XIV.—*The Spectra of Blue and Yellow Chlorophyll, with some Observations on Leaf-green.*

By W. N. HARTLEY, F.R.S., Professor of Chemistry, Royal College of Science, Dublin.

It has long been known that leaf-green is composed of at least two colouring matters, called blue and yellow chlorophyll, the optical properties of which have been separately studied (G. G. Stokes, *Proc. Roy. Soc.*, **13**, 144; and H. C. Sorby, *ibid.*, **21**, 442). Chautard made an elaborate study of the spectra of mixed colouring matters of green vegetation (*Compt. rend.*, **75**, **76**, **77**); so also has Timiriazeff (*Ann. Chim. Phys.* [5], **12**, 355), and lately Reinke has studied very minutely the spectra of green living tissues (*Ber. deutsch bot. Ges.*, **1**, 395). The subject has further engaged the attention of Lommel (*Ann. Phys. Chem.*, **143**, 568), Schönn (*Ann. Phys. Chem.*, **144**, 166), Vogel (*Sitzungsberichte der K. Akademie, München*, 1872, 133), Gerland, Wiessner, Filhol, Liebermann, Sachsse, and many others. A recent paper by Tschirch is published in the *Trans.*, 1884, **45**, 57. The most recent researches are those of Schunck (*Proc. Roy. Soc.*, **39**, 348; **41**, 465; **42**, 184), which deal very largely with the decomposition product of chlorophyll called phyllocyanin, and its derivatives

and combinations with basic substances and with acids. Also Wollheim (*Ann. Agronom.*, **14**, 141—143) has elaborately investigated such compounds (Abstr., 1888, 723). The colouring matters of green leaves are substances of a particularly interesting character, first, on account of the connection which seems now to have been established between the absorption spectrum of chlorophyll and its physiological functions, and, secondly, because of the intimate relation which must exist between its absorption spectrum and its chemical constitution.

While endeavouring to study the chemical constitution of chlorophyll, by means of its absorption spectrum, it has been found necessary to re-examine the substance, so as to establish the relationship between the visible and the ultra-violet spectra.* H. Becquerel has examined the action of an alcoholic solution of chlorophyll upon the infra-red rays, but he finds no strong band of absorption, analogous to those in the luminous region (*Ann. Chim. Phys.* [5], **30**, 40). Abney, moreover, by his photographic process, has also failed to find any infra-red band.

The subject matter of this paper may be conveniently described under the following heads:—

- a. Observations on the spectra of chlorophyll contained in living tissues.
- b. The spectrum of the chlorophyll as seen in dried leaves.
- c. Mode of extracting leaf-green unchanged, and separating the blue from the yellow chlorophyll.
- d. Measurements of the spectra of the chlorophylls.

Observations on Living Tissues.

In order to ascertain how far the extracted colouring matters differed from, or were identical with those contained in living tissues, a series of observations were made upon green tissues, both by the visible and the ultra-violet spectra. In order to avoid the absorptive action for the ultra-violet rays which is known to be exerted by substances other than those which constitute the cellular tissue and the colouring matter of plants, it was considered desirable to make use of leaves not likely to contain any vegetable acids, aromatic substances, alkaloids, or glucosides, and, accordingly, aquatic plants were chosen, *Anacharis* and a filmy green sea-weed being taken as examples. According to Sorby, yellow chlorophyll is absent from green sea-weeds. As the observations on living tissues did not give different results with different plants, whether ferns, of which some

* Since this paper was written, A. Hansen (*Die Farbstoffe des Chlorophylls*, 1889, p. 82) has examined the ultra-violet spectrum by Soret's method, but only incompletely.

six species were examined, palms, three species, or ivy, two species, the character of the spectrum, as seen in the spring time, February, March, April, and May, may be briefly stated to be as follows :—The thickness of one leaf or frond showed the dark band overlying B, with an extension, consisting of a second weaker and closely-adjacent band, nearer the orange, and overlying C. There were no traces of a band in the yellow, close to D, and no indication of one in the green. The spectrum ended just beyond the magnesium green triplet *b*. Two thicknesses caused the bands to be much increased in density; there was no band, but only a dimness in the yellow at D; the green was practically unaltered, and the end of the spectrum was approached at *b*. Three and four thicknesses transmitted no trace of red, but only the green and part of the yellow rays, no band was observed in the green at all. Care was taken that no alteration should be caused by exposing the colouring matter to a beam of light, concentrated by a lens, only the direct sun's rays or such as were reflected from a mirror, being employed. The leaves and fronds were pressed between plates of glass. No living green tissue of any kind was found to be capable of transmitting the ultra-violet rays. Ivy leaves, gathered in February, are so rich in green substances that even in strong sunlight one thickness transmitted nothing but the green rays, and showed no sign of any absorption band.

