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hardly possible at this date to give an accurate list of all the shrubs and evergreens which have either proved hardy or unable to resist the severity of such a winter as that of last year; but it is to be hoped that the list already given of such specimens as the frost appears to have seriously affected, will not require to be augmented by further experience; while, on the other hand, we trust that with a mild and genial spring, and an absence of easterly frosty winds, many of those species already reported as in a state of dubious vitality may yet recover.

It should have been stated that the lowest temperature reached during last winter was on New Year's Day morning, January 1, 1875, when it registered here 4° below zero at 4 feet from the ground. On the ground, 8° below zero was the minimum point reached.

III. *On the Absorption Spectra of Chlorophylline Chromules.*

By N. PRINGSHEIM. Communicated by J. F. DUTHIE, B.A.*

From a somewhat extensive series of experiments on Chlorophyll, the author has here collected some results obtained from the optical examination of those yellow chromules,† which, besides chlorophyll, or as representing it, are generally diffused throughout the leaves and flowers of plants.

The phenomena of absorption and fluorescence peculiar to these chromules are imperfectly known. Their spectra, however, show a very noteworthy agreement with that of chlorophyll. This agreement, hitherto overlooked, reveals the close affinity of these chromules to chlorophyll, and leads to rather important physiological inferences, which being only indicated here will be further carried out and established in future communications.

The author divides his subject into the following heads:—I. The yellow chromule of etiolated plants. II. The

* This paper, by permission of the Council of the Society, appeared in the "Journal of Botany" for April 1875.

† The term "chromule" is used throughout in the general sense of colouring matter.

chromule of yellow blossoms, with appended remarks on the spectrum of the yellow chromule of autumn leaves. III. The yellow ingredient in the green leaf (chlorophyll).

The following is the substance of these investigations:—

I. *The yellow chromule of etiolated plants.*

Plants grown in the dark, more particularly Angiosperms, do not become green, and so it has been generally supposed that chlorophyll is developed under the influence of light. If, however, plants develop for some time in the dark, they do not appear colourless, but intensely yellow.

The relations between this yellow chromule and chlorophyll are not yet known; and although it may seem natural to suppose, when etiolated plants become green on exposure to the light that it is this yellow chromule which changes to chlorophyll, still no facts have yet been brought forward to warrant such a conclusion.

The optical characteristics of chlorophyll, its monochromatic fluorescence, and very peculiar spectrum of absorption easily distinguish it from other chromules. There exist however, several modifications of chlorophyll. I wish now to confine myself to those properties of the chlorophyll-spectrum which are directly involved in a comparison with the other spectra to be discussed. I refer particularly to the seven known dark bands in the chlorophyll spectrum corresponding to the regions of greater absorption; and as usual I count them as they appear in an alcoholic solution in the direction from red to violet.

It is important to notice the relative amount of absorption in the darkened regions of the several forms of chlorophyll as indicated by the order in which the bands of absorption become visible according as the amount of chlorophyll contained in the layer increases.

The spectrum given by solutions of moderate concentration of chromule shows bands I., II., III., IV., or in some cases I., II., IV., in the first or less refrangible half of the spectrum; in the second and more refrangible half a continuous absorption takes place consisting of bands V., VI. VII., joined, beginning either at F (Frauenhofer), or at varying distances before F, according to the richness of

the layer. Layers containing extremely little chromule show only a trace of band I. at first, which becomes more sharply defined on increasing the chlorophyll matter; and with a further increase the bands of the second half—V., VI., VII.—appear; after which II. and IV., and finally III. become visible.

Various conditions may interfere with the above order of appearance of these bands, such as—slight chemical changes in the chromule; the influence of more than one solvent; the varying intensity of transmitted light, &c. Nevertheless it may be taken as a general rule that bands V., VI., VII., in the blue, also bands II. and IV., observe with regard to I. and III. the order of succession as stated above; and we may consider the invariable predominance of band I. as the characteristic mark of all *green* chlorophyll. It is also the most persistent band of those of the first half.

It has always been supposed that the 7-banded spectrum here described belonged exclusively to the green chlorophyll of leaves, and could thus be distinguished from all other chromules, including that of etiolated plants, which latter was believed to have absorptions only in the blue region. Both Askenasy and Kraus held this opinion. A closer examination shows, however, that the chromule of etiolated leaves, which the author proposes to call Etiolin, gives a spectrum nearly identical with that of chlorophyll, only differing as to the *amount* of absorption in the places corresponding to the chlorophyll bands. This correspondence between etiolin and chlorophyll may be observed by using a more concentrated solution of the former.

