snowdrop (*Galanthus nivalis* L.) which embodies the results of a careful series of observations extending over several years, and the recent work at Rothamsted of Davis, Daish and Sawyer who have called attention to several sources of error in the methods of earlier workers. With the results obtained by these different investigators we shall deal in the next section of this chapter. We shall here devote a little space to the description of the methods evolved by these various workers for this extremely difficult analysis.

1. *Preparation of Material.*

In order to obtain correct results in the estimation of substances so liable to change by enzyme action as carbohydrates, special care has to be taken to avoid such change in the preparation of the material for analysis.

Brown and Morris (1893) therefore dried the leaves rapidly at from 75°C to 80°C before estimating starch, and for estimating sugars the leaves were dried on wire-bottomed trays in a steam oven. Parkin (1911) used a similar method. The leaves were air dried at a temperature sufficiently low to prevent discoloration. In both cases the dried leaves were then powdered. That the sugars are extracted unchanged by this method was shown by Parkin by estimating them in material so prepared, and in leaves killed by immersion in liquid air which were subsequently ground up while frozen and then thrown into boiling water (containing a few drops of ammonia to neutralise any acid from the leaf) in order to kill the enzymes.

The following table shows that the two methods give almost identical results.

The numbers for two separate examples (I and II) are given.

TABLE XXIX.

Comparison of Sugars in Air-dried Leaf and in Leaf treated with Liquid Air.

—	Leaf treated with liquid air.		Air-dried Leaf.	
	I	II	I	II
Sucrose	12·84	10·46	12·74	10·42
Reducing Sugars ...	5·94	12·87	5·67	12·38
Total Sugar	18·78	23·33	18·41	22·8
$\frac{\text{Sucrose}}{\text{Hexose}}$	1 : 0·46	1 : 1·23	1 : 0·45	1 : 1·19

From the leaf powder of *Tropæolum* Brown and Morris extracted fat and chlorophyll with ether. The residue was then twice extracted for 24 hours with 80% alcohol at 40°C. The alcoholic extract was used for the estimation of sugars, the residue contained the starch.

In the case of the Snowdrop where the leaf contains no starch, Parkin extracted the sugars by four extractions with cold water

which removed about 97% to 98% of the sugars.

Davis, Daish and Sawyer (1916) consider this method of treatment is unsatisfactory in the case of moderately thick leaves such as that of the mangold, where heating up may be slow and a certain amount of enzyme action is possible before the enzymes are destroyed. They therefore adopt the following method in their work. About 1 kilo. of freshly picked leaf material is dropped in small quantities at a time into 2 litres of boiling 95% alcohol contained in a large zinc beaker to which 20 c.c. of ammonia of S.G. 0·880 is added in order to neutralise the acids present in the leaf. After boiling the alcohol for half an hour the further extraction of the alcohol-soluble contents of the leaf is carried out in an extraction apparatus on the principle of the Soxhlet extractor. The extraction is complete after 12 or 18 hours. The final separation of the extract from the residue is effected in a Buchner press. The residue is dried on paper trays in a steam oven for 18 hours and from it the total insoluble matter, the starch and pentosans are estimated. The alcoholic extract is analysed for total soluble matter, sucrose, maltose, glucose, fructose, and pentoses. It can be kept in a waxed-corked bottle for 3 to 6 months without any change occurring in the sugars if about 10 c.c. to 20 c.c. of toluene are added.

2. *Estimation of Starch.*

Brown and Morris estimated the starch in their dry leaf powder by O'Sullivan's method (1884) which consists in converting the starch into a mixture of dextrin and maltose by means of diastase. The leaf material usually contains tannins, amino-acids, etc. which influence the optical activity and reducing power of a solution and these accompanying substances have therefore to be removed by precipitation by means of basic lead acetate. Davis and Daish (1914) find that this method does not give correct results because some of the dextrin is carried down with the precipitate and so is lost to the analysis. It is estimated that as a result of this the starch estimations in leaf material made by O'Sullivan's method may be 15% to 20% below the actual starch content.

Davis and Daish therefore treat the plant material with taka-diastase which converts starch wholly into maltose and dextrose which are then estimated by measurement of the cupric reducing power and the optical activity. The leaf material is first treated for 24 hours at 38°C with about 20 times its weight of water con-

taining 1% by volume of toluene which removes certain optically active leaf substances, among them dextrin if it is present. The residue, say 10 grams, containing the true starch, is then boiled with 200 c.c. of water to gelatinise the starch. It is then left for 24 hours at 38°C after addition of 0·1 gram of taka-diastase and 2 c.c. of toluene. After destroying the enzyme with 2 drops of concentrated sodium hydroxide and filtering, basic lead acetate is added (about 2·5 c.c.) and the volume made up to 500 c.c. The slight excess of lead is removed by the addition of the exact quantity of solid sodium carbonate necessary. After filtration the reducing power and optical rotation of the solution are determined. From these values the quantity of starch is calculated on the assumption that the only reducing and optically active substances present are glucose and maltose. In measuring the cupric-reducing power of all sugars examined the standard conditions laid down by Brown, Morris and Millar (1897) are employed, and their tables of the reducing power of maltose, glucose and fructose used. Similar tables for *l*-arabinose and *l*-xylose have been compiled by Daish (1914).

3. *Estimation of Dextrin* ("*Soluble Starch*").

It was found by Davis and Sawyer (1916) that the leaf material from potato, after extraction with 80% alcohol contains large quantities of a substance readily soluble in water and having a high positive optical rotation. This and the reducing power were determined, and again after treatment with taka-diastase and basic lead acetate, and from the change in reducing power and rotation thus brought about, the dextrin was calculated.

4. *Estimation of Pentosans.*

These were estimated by Davis, Daish and Sawyer by distilling 1·0 to 1·5 gram of the oven-dried leaf material with hydrochloric acid by the Kröber-Tollens method and weighing the furfural produced as phloroglucide.

5. *Preparation of the Leaf Extract for Estimation of Sugars.*

Before estimating the sugars in the leaf extract containing them, the alcohol in the case of an alcoholic extract is replaced by water by evaporation of the alcohol and subsequent dilution with water. Davis, Daish and Sawyer evaporate the alcohol under reduced pressure (20–30 mm.) in a special distillation apparatus (Davis, 1913). By this means 3 litres of extract is reduced to 150 c.c. and diluted with water to 500 c.c., a little hot alcohol or toluene

being used to wash out the flask if much chlorophyll or fat is present.

It is now necessary to remove tannins, amino-acids, basic substances, etc. This is effected by addition of the exact quantity of basic lead acetate required to precipitate the whole of these substances. Any excess of lead is removed by hydrogen sulphide (Brown and Morris) or by solid sodium carbonate (Davis, Daish and Sawyer). A solution so prepared can be kept for several weeks if a little toluene is added, provided no excess of basic lead acetate is present and that the solution is *just* alkaline. Any excess of basic lead acetate or much alkali brings about a rapid destruction of fructose.

6. *Estimation of Sugars.*

The methods employed by various workers for the estimation of sugars vary in details, but the general principles underlying all of them are the same. We will consider first the method used by Parkin, for as this worker concluded that sucrose, glucose and fructose are the only sugars present in the snowdrop leaf, his analysis is simpler than that of Brown and Morris, and Davis, Daish and Sawyer who analysed their extracts for other sugars as well.

(*a*) *Parkin's Method.* The cupric-reducing power and optical rotation of a definite volume of the purified extract is first measured. The cupric-reducing power is due to the hexoses alone, the optical rotation to the hexoses and sucrose together. A further volume of the extract is inverted with invertase and the cupric-reducing power and optical activity again measured. The increase in reducing power and change of optical activity must be due to the inversion of the sucrose, and from these numbers the quantity of sucrose is obtained. From the reducing power and optical activity due to the hexoses, the quantities of fructose and glucose can be calculated.

Parkin also found that after fermentation with brewers' or bakers' yeast, that the cupric-reducing power and optical activity became negligible. This indicates the absence of pentoses and the *l* forms of the hexoses.

(*b*) *Method of Brown and Morris.*

(i.) The cupric-reducing power and optical rotation are first measured.

(ii.) For the estimation of cane sugar the solution is inverted with invertase at $50°$ to $55°C$. The increase in cupric-reducing power and change in optical rotation will both give the quantity of sucrose.

(iii.) 50 c.c. of the 1% solution is heated with 3 c.c. of concen-

trated hydrochloric acid for 3 hours on a boiling water bath. This results in the hydrolysis of both the cane sugar and maltose, and the increase in cupric-reducing power and change in optical rotation as compared with the numbers obtained after inversion of cane sugar, give the quantity of maltose.

The cupric-reduction and optical rotation methods do not give concordant numbers for maltose. Davis and Daish (1913) suggest that this is due to the destruction of fructose.

(iv.) The cupric-reducing power and optical rotation of the original purified extract not accounted for by sucrose and maltose are due to glucose and fructose. the quantities of which can be calculated from these values. We discuss the reliability of the glucose and fructose numbers later (pp. 215–216).

(c) *Method of Davis, Daish and Sawyer.* These workers estimate sucrose, maltose, glucose, fructose and pentoses. Their methods are essentially the same as those of Brown and Morris, but they eliminate several sources of error and introduce some important modifications.

(i.) As with previous workers the cupric-reducing power and optical rotation of the purified extract is measured. The cupric-reduction is due to glucose, fructose, maltose and pentoses.

(ii.) For the estimation of cane sugar the solution is inverted with dilute acid or invertase. But Davis and Daish (1913) find that with 2% citric acid, inversion of cane sugar is not complete in plant extracts. For this reason they conclude that the earlier results of Campbell (1911), for example, must be completely withdrawn, as an error in the estimation of cane sugar results in an error in maltose and hexoses as well. In the case of Parkin's experiments, however, they consider that any error arising from this cause was small.

Davis, Daish and Sawyer therefore invert the slightly acid solution by boiling it with 10% citric acid for 10 minutes, or by treating it with 1–2 c.c. autolysed yeast (containing invertase) for 24 hours at 38°–40°C. The increase of reducing power or the change of optical rotation both give the quantity of sucrose present. As the two numbers do not give approximately the same value for sucrose, it is assumed that there are optically active substances other than sugars which vitiate the values obtained from optical rotation data. The values obtained by cupric-reduction are therefore the ones assumed to give the true value for cane sugar.

(iii.) In order to estimate maltose, the lead in the extract (p. 213) is completely removed by treatment with hydrogen sulphide. The excess of this is removed with ferric hydroxide. The solution

becomes acid owing to the presence of free acetic acid which is removed by the addition of dilute sodium carbonate solution until the extract is faintly acid to litmus.

Portions of the extract are then fermented with yeasts which do not contain the enzyme maltase, namely, *Saccharomyces marxianus, S. anomalus, S. exiguus.* Two other portions are fermented with bakers' yeast. The yeast is allowed to incubate for 21 to 28 days at 25°C, by which time fermentation is complete. After addition of alumina cream and filtration, the cupric-reducing power is measured. The differences between the cupric-reducing power of the extracts fermented with maltase-free and maltase-containing yeasts, must be due to the glucose resulting from the hydrolysis of maltose, and so the proportion of maltose in the extracts may be calculated.

(iv.) The pentoses are estimated by distillation with hydrochloric acid and weighing the furfural produced as phloroglucide.

(v.) The reducing power of the maltose and pentose is calculated, and from the reducing power of the original extract, the reducing power of the hexoses can be calculated. The optical activity of the sucrose and maltose is calculated, and that of the pentoses on the assumption that they consist of *l*-xylose and *l*-arabinose in equal proportions. By comparison of these data and the optical activity of the original solution, the optical activity due to the hexoses can be calculated. From this and their cupric-reducing power, the quantities of glucose and fructose can be calculated on the assumption that these are the only hexoses present.

It will be observed that the accuracy of the determinations of glucose and fructose depend upon the accuracy of the following assumptions :—

(i.) That sugars are the only cupric-reducing and optically active substances in the purified extracts.

(ii.) That the only sugars that can be present in the extracts are sucrose, maltose, *d*-glucose, *d*-fructose and pentoses.

(iii.) That the pentoses present are only *l*-arabinose and *l*-xylose, and that these are present in equal quantities.

In regard to this last assumption, Davis, Daish and Sawyer show that the error involved is not very large (about 7%) if the whole of the pentose is either *l*-arabinose or *l*-xylose.

The accuracy of the glucose and fructose estimations also depends upon the accuracy of the following operations :—

(i.) The completeness of the extraction of sugars.

(ii.) The completeness of the inversion of cane sugar.

(iii.) The completeness of the fermentation of sugars other than pentoses by bakers' yeast.

(iv.) The completeness of the fermentation of sugars other than maltose and pentoses by maltase-free yeasts.

Finally the accuracy of the glucose and fructose determinations depends upon the accuracy of the following determinations:—

(i.) The reducing power of the original plant extract.

(ii.) The optical rotation of the original plant extract.

(iii.) The reducing power of the extract after inversion.

(iv.) The reducing power of the extract after fermentation with maltase-free yeasts.

(v.) The reducing power of the extract after fermentation with bakers' yeast.

(vi.) The estimation of pentoses by the Kröber-Tollens method.

As the accuracy of the glucose and fructose determinations thus depends on the accuracy of 13 separate assumptions, operations and determinations, it is not to be expected that the results given for glucose and fructose are likely to have a high order of accuracy. Indeed, Davis (1916) points out that the extracts probably contain optically active substances other than sugars, *e.g.*, amino-acids and amides. As Davis himself says, "the values given as dextrose and lævulose probably do not, in most cases, represent real values"; he therefore prefers to designate them as "apparent dextrose" and "apparent lævulose."

A method by which it may be possible to make more satisfactory estimations of fructose is suggested in a recent publication of Miss Wilson and Atkins (1916). The sucrose is first estimated by measuring the reducing power and optical rotation of the solution of mixed sugars before and after treatment with invertase. After inversion, fructose may then be estimated by oxidising other sugars (glucose and maltose) by means of bromine. Under certain definite conditions, the glucose and maltose are destroyed by this means, and the fructose remains almost entirely unchanged. The method is not very exact, but it is possible that further research on it may render it more accurate, and in any case, the results obtained by its means are not open to all the objections of the indirect method previously described. It is, moreover, considerably more rapid.

Although Miss Wilson and Atkins worked out the method in order to apply it to the analysis of leaf extracts, no account of work involving its use is as yet available.