The bands mentioned above are clearly shown in the diagrams representing the absorption curves of blue and yellow chlorophyll. The reason why the band *Ib* in the red is not seen distinctly, but only as a continuation of *Ia*, will be understood if a combination of the two curves is observed, as this shows the effect of the mixed chlorophylls. Overlying B and C there are actually three bands, two belonging to the blue, and one to the yellow chlorophyll.

A valuable abstract of a paper by Dr. Husemann appears in *Nature*, **30**, 224, for which we are indebted to Dr. C. A. MacMunn. It is there stated that yellow chlorophyll shows no fluorescence and no absorption band in the red, statements to the contrary being based on imperfect methods of separation. I think I shall be able to show that a yellow chlorophyll (that is to say, a yellow substance extracted from leaf-green), *containing no trace of the blue chlorophyll*, has a feeble but distinct and different band of absorption in the red, and a distinct fluorescence (see p. 118 and plate, p. 122). It is true that the band is very indistinct in an alcoholic solution, but this is by no means the case with the solution in benzene, if the solution be strong. When young leaves, gathered in the month of June from ivy, Virginia creeper, borage, rose trees, or blades of grass, are examined by sunlight, concentrated with a lens or concave mirror, the absorption band in the red is seen to occupy the space between B and C,

and to overlies those lines, the termination of the spectrum being about wave-length 5000. Under the concentrated rays of the sun the tissues and the colouring matter are soon altered.

The Spectrum of Colouring Matters obtained from Dried Leaves.

To ascertain whether leaf-green is altered by drying, observations were made on *Anacharis* dried with blotting paper and subsequently heated in a water-bath to 100°; ivy leaves dried by exposure to dry air, and also over sulphuric acid in a vacuum; green sea-weed dried in a water-oven. Perfectly pure and dry ether, expressly prepared for the purpose, extracted in each case a green colouring matter, which showed two bands of absorption, one in the orange the other in the green, which I have not observed in living tissues. These are, however, figured in the beautiful drawings by Tchirsch, illustrating Reinke's paper (*Ber. deutsch. bot. Ges.*, 1, 14). These spectra were observed in leaves by means of a microspectroscope, and it is not unlikely that the bands were caused by an alteration in the colouring matter effected by exposure to intense light, but it is also possible that at the time of the year when the plants were examined a change had taken place in the colouring matter by a normal process. It appears that leaf-green also undergoes some chemical change when dried, even if the drying be effected in a vacuum at the ordinary temperature.

Mode of Extracting Leaf-green Unchanged, and of Separating the Blue from the Yellow Chlorophyll.

Solvents which are of service in separating blue and yellow chlorophyll are the following:—Light petroleum, benzene, chloroform, alcohol with not more than 2 per cent. of water, alcohol of 50 per cent., and dry ether. The hydrocarbons and chloroform dissolve the yellow chlorophyll in preference to the blue, when the colouring matter is dry. When chloroform has been used to make a solution, it should be distilled off as soon as possible, otherwise the colouring matter is apt to change in contact with air, and an absorption band appears in the green, the liquid becoming brownish. Turpentine is wholly unsuitable for the same reason, although at first the solution of blue and yellow chlorophylls is very brilliant. Hot turpentine yields a solution of an olive-green colour; on exposure to the air and diffused daylight, this is changed to a dull yellow, and finally becomes decolorised. A strong band of absorption in the green is seen in this solution. Other solvents of no particular interest are amyl alcohol, isopropyl alcohol, ethylene dibromide, and aldehyde.

To extract the colouring matter, ivy leaves were rolled into bundles and rasped on a bread-crumb grater, or minced finely with a knife on a chopping-board. Sometimes the shreds were steeped in cold water for 12 hours, whereby a good deal of a yellow or orange-yellow colouring matter was removed along with pectose, glucosides, and resin. As soon as no more of this colour was yielded to fresh water, the material was squeezed between linen cloths and folds of dry blotting paper. The colouring matter was then extracted by triturating with broken glass or fine quartz-sand in a porcelain mortar, together with the solvent to be used. When ivy leaves are boiled previous to extracting the colouring matter, and subsequent to washing with water, the shreds of leaf are of a dull olive-green in the centre and bluish at the edges. Extraction with cold alcohol then gives a solution showing the absorption band in the green strong, as in the turpentine solution; and no doubt the colouring matter has undergone a change.

When a solution of leaf-green is made with cold alcohol of 98 per cent., by extracting the colour from mixed ivy leaves, either with or without washing, its properties are seen to be those of the ivy leaf, in that it absorbs nearly all the red rays and the green rays near to the termination of the spectrum, the yellow and orange rays are dimmed, but the green is wholly transmitted. I prefer to extract the colour from the leaves immediately after mincing them, and as rapidly as possible, the whole of the green matter being immersed in spirit in a stoppered earthenware jar, and so protected from air and daylight.