The etiolin solutions were obtained from various plants which had germinated in the dark, until the cotyledons had appeared and developed. Even with moderately thick layers, generally before the three bands in the blue have united into a continuous absorption band, the chlorophyll band I. is seen in its right place, very distinctly defined. Further, when the illuminated layers increase in thickness, bands II. and IV. gradually show themselves, and when sufficiently concentrated, band III. also appears.

It is an important fact that this spectrum of etiolin possesses all the seven bands of the chlorophyll spectrum.

and that bands I., II., III., IV. lie exactly in the same places as in the case of chlorophyll; whereas bands V., VI., VII., appear somewhat displaced and nearer to the red. A further difference is, that if the solution be thick enough, band II. invariably appears split. These differences, however, are of minor significance, seeing that similar variations may be produced in any chlorophyll solution under certain definite conditions. We are thus justified in considering etiolin merely as a slight modification of chlorophyll produced under different physiological conditions. Etiolin also shows the same red monochromatic fluorescence peculiar to chlorophyll.

It might, perhaps, be assumed that in etiolated plants there exist two substances, viz., a yellow one without absorption in the first half of the spectrum being present in larger quantity, and the normal green chlorophyll in a less degree, according to which view chlorophyll would be produced without the agency of light. There are no facts to support this theory, however, and it seems much more probable that etiolin is an unmixed substance, being a yellow modification of chlorophyll. For we have never been able to separate etiolin into green and yellow components, as in the case of chlorophyll.

According to this view, therefore, concerning the relation between etiolin and chlorophyll, the proposition respecting the influence of light requires qualifying, by saying, that while the formation of chlorophyll in general is independent of light, it is a yellow form of chlorophyll that is produced in the dark, but a green modification in the light.

II. *The chromule of yellow blossoms.*

The changes in colour which the parts of yellow blossoms and fruits undergo during their development, point to a close connection with the chromule of chlorophyll.

Its optical properties differ only in having weaker absorptions in the first part of the spectrum. Kraus seems to have been the only one who has subjected the chromules of yellow blossoms to spectrum analysis. From not using layers sufficiently rich in chromule he failed to detect the

chlorophyll bands of the first half of the spectrum, just as he had done in the case of etiolin.

This weakening of some of the chlorophyll characteristics varies in different flowers. The strength of the bands, however, seems to remain constant in plants of the same Natural Order. In some of the examples which showed the weakest absorptive power, such as a kind of yellow rose, *Carthamus tinctorius*, and various yellow Dahlias, even with concentrated layers 370mm. in thickness, there was scarcely a trace of band I. to be seen. The yellow chromules of blossoms accordingly form a continuous series of gradations of chlorophyll. Even those which differ most betray their genetic connection by presenting similar absorption bands in the blue.

In this anthoxanthin series the red fluorescence is more or less apparent in proportion to the strength of the chlorophylline absorption phenomena.

There is a further important fact, viz., that in proportion as the peculiar optical characteristics of chlorophyll vanish, the solubility of the anthoxanthin in water increases. This fact obviously throws light on the nature and origin of the yellow chromules soluble in water which occur associated with chlorophyll in the green parts of plants and which finally lose even the last spectrum marks of chlorophyll.

Special precautions were taken to select for anthoxanthin examinations examples entirely free from any accidental admixture of green chlorophylline particles. The optical qualities of anthoxanthin cannot be inferred from the forms of these particles. They most frequently occur as very minute, round or oval, elongated, pointed, or spindle-shaped corpuscles; sometimes solid and sometimes hollow. Frequently it pervades a protoplasm entirely amorphous, and in rarer cases the material basis exhibits the more definite forms belonging to true chlorophyll globules.

Sometimes yellow-coloured drops occur in the cells, the chromules being dissolved in an oleaginous substance, whilst cases have been observed, but rarely, where the entire cell was filled with this liquid. In some examples of this the author remarks how the liquid frequently shows a

changing hue, so as to make it doubtful sometimes whether the cell is filled by a yellow or a green liquid.

The optical qualities of this chromule in regard to its greater or less approach to green chlorophyll is by no means in proportion to the form of its basis. The chemical changes which the chromule itself suffers, cannot, however, on the whole, be very considerable, seeing how slightly their optical characteristics vary. It might be assumed that this close optical correspondence arose from the presence of unchanged chlorophyll in the yellow blossoms employed. This, however, cannot in any way be anatomically demonstrated, and it is impossible, after the manner of the attempted separation of chlorophyll, to separate the anthoxanthin (possessing chlorophylline properties) into a green chlorophyll element, and a yellow element without the characteristic optical marks of chlorophyll.

On the spectrum of the yellow chromule of autumn leaves.