E. Variations in the Carbohydrate Content of Leaves.

1. Garden Nasturtium (Tropæolum majus) (Brown and Morris).

Tropæolum majus possesses a leaf which forms much starch. Brown and Morris analysed three sets of leaves of *Tropæolum majus* by their methods indicated in the last section. One set of leaves was picked at 5 a.m. and quickly dried in the steam oven; the second set was picked at the same time and kept in sunshine for 12 hours with the petioles in water before drying; the third set was picked at 5 p.m. after 12 hours insolation. The results of the analysis are given in the following table.

Table XXX.

Variation in Starch and Sugar Content of Tropœolum *Leaves, August 23rd.*

The values are given in percentages of the dry weight.

Carbohydrate.	Picked and dried 5 a.m.	Picked 5 a.m. Kept insolated in water until 5 p.m.	Picked and dried 5 p.m.
Starch	1·23	3·91	4·59
Sucrose	4·65	8·85	3·86
Glucose	0·97	1·20	0·00
Fructose ...	2·99	6·44	0·39
Maltose	1·18	0·69	5·33
Total Sugars ...	9·69[1]	17·18	9·58

In a further experiment one set of leaves was picked and dried at once while another set was placed in water in the dark for 24 hours after picking. The results of the carbohydrate analysis were as follows :—

Table XXXI.

Carbohydrate Content of Leaves before and after 24 hours in the Dark.

Carbohydrate.	Leaves picked and dried at once.	Leaves kept in the dark for 24 hours after picking.
Starch	3·693	2·980
Sucrose	9·98	3·49
Glucose	0·00	0·58
Fructose	1·41	3·46
Maltose	2·25	1·86
Total Sugars	13·64	9·39

[1] This is the number given by Brown and Morris.

From these results Brown and Morris conclude that cane sugar is the first sugar formed in the leaf and that this functions as a temporary reserve which accumulates during active assimilation. When the concentration of cane sugar reaches a certain amount, any excess of sucrose is converted into starch in the chloroplast. The cane sugar, on being translocated from the leaf, is inverted into glucose and fructose, while the starch is hydrolysed and translocated as maltose. That it is not hexoses that are the first sugars formed in the assimilatory process is indicated by the fact that after assimilating all day, leaves still attached to the plant contain no glucose and very little fructose. The cane sugar, on the other hand, has remained almost constant while starch and maltose have both decreased. In the case of the cut insolated leaves it is supposed that translocation is to all intents stopped. Under these circumstances the cane sugar and starch both increase greatly, but the glucose very little.

The results given in Table XXXI indicate that in the dark the cane sugar and starch both decrease in amount, while the glucose and fructose have both increased in amount. As presumably the sucrose is hydrolysed into equal quantities of glucose and fructose, and the latter appears much in excess of the former, Brown and Morris conclude that glucose is largely used for respiration in the leaf.

However, from what we have already said on the reliability of the measurements of glucose and fructose, it is extremely doubtful whether the recorded values of glucose and fructose have any meaning. Moreover, Davis and Sawyer (1916) have been unable to find maltose in *Trcpæolum majus* and they conclude that the maltose found by Brown and Morris in their extracts resulted from the degradation of starch by diastatic enzymes after maltase in the leaf had been destroyed.

2. *Snowdrop (Galanthus nivalis, L)* (Parkin).

The snowdrop possesses the very usual monocotyledonous characteristic of not forming starch in the leaves. Hence as already indicated, the analysis of sugars is simplified. Parkin's results were obtained from observations made over a number of years. In most cases only the values of sucrose and hexose are given, no attempt being made, except for a special purpose, of distinguishing between the hexoses. Parkin's results are the most clearly stated of all the accounts we have of leaf carbohydrates and it is possible from the numbers he gives, to

realise the degree of accuracy of the results. He realises, for instance, that " this branch of physiological chemistry is as yet in the tentative stage." He prefers to make a large number of analyses with a moderate degree of accuracy to a few with many precautions taken, and he draws conclusions only from wide differences in sugar contents.

As a result of his analyses, Parkin finds that during any single day in spring the percentage of hexose sugars in the leaf remains fairly constant, whereas the sucrose fluctuates greatly, increasing during the day and diminishing at night. Tables XXXII and XXXIII exhibit some of the actual numbers obtained. The values are given in percentages of the dry weight.

TABLE XXXII.

Comparison of Sugars in Snowdrop Leaves picked in the early Morning and in the late Afternoon.

March 7th, 1906, Cambridge.

Maximum shade temperature 19·4⁰C.
Minimum temperature, previous night 6·1⁰C.

	9 a.m.	3.30 p.m.
Sucrose	11·22	14.65
Hexose	6·35	5·48
Total Sugars	17·57	20·13

TABLE XXXIII.

Comparison of Sugars in Snowdrop Leaves in the Evening and the following Morning.

March 30th and 31st, 1905, Carlisle.

Maximum shade temperature 9·7°C.
Minimum temperature 3·3°C.

	5·30 p.m.	8 a.m.
Sucrose	15·46	10·84
Hexose	11·41	12·64
Total Sugar	26·87	23·48

As the season advances, the hexose sugars in the leaf increase in proportion to the sucrose, as the following table shows.

TABLE XXXIV.

Seasonal Variation of Sugars in Snowdrop Leaves.

Date of picking.	Time of picking.	Max. shade temp.	Per 100 g. dry leaf.		Sucrose. Hexose.
			Sucrose.	Hexose.	
Feb. 16, 1906	3 p.m.	9·4°C	19·8	3·56	1 : 0·2
,, 26, 1907	4–5 ,,	7·2 ,,	15·07	2·53	1 : 0·2
Mar. 7, 1906	3.30–4 p.m.	19·4 ,,	14·55	5·69	1 : 0·2
,, 30, 1905	5–6 ,,	9·6 ,,	15·5	11·4	1 : 0·7
Apr. 5, 1906	4–4.30 p.m.	15·6 ,,	14·64	11·17	1 : 0·8
,, ,, 1907	,,	14·4 ,,	14·64	11·61	1 : 0·8
,, 24, 1905	,,	10·6 ,,	14·84	17·29	1 : 1·2
May 4, 1905	3–3.30 ,,	11·7 ,,	10·3	12·78	1 : 1·2

Parkin considers that his results strongly support Brown and Morris' view that sucrose is the first recognisable sugar to appear in the leaf, and that glucose and fructose arise from it by inversion. He also brings forward evidence in support of Brown and Morris' contention that fructose is generally present in excess of glucose. Thus, out of 54 analyses, in 47 cases fructose was in excess of glucose, the proportion $\frac{\text{fructose}}{\text{glucose}}$ varying from $\frac{1}{0·4}$ to $\frac{1}{0·76}$, while in only 7 cases was the reverse the case and then the glucose was only slightly in excess, the fructose : glucose ratio varying from 1 : 1·01 to 1 : 1·06. As there is a greater tendency for the fructose to be destroyed by the careless use of basic lead acetate, these 7 results are probably due to experimental error. Parkin considers therefore with Brown and Morris, that glucose contributes more readily than fructose to the needs of the leaf.

3. Mangold (Beta vulgaris, *L., var. Sutton's Yellow Globe*).

Davis, Daish and Sawyer have attempted to obtain information in regard to the sugars in leaves and leaf stalks of the mangold at different times of the day and night and at different seasons by an extensive series of analyses carried out at Rothamsted.

Collections of leaves were made from plants growing in the field at 2-hourly intervals over a 24 hour period. Such series of measurements were made at three different times.

I. Stage of early growth. 6 a.m., August 26—4 a.m., August 27, 1913.

II.   Stage of intermediate growth.   10 a.m., September 10—
8 a.m., September 11, 1912.

III.   Final stage of growth. 9 a.m., October 11—7 a.m.,
October 19, 1912.

In each case, the leaves and leaf stalks were treated separately.
In the first series the upper and lower parts of the leaf stalk were
dealt with separately; in the second series, the midribs of the leaves
were subjected to a separate analysis ; in the third stage of growth
the midribs and leaf stalks were treated together.   Their results
are all calculated in terms of the total vacuum dried matter of the
leaf.

It may be mentioned at once that Davis and his collaborators
found starch was absent from the leaves and petioles of the mangold
at all stages of growth except the very earliest.   Similarly, no maltose
was ever found in either leaves or petioles of this plant at any time.
The quantities of other carbohydrates present in the leaf are
indicated in the accompanying figures, which are based on the
numbers and curves given in Davis, Daish and Sawyer's paper.

Fig. 14 shows the variation in content of the sugars of the
mangold leaf found during 24 hours on August 26–27, 1913.   The
most noteworthy features of these results are :—

1.   Both hexoses and sucrose increase rapidly in quantity
after daybreak and reach a maximum about mid-day, after which
the quantity present falls off fairly regularly and rapidly until the
following dawn.   Practically the whole of the hexose sugar disappears
and about half the sucrose.   These changes are closely parallel to
the temperature curve (and probably also to the curve of light
intensity).

2.   The quantity of sucrose is always greater than that of
hexose.

3.   The variations in the quantity of cane sugar are small, the

4.   The quantity of pentosan remains practically constant
limits between being 3·11% and 1·5%, whereas the hexoses vary
between 0·77% and 2·16%.

throughout the day, the fluctuations being probably within the
range of experimental error.   The same holds for the matter
insoluble in alcohol.   Davis and his co-workers consider the
increase they found in the values of these substances to be really
significant, but if this were so, there should be either a sudden fall
in these values at sunrise, or the proportion of them in the leaf
should go on increasing from day to day.   As a matter of fact, in

the second series of these workers, measured a fortnight later in the season, the proportion of pentosan is exactly the same as at the earlier period, while the proportion of matter insoluble in alcohol has actually decreased from about 60% to 50%. Nor is there any better evidence for the second alternative, for at this intermediate stage, where the measurements were taken before and after sunrise, the results obtained show actually a slight increase of these substances after sunrise. The most reasonable explanation is therefore that the differences recorded are simply within the range of experimental error. Similar considerations apply to the variations in the pentose content.
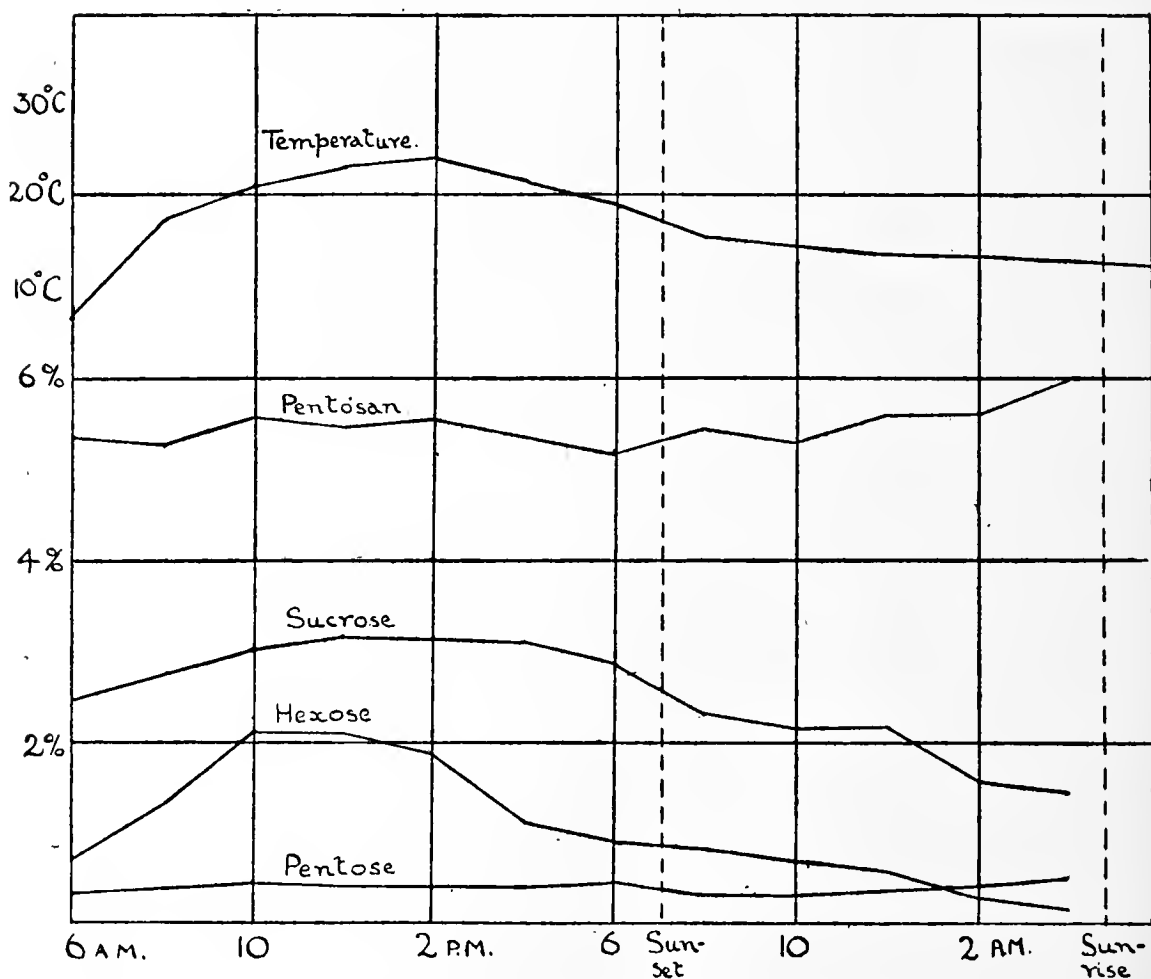

Fig. 14. Variation in Content of Various Carbohydrates in the leaf of Mangold during 24 hours, Aug. 26—27, 1913 (After Davis, Daish and Sawyer).

It is indeed regrettable that Davis, Daish and Sawyer give no data which enable one to judge the likely limits of error of their determinations. They do indeed, under the heading " Probable Error of the Analyses and Methods of Sampling," show that the reduction method and optical rotation method give values for sucrose which differ by about 20%, and they also give the analyses of hexoses

and sucrose in two samples collected at the same time, in which the hexose determinations in the two cases differ by 6%. The only information this gives us is that there is possibly a considerable error due to the variability of different samples, but two samples alone can give us no idea whatever of the actual magnitude of the probable error, which Davis and his co-workers have not determined. It is therefore misleading to give these two analyses under the sub-heading " Error of Sampling."