To the alcoholic solution, a warm saturated solution of barium hydrate is then carefully added, until no further precipitation takes place, whereby a rich green precipitate is formed, leaving the solution yellow. After filtration and repeated washing on the filter with water and alcohol, which latter may be boiling, some of the yellow colouring matter still stains the filter. This is easily removable by washing with either cold or boiling chloroform. After a time, the washings begin to run with a greenish-yellow tinge. At this stage, the filter may be washed with boiled alcohol, and a repetition of the process causes the spirit to run through colourless. In order to separate the blue chlorophyll from the baryta compound, the precipitate is treated with a warm solution of boric acid in glycerin, to which alcohol is added, when a solution of blue chlorophyll is obtained. In this state it is very stable provided no acid be added to it; a few minutes boiling causes no alteration in its spectrum. It exhibits only two absorption bands in the red, very close to each other, the less refrangible being the sharpest in definition and the most intense; the spectrum ends in the blue.

A precisely similar substance is obtained by precipitating blue

chlorophyll by the addition of water to an alcoholic solution of leaf-green, and shaking the liquid with benzene. In this case the benzene takes up the blue chlorophyll, while the yellow remains dissolved in the weak alcohol. This method of separation is similar to that first employed by Sorby. From the solution of yellow chlorophyll in weak alcohol, a portion of the spirit was distilled off, and the aqueous liquid was shaken with chloroform, which was separated and distilled; the substance was then redissolved by treating the residue with dry ether or with benzene.

A considerable quantity of a baryta compound of yellow chlorophyll was formed; this was of course separated by filtration, and the colouring matter was liberated by boric acid, as in the case of the bluish-green substance. It was proved that boric acid did not affect the spectrum of the yellow substance.

In filtering a large quantity of a strong alcoholic solution of leaf-green, it was noticed that there was a tendency in the yellow colouring matter to creep away from the blue chlorophyll. This separation of the two substances by the action of capillarity was promoted by using a filter 16 inches in diameter, and 2 inches too large for the funnel, and pouring the coloured solution at once on to the filter without previously moistening it. By this means a separation of the yellow substance was obtained, and its properties were studied. The portions of the filter stained yellow were removed, and treated with alcohol or ether, and the solution so obtained was submitted to the same reaction which the yellow chlorophyll had been subjected to on a larger scale; it was found that such treatment left it unchanged. Four preparations of blue chlorophyll and three of yellow chlorophyll were made from ivy leaves by the method described. Those specimens of the blue substance which I consider to be the purest were prepared from a well purified baryta precipitate, and gave a beautiful, red fluorescence. Two other specimens gave, one a brown, the other a chocolate-coloured fluorescence. The reason of this variation was ascertained, and it may be here stated that the true fluorescence is red.

The yellow chlorophyll in concentrated solutions in benzene or alcohol is brownish, its fluorescence is a magnificent red; a dilute ethereal solution of the yellow chlorophyll was seen to change from a yellow to a greenish-yellow in the bottle in which it was kept during the period it was under examination by the spectroscope; at last it became quite colourless. This specimen had been dissolved in ether which was not perfectly dry, and the trace of moisture may have promoted the change. The following reactions of blue and yellow chlorophyll are to be noted. *When the barium compound of blue chlorophyll is treated with an aqueous solution of boric acid, the substance instantly changes to a yellow colour upon boiling.*

Agitation with hydrochloric acid and ether yields with blue chlorophyll a brownish coloration in ether, and the blue characteristic colour in water. With a small quantity of the chlorophyll, there is no brownish coloration, and the ether remains colourless. When yellow chlorophyll is treated in a similar manner, the ethereal solution is yellow and the aqueous of a greyish tint. It appears, therefore, that the blue chlorophyll produces the blue colour in Frémy's reaction. When blue and yellow chlorophyll are separately treated with formic acid and ether, there are produced two new substances, showing absorption bands in the green.

M. Guignet has described a method of extracting the green colouring matter from leaves, and has recorded the existence of a definite crystalline compound of blue chlorophyll with sodium (*Compt. rend.*, **100**, 434). His preparations are made in the following manner:—Fresh leaves of spinach are treated repeatedly with boiling water containing $\frac{1}{2}$ per cent. of soda crystals; in this way, tannin and pectose are removed. The residue is then strongly pressed and digested with alcohol of 95 per cent. If dried leaves are extracted with light petroleum, and subsequently with alcohol, the products are purer. He states that no blue chlorophyll is yielded to light petroleum, but only a mixture of the yellow and colourless matters. Finally, a small quantity of green colour is dissolved by the light petroleum.