In the examination of these leaves my object was to test whether any chlorophylline chromule could be found which did not exhibit the first four bands of the chlorophyll spectrum. Here certainly the possibility of an admixture with chlorophyll might be much sooner suspected than in the case of anthoxanthin; and accordingly perhaps less value may be attached to the circumstance that in yellow autumn leaves I have likewise failed to discover any purely yellow chromule without chlorophyll band in the red. These bands, however, are not nearly so clearly seen as in the case of anthoxanthin and etiolin: a result hardly to have been expected on the supposition of the chromule of autumn leaves containing any possible admixture of chlorophyll.

The author then gives the results of some experiments with the alcoholic solutions made from faded oleander leaves and old rye straw, from which he concludes that their chromules possess the properties of chlorophyll but only in a slight degree, only band I. having been as yet seen, to show its origin from chlorophyll.

III.—*The yellow ingredient in the green leaf (chlorophyll).*

Since Fremy succeeded in separating an alcoholic solution of chlorophyll into a yellow and blue component by means of muriatic acid and ether, the view of the older botanists with regard to its green colour depending on its containing two substances, has to some extent been verified. It was, however, very soon demonstrated that acids and alkalis have the power of changing the constitution of chlorophyll, for it was proved that at least one of the two chromules obtained by Fremy, viz., that taken up by the muriatic acid, is not an original constituent of chlorophyll, but a result of its disintegration. A better method of separating the component parts of chlorophyll has since been made known, viz., that of using two non-miscible solvents, generally a normal green alcoholic solution of chlorophyll, whilst the other solvent should be one that will not chemically unite with alcohol, such as benzol, carbon, disulphide, or some fatty or ethereal oil. The liquids are intimately mixed by shaking. After separation the alcohol of the original solution will no longer appear green, but more or less yellow, whilst the other solvent will show a grass-green or glaucous colour. Those two chromules are looked upon as the pure components of leaf-green. The yellow portion dissolved in the alcohol does not possess the optical marks of chlorophyll, whilst the glaucous component, though supposed to possess the essential characteristics of chlorophyll, is still believed to distinguish itself from the original solution.

The opinion that chlorophyll is a compound, its colour being due to the two constituent elements, has received important support, and indeed seemed completely confirmed by the yet unrefuted assertion of Kraus, that the absorptions of the two components are combined in the chlorophyll spectrum. Kraus also affirms that the yellow component has absorptions only in the blue, and the green or glaucous component in both halves of the spectrum, which is undeniable; yet those in the second half are obviously displaced. He believes that the first four bands in the chlorophyll spectrum are entirely due to the glaucous component, and that the continuous absorption shown by

Separated chlorophyll is simply a combination of the in what differently situated bands of the two components. These assertions, however, require considerable correction.

Certain conditions are necessary for the proper observation of these phenomena. The first to be mentioned is that the added solvent must not be perfectly miscible with the alcohol of the chlorophyll solution, which depends on the dilution of the alcohol. Thus, *e.g.*, benzol mixes freely with alcohol of 90 per cent. strength. With weaker alcohol the phenomenon always presents itself, but its distinctness depends on the degree of dilution of the alcohol, on the relative proportions of alcohol and benzol, and also whether more or less benzol is retained in solution by the alcohol. A certain amount of dilution of the alcohol is an advantage therefore by keeping the alcohol and benzol portions as separate as possible; too much water, on the other hand, is apt to produce turbidity on the addition of the benzol.

As to the colours which make their appearance, it is important to remember that benzol, carbon disulphide, various fatty and ethereal oils, certain acids, &c., are able to absorb many times more chlorophyll than even high-percentage alcohol, and are accordingly tinged much more deeply.

The disregard of these conditions has given rise to much dispute as to the proper *colour* of the green component.

The yellow colour of the alcoholic portion is also influenced, but in a reverse manner, of course. As already remarked, the yellow alcoholic portion of chlorophyll is believed to have absorptions only in the second half of the spectrum, but a closer examination reveals also the characteristic chlorophyll bands in the first half. In a few cases Kraus appears to have noticed a trace of the chlorophyll band I., from which he inferred that the chlorophyll had not been completely eliminated from the yellow chromule. But we have never succeeded in obtaining a yellow alcoholic portion which had no chlorophyll bands in the first half. When the chromule is abundant it is not difficult to produce the entire chlorophyll spectrum.

It is an important fact too, that band I. is visible longer than the bands in the blue. This affords a proof that the chromule retained by the alcohol consists at least in part of normal green chlorophyll matter. Which of the yellow chlorophyll chromules may in addition be also present in the leaf-green—whether etiolin, zanthophyll, or both—can scarcely as yet be determined by spectrum analysis.