In Fig. 15 are summarised the results of analyses of leaves in the second period. Here the hexoses are in excess of the sucrose Both curves show synchronising maxima at 2 p.m., 6 p.m. and 2 a.m. Whether these maxima have any meaning, or whether they are merely the result of differences in sampling, it is impossible to say. The fact that the hexoses and sucrose always show unusually high
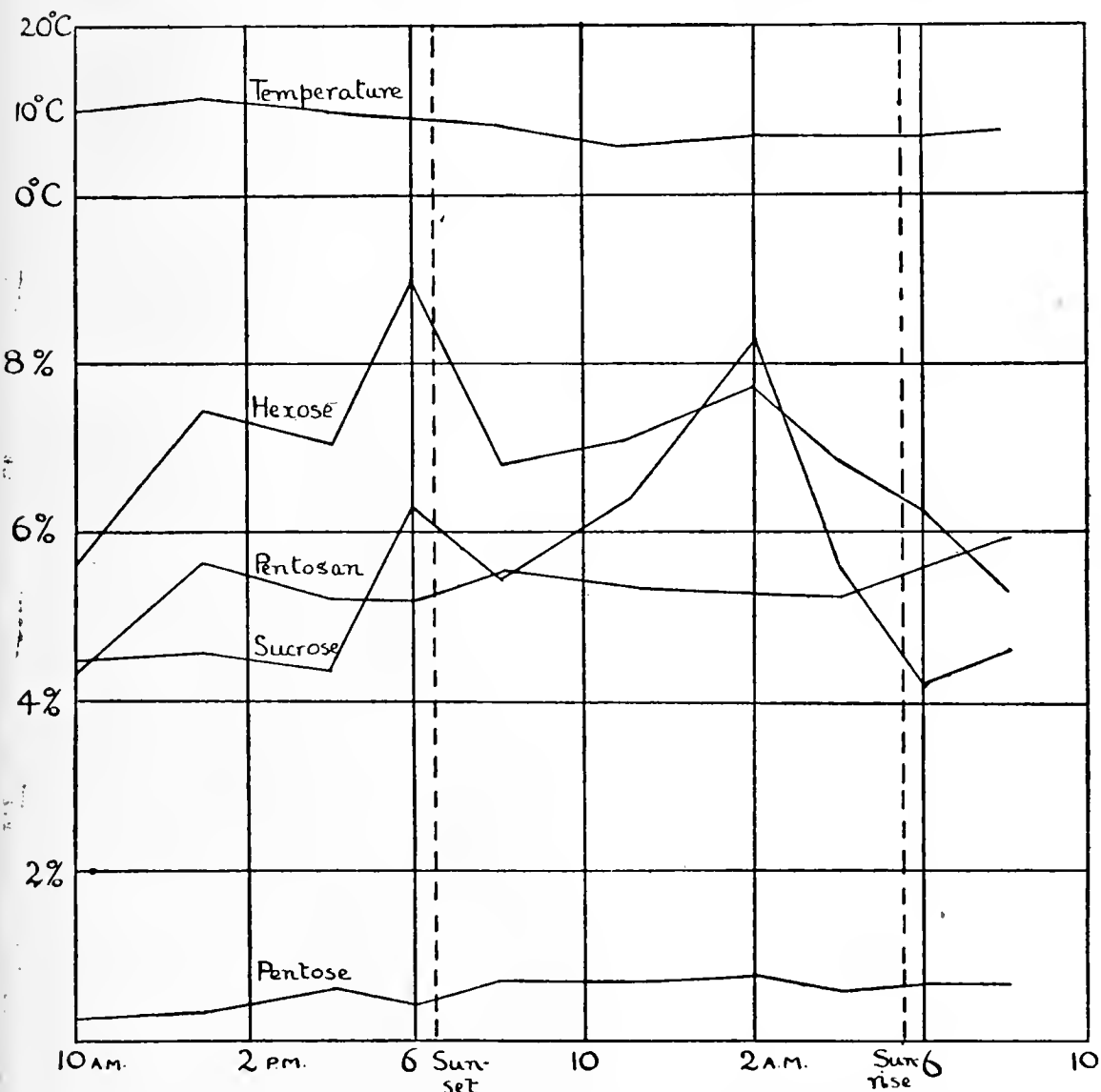

FIG. 15. Variation in Content of various Carbohydrates in the Leaf of Mangold during 24 hours, Sept. 10--11, 1912 (After Davis, Daish and Sawyer).

values in the same samples is suspicious, and the most likely explanation is that in some cases, at any rate, they are apparent maxima and minima, due to errors in sampling. This is especially so in the case of the maxima at 2 p.m., which appear owing to the minima at 4 p.m. Thus, if the minimum value found for hexoses in this case were increased by 7%, the minimum on the hexose curve would disappear, and we have already cited the instance in which the hexose content of two samples collected under similar conditions differed by 6%.

It seems reasonable to conclude from Davis, Daish and Sawyer's figures that the hexose and sucrose in the leaf increase during the day and then gradually decrease during the night. The maximum in these sugars in the middle of the night, at 2 a.m., is extremely difficult to account for on any other ground than error in sampling, for the leaf manufactures no fresh material, and yet the total carbohydrate in the leaf (pentosan, sucrose, hexose and pentose) has increased from 19·53% to 22·13% of the total dry matter of the leaf according to Davis, Daish and Sawyer's complete analysis. These authors suppose this increase is due to the breaking down of a water soluble gummy substance in the leaf into carbohydrates.

The relative variations in sucrose content are similar to those in August, although the percentage of sucrose is more than twice as great. The total hexoses present is about the same amount by weight as sucrose, and is much more than is present earlier in the season.

The pentose content varies little throughout the day; it appears to diminish somewhat during the night.

The results obtained for the last stage of growth are similar to those obtained for the intermediate stage. They are shown graphically in Fig. 16. As before, the sugar content is greater during the day than during the night. In this case, hexoses and sucrose show two maxima during the night, at 7 or 9 p.m. and at 3 a.m. As we have already indicated, the data furnished by the experiments of Davis, Daish and Sawyer are insufficient to enable us to judge whether such night maxima in sugar content actually exist in mangold leaves, or whether their appearance in the curves is simply due to a sampling error.

The results obtained by Davis, Daish and Sawyer in regard to carbohydrates in the mangold leaf may be summarised as follows :—

(i.)   All the sugars in the leaf increase in quantity from the first to the final stage of growth.

(ii.) The pentosans form a larger and larger proportion of the matter insoluble in alcohol as the season advances.

(iii.) Of the total sugar, the hexoses form a progressively increasing proportion as the season advances. This point has already been brought forward very clearly by Parkin in the case of the snowdrop (see Table XXXIV).
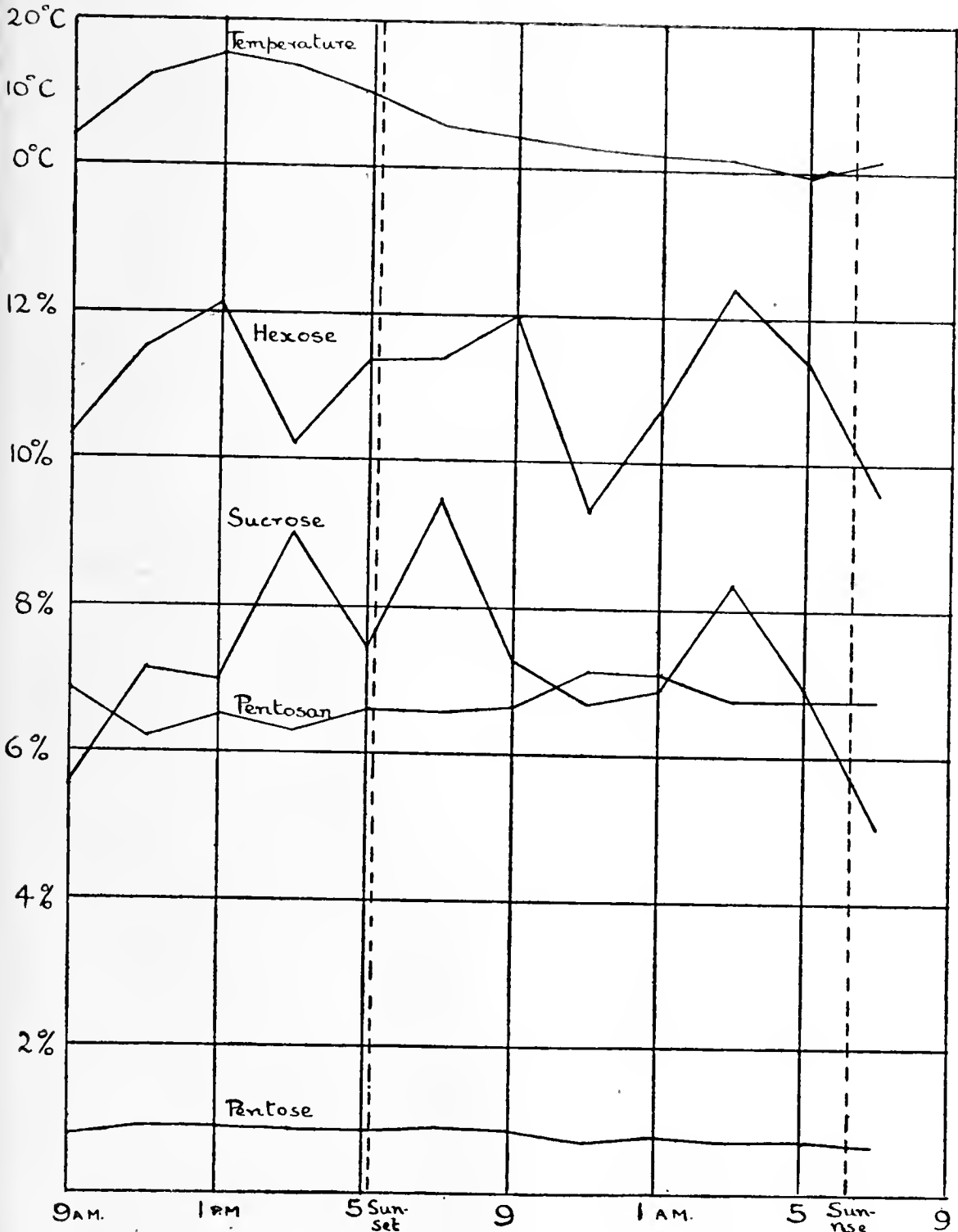

FIG. 16. Variation in Content of various Carbohydrates in the Leaf of Mangold during 24 hours, Oct. 11—12, 1912 (After Davis, Daish and Sawyer).

(iv.) In the first stage of growth, practically all the reducing sugars and about half the sucrose disappear from the leaf during the night. As the season proceeds, a less proportion of the sugar in the leaf disappears each night. It is especially the hexoses which increase in the leaf owing. to this. These results are summarised in the following table.

TABLE XXXV.

Seasonal Variations in Carbohydrate Content of Mangold Leaves.

Date.	Temp.	Sucrose.	Hexoses.	Pentoses.	Pentosans.
Aug. 26–27	7·2—23·9°C	1·50—3·11	0·20 —2·16	0·36—0·52	5·19—5.96
Sept. 10–11	6·1—10°C	4·24—8·27	5·38—8·90	0·34—0·76	4·42—5·90
Oct. 11–12	-0·6 - 16·1°C	4·98—9·52	9·39—12·41	0·61—0·92	6·21 – 7·15

The observations made by Davis, Daish and Sawyer on the sugars of midribs and petioles, show that these always contain a higher percentage of sugars than the leaves, and this percentage increases with the season. The hexoses are always much in excess of the sucrose, and the ratio of hexoses to sucrose is always much greater in the petioles than in the leaf lamina. These results are comparable with Parkin's observations that the sugar content of the snowdrop leaf increases from above downwards, and that the ratio of hexose to sucrose also increases. The conclusion drawn from this by both Parkin and the Rothamsted workers is that sucrose is the first sugar formed in the leaf and that this is converted into hexoses for translocation purposes. In support of this they also adduce the fact that the cane sugar is always present in relatively high proportion in the leaf, especially early in the season when it is present in excess of the hexoses. They suppose the cane sugar is gradually inverted by means of the enzyme invertase which is secreted or distributed on the surface of the sieve tubes.

We have already referred to the unreliable character of the determinations of glucose and fructose, an unreliability which is quite realised by Davis, Daish and Sawyer. As, therefore, it is not at all clear what the quantities they term " apparent dextrose " and apparent levulose " really represent, we do not think any useful purpose would be served by discussing the values they obtain for these quantities.

4. Potato (Solanum tuberosum, *var. King Edward VII*).

Davis and Sawyer have made analyses also of the carbohydrates of a leaf which forms starch, that of the potato. The samples were gathered at 2-hourly intervals, from 6 a.m., July 16th to 4 a.m., July 17th, 1914. Separate analyses were made of the stalks. Their results are summarised in Fig. 17. It will be observed that sucrose is the chief sugar present, as was found to be the case also in the early stage of growth of the mangold. The sucrose content of the leaves rises during the day until 2 p.m., and then falls off regularly until dawn the next day. The hexoses show much variation throughout the day and night, and here again it is impossible through absence of data, to judge whether these variations are anything more than differences due to sampling. The hexose content is, on the whole, higher during the day than at night. The pentoses show little variation.
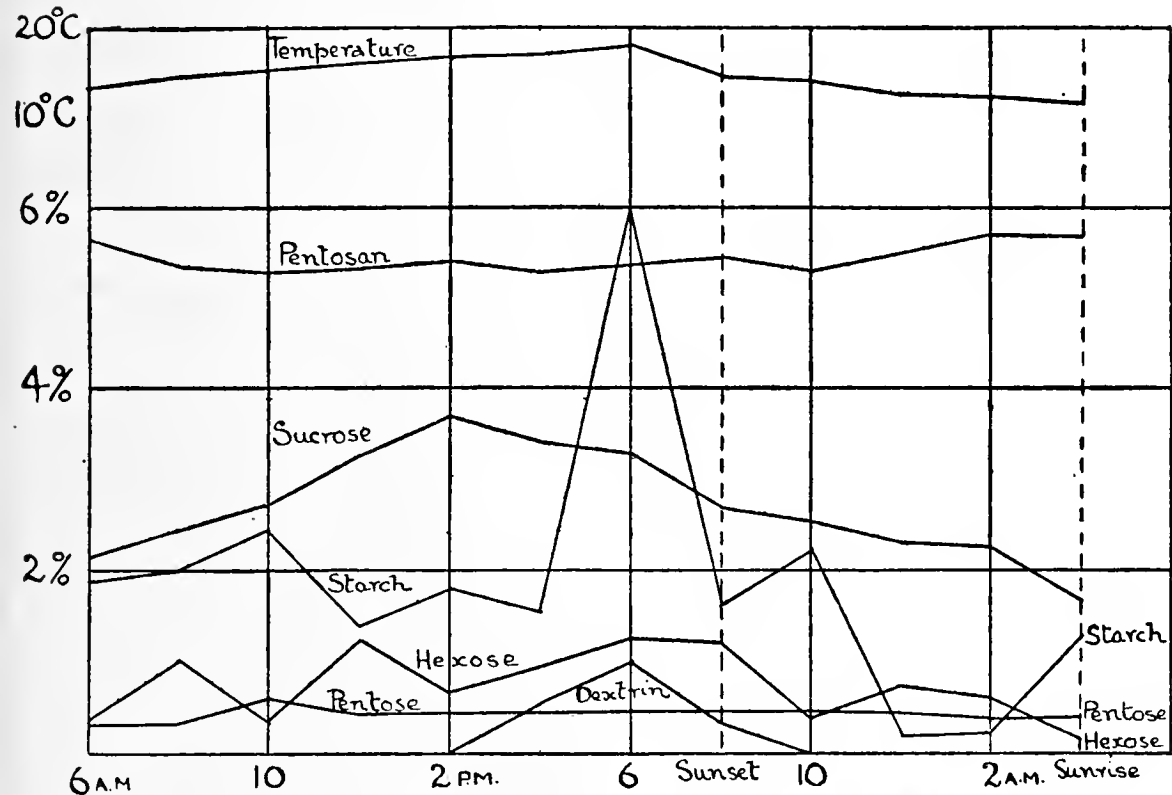

Fig. 17. Variation in Content of various Carbohydrates in the leaf of Potato during 24 hours, July 16—17, 1914 (After Davis, and Sawyer).