My experience of the action of light petroleum upon air-dried, minced ivy leaves showed that at first there was extracted only a yellow chlorophyll; after a prolonged extraction a green substance became dissolved, and in forty-eight hours a deep-green solution was obtained, which showed two strong absorption bands in the green, and therefore did not consist of pure blue chlorophyll. The third extract was greenish-yellow; it showed only two contiguous absorption bands in the red, and a red fluorescence characteristic of yellow chlorophyll when mixed with a little blue. The fourth extract was at first, as nearly as possible, colourless, but in 12 hours it became yellow, with a very faint, greenish tinge; even the twelfth extract was yellow, and hitherto I have failed to get the solvent to remain colourless. The quantity of the solvent employed in this treatment amounted in all to at least 16 times that required to completely immerse the chopped dried leaves. On keeping the several extracts until a large accumulation should render it convenient to distil off the petroleum, the second solution of a greenish colour became bleached, probably by the action of light. Hence it appears that the action of light petroleum is not in all cases the same, and that this method is more conveniently applicable to the treatment of spinach than of ivy leaves, since the latter require to be cut up, if not minced finely. It was found that an alcoholic extract of fresh leaves became altered,

notwithstanding that it was preserved in a bottle filled to the stopper, so as to leave not more than 1 or 2 c.c. of air-space in a litre of liquid, and in a fortnight, although the vessel was kept in a dark cupboard, the rich green tint had taken somewhat of an olive tinge, and the bands of absorption in the yellow and green were seen to be strongly developed (compare Russell and Lapraik, *Trans.*, 1882, **41**, 334). Hence it is best first to extract freshly minced leaves without drying and with very strong alcohol, and then to precipitate the green colouring matter as rapidly as convenient. It might be advantageous to add a little ammonia to the alcohol, in order to neutralise any acid existing in the juices of the plant or likely to be formed by the oxidation of aldehydes. Russell and Lapraik have studied the changes in the chlorophyll spectrum caused by acids, &c. (*loc. cit.*).

The fresh extract of green leaves, and even that which has been kept for some little time, readily reduces nitrate of silver to a mirror when boiled in a flask, but as the quantity of reducing substances is very small, the mirror is thin. Hence, we may infer that the aldehyde is by keeping oxidised to formic acid, and that this alters the chlorophyll. Chlorophyll, altered by a trace of formic acid, is precisely like that altered by keeping.

The ivy leaves which had been treated 16 times with light petroleum were allowed to stand for some weeks with that solvent. The liquid acquired a deep olive-green colour. It was kept all the time in the dark, so that light could have had no effect upon it; air, too, was excluded.

Comparison of Various Chlorophyll Spectra.

Wave-length measurements of the bands visible in thick and thin layers of the solution are given on p. 114 (A). The bottles were then exposed to diffused daylight for a week; the contents of the small bottle lost much of the colour, and, indeed, the solution was only very faintly greenish in hue; its spectrum is shown (B).

Spectrum C is that of Tschirch's chlorophyll (*Trans.*, 1884, **45**, 57). The process by which this, which was believed to be *pure chlorophyll*, was obtained, appears to have been previously described by Church and the substance itself is probably a zinc compound. Spectrum D is that of Russell and Lapraik's alcohol and ether-extract of various leaves. In spectrum A there were two bands overlapping in the red, numbered Ia and Ib; of course, only the more refrangible edge of Ib could be measured.

If a careful comparison be made of these measurements, it will be observed that in Tschirch's spectrum C, band I corresponds to bands Ia and Ib, and the same remark may be applied to Russell and Lapraik's

Comparison of Various Chlorophyll Spectra.

		Wave-length in millionths of a millimetre.						
		Red end of spectrum.	Red.		Orange.	Green.	Blue.	Blue.
			Overlapping bands.					
			Band Ia.	Band Ib.				
A. Colouring matter of ivy leaves, extracted with petroleum spirit. Air and light excluded.	—	739	695—656	—640	623—607	570—558	541—530	515 end
B. The same, a similar preparation	Thin layers Thick layers	— —	690—663 685—658	— —	620—607 620—606	None 570—557	542—533 540—533	514—594 514 end
C. Tschireh's preparation, probably a zinc compound..... (Schunek, <i>Proc. Roy. Soc.</i> , 39, 360).	Thin layers Thick layers	— —	680—630 685—	— —	620—595 —	585—557 —555	540—525 535—520	500 end 510 end
D. Russell and Lapraik's alcohol and ether extract of green leaves. Bands caused by the action of acids. The same action, increased in intensity	— — —	— — —	678—640 — —	— — —	628—600 — —	589—573 573—558 —	545—532 — 544—532	510 end 510 end 510 end

spectrum D, if allowance be made for the difference in strength of the solutions, and, above all, for the shifting of the bands, by reason of the solvents being different.

Band V, which has been observed by Krauss, does not seem to have been measurable in the preparations examined by Russell and Lapraik and by Tschirch. Band III in B seems to have been destroyed by exposure of the solution to the light. This band appears to have been present in Tschirch's substance, though the experiments of Russell and Lapraik show that it is caused by acids, which is entirely in accordance with my own observations, whether the acid be produced by oxidation of the aldehyde in the plant tissues, or in the chlorophyll extract, or whether the acid be added to the substance.