Kraus' statement regarding the glaucous, or grass-green, ingredient is certainly correct. The conclusion which he draws from this is, however, not admissible; for he supposes that the relatively displaced bands in the blue reveal a special component which he calls Kyanophyll, and that bands V., VI., VII., as situated in kyanophyll, together with the similar bands of the yellow alcoholic portion, combine to form the terminal absorption of the normal chlorophyll spectrum. The spectrum from chlorophyll solutions of medium thickness might possibly be thus explained; but in weaker solutions the three bands of kyanophyll do not present themselves, whereas in equally diluted solutions of kyanophyll they come out distinctly. Apart from this, however, this view is already refuted by the fact that the altered position of bands V., VI., VII., depends on the solvent.

Kraus was aware of the influence of the solvents, and thought that the displacement of the bands depended on their specific gravity; but Kundt shows that it depends on their dispersive power. I have found that some solvents of chlorophyll not only cause displacements of the bands, but also remarkable changes in the breadth and intensity of the bands. This would indicate, at least with regard to chlorophyll, that these solvents have even a chemical influence on the chromule.

The author next proceeds to a description of the peculiarities presented by the spectrum of a solution of chlorophyll in benzol, in which the displacement of bands I., V., VI., and VII., are easily observed, and in addition to this a splitting of band I. first into two and then into three bands is very distinctly to be seen when medium thicknesses of chromule are used. A similar splitting of band I. has already been observed by Schöm, Gerland, and Rauwenhoff in ethereal solutions of chlorophyll, and this splitting of

I and I may under favourable circumstances be noticed also in alcoholic solutions. Turpentine chlorophyll also shows this splitting.

The situation of the bands characteristic of benzole chlorophyll, of course comes out also in the benzole portion of separated chlorophyll, and constitutes in fact the displacement of the bands in the blue, which Kraus saw and described quite correctly; but his assumed composition of the chlorophyll spectrum cannot be inferred from this, simply because the bands of his kyanophyll are not present at all in unseparated alcohol-chlorophyll. A further study of these alcoholic and benzole portions will occasionally reveal slight deviations in the respective situations of the bands as described by me. These may very easily be explained if we only bear in mind that in most cases of separation we do not obtain pure solutions of chlorophyll in this or that solvent, but in a mixture of solvents. By judiciously regulating the relative proportions of the solvents, we may sometimes succeed in producing the observed medium.

Conclusion.

In order to define more accurately my views concerning these chlorophylline chromules, I will now compare the distinguishing characteristics of the various groups, and add a few remarks on the diverging views of other physiologists.

In the course of his recapitulation the author, after alluding to the varying degrees of solubility in alcohol and in water, presented by the anthoxanthin group, remarks that in addition to these we very generally meet with other yellow chromules often associated with the cell-walls of plants which do not even possess the three chlorophyll bands in the blue, but in their place an absorption beginning from the violet end and spreading continuously over the spectrum. This absorption might possibly be conceived as an intensification of the chlorophyll band VII., in which case these chromules would have to be regarded as the last members in the chlorophylline series.

It must now appear evident why I could not agree with Frémy, Fülhöl, or Kraus in their descriptions of the green and yellow chromules, and still less with Sorby respecting the

several chromules which he alleges to have obtained from various plants, and which he regards as distinct and undecomposed substances pre-existing in the plants and capable of definite analysis.

It is certain that many of these chromules must have been deprived of their original spectrum characteristics by the treatment to which they were subjected. In the determination of the spectra, moreover, the influence of the solvents, that of concentration, and of the thickness of layers, seem to have been equally disregarded. It is clear that a single spectrum can give us no adequate information as to the absorption phenomena of any chromule, unless we are acquainted beforehand with its phases of absorption, and know to which phase of the bands it corresponds. Sorby has nowhere stated, with reference to his yellow chromules, to which he ascribes two separate bands in the blue, to what extent the various positions of the bands are influenced by the solvents, nor how they depend on the thickness of the layers; nor does he state, that with an increase of the chromule additional bands do not appear. I doubt not that the whole, perhaps, of his yellow chromules—one only excepted, would suddenly reveal also the chlorophyll bands of the first half, if my method were applied.

If in future more accurate distinctions are attempted to be drawn, it will be necessary in every case to demonstrate carefully to what extent the observed difference in the spectra might or might not be explained by the solvents employed, or whether they do not depend on some cellular contents of the plants examined; and finally, it would have to be shown that they are more than particular phases of the same absorption bands.

[Abstract of paper in the "Monatsbericht" of the Royal Academy of Berlin, October, 1874.]