The starch content shows a decided maximum in the late afternoon (6 p.m.) and at the same time a quantity of dextrin (soluble starch) is present. By sunset this has almost disappeared and the starch content has rapidly fallen.

As in the mangold, in the leaf stalks of the potato the hexoses are much in excess of the sucrose, and it is reasonable to suppose

that the sucrose is translocated away from the leaves in the form of hexoses.

Even in this plant where the leaves contain abundant starch no maltose is found, either in the leaves or petioles. Davis and Sawyer therefore conclude that the starch, on utilisation by the plant, is broken down into hexoses by a mixture of enzymes similar to that of *Aspergillus oryzæ* which yields taka-diastase. They consider that dextrin and maltose are intermediate stages in the degradation of starch to glucose.   Dextrin indeed appears at the period when starch is in large quantity in the leaf. They suppose the enzyme maltase is always present in relative excess in the leaf, and Daish (1916) has shown the presence of maltase in a number of different leaves.

5.   *Vine* (Vitis vinifera).

In the leaf of the vine, a plant which stores its carbohydrate as glucose, Deleano (1912) was unable to detect sucrose in the leaf. Davis and his co-workers, on the other hand, state that after taking special precautions in sampling to prevent the leaf enzymes from acting, sucrose is found to be the principal sugar of the leaf.   This they regard as supporting their view that sucrose is the first sugar formed in assimilation.

F.   Carbohydrate Transformations in the Leaf.

The pioneer researches of Sachs indicated the formation of starch in the chloroplasts of the leaf as a result of carbon assimilation, and it was this investigator who showed the dependence of starch formation on light and chlorophyll.   The proof was completed by Godlewski (1873) and Pfeffer (1873) who showed the necessity for an outer atmosphere containing carbon dioxide.

Sachs held the view that starch was the first visible product of assimilation, and he bound himself to no theories concerning possible intermediate products in its formation.

Kayser's work (1883) established the presence of sucrose in the leaf of the vine.   It was supposed that starch was converted into cane sugar by diastatic enzymes and that the cane sugar was inverted in the conducting tissue of the leaf. · Sachs (1884) also expressed the opinion that the starch is translocated in the form of sugar.

That starch could not always be the first visible product of carbon assimilation became obvious from the researches of A. Meyer (1885) who showed that different species varied greatly in their

capacity for forming starch, many plants not forming it all. In these cases it was shown that the absence of starch was not due to rapid translocation, for no starch was formed even under conditions most favourable to rapid assimilation and accumulation of products. The same investigator showed later (1886) that leaves depleted of starch floated on sugar solutions could form starch from the sugars. Thus almost all leaves formed starch from a 10% solution of fructose, a few from glucose and a very few from galactose. It became reasonable to suppose that starch might possibly be formed in the leaf from sugar.

Confirmatory evidence of this theory was derived from researches on starch formation in the plant, carried out by Boehm (1874, 1876, 1877) and notably by Schimper (1880). Boehm showed that not all starch is the direct result of carbon assimilation. Thus he showed the formation of starch in the leaf as a result of transference of reserve material from other tissues under feeble light intensity, and in an atmosphere devoid of carbon dioxide. Schimper investigated the development of the starch granule. He showed that starch is always formed in a plastid, which might be colourless or green, and although he held that the mesophyll chloroplasts could not elaborate starch from other carbohydrates, further work of Boehm (1883) and that of A. Meyer mentioned above, showed that these chloroplasts could elaborate starch from sugars. These considerations lead to the conclusion that there is no difference between the chloroplast and the colourless amyloplast in regard to their powers of elaborating starch, and it is at least possible that starch formed normally in the chloroplast is formed secondarily and not as a direct result of assimilation.

These considerations were generally held to support Baeyer's theory, to which we shall refer in detail later, that the first part of the assimilation process consisted in the formation of formaldehyde which polymerised to hexose, and then gave rise to sucrose or starch. This theory, which involves the view of hexose as the first sugar formed in assimilation, has recently received support from the work of Strakosch (1907), who investigated the distribution of sugars in the leaf and other parts of the sugar beet, by means of microchemical tests depending on the production of osazones. He concludes that glucose is the only sugar present in the mesophyll cells of the leaf. In the veins fructose appears as well, and later, sucrose. Maltose also occurs only in the petiole. Strakosch therefore concludes that glucose is the first sugar formed in assimilation and

sucrose is a later formed product, and he supports this conclusion with an analysis of leaf extract which shows only a negligible quantity of sucrose in the leaf (about one-sixth of the quantity of glucose) while the veins contain nearly five times as much sucrose as hexoses.   Strakosch's results are in direct contradiction to those of the English workers, whose work we have summarised in the preceding section of this chapter.   Davis, Daish and Sawyer point out that Grafe's test (1905) for fructose, used by Strakosch, is of doubtful applicability in presence of glucose, while the method used to localise glucose and sucrose, which is due to Senft (1904), is, according to Mangham (1915), untrustworthy when sucrose is present together with its hexose constituents.   Davis, Daish and Sawyer criticise Strakosch's results as well as Mangham's identification of maltose in plant tissues, on the general ground that little reliance can be placed on such microchemical tests as a means of identifying one sugar in presence of others in plant tissues.   The fact that Mangham should claim to distinguish between d-glucose and d-fructose in the plant by means of the osazone test, when their phenyl osazones are of course identical, is not very reassuring as to the degree of reliability of his results.

Such qualitative observations as those of Strakosch and of Dixon and Mason (1916) in any case cannot have the value of the quantitative researches recorded in detail in the preceding section of this chapter, and we find in all detailed quantitative examinations made of the carbohydrates of the leaf, that cane sugar is always present in the leaf in considerable quantity.   We have already pointed out that all the workers who have obtained quantitative data have expressed the opinion that sucrose is the first sugar formed in assimilation, and that this is inverted into hexoses for translocation. When carbohydrate accumulates in the leaf in consequence of assimilation taking place at a greater rate than translocation, the excess of sucrose is supposed to be transformed into starch in *Tropæolum* (Brown and Morris).   In the potato, which also forms starch in the leaf, Davis and Sawyer (1916) appear to regard the starch as formed from hexoses, soluble starch, which appears in appreciable quantity in the leaf at the period when starch content is at a maximum, being an intermediate product.   This view is based on the intimate relation between the content of hexoses and starch in the leaf throughout the day, a relation, however, which is not very obvious from Davis and Sawyer's published curves (cf. Fig. 17). It must be admitted that the evidence in favour of the production of

either hexoses or sucrose as the first sugar produced in assimilation is scarcely adequate for discussion. In any case Lundegårdh (1914) regards the transformation of sugar into starch and the reverse process as a very complicated one, depending not merely on the concentration of the sugar in the cytoplasm, but also on the quantity of an enzyme, the concentration of which depends on factors at present unknown.

The evidence that hexoses are in excess of other sugars in the conducting tissue of the plant seems definite enough. From this it is concluded that sucrose is converted into hexose by means of invertase in the conducting cells of the plant, and is translocated as hexose sugars. The percentage of sucrose in the petiole is less than in the midribs of the leaf, from which one could expect a diffusion of sucrose away from the leaf. Such a state of affairs would, of course, be produced if the sucrose were inverted in the manner suggested by Davis, Daish and Sawyer in the veins of the leaf and stem, and they cite in support of this view the observation of Robertson, Irvine and Dobson (1909) that invertase is abundant in the leaf and stem of the beet, although absent from the root. The migration of sucrose, therefore, from a place of higher concentration in the leaf to a place where its concentration is kept constantly lower by the invertive action can be readily understood, and it is more likely that the simpler monosaccharides would diffuse through the plant more rapidly than the more complex disaccharide. The difficulty arises from the fact that the published analyses of Davis, Daish and Sawyer show generally a higher percentage of hexoses in the petioles than in the leaf veins, and one would therefore expect a flow of hexoses towards the leaf and not away from it. On the other hand there is no definite information as to the actual *concentration* of sugars in the actual conducting cells, and as the hexoses are elaborated into sucrose in the root, it would seem that the concentration of hexoses in the cells of the leaf must rise above that in the root, so that diffusion of hexoses towards the root will take place. There is no doubt that the mechanism of translocation is complex, depending probably on differences of enzyme concentration and possibly also on permeability changes, of which we are at present not merely ignorant of the causes, but also of the nature.

But whether carbohydrates are translocated as hexoses or as sucrose has little bearing on the question of carbon assimilation itself. The inversion of sucrose into hexoses for purpose of translocation, Davis, Daish and Sawyer regard as evidence that

sucrose is a primary product in assimilation. Although it is possible that all the sugar in the mesophyll cells of the leaf is sucrose, and that the hexoses are confined to the vascular bundles, there is no direct evidence of this, and there seems no sufficient reason to conclude that sucrose is the first sugar formed rather than that glucose or other hexoses first appear and that cane sugar is formed from them. It is no more unreasonable to suppose that sucrose should be formed from hexoses in the leaf when these latter reach a certain concentration, that to suppose starch should be formed as a temporary reserve carbohydrate in such leaves as those of *Tropæolum* or Potato, for as starch is a storage form in the potato tuber, and is similarly formed in the leaf, so since sucrose is the storage carbohydrate in the root of *Beta*, being formed from hexoses according to Davis, Daish and Sawyer, there is no reason why it should not also be formed from hexoses in the leaf. The value of evidence in regard to the first sugar formed in the leaf, derived from considerations of the variation in amount of the different sugars, is indicated by the fact that Brown and Morris, Parkin, and Davis, Daish and Sawyer all conclude that sucrose is the first sugar formed in assimilation, while their results in regard to the variation of different sugars through the day differ absolutely. Thus Brown and Morris found that both the sucrose and hexose content diminished during the day; Parkin found the hexose content remained practically constant, while the sucrose varied; Davis, Daish and Sawyer found both the sucrose content and hexose content varying in the leaf, while in the veins the sucrose content remains approximately constant, the hexose varying widely.

We do not wish it to be supposed that we therefore support the view that glucose is the first sugar of carbon assimilation. We hold that the data so far produced from analyses of carbohydrates in leaves and from microchemical examination provide insufficient evidence in favour of or against either theory. While we may regard starch as a secondary product of assimilation, and while also there is good evidence that carbohydrates are translocated, in some cases, or to some extent at any rate, as hexose sugars, and while there is strong evidence that sugars are the first *definitely* known products of the assimilatory process, there is no evidence at present as to which particular sugar is the first one to be produced in the leaf.

<p style="text-align:center">(To be continued.)</p>

THE ALGAL ANCESTRY OF THE HIGHER PLANTS.[1]

By F. E. Fritsch, D.Sc.

[With Two Figures in the Text].

IN all of the groups, above the level of the Thallophyta, the life-cycle is characterised by the succession of two individuals—the one sexual, the other asexual—in regular alternation, and the origin of these two alternating generations has long been the subject of discussion. One group of Botanists, with whom we may couple the names of Pringsheim and Scott,[2] have held that the two generations are homologous, having arisen by gradual differentiation from an indifferent generation bearing both asexual and sexual organs, such as is commonly found in the Algæ at the present day.

Other authorities, especially Celakovsky and Bower,[3] hold that the sporophyte is a new intercalation in the life-history, originating by a gradual elaboration of the zygote, *i.e.*, it is antithetic to the gametophyte.

Such evidence as has been adduced speaks neither for the one nor for the other view; in the case of the Pteridophyta the balance is, perhaps, in favour of the homologous theory, in the case of the Bryophyta more in favour of the antithetic. Nor does it seem necessary to postulate the same mode of origin for the two groups, a point of view that has not, perhaps, received sufficient considera-tion.[4] The differences between the sporophyte in Bryophyta and Pteridophyta are very marked; in fact, the only important resem-blance is the differentiation of stomata of a similar type.[5]

In 1909, Lang [6] put forward a theory of alternation, the essence of which is to explain the differences between the two generations as being due to the retention of the spore (whether asexually or sexually produced) within the body of the parent-organism for a longer or shorter space of time. Lang's theory would appear to

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[1] Presidential Address to the Intercollegiate Botanical Society, University of London.

[2] cf. Presid. Address, Bot. Sect., Brit. Assoc., Liverpool, 1896.

[3] Annals of Botany, IV., 1890, p. 347.

[4] cf. however Tansley, in New Phytologist, XI., 1912, p. 216.

[5] Recalling the development of structures closely resembling the sieve-tubes of the Angiosperms in such Laminariaceæ as *Macrocystis*, it is evident that like demands may lead to the differentiation of similar structures, with-out implying any close relationship.

[6] W. H. Lang. A theory of alternation of generations in Archegoniate plants based upon the ontogeny. New Phytologist, VIII., 1909, p. 1.

favour essentially the homologous view of alternation, and obviously opens up many possibilities for experimental research into this vexed question.