Both Tschirch and Russell and Lapraik seem to have regarded the overlapping bands Ia and Ib as only one band. Band Ib appears to lie upon that due to yellow chlorophyll. Tschirch states that in his examination of living leaves he used sun-light concentrated by a lens, and passed the rays through two alum cells to intercept the infra-red spectrum, but he appears to have overlooked the fact that the violet and ultra-violet rays are capable of intense chemical action, and therefore it is rather doubtful whether the colouring matter did not undergo some change in the living tissue while under observation.

Measurements of the Spectra of the Chlorophylls.

The measurements which follow are those of the visible and of the ultra-violet region. The spectroscope used for the visible rays was furnished with a compound prism of Grubb's construction, capable of separating the two D lines; the telescope moved on a divided arc, and a pointer in the eye-piece, shaped like one of the nibs of a steel pen, that is, with one straight and one curved edge, served to fix the position of the bands. Readings were taken when the point and the straight vertical edge were no longer visible in an absorption band. The metallic lines in flame and spark spectra given in the British Association Report for 1878 were employed for converting arc measurements into wave-lengths and oscillation frequencies. The ultra-violet spectra were examined by a method already described (*Proc. Roy. Soc.*, 38, 1). The absorption curve obtained from a solution in ether of colouring matter from dried *Anacharis* resembles that which may be drawn from Reinke's spectra (*loc. cit.*), with the addition of a feeble extension into the ultra-violet, which in very dilute solutions shows an absorption band. Specimens of blue and yellow chlorophyll were separately examined and absorption curves drawn from the measurements; the solutions of the three specimens of yellow chlorophyll were not of the same strength, and on this

account, and from different solvents being used, the curves differ slightly.*

It is necessary to mention that it was proved by direct experiment that the solution of boric acid in glycerine, when mixed with an equal volume of alcohol, absorbed none of the ultra-violet rays less refrangible than wave-length 2200, so that solutions, which might contain these substances, could not be optically affected thereby.

Reinke (*Bied. Centr.*, 1884, 692—696; and *Abstr.*, 1885, **48**, 182) states that the spectra of living leaves and solutions of chlorophyll in alcohol were found to be somewhat different. Solutions of *fresh*

THE SPECTRUM OF BLUE CHLOROPHYLL.

Solution in Alcohol.

Thickness of layer of liquid.	Description of spectrum.	Oscillation-frequencies.	Wave-lengths.
5 mm.	Spectrum commences at	127	788
	Very strong absorption band	140—155	714—645
	Weak absorption band	169—176	589—565
	Spectrum ends at	214	467
4 mm.	Spectrum commences at	130	764
	Very strong absorption band	139—147	719—678
	Weaker absorption band , almost continuous with the less refrangible band, but more diffuse.....	149—157	669—637
	Very weak absorption band	169—175	589—570
3 mm.	Spectrum ends at	214	465
	Commencement in the red	132	755
	Strong absorption band	141—146	709—682
	Weaker absorption band , measurements rather doubtful	151—155	661—645
2 mm.	Spectrum ends in the blue	215	463
	Commencement in the red	133	749
	Absorption band	141—146	706—682
	Spectrum ends	218	
1 mm.	Commencement in the red	134	743
	Absorption band	142—145	701—686
	Termination in the blue about	222	450
	Rays feebly transmitted at	306	326
	and at	334	299

This solution was diluted five times; 2 c.c. were made up to 10 c.c.

* The curve of the alcoholic solution of yellow chlorophyll is represented in the plate, p. 122, by a dotted line.

leaves show no fluorescence; ordinary solutions, as is well known, do. Hence, he believes that the solutions change in properties by a disaggregation of the chlorophyll molecule. In the *Bot. Zeit.*, 1885, Nos. 5 and 9 (also Abstr., 48, 991), he shows that the maximum decolorising action of light on chlorophyll in alcohol and benzene lies between wave-lengths 650—610, between B and C, and the next between 450—410, which measurements agree with the relative intensities of the two most important and persistent bands, as shown by my curves, and which, therefore, correspond with vibrations of greatest amplitude at opposite ends of the spectrum.

Thickness of layer of liquid.	Description of spectrum.	Oscillation-frequencies.	Wave-lengths.
5 mm.	Commencement in the red	134	743
	Absorption band	141—144	706—692
	Visibility of rays terminates in the blue; rays photographed.....	208	480
	Very faint, not continuous	222	450
4 mm.	Commencement in the red	134	743
	Absorption band directly overlying C ..	145	689
	Rays transmitted to	208	480
	Faint and not continuous to	227	440
	Absorption band	227—277	440—361
3 mm.	Rays transmitted, but not continuously, to	353	283
	Commencement in the red	134	743
	Absorption band at C still visible	145	689
	Rays transmitted to	227	440
	Absorption band	227—251	440—398
2 mm.	Rays transmitted, weak but not continuous, to	364	274
	Commencement in the red	134	
	Rays fully transmitted to	208	480
	Rays continuous, but weak to	364	274
1 mm.	Commencement	134	
	Rays transmitted continuously to	364	274
	Rays not continuous at	370	270
	” ” to	389	256
	Absorption band	389—433	256—231
	Spectrum ends at	433	231

The solution was again diluted five times; 2 c.c. were made up to 10 c.c.

Thickness of layer of liquid.	Description of spectrum.	Oscillation-frequencies.	Wave-lengths.
5 mm.	Precisely the same as with 1 mm. of the previous solution.		
4 mm.	Commencement in the red	134	743
	Continuous spectrum, strong to	353	283
	" " weak to	364	274
	" " very weak to	389	256
	Absorption band	389—413	256—242
	Rays transmitted, but not continuous to end, at	442	256
3 mm.	The spectrum is continuous to	389	256
	Much weakened; it extends to	442	256
2 mm. } 1 mm. }	Spectrum becomes stronger, but is not of normal strength; it extends to	447	223

The solution was again diluted five times, and yet 1 mm. of the diluted solution only transmitted the ultra-violet rays feebly beyond 389 (256).

The absorption band seen in this solution between oscillation-frequencies 222 and 288, when 5 mm., 4 mm., and 3 mm. were examined, does not appear in the benzene solution of yellow chlorophyll, but a band appears between oscillation frequencies 226 and 246, but only at one thickness 2 mm. This might give rise to a suspicion that it is due to a slight admixture of blue chlorophyll with the yellow solution, but for the fact that, if it were, the absorption band in the red would be visible along with it.

THE SPECTRUM OF YELLOW CHLOROPHYLL.

Solution in Alcohol.

It must be noted that these spectra were measured in two halves, 1st, by the eye from A to beyond G, 2nd, by photography from F to beyond U. In such cases where the spectrum ends at about F, and the photographs show no rays between F or G and H, it is evident that an absorption band occurs, because the visible and the photographic spectra overlap to a considerable extent.

Thickness of layer of liquid.	Description of spectrum.	Oscillation-frequencies.	Wave-lengths.
15 mm.	Spectrum begins in the red between A and B Very weak absorption band , not easy to measure, the edges being too diffuse. Centre of band about C at.....	139 151	719 660
10 mm	Spectrum ends in the green at the B group, at Spectrum begins in the red Spectrum ends in the blue at	193 139 204	518 719 490
5 mm. } 4 mm. } 3 mm. }	These are photographed spectra, and there is a very feeble transmission of some of the strong bright lines. Ill-defined absorption band , strong	193—213	518—467
2 mm.	Spectrum begins at (between F and G).... Continued very feebly to Absorption band , strong	213 226 226—246	467 440 440—407
1 mm.	Continuous weak spectrum from near F to This solution was diluted five times; 2 c.c. was made up to 10 c.c.	208 389	480 256
5 mm. } 4 mm. } 3 mm. }	A continuous weak spectrum to	389	256
2 mm.	Continuous spectrum weak to	433	231
1 mm.	„ „ strong to	353	283
	„ „ weak to.....	442	226

Solution in Alcohol. Another Preparation.

20 mm.	Spectrum begins in the red at..... Weak absorption band Spectrum ends in the green.....	139 149—152 199	719 670—658 502
--------	--	-----------------------	-----------------------

Solution in Benzene.

Thickness of layer of liquid.	Description of spectrum.	Oscillation- frequencies.	Wave- lengths.
15 mm.	Red rays commence between A and B Weak absorption band from near B to C Visible spectrum ends near E in the green	139 144—151 188	719 694—660 530
5 mm. } 4 mm. } 3 mm. }	No absorption from near B to C visible. Rays transmitted as far as the green, but all beyond absorbed.		
2 mm.	All rays beyond the green absorbed with the exception of certain strong lines in the ultra-violet. Absorption band from near E to near M Spectrum weakly transmitted from be- tween K and M to beyond U	188—264 264 353	530—378 378 274
1 mm.	Feebly continuous spectrum from to This solution was diluted five times; 2 c.c. was made up to 10 c.c.	264 364	378 274
5 mm. } 4 mm. } 3 mm. } 2 mm.	The same effect as with 1 mm. above, the spectrum increasing in strength pro- gressively. Spectrum begins feebly at and extends to	213 370	467 270
1 mm.	Spectrum begins a little beyond F at..... and extends to..... This solution was diluted five times; 2 c.c. was made up to 10 c.c.	208 370	480 270
5 mm. } 4 mm. } 3 mm. }	The same effect as with 1 mm. above.		
2 mm. } 1 mm. }	Spectrum feebly visible at Continuous, fairly strong, from " " " to	208 277 308	480 361 324

THE SPECTRUM OF YELLOW CHLOROPHYLL.