The writer has no intention of discussing the relative merits of these different theories. The purpose of the present communication is, in the first place, to sift the ground for the characters of the ancestral group from which the higher plants may have arisen. No one is probably prepared to dispute that the latter have a common origin, and that their ancestors were of the type of the present-day Thallophyta. Since all the groups above the level of the latter have pure green chloroplasts, with starch as a customary product of assimilation, it is to be presumed that their ancestors exhibited these features. Moreover, the spermatozoids appear invariably to be isokontan. This leads us inevitably to look for the ancestry of the higher forms among the Isokontæ.[1] The two generations in the higher groups are in general characterised by certain prominent features, in considering which we may leave the highly specialised Phanerogams out of the realm of our discussion.

The gametophyte is evidently typically prostrate and dorsiventral, the assumption of a radial construction being rare, and a mark of specialisation. The sexual organs seem primitively to have been borne on the upper surface, as we see it at the present day in most of the Hepaticæ, although tending to shift to the lower side, where more ample protection is obtainable. In practically all the Pteridophyta and all those Bryophyta regarded as the more primitive, the gametophyte retains a thalloid differentiation.

The sporophyte, on the other hand, is typically upright, and radial in organisation. This is quite patent in the Bryophyta, and it appears to be generally accepted that the sporophyte, in the Pteridophyta, was primitively radial.[2] A second feature of the sporophyte, in the Archegoniatæ, is the tendency to differentiate a main axis, with lateral appendages subservient to assimilation and the production of the asexual cells.

The two generations of Archegoniatæ may, therefore, be briefly characterised as follows :—The gametophyte a dorsiventral prostrate thallus, with the reproductive organs primitively on the upper surface ; the sporophyte an upright structure, with radial organisation and a tendency towards peripheral placing of the assimilatory

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[1] For a discussion of Schenck's hypothesis as to the origin of the Archegoniates from the Phæophyceæ, see p. 13.

[2] cf. Bower, The Origin of a Land Flora, 1908, pp. 363 and 625.

and reproductive tissues and gradual assertion of a main axis. A likely algal ancestry might, therefore, be expected to display these tendencies:— Differentiation of prostrate dorsiventral and radial upright systems, assertion of a main axis in the latter, and restriction of sexual organs to the prostrate portion, and of asexual organs to the appendages of the upright system. We may, perhaps, add to this, evidence of a terrestrial tendency, with corresponding adaptations.

The filamentous Algæ among the Isokontæ, to which we may now turn, include a number of separate series, viz., Ulotrichales, Chætophorales, Siphonales, Œdogoniales, and Conjugatæ. The last three are obviously specialised along lines of their own, in fact, some algologists are inclined to regard the last two as originating from ancestries independent of that of the remaining Isokontæ, a view which the writer does not share. Ruling out these three groups we are left with the Ulotrichales and Chætophorales, which are probably best regarded as distinct, though closely related, series.[1] The former are, on the whole, very uniform, and display little morphological differentiation, and what there is is scarcely relevant in the present connection. In the Chætophorales, on the other hand, we have a group with very considerable morphological differentiation and one, moreover, showing a very wide range of construction and a great diversity of habitat.

As a relatively primitive and central member of this group, we may regard the genus *Myxonema* (*Stigeoclonium*). In this genus the typical thallus consists of two portions, a prostrate system attached to the substratum (in the following, briefly referred to as the creeping base), and a more or less elaborate, branched, upright part, arising from the base and extending out into the water (Fig. 1, *a*); the former is dorsiventral, the latter essentially radial. Among the known species of *Myxonema* there are all possible variations in the degree of relative development of the base and the upright system. In some (*e.g.*, *M. tenue* (Fig. 1, *a*), *M. falk-*

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[1] It will probably be useful to give the following outline sketch of the classification of the Chætophorales :—

(*a*) *Chætophoraceæ* :—Myxonema (Stigeoclonium), Chætophora, Draparnaldia, Gongrosira, Protoderma, Endoderma, Gomontia, Tellamia, Acrochæte, Ectochæte, Pseudochæte, Ochlochæte, Pringsheimia, Aphanochæte, etc.

(*b*) *Chætosphæridiaceæ* :—Chætosphæridium, etc.
(*c*) *Chætopeltidaceæ* :—Chætopeltis, etc.
(*d*) *Coleochætaceæ* :—Coleochæte.
(*e*) *Chætosiphonaceæ* :—Chætosiphon.
(*f*) *Trentepohliaceæ* :—Trentepohlia, Cephaleuros, etc.

landicum), the base is relatively insignificant, whilst the upright portion attains to considerable development ; in others (*e.g.*, *M. farctum*, Berthold[1]) the base (Fig. 1, *b*) is predominant and of considerable extent, whilst the upright system is reduced to short filaments and hairs, the filaments being but little branched. The writer has recently described a *Myxonema prostratum*,[2] in which the creeping base (Fig. 1, *e*) is of still greater extent (covering several square millimetres of the substratum) ; large areas of this base are without any upright system whatsoever (apart from very scanty hairs), but at remote intervals there arise tufts of 3-5-celled branchlets.

From the available evidence it seems that one and the same species of *Myxonema* may exhibit a very varied relative development of base and upright system under different circumstances, *i.e.*, a species may be prevalently upright or prevalently prostrate, according to the conditions of the habitat. An investigation of the methods of germination of the zoospores in this genus has shown that whereas some first form a creeping base, from which the upright threads subsequently grow out, others only form upright threads, either lacking the base altogether, or only producing it at a later stage,[3] phenomena which again illustrate the varying importance or the two parts of the thallus in this genus.

Before proceeding, it will be well to refer to one feature of the Chætophorales, to which undue value might be attributed, viz., the production of hairs. These are, undoubtedly, a marked characteristic of the group, although completely lacking in some forms, and of very varying development in many. The tendency towards differentiation of the ends of the branches as hairs may be regarded as one indication of morphological elaboration. We can, however, also look upon it as an expression of the tendency towards reduction of the upright system, of which the hairs become the sole representatives in the almost completely prostrate forms to be dealt with in the following. It is questionable whether the peculiar sheathed hairs, characteristic of such forms as *Coleochæte* and *Chætosphæridium*, are to be considered as belonging to the same morphological category as the simple hairs of other Chætophorales. It is not out of the question that the different types of hairs may

[1] Nov. Act. K. Leop.-Carol. Ak. d. Naturf., XL., No. 5, 1878, pp. 201, 202.

[2] F. E. Fritsch, in Annals of the S. Afr. Museum, IX., 1917,

[3] cf. Berthold, *loc. cit.*, also Fritsch, in Beih. Bot. Centralbl., XIII., 1903, p. 372, etc.

have some biological significance that has as yet escaped recognition, but in any case we must regard them as a special development, which may not interfere with our perspective in estimating the remaining morphological characters of this group.
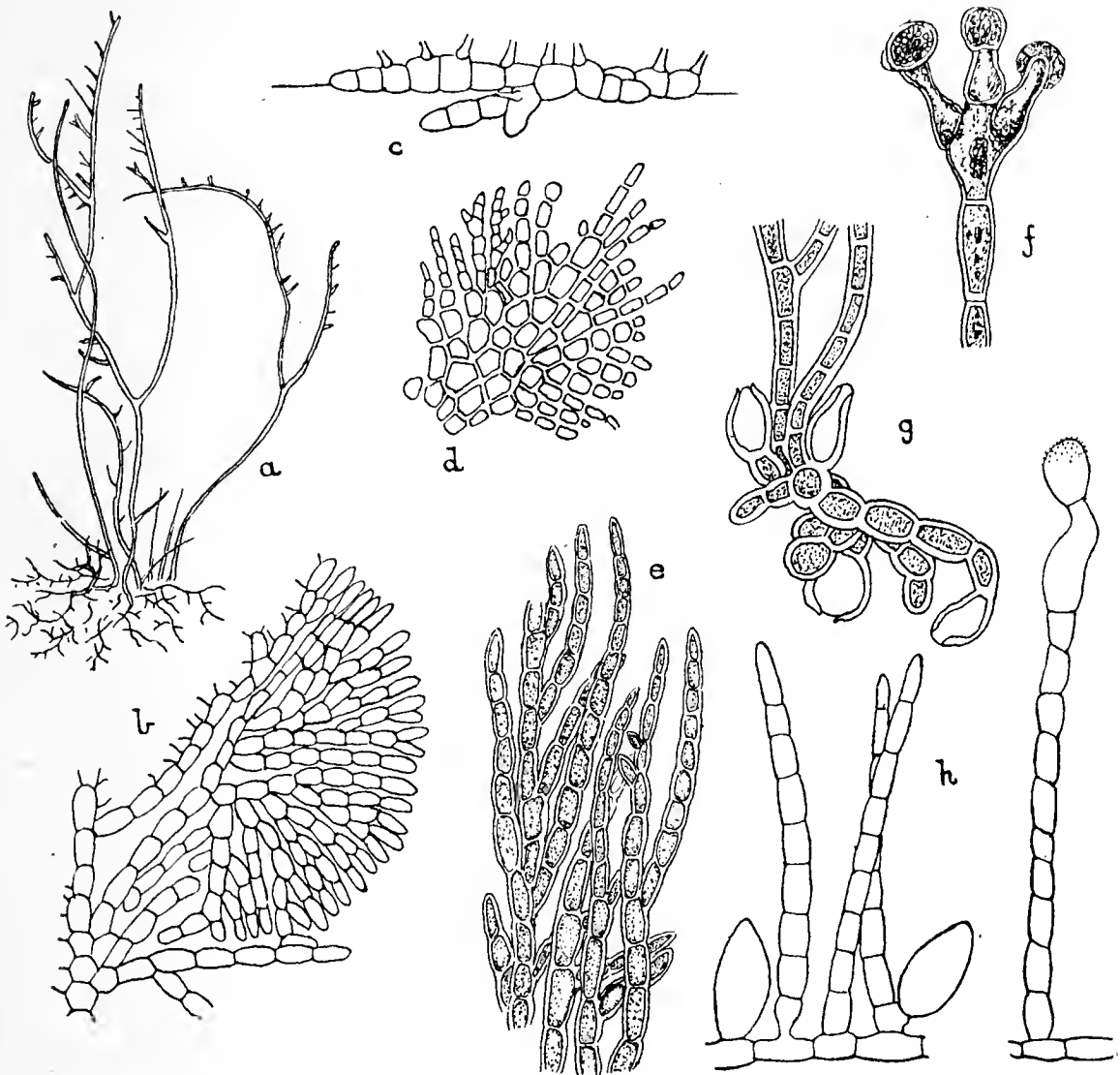

Fig. 1. *a*, *Myxonema tenue*, habit (after Huber) ; *b*. *M. farctum*, small portion of the creeping base (after Berthold) ; *e*, *M. prostratum*, a portion of the base ; *d*, *Protoderma viride* (after West) ; *c*, *Aphanochæte repens* (after West), only the bases of the hairs are shown ; *f*, *Trentepohlia umbrina*, end of an upright thread with three zoosporangia (after Oltmanns) ; *g*, *T. aurea*, base and erect system, the former with three gametangia (after Brand) ; *h*, *T*, *ellipsicarpa*, Schmidle, var. *africana*, Schmidle (after Schmidle).

The height of morphological differentiation among the Chæto-phorales, in fact among the filamentous Isokontæ generally, is attained by the genus *Draparnaldia*, a fact first brought out clearly by Berthold.[1] The elaboration in this case concerns the upright system, and, in correspondence with this, the creeping base is poorly developed ; in some cases, according to Berthold, it is com-

[1] Berthold, *loc. cit.*, p. 202 *et seq.*

pletely wanting, the upright filaments being attached merely by the basal cell, strengthened by the outgrowth of rhizoids from some of the lower cells of the upright axes. The radial upright system exhibits a sharp differentiation into long and short branches. The former consist of large, slightly barrel-shaped cells, with a small chloroplast forming an equatorial girdle in the otherwise colourless cell. The short branches arise in dense, often more or less whorled tufts, are richly branched, and consist of short cells, each occupied by a large chloroplast; the different branches terminate in more or less marked hairs. It is plain that the short lateral branch-systems are the seat of the main assimilatory activity, and the production of swarmers and other reproductive cells appears to be confined to them. The large-celled long axes are probably in the main supporting and possibly also serve for purposes of storage. The differentiation of this Alga into main axes and lateral assimilatory and reproductive appendages is most marked.

In contrast to *Draparnaldia*, with its elaborate upright system, a large number of Chætophorales exhibit more or less complete reduction of the erect portion, whilst the dorsiventral creeping base remains highly developed. In some (*e.g., Gongrosira*) there is still a considerable development of upright branches, although these are short and commonly combine with the densely branched pseudoparenchymatous base to form a convex cushion. In others, the upright system is represented only by hairs (e.g., *Aphanochæte,* Fig. 1 *c, Ochlochæte, Ectochæte*). Lastly, there are forms in which the upright portion of the thallus is completely suppressed, the whole consisting merely of the creeping base (e.g., *Protoderma,* Fig. 1 *d, Pringsheimia*). It should be pointed out that, in referring to these forms in this order, no probable line of evolution whatsoever is implied; in fact, it seems likely that there are several distinct series of reduction of the upright system in the Chætophorales. The instances are merely given as examples of varying morphological differentiation.

Among the terrestrial Trentepohliaceæ we encounter similar features. The genus *Trentepohlia* (*Chroolepus*) has a thallus differentiated into creeping base and upright threads (Fig. 1, *g*), the two varying somewhat in relative development in the different species, whilst in *Phycopeltis* and *Cephaleuros* the upright system is much reduced.

In all the more extreme prostrate forms the base shows a tendency to become pseudoparenchymatous, a feature well seen in

Pringsheimia and *Protoderma* (Fig. 1, *d*), but, in both of these cases, the origin from a system of branched threads is plainly evident, and this is also quite distinct in the discoid *Coleochætes*. There are, however, a number of Algæ which probably belong to this series (many of them not yet properly described), in which this origin from a filamentous condition is unrecognisable in the mature state.

Returning for a moment to our original statement as to what we may expect to find in the algal ancestry of the higher plants (p. 4), it will be seen that the Chætophorales satisfy all requirements, with one exception. They are a group displaying differentiation of the thallus into prostrate dorsiventral and radial upright systems ; one genus (*Draparnaldia*) shows a predominant upright system, with a distinct main axis and laterals subservient to assimilation and reproduction ; a considerable number of forms show reduction of the upright system, so that the thallus comes to consist largely or entirely of the prostrate portion. The only one of our expectations that is not fulfilled is the existence of forms, having sexual organs on the prostrate base and asexual organs on the upright threads. As regards this latter point it may first be noticed that, in the aquatic Chætophorales, as long as there is a properly developed upright system, the reproductive organs are usually confined to it ; but, with the reduction of the upright system, they are relegated to the prostrate base (cp. for instance, *Coleochæte pulviuata* with *C. scutata*).