Solution in Ether.

Thickness of layer of liquid.	Description of spectrum.	Oscillation-frequencies.	Wave-lengths.
20 mm.	The spectrum was the same as that measured from the solution in benzene. Red rays begin between A and B Weak absorption band from near B to C Spectrum ends in the green near E at	139 144-148 188	719 694-670 530
5 mm. } 4 mm. }	No spectrum transmitted in the { ends at blue, violet, or ultra-violet { ends at	206 214	
3 mm.	A faint, discontinuous spectrum, ends at ..	221	
2 mm.	Spectrum commences on the photographs at Continuation <i>very feeble</i> to..... Absorption band from between F and G to H..... Rays transmitted, but not continuously, to	213 226 226-251 307	467 440 440-398 325
1 mm.	Weak continuous spectrum to	288	346
	The original solution was diluted five times; 2 c.c. made up to 10 c.c.		
5 mm. 4 mm. 3 mm. 2 mm. 1 mm.	The same effect as with 1 mm. above. Continuous spectrum, weak to The same. Continuous spectrum, weak to Continuous spectrum, weak to	288 308 353	325 324 283
	This solution was diluted five times; 2 c.c. made up to 10 c.c.		
5 mm. 4 mm. 3 mm. 2 mm. 1 mm.	The same as with 1 mm. above. " " " Continuous spectrum, weak to The same. Continuous spectrum, weak to	370 433	270 231

This solution, which originally was prepared with absolute alcohol, was of a deep yellowish-brown colour; it was evaporated to dryness in a vacuum and taken up with ether. Its brownish colour remained for a few days, but during examination by sunlight, it became a greenish-brown, later of a pale-green, and finally it became de-colourised.

The yellow chlorophyll has an absorption band in the orange-red, and a very powerful absorption of all rays beyond the *b* group. When

very thin layers of a dilute solution are examined, there is a powerful absorption band extending from *b* to K. The absorption band in the orange-red is different from either of those belonging to blue chlorophyll, and the curves offer further evidence that the specimens of yellow chlorophyll were quite free from any admixture of the blue, because, if they had not been so, the least refrangible and most persistent absorption band of the latter would have been visible. It is only the *alcoholic* solutions of blue and yellow chlorophyll which are strictly comparable, because the displacement of the bands by the difference in the refraction index of the solvents interferes with the comparison of a solution in alcohol with one in benzene or ether even of the same substance. But in all three spectra there is a powerful band of absorption extending from the *b* group to K, even in thin layers of a weak solution. In thick layers, no rays beyond *b* are transmitted.

It will be seen that the leading characteristics of unaltered leaf-green are those of blue chlorophyll, namely, an intense absorption in the red, somewhat stronger even than in the violet and ultra-violet.

We learn from the absorption bands that the vibrations within the molecule of the blue chlorophyll are both of the slowest and the most rapid, and that they are also of very great amplitude.

The rays transmitted by leaf-green, namely, the yellow and green, are barely capable of any photochemical action either on silver salts or organic substances. Such rays, therefore, are not likely to be effective in decomposing carbonic acid.

Blue chlorophyll, judging from its chemical composition and its spectrum, is very probably a derivative of a benzenoid hydrocarbon. It is certainly neither a wax, a fatty acid, a carbohydrate, nor an albuminoid from grain, nor, in fact, any other substance derived from an open chain of carbon atoms.

It has been clearly established by the experiments of Lommel, Timiriazeff (*Compt. rend.*, **100**, 851), Reinke (*Bot. Zeit.*, 1885, Nos. 5 and 9), Bonnier and Mangin (*Compt. rend.*, **102**, 123), and Paul Bert (*Compt. rend.*, **87**, 695), that the rays absorbed by blue chlorophyll are those which are necessary to plant life, and which cause plants to grow; and that plants die when they can get no other daylight than such as has passed through a weak solution of chlorophyll.

By Regnard (*Compt. rend.*, **101**, 1293), and also Timiriazeff (*Compt. rend.*, **102**, 686), it has been shown that outside the cell walls, a colourless substance derived from chlorophyll by the action of nascent hydrogen, chlorophyll is capable of decomposing carbonic acid when exposed to light. Regnard showed that even wool dyed with chlorophyll is capable of liberating oxygen from carbonic acid.

It seems, therefore, that the molecule of chlorophyll, or one of its

Journ. Chem. Soc. Feb. 1891.

HARTLEY.

ABSORPTION CURVE OF BLUE CHLOROPHYLL ALCOHOLIC SOLUTION.

*The dotted line indicates the curve of yellow chlorophyll.**Scale of Oscillation-frequencies.*

transformation products, is actually capable of reducing carbonic acid.