It is, however, among the terrestrial Trentepohliaceæ that we meet with the most important indications in this connection. In the genus *Treutepohlia* the zoosporangia are pedicellate, being provided with a stalk-cell of a characteristic knee-like form (Fig. 1, *f*), whilst the gametangia are sessile (Fig. 1, *g*). The two kinds of reproductive organs are, therefore, readily distinguishable, but it should be added that it is not yet certain that these structures are definitely asexual and sexual respectively in all the species of the genus ; in several, however, this has been established beyond doubt, and it is to be presumed that it obtains in most, if not in all cases. In many species both kinds of reproductive organs are borne on the upright system, but especially in those forms in which the base is strongly developed, the gametangia tend to arise from the base (Fig. 1, *g*), whilst the sporangia are found on the upright threads [1] (Fig. 1, *f*). Very good instances are afforded by *T. diffusa*, De

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[1] Oltmanns, Morph. u. Biol. d. Algen, I, 1904, p. 252.

Wildeman,[1] and *T. ellipsicarpa*, Schmidle var. *africana*, Schmidle[2] (Fig. 1, *h*).

We can see in this phenomenon a necessary result of the terrestrial habitat. The zoosporangia are detached as a whole, dispersed by the wind, and only liberate the contained swarmers when they come to lie on some moist substratum. The gametangia, on the other hand, liberate their gametes *in situ*, and thus the most favourable position for them will be on the creeping base, in close contact with the substratum, where inundation must be a frequent phenomenon.

It should also be noted that zoosporangia and gametangia are in some cases found on distinct, though similar, individuals.[3] There are thus all the necessary indications for the gradual differentiation of two alternating generations, of which the one bears the asexual organs on the upright system, the other bears the sexual organs on the creeping base. Disappearance of the base in the former, and of the upright system in the latter (both phenomena which are known to occur among the Chætophorales) will give two different generations, resembling those of the Archegoniatæ in all essential respects (cf. also the case of *Cutleria* discussed on p. 15).

From such a group as the Chætophorales, then, we could suppose two alternating generations like those of the higher plants to have arisen. We have to picture an ancestor, with well-developed base and upright system, from which the two generations gradually diverged in the way just indicated. Such an origin, of course, amounts to an homologous one, although presumably of a somewhat different kind to that in the minds of the adherents to the homologous theory; and the writer may appear, after taking an impartial attitude at the beginning, to be caught in the delinquency of favouring this view. It does not seem to him, however, that the above mode of origin is applicable to the Bryophyta, with their completely dependent sporophyte, without forced assumptions. It is propounded for the case of the Pteridophyta, and the writer realises that he, as little as anyone else, is able to bridge the gap between them and their ancestry.

There is, however, no necessity to seek a separate algal ancestry for the Bryophyta, as the group of the Chætophorales also

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[1] Les Algues de la Flore de Buitenzorg, 1900, p. 72.

[2] Schmidle, in Engler's Bot. Jahrb., XXX., 1902, p. 63, Tab. II., Fig. 8-10.

[3] cf. J. Bonnet, in Progressus Rei Botanicæ, V., 1914, p. 101 ; Oltmanns, *oc. cit.*, p. 253.

affords indications of another possible mode of origin for the two alternating generations, an antithetic one, that seems most applicable to the case of the Bryophyta. This concerns the much-discussed *Coleochæte*. Here alone among the Isokontæ do we get any pronounced indication of an intercalated phase, due to the elaboration of the zygote, although minor instances are afforded by several other filamentous members (e.g. *Ulothrix, Oedogonium*). Comparisons between *Coleochæte* and *Riccia*, the Liverwort with the simplest type of sporophyte known, have been instituted by many Botanists, and it is unnecessary to enter into details here.

The value of *Coleochæte*, in relation to the origin of the two alternating generations in the higher plants, has been called into question by many in recent years, because the cytological features are not in accord with those obtaining in the latter. Allen[1] has shown that reduction in chromosome-number takes place on the germination of the oospore, so that the 16-32-celled plantlet arising from the latter corresponds cytologically with the ordinary sexual-organ-bearing thallus, and not with the diploid sporophyte of the higher plants. Should this, however, be taken as in any way invalidating the value of *Coleochæte* as an instance of the intercalation of a new generation in the life-cycle by the elaboration of the zygote? In the group of the Algæ everything is still in a condition of fluctuation, and we know that reduction in chromosome-number occurs at varying points in the life-cycle, sometimes on gametogenesis, sometimes after sexual fusion.[2] Thus reduction rendered necessary by the occurrence of sexuality will have taken place at different stages and will only gradually have become fixed at the stage of sporogenesis. Perhaps it was only with the establishment of the tetrad-division, so typical for the production of spores in the higher plants (as well as in *Dictyota* and many Rhodophyceæ), that reduction became located at a definite point in the life-cycle.

In considering the possible origin of the sporophyte in Bryophyta by gradual elaboration of the zygote, as we see it indicated in *Coleochæte*, it is not even necessary to ignore the essential construction of the thallus in the Chætophorales as a whole. The two main groups of the Bryophyta appear to have diverged at a

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[1] Ber. deutsch. Bot. Ges., XXIII., 1905, p, 285.

[2] Reduction takes place on gametogenesis, for instance, in Fucaceæ. To regard the thallus in this group as being a sporophyte, on this basis, is surely the height of absurdity! cf. also Farmer, in NEW PHYTOLOGIST, VIII., 1909, pp. 112-114 ; and Tansley, in NEW PHYTOLOGIST, XI., 1912, p. 216.

very early stage, the thallus of the Hepaticæ arising from the creeping base, that of the Musci from the upright system. The Moss-protonema appears as a relict of the original creeping base, bearing, as it does, the upright and radial Moss-plant as a lateral branch. There is no difficulty at all in making these assumptions with the facts given in the foregoing pages before us.

The derivation of Bryophyta and Pteridophyta from one common ancestral algal group, although their further development is presumed to have proceeded on very different lines, is quite sufficient to explain the resemblances between sexual organs, etc. in the two cases. Moreover it should be noted that these resemblances are much more pronounced in the gametophyte (whose origin is assumed to be very similar in the two groups) than in the sporophyte (whose origin is regarded as very different in the two cases).

Looked at from the point of view of a group betraying characteristics indicative of the probable origin of the higher plants, it is significant that the Chætophorales exhibit more potentialities than any other group of the Isokontæ. A brief summary will suffice to illustrate this point :—

1. Great range of morphological construction.

2. The most highly differentiated sexual organs encountered among the Isokontæ (in *Coleochæte*).

3. The most marked development of the zygote as an independent generation (in *Coleochæte*).

4. Although the bulk of the present-day forms are found in freshwater (a fact which is not insignificant when we consider that the higher plants are likely to have had an origin from freshwater forms), a certain number are marine (e.g. *Pringsheimia*).

5. One whole series, the Trentepohliaceæ, are terrestrial.

6. A number of forms have become endophytic, being either subcuticular (*Endoderma*) or intercellular.

7. There is further great diversity of habitat; thus, *Tellamia* penetrates into the shells of Molluscs, *Gomontia* into calcareous substrata, whilst *Dermatophyton* grows on the testa of freshwater tortoises. The species of *Trentepohlia* play a part in the formation of Lichens.

8. Some forms (e.g. *Cephaleuros, Acrochæte parasitica*) are parasitic.

9. A number become encrusted with carbonate of lime (e.g. *Gongrosira*).

It must be confessed that, in this series, almost every conceivable type of growth-form and habitat has been realised. It does not, therefore, appear a forced assumption to seek in a group with such unlimited potentialities those evolutionary tendencies which gave rise to the higher plants.

In discussing the alternation of generations of the Archegoniatæ, much use has been made of representatives belonging to other groups of the Algæ, in particular of the Phæophyceæ. The latter show many interesting parallels with the Isokontæ and afford very useful data in connection with the probable mode of origin of the higher plants, but in utilising the information derived from this group it is necessary to maintain the proper perspective and to realise that the Brown Algæ cannot be regarded as anything else than a side-line of evolution (cf. below). In many respects, morphological and anatomical complexity of the thallus, differentiation of the sexual organs, and development of well-marked alternation, they have gone much further than the Isokontæ, and it is just in the evidence to be derived from these more advanced characteristics that the value of the Brown Algæ lies. We may suppose that the more advanced types among the Isokontæ were lost during the vicissitudes that must necessarily have accompanied establishment on dry land, whilst the Phæophyceæ, which have remained a marine group, have preserved numerous traces of this more advanced development. It is certainly significant that the Isokontæ show but little of the morphological elaboration seen in the Brown and Red Algæ.

At this point reference may be made to Schenck's attempt to derive the Archegoniatæ and the Characeæ from a Phæophyceous ancestry.[1] Whilst in no way underestimating the value of the detailed comparisons which he makes between vegetative and reproductive organs in the Brown Algæ and the higher groups, the writer cannot go with him to the extent of actually deriving the latter from this group of Algæ. The Phæophyceæ as a whole are distinguished from the Isokontæ and the higher plants alike by different pigmentation of the chloroplasts, different products of assimilation, and especially by a very characteristic type of motile element. In particular stress may be laid upon the motile element (whether zoospore or gamete) which is characterised by its laterally attached cilia, the one pointing forwards, the other backwards, and the close relation between their position and that of the

[1] Engler's Bot. Jahrb. XLII., 1909, pp. 1-37.

chloroplast and eye-spot. Moreover the Phæophyceæ are essentially marine. If we look at Schenck's work from the point of view of parallel development, however, it is exceedingly instructive. A detailed discussion is unfortunately impossible.

Before dealing with the established cases of alternation among the Brown Algæ, it will be well to examine a little more closely the lowest series of this group, viz., the Ectocarpales. This may probably be regarded as displaying many of the more primitive characteristics of the Phæophyceæ, and in many respects affords an astonishing degree of parallel with the Chætophorales. The simple filamentous *Ectocarpus* has a thallus differentiated into the same two portions as a *Myxonema*, i.e., there is a creeping base and an upright system, the branches of the latter often terminating in hairs. There are a large number of forms (*Ascocyclus*, Fig. 2, *d*, etc.) in

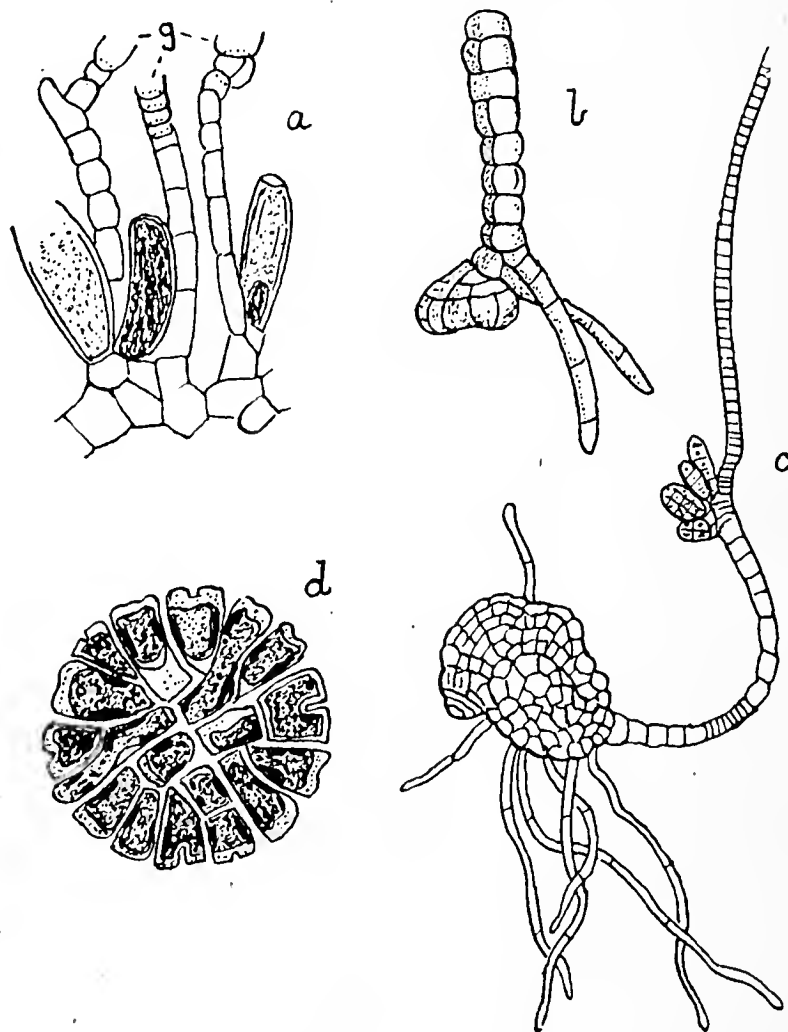
Fig. 2. *a*, *Myrionema vulgare*, showing zoosporangia and gametangia (g) (after Sauvageau) ; *b*, young *Aglaozonia*-thallus (from Oltmanns) ; *c*, abnormal *Cutleria* (from Oltmanns) ; *d*, *Ascocyclus secundus*, a young thallus (after Reinke).

which only the prostrate portion is developed, and there is the same diversity of habit as in the Chætophorales, though a terrestrial series is lacking.   In many of the forms with a well-developed base, there is a tendency for the asexual (unilocular) sporangia to appear as lateral branches from the base of the upright threads, whilst the gametangia (plurilocular sporangia) are borne near their apex ; this is well seen in *Myrionema vulgare*[1] (Fig. 2, *a*).   It will be noticed that this arrangement is just the reverse of what obtains in *Trentepohlia*.

Throughout the Phæosporeæ the thallus betrays a more or less marked differentiation into prostrate and erect portions, although this is very much obscured in some cases.   It appears, however, to be a safe assumption that this type of construction is the primitive one for the whole group.

The cases of alternation among the Brown Algæ that have been fully studied are those of *Cutleria*, *Zanardinia* and *Dictyota*, and a consideration of these will suffice.   In *Dictyota*, the two generations,[2] indubitably identical in morphological construction as they are, could in no way be supposed to have arisen from different parts of an ancestral thallus ; rather, they appear as two similar individuals, one of which has become sporogenetic, the other gametogenetic. The creeping base seems to have become completely suppressed in this case, although traces are seen in allied forms.   *Dictyota* furnishes us with an instance of *strictly* homologous alternation, and the writer would put this case in a special category and does not regard it as directly comparable to the kind of homologous alternation that is supposed to have arisen in the line of evolution of the Pteridophyta.   *Zanardinia*[3] seems to display the same kind of alternation as *Dictyota*, although here, possibly, it is the creeping system that has persisted and that has furnished the two homologous individuals.   The two cases just cited are probably instances of the numerous modifications in the method of alternation which we may expect to find in so plastic and primitive a group as that of the Algæ.

The case of *Cutleria* is different.   Here, apparently, we have a good instance of antithetic alternation, but appearances are deceptive.   The life-cycle, whose cytology has been investigated by

[1] cf. Oltmanns, *loc. cit.*, p. 383, fig. 235 ; Sauvageau in Ann. sci. nat., Bot., 8 ser., V, 1898.

[2] cf. especially Lloyd Williams, in Annals of Botany, XVIII, 1904, pp. 141, 183 ; Hoyt, in Bot. Gaz., L, 1910, p. 55.

[3] cf. Yamanouchi, in Bot. Gaz., LVI, 1913, pp. 1—35.

Yamanouchi,[1] with the result that the features of reduction correspond closely with those obtaining among the higher plants, includes the erect sexual *Cutleria*-plant and the prostrate asexual *Aglaozonia*-plant, normally following one another in definite alternation. Parthenogenesis is a rather frequent phenomenon, the parthenospores, however, giving rise to more or less distinct *Aglaozonia*-stages. But there are numerous other important abnormalities. Thus, the zoospores, whilst often growing into typical *Cutleria*-thalli, occasionally[2] afford dwarfed thalli or even only simple, or more rarely branched, threads; these abnormal individuals (Fig. 2, *c*) sometimes produce gametangia on the erect system, but almost invariably grow out into *Aglaozonia*-like discs at their base. Oltmanns[3] interprets such stages as instances of a secondary formation of *Aglaozonias* by budding from the *Cutleria*-plant, but the writer would like to place a totally different interpretation upon them.

It seems likely that the ancestor of these forms had a thallus differentiated into the usual prostrate base and erect portion (cf. p. 14), in fact in the normal *Aglaozonia*-stage this is quite apparent. the base being represented by the *Aglaozonia*-disc and the upright system by the small erect column which invariably arises in the germination of the *Aglaozonia*-plant (Fig. 2, *b*). Young *Cutleria*-plants also seem to display some indications of a creeping basal system. It may be suggested, therefore, that the ancestor of *Cutleria* had a thallus bearing asexual organs near the base and sexual organs on the upright system, as we find it in *Myrionema*, etc., among the present day Ectocarpales (p. 14). With the development of these organs at different periods, they became segregated on distinct individuals, the asexual ones practically losing the upright system and the sexual ones the prostrate portion. The erect column of the *Aglaozonia*-stage would thus be a relict of the original erect system, whilst the abnormal stages above described would merely be a return to the ancestral condition. Sauvageau[4] has described stages which appear as transitions between young *Cutleria*-plants and the column of the *Aglaozonia*-stage, and these are quite in accord with the above hypothesis. All the evidence seems to point to the conclusion that the two generations of *Cutleria*

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[1] cf. Yamanouchi, in Bot. Gaz., XLVIII, 1909, pp. 380—387.

[2] Church, " Polymorphy of *Cutleria multifida*," Ann. of Bot., XII, 1898, p.75; Kuckuck, in Wiss. Meeresunters. Abt. Heligoland, III, 1900, pp. 61—79; cf. also Oltmanns, *loc. cit.*, p. 403.

[3] *loc. cit.*, p. 403.

[4] Ann. sci. nat., bot., 8 sér., X, 1899, p. 265.

are merely diverging developments from a common ancestral sporogenetic and gametogenetic thallus.

Such a view is, moreover, quite in accord with the hypothesis put forward above in connection with the origin of the two generations of the Pteridophyta from a Chætophoraceous ancestry, and we have here a good illustration of how diverse the two diverging generations may soon become. It is interesting to note that the morphological origin of the two generations in *Cutleria* is just the reverse of that postulated in the Chætophorales, the sporophyte being prostrate, the gametophyte erect, a variation only too likely to occur in groups where alternation was in the making.

Finally, we may briefly consider the alternation that has been observed in the Red Algæ. In this group also, differentiation of the thallus into a creeping basal portion and an upright system is frequently observed, especially in the simpler forms (e.g., *Batracho-spermum, Chantransia*), but in the vast majority it is the upright system that has been elaborated, with more or less complete elimination of the basal portion. Two types of alternation are known in the Red Algæ, *viz.*, the type characteristic of *Nemalion* and presumably of many of its immediate allies, and the complex alternation typical of the more advanced forms.

The case of *Nemalion* is very similar to that of *Coleochæte*, the sporophyte (as constituted by the sporogenous threads forming carpospores) appearing as an intercalated stage. Wolfe[1] came to the conclusion that reduction occurred at the end of this intercalated phase, *viz.*, on the formation of the carpospores. Svedelius[2] has, however, recently investigated *Scinaia furcellata*, another member of the Nemalionales, and finds that the oospore undergoes reduction-division at the inception of germination, three of the four nuclei produced aborting, whilst the remaining one is used in the formation of the sporogenous threads. It seems probable that this is the usual course of events among the Nemalionales, which therefore, as regards their life-cycle, are in almost complete agreement with *Coleochæte*. Presumably matters are the same in *Batrachospermum*; here, however, the carpospores, on germination, give rise to the well-known *Chantransia*-stage, that is a mainly prostrate system of creeping threads, from which the *Batrachospermum*-plant arises as a side-branch. This sequence is very similar to that occuring in Mosses, and here we have the state of affairs postulated on p. 12.

[1] Annals of Botany, XVIII, 1904, p. 608.

[2] Nov. Act. Reg. Soc. Scient. Upsala, sér. IV, Vol. IV, No. 4, 1915.

In the case of the more advanced Floridæ, several of which have been fully studied,[1] we have the most complicated instances of alternation known among the Algæ. We may take *Polysiphonia* as a type. Here we have first the production of a phase, which arises by the elaboration of the zygote and is constituted by the cystocarp. The carpospores of the latter give rise to a *Polysiphonia*, identical in all respects with the thallus bearing the sexual organs, except that it is diploid and produces only tetraspores. The latter again give rise to the sexual *Polysiphonia*, reduction having taken place during the tetrad-division in the tetrasporangium.[2] The numerous complications occurring in many forms and disclosed by the work of Oltmanns and others can be neglected here, where we are only concerned with the main facts.

In the life cycle of these Floridæ we therefore have two spore-bearing generations, *viz.*, (a) the cystocarp, parasitic on the gametophyte and propagating by carpospores ; and (b) the ordinary *Polysiphonia*, independent and propagating by tetraspores. That reduction, which can only take place once in such a life-history, occurs on the second spore-producing generation is probably associated with the acquisition of a definite tetrad-stage in spore-formation (p. 11). The writer would regard these advanced Floridæ as exhibiting both an antithetic spore-producing generation and one which is strictly homologous.[3] If, as he has attempted to show, both antithetic and homologous alternation can occur among the Algæ, it is quite plausible that the two phenomena should be combined in the same life-cycle.[4]

Amongst all the cases of alternation among Algæ that have been considered, that of *Cutleria* certainly stands out as the most illuminating in connection with the main theory here propounded. Though the result in this case is just the opposite, *viz.*, a prostrate sporophyte and an erect gametophyte, it fully illustrates the course of events here suggested.

In conclusion, some of the main features may be briefly summarised as follows :—

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[1] cf. Yamanouchi, in Bot. Gaz. XLI, 1906, p. 425 and XLII, 1907, p. 401 ; Lewis, in Ann. of Bot., XXIII, 1909, p. 639 ; Svedelius, in Svensk. Bot. Sidsskrift V, 1911 ; VI, 1912 ; VIII, 1914 ; also Ber. deutsch. bot. Ges. XXXII, 1914.

[2] cf. Yamanouchi, *loc. cit.*

[3] The strict homology of the sexual and tetraspore-bearing generations is shown by the occurrence of abortive tetrasporangia on sexual plants and of abortive carpogonia on tetraspore-bearing plants ; for details, see Svedelius and Lewis, *loc. cit.*

[4] cf. also Tansley, in New Phytologist, XI, 1912, pp. 213—216.

(1) In all the more advanced groups of the Algæ, the thallus exhibits frequent differentiation into a creeping base and an upright system.

(2) Among the Chætophorales, which plainly show such a differentiation of the thallus, there are evidences of unlimited potentialities; we also find in this group (a) a whole series of terrestrial forms, (b) the only member of the Isokontæ with a distinct main axis bearing laterals which carry on assimilation and reproduction and (c) in the species of *Trentepohlia*, forms showing relegation of the sexual organs to the base and of asexual organs to the upright system.

(3) The available evidence is regarded as pointing to the Isokontæ for the ancestry of the higher plants, and for the reasons mentioned under (2) this ancestry is thought to lie among forms resembling the Chætophorales.

(4) The Pteridophyta are supposed to have arisen from such forms by the gradual divergence of two generations, the sexual derived from the creeping base, the asexual from the upright system. The Bryophyta are supposed to have arisen from forms resembling *Coleochæte* by gradual elaboration of the zygote.

(5) *Cutleria*, in a side-line of evolution, fully illustrates the way in which two generations can be derived from the type of thallus common in all the main groups of the Algæ, after the manner postulated for the case of the Pteridophyta.

(6) The cases of alternation among the Algæ may be distinguished as follows :—

(a) The two generations arise from different parts of the ancestral sporo- and gametogenetic thallus (*pseudo-homologous alternation*) :—

> (1) The gametophyte is prostrate, the sporophyte erect (Pteridophyta, possible cases to be found among the Chætophorales).
>
> (2) The gametophyte is erect, the sporophyte prostrate (*Cutleria*).

(b) The two generations arise from the same part of the ancestral thallus, the other portion aborting (*strictly homologous alternation*) :—

> (1) The two generations arise from the upright system (*Dictyota*).
>
> (2) The two generations arise from the prostrate system (*Zanardinia ?*).

(c) The sporophyte arises as an intercalated stage in the life-history, due to the elaboration of the zygote (*antithetic alternation*):—

> (1) The gametophyte arises from the prostrate system (*Coleochæte*, Hepaticæ).
>
> (2) The gametophyte arises from the erect system (Nemalionales).
>
> (3) The gametophyte retains both the prostrate and erect systems (*Batrachospermum*, Musci).

(d) There are two spore-producing generations, the one an intercalated phase produced from the zygote, the other strictly homologous (*Polysiphonia*, *Griffithsia* and other advanced Florideæ).

East London College,
November 28th, 1916.

THE ORIGIN OF SPHAGNUM ATOLLS.

By Harvey Stallard.
(*University of Minnesota*).

IN connection with the study of the development of the climax vegetation of Minnesota, opportunity has been afforded the writer to investigate the sphagnum atolls of the state and particularly the Anderson and Ballard atolls of Crow Wing County which MacMillan (1) described in 1894. A sphagnum atoll is any bog surrounded by a trench of stagnant water. In the centre of the bog the remains of the original pond may be present in which case the name atoll is quite applicable. Such bogs are to be found in northern and central Minnesota and are said to occur in Wisconsin, Michigan, and New York.

MacMillan (1) endeavoured to explain their development and suggested probable causes of their formation. He attributed their development primarily to the rise and fall of the water level of the original pond, and summarises the process as follows: "The origin of the sphagnum atolls in the cases studied may be ascribed to a season of gradual recession of the waters of the pond, followed by a season of comparatively rapid increase in area and level. The atolls first appear as annular floating bogs separated from the shoreward turf as a result of the original zonal distribution of littoral plants and the rise of the waters together with the favourable concurrence of a group of special and necessary conditions. Some of the apparent conditions of the atoll-formation are (*a*) a definite maximum size and depth of the parent pond; (*b*) considerable

height and regularity of the banks of the parent pond; (c) a regular and gentle slope of the pond bottom from shore to centre; (d) a definite original character of littoral vegetation when the pond was at low level; (e) a reduction within minimum limits of the lateral pressure and tension of winter ice; (f) a comparatively prompt anchoring of the atoll upon the bottom."

Atkinson (2) states that the shade of overhanging trees of the banks is probably responsible for the marginal trench. Shaw (3) describes some curious ponds near Woods Hole which are not to be understood as sphagnum atolls and in all probability their development is due to the factors that he mentions.

From the description of MacMillan, the Ballard atoll in 1894 was surrounded by a narrow zone of *Carex* with such subdominants as *Juncus, Iris, Utricularia,* and *Potentilla.* The atoll was covered with *Chamædaphne, Andromeda, Oxycoccus, Kalmia* and characteristic herbs of this associes, such as *Sarracenia, Eriophorum,* and *Menyanthes.* This bog is at present a *Carex-Calamagrostis* meadow in which a few small spruce and tamarack trees remain on sphagnum hummocks near the north end. The outer zone of the Anderson atoll, as MacMillan reported it, consisted of " a luxuriant growth of *Panicularia fluitans* (Linn.) OK. mingled with the following in less abundance: *Typha, Potamogeton, Sagittaria, Phragmites, Polygonum,* and *Utricularia.* The vegetation of the atoll itself, except for the presence of the same three species of *Sphagnum* and a very abundant growth of *Limodorum,* differed entirely from that of Ballard's atoll. The most conspicuous plant was *Picea mariana* (Mill.) B.S.P. Twenty-seven young trees of this species—the black spruce—had established themselves upon the atoll. The largest was but four and one-half feet in height, while the smallest noted was not over eight inches. These trees, evenly distributed, occupied the middle of the ribbon of sphagnum and presented a most attractive and unusual appearance, forming as they did an almost perfect ring about the open, placid and central lagoon. Next in importance, as giving character to the atoll, was a dense growth of *Ledum latifolium* Ait.—Labrador tea—which covered almost the entire island. " The lagoon of this atoll, unlike that of the other, was somewhat invaded with floating vegetation— mostly *Utricularia intermedia* Hayne, with a few plants of *Panicularia.*"

The Anderson bog is to-day a wet meadow with a few sphagnum hummocks here and there, which vary in size from a few

square yards up to a few square rods. In the spring these bogs are covered with water varying in depth from eighteen inches to four or five feet. Later in the summer on account of evaporation and drainage the water level falls to such an extent that the surface of the bog becomes firm. From such evidences as fire scars on the stumps around the edge of the bogs and on some of the remaining hummocks, it became apparent that fires had disturbed the vegetation and had initiated secondary succession which in the main has reached the *Carex-Calamagrostis* associes.