From the researches of Gerland and Rauwenhoff, and especially Reinke, it is known that leaf-green contains some aldehydic substance, and that formic aldehyde is constantly present in living green tissues. As formic aldehyde is the first product when a molecule of oxygen is removed from carbonic acid,

and as it is very easily polymerised and converted into a sugar, its further conversion into starch $10(\text{H}_{20}\text{C}_{12}\text{O}_{10})$ by the removal of 20 mols. of water is easily understood by all who are familiar with the chemistry of the carbohydrates. Such a course of chemical change is not a purely speculative one, for E. Laurent has shown that the etiolated shoots of potatoes, when kept in the dark, can form starch from glycerol, and carbohydrates of the glucose and saccharose groups. Moreover, a glucoside has actually been separated from chlorophyll. Baeyer discovered a series of substances formed by the union of aldehydic compounds, and particularly formic aldehyde with aromatic derivatives of the most diverse nature (*Ber.*, 5, 1094; and 6, 220). I have shown the probability of the chlorophylls being aromatic substances, and we can thus understand how they could combine with formic aldehyde; therefore it is quite intelligible how such a combination could be split up into a sugar or starch, and chlorophyll.

R. Sachsse supposes that chlorophyll is the first product of the reduction of carbonic acid, and this is itself converted by further change into starch and other carbohydrates. It is difficult to conceive of even the bare possibility of this, but there is no difficulty in reconciling the evidence which has led to this conclusion with facts consistent with a process which I have here sketched in outline.

The first step in the reduction of carbon dioxide or of carbonic acid, H_2CO_3 , is a process of dissociation, for it has been observed by Godlewski that in a bright light an increase of carbon dioxide in the air, up to 8 per cent., causes a rapidity in the formation of starch which is fourfold the ordinary amount. In other words, the rate of chemical change (starch formation) appears to be a function of the intensity of the light and the pressure of the carbon dioxide, and inversely as the pressure of the oxygen. The formation of starch under these conditions is a photographic process, that is to say, one dependent on radiant energy, acting upon carbonic acid.

Latterly, Hansen (*Abstr.*, 1888, 867) states that the colouring matter of leaves absorbs carbon dioxide, and forms an unstable compound therewith; the absorption increases with the rise of temperature and the pressure of the gas. He believes that the chlorophyll,

124 THE SPECTRA OF BLUE AND YELLOW CHLOROPHYLL.

after it has combined with the CO_2 , hands it over to the plasma of the chlorophyll grains. It is, however, much more probable, on chemical grounds, that the compound of chlorophyll with carbon dioxide is entirely decomposed, first, by the elimination of oxygen, and secondly, by the elimination of water, so that there are successively formed compounds of chlorophyll (1) with carbon dioxide; (2) with formic aldehyde; (3) with glucose; and finally, starch, completely formed, is split off the molecule.

NOTES.

(1.) According to Draper, Sachs, Pfeffer, and Gerland, the curve of assimilation is in the green and yellow. Later experiments, as, for instance, those of Timiriazeff, show that it is in the red overlying B. Paul Bert's experiments conclusively prove that it is not in the green and yellow.

(2.) When one compound enters into combination with another, the resulting molecule may be altogether different in its mode of vibration, and become susceptible of decomposition by vibrations to which it was previously insensible; the evidence of this is afforded by organic bases and their salts; thus, molecular weights of paratoluidine and hydrochloric acid when submitted to the action of rays lying between the red and extreme ultra-violet yield a spectrum which is that of the base alone, for the hydrochloric acid has no action on these rays; but when the molecules are combined, the spectrum is greatly modified.

(3.) Some writers have apparently considered vibrations of great wave-length as vibrations of great amplitude. They are not necessarily so. Vibrations of great wave-length are slow vibrations, but they may be either of large or small amplitude. Amplitude is measured by the *intensity of the rays*. Amplitude in the vibrations of a molecule is measured by the *intensity of the absorption* of the rays.

(4.) Reinke has calculated the rate of vibration of the molecule or of the atomic group within the molecule which causes the reduction of carbonic acid. This is an application of the method by which I calculated the rates of vibration of the molecules of ozone, benzene, naphthalene, and anthracene. Reinke's calculations are to some extent inaccurate, inasmuch as the absorption of the ultra-violet rays has not been taken into account.

(5.) It will be recollected that according to Pringsheim the function of chlorophyll is merely a physical one, due to its colour. It acts as a screen, diminishing the intensity of the light, and thereby the amount of oxidation in the cell ("Researches on Chlorophyll," *Quarterly J. Micr. Sci.*, 1882, 110). If this were correct, any other green screen would answer the purpose, and best of all would be a solution of chlorophyll; but it has been shown that under such protection plants die.

(6.) To be strictly accurate 0 should be added to the number in the columns headed "oscillation-frequencies." W. N. H.