Numerous other bogs in the vicinity of Hubert were visited and among these was found north of Hubert Lake in the centre of the N.W. ¼ of section 24 an atoll whose outer trench has been formed recently. The bog differs from the two mentioned above in that it has no outlet. In the spring, when the water-level is high, the centre is occupied by a small pond varying in depth from eighteen inches to three feet. The outer trench of water varies in width from a few feet to one or two rods, and in depth from one to three feet. On the south-west side the trench has not been formed, and here the *Sphagnum* with the heaths join directly with the vegetation of the bank. The vegetation of the central pond is composed largely of *Carices*, *Nymphæa*, and other plants character-istic of the *Carex-Nymphæa* associes. On the sphagnum areas are to be found the usual species of *Vaccinium*, *Chamædaphne*, *Ledum*, *Andromeda*, *Eriophorum*, *Comarum* and *Menyanthes*. The vegeta-tion in the outer trench consists of species of *Carex*, *Calamagrostis* and the subdominants which are generally found in the *Carex-Calamagrostis* associes. The banks are sparsely covered by jack pines, underneath which is a mat of *Arctostaphylos* and *Vaccinium*.

The total area of the bog is about twenty-five acres, its average width and length being respectively about fifty rods by eighty. The banks of the north and west sides rise gradually to perhaps twenty or thirty feet. On the east they rise not higher than ten or fifteen feet. By means of the peat-sampler the depth of the centre was found to be about twenty-two feet.

A third very characteristic atoll is located near the Wild Flower Garden in Glenwood Park of Minneapolis. This bog is narrow and long. It is surrounded on three sides by high banks which rise rapidly and reach a maximum height of perhaps fifty feet. The north bank is lower but does not afford an outlet. The vegetation of the banks consists of a bur oak and red oak grove with a mat of blue grass beneath. The soil is a sandy morainic

till. A very narrow trench varying in depth from a few inches to four feet completely surrounds the bog. Inside the trench where *Sphagnum* has been killed, *Typha* and sedges have become established. The trench in the shallower places is being invaded by *Typha*, species of *Carex*, *Sagittaria*, and *Alisma*. The maximum depth found in the peat is about eight feet, and there is no remnant of the parent pond in the centre.

The fact that the change of vegetation of the Ballard and Anderson atolls was evidently due to burning, suggested that perhaps the cause of the atoll's outer zone of water was due to fire. In pursuit of this study evidences of fire were searched for in the surface layer of all the atolls and in the Anderson and Glenwood atolls at various depths beneath the surface. The character of the peat beneath the surface was explored by studying the samples taken with a peat-sampler at various intervals in a line across the bog. In all the atolls studied fire scars were found in or near the trench on stumps and fallen logs. In samples taken from the Anderson bog charcoal was found at two, three, four, five, and six feet below the surface, which indicates that fires occurred at those levels. By digging with a spade in the trench of the Glenwood atoll quantities of charcoal were found, and upon inquiry of the park attendants it was learned that the bog had been burned several times. The burning of the Hubert atoll was so superficial around the inner edge of the trench and so recent that surface fire scars indicated clearly the course and extent of the fire.

After finding that the marginal zone of water of the atolls just mentioned is beyond any doubt due to deep burning during dry seasons, the question arose as to whether it is ever formed as the result of other causes, such as the method suggested by MacMillan, or by shade, or by such processes as the inwashing of toxic substances from the banks.

In considering the conditions assumed by MacMillan, it is true that the water-level does rise and fall, but the vegetation of the shore is so firmly anchored by roots and rhizomes that only on windward shores of moderately large lakes is it ever detached. Furthermore, if the turf did float upward with the rise of water, which in small ponds perhaps never happens, the slight wave and wind action would inevitably set the débris further shoreward and not toward the centre. In the Anderson and Ballard atolls an excessive rise of water-level can only be temporary since both drain rapidly by direct outlets. All the conditions prescribed in the hypothesis

are not attained in any of the bogs studied. Variations in the size, shape, depth, and situation of the atolls indicate that their formation, is independent of such factors.

The primary development of a bog is not as one would infer from the hypothesis set forth by MacMillan, and before entering upon a discussion of the effects of shade and toxic substances it will be necessary to state briefly the development of the bog. This development has been sketched in the " Development of Climax Formations of Northern Minnesota " (4), and it seems sufficient to mention here those stages of the primary hydrarch succession of which the bog is a part. The following associes are usually representative stages of the bog's development:—

1. *Chara-Philotria* Associes.
2. *Castalia-Nymphæa* Associes.
3. *Scirpus-Zizania* Associes.
4. *Carex* Associes.
5. *Andromeda-Chamædaphne* Associes.
6. *Larix-Picea* Associes.

Sphagnum enters with or after *Carex* and along with *Andromeda* and *Chamædaphne*. Just how much the development of the bog depends upon the *Sphagnum* which grows in the water preceeding *Carex*, has not been determined. In most cases it is the *Sphagnum* which follows the *Carex* that plays the most important role in filling up the pond.

The succession begins naturally with the shore of the pond, and its centripetal advance if not disturbed presents a zonation corresponding to the stages of succession as long as there is a remnant of the pond. In undisturbed areas there is no zone of water near the margin of the parent pond. The advance of the shade-casting trees is never so rapid that it halts the succession by killing out *Sphagnum*. The latter must develop to prepare the way for the trees, and the growth of the tamarack and spruce is so slow that several inches of peat may be deposited before there is sufficient shade to destroy the *Sphagnum*. The toxic substance supposed to interfere with the development of the outer part of the bog is calcium carbonate. The presence of calcium carbonate however does not seem to interfere with the normal sequence of the associes given below. Lakes and bogs containing large deposits of calcareous marl present the same prisere as those devoid of lime. *Sphagnum*, which lime salts affect, enters the succession after the *Scirpus-Zizania* and the *Carex* associes have deposited a

layer which is impervious to the upward diffusion of the calcium carbonate. Hence from the nature of the succession it seems unlikely that shade or lime can ever be responsible for the trench of an atoll.

From the observations on occurrence of atolls it has been noted that they are numerous in sand areas. In fact none have been reported or observed in clay areas. The soil of Crow Wing County near Gull Lake is notably deficient in lime, and in such an area it is impossible to assume that lime in the run-off water of the bank has any effect on the outer zone of a bog. The fact that the *Sphagnum* and heaths of the Hubert atoll join with the vegetation of the bank where the fire was absent is proof that there is nothing in the run-off water of the bank to interfere with the growth of these plants.

The fact that fire is the cause of the marginal trench has not been apparent because the bog assumes its atoll character only when the water-level is high enough to obscure or conceal the fire scars. Moreover very dry seasons are rare where bogs are formed, especially in clay soil areas. Since the water-content of bogs is always high it seems almost incredible that they should burn. Fire in an undrained bog usually burns very slowly, and the heat dries out the surrounding medium which is then consumed. If the burning, starting from the edge of the bog, is left undisturbed it consumes the organic matter as far down as the bottom of the parent pond. Apparently the centripetal progress of the fire is checked when it reaches the peat that directly overlies the water. When the water rises again to the normal level the trench appears and the bog seems mysteriously isolated from the bank.

The nature of the succession in the trench depends on the depth of the water. It is possible for the depth to be sufficient to initiate a prisere. However, in the bogs studied the succession of the trenches is typically secondary. The Ballard and Anderson atolls are now for the most part in the *Carex-Calamagrostis* associes which is being slowly followed by *Sphagnum* and *Andromeda-Chamædaphne* of the prisere. In the deeper places of the ditch of the Glenwood atoll the succession is beginning with *Typha* which will probably be followed by a *Carex-Calamagrostis* associes, and this by *Andromeda-Chamædaphne*. The outer margin of the Hubert atoll is in the *Carex-Calamagrostis* associes, which in the shallower places is already being invaded by representatives of the *Andromeda-Chamædaphne* associes.

From the description and photograph of the bog described by Atkinson (2) the zonation is such as to indicate that a secondary succession has been initiated in the outer edge of the bog. The initial cause is undoubtedly fire, since this is the only agency that can remove the peat. The succession in the bog seems essentially like that of Minnesota.

In concluding it may be stated that a sphagnum atoll is a bog whose outer edge has been partially destroyed. The only agency capable of both denuding and destroying the edge is fire. Fire occurs during or at the end of a prolonged period of drought when the water-level is low. The surrounding soil has no direct influence on the atoll formation, but since desiccation occurs more frequently and to a greater degree in sandy areas than in clay, atolls are more frequent in the former because fires are more likely to occur there.

Grateful acknowledgment is due to Professor H. F. Bergman, who assisted in securing the samples of the atoll studied and who discovered and pointed out specifically to the writer the nature and location of the Hubert and Glenwood atolls.

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THE BRITISH ASSOCIATION AT NEWCASTLE.

THE third "War" Meeting of the British Association was held at Newcastle, September 5-9. The botanical section met both morning and afternoon on Wednesday, Thursday and Friday, and, true to tradition, proceeded to Alnmouth for a field expedition on Saturday.

One of the most important papers presented was that by Dr. Kidston and Professor Lang on a new fossil, *Rhynia Gwynne Vaughani*, from the sandstone horizon opened up by Dr. Mackie. This is fully described in the Transactions of the Royal Society of Edinburgh, and it is therefore sufficient to call attention to the special interest attaching to this curiously simple, much-branched Pteridophyte from so ancient an horizon.

Miss Prankerd gave two papers on special aspects of her extensive statolith investigations. The first entitled "On the movements executed by young fern fronds," with special reference to geotropism, established the possession by ferns of statenchyma and the correlation between its presence or absence and the possession or loss of geotropic irritability. The second gave an account of the distribution in time and space of statolith starch in the branches of trees.

Dr. Willis made a contribution bearing on his well-known hypothesis of correlation between age and distribution of species, and Mr. Small presented an account of the distribution of the Composites.

Professor Bower gave a very full exposition on Leaf Architecture, tracing the evolution of the vascular system of leaves throughout the Ferns.

It is not the custom for the Presidential Address to bear any title but, had it been otherwise, Dr. Rendle's discourse might well have been termed Botany in the service of Man, Botany and its Applications, etc., and indeed, the President summed up the scope and intention of his address in the concluding paragraph, " Botany is the *alma mater* of the applied sciences of agriculture, horticulture, forestry, etc., but the *alma mater* who is to receive the due affection and respect of her offspring must realise and live up to her responsibilities."

He dwelt upon the desirability, nay the necessity, for co-operation between the scientific worker and the practical man, which should differ radically from the well-meant efforts in the London County Council lecture halls of thirty years ago, when a young man, fresh from the University, shouted ineffectually across the inevitable gulf which separated his view point from that of the practical man in his audience. It should be a natural thing for the students of the schools to work also at the applied institutions of Horticulture, Agriculture, etc., and as an introduction to this interchange it might be well if advanced students of Botany spent their long vacations in a nursery or other practical centre.

Workers thus trained in the science and art of their subject would find the fields ripe for the harvest of their discoveries in connection with plant disease, plant breeding, medicinal plant rearing, utilization of waste land, etc.

Dr. Rendle spoke also of the desirability of closer cooperation between investigators themselves, to obviate wasteful overlapping and hurried publication. In particular he dwelt upon the necessity for the development of the wealth of material of economic value, contained in our tropical possessions. " If we are to make the best use of our resourees, botanical research stations in different parts of the empire, adequately equipped and under the charge of a capable trained botanist, are a prime necessity. We seem to have been singularly unfortunate, not to say stupid, in the management of some of our tropical stations and botanical establishments."

Some of the practical problems referred to in the presidential address formed the bases of organized discussions and papers during the course of the meeting. As the result of the contributions

on Plant Disease, a committee was appointed to consider the best means of promoting the advance of Phytopathology, and following the discussion on the relation between the researcher in Genetics and the practical breeder and horticulturist a committee was formed representing Botany, Zoology, and Agriculture. Miss Saunders had suggested in her report that the trades concerned should be encouraged to establish research departments, while research would be promoted by the formation of a Genetics Association. These suggestions were warmly supported by Mr. Bateson who, however, negatived the idea of a new publication at present.

The discussion on Plant Disease was opened by Professor Potter, who laid stress on the enormous annual loss entailed by the depredations of disease, that of Rust alone having involved in Australia a loss of 2½ millions sterling on one year's wheat crop. In this paper and in the subsequent ones by Mr. Brierley and by Mr. Ramsbottom the need for the establishment of a Central Institute was elaborated, as well as the urgency of providing better training for the investigator. Mr. Salmon and Dr. Eyre testified to the willingness of the farmer to apply well tested scientific results.

One of the most important series of papers was that on Utilization of Waste Lands, introduced by Professor Oliver. He showed that in many cases two courses are open (*a*), to encourage and develop the natural product, as for instance, maritime grasses, utilizable for paper-making; or, (*b*) to reclaim and convert to normal fertile soil. The labour required for this might well give particularly suitable provisional employment to soldiers at the end of the war. Thus sand dunes might add profitably to our timber area, and Mr. Martineau, of the Reafforesting Association showed that even pit mounds may be successfully planted.

Dr. W. G. Smith gave an excellent account of the difficulties attendant upon treatment of mountain and heath land, but suggested that more frequent burning and inclusion of a greater number of cattle in the grazing would increase their productiveness. The encouraging results obtained by treatment of moorland with bacterized peat were reported by Professor Bottomley.

Sir John S. Stirling Maxwell delivered an interesting paper on Reafforestation after the War, maintaining that the British Empire as a whole should aim at being self-supporting in the matter of timber.

Dr. M. C. Stopes, in a paper on The Botanical Aspects of Coal, urged the importance of the cooperation of the Palæobotanist, Chemist, and Ecologist, and referred to the probable association of spore-content with certain chemical properties of particular coals. It was essential not to confine palæobotanical investigation to the Carboniferous epoch in view of the Tertiary origin of Indian coal.

An industrial problem of immediate import was handled in the discussion on the Collection and Cultivation of Medicinal Plants. Professor Greenish, of the Pharmaceutical Society, gave an account of the present position of the English market and showed that serious steps were being taken greatly to increase home production. Sir Sidney Olivier, the Secretary of the Board of Agriculture and Fisheries emphasized the importance of establishing the industry on sound commercial lines if it were to meet with success.

E. N. T.