

PLATE VI.

This Plate gives various very beautiful transformations of the circular solar spectra as seen by viewing the solar disk of the mercurial globule portrayed obliquely on the stage, which vary in their forms according as the glasses are under- or overcorrected, as depicted in the figures, which it is needless here to describe in detail.

Fig. 34 is another form of mercurial globule, beautifully defined.

The figures displayed by the magnified artificial star for oblique reflection render it probable that the obliquely illuminated mercury globule, viewed directly in close proximity to the front glass of the microscope upon the stage, is a very imperfect test; and the methods here described are submitted as possessing very superior delicacy and convenience.

XXII. "On Comparative Vegetable Chromatology." By H. C. SORBY, F.R.S. &c. Received June 9, 1873.

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Introduction.

The study of colouring-matters as described in the following paper requires the use of a somewhat special kind of chemistry and of spectro-

scopic instruments designed for the purpose, but yet it cannot be looked upon as being either chemistry or spectrum-analysis. It is also requisite to make use of light as a reagent, and yet the subject could not be called photography or even photo-chemistry; and though most intimately connected with biology, it could scarcely be regarded as a branch of that science. It appears to me most desirable to adopt some name for the whole subject, including whatever modifications of other branches of science are requisite for its efficient study, and none would meet the requirements of the case better than *Chromatology*. I trust that the facts I am about to describe will be sufficient to show that we have such a wide branch of science before us that it deserves some special name. I then, again, since I now purpose to describe the points of agreement and difference in the colouring-matters of various classes of plants, it appears to me that no better title could be adopted than the one I have chosen, viz. *Comparative Vegetable Chromatology*.

My attention has been for a long time directed to the colouring-matters of plants, but it is only within the last year that I have discovered the means of carrying out the inquiry in a satisfactory manner, and convinced myself that the most valuable conclusions are to be learned, not by looking for the rarer or more attractive substances which give remarkable spectra, but from the qualitative and quantitative determination of the different coloured constituents of complicated mixtures, some of which substances have no striking properties, and might easily be overlooked, though perhaps of great importance in connexion with the life of particular plants. The successful carrying out of this inquiry will necessarily be the work of years; for not only is the whole field comparatively unexplored, but it will be requisite to examine all classes of plants and many of animals, at various seasons of the year and when growing in different natural and artificial circumstances, and also to study the relation of the different colouring-matters to one another, and the action on them of light and chemical reagents. In every department of the subject very interesting questions remain to be answered; and the following paper must be looked upon merely as a general outline of what I have hitherto been able to learn, and as an indication of what may be expected to result from further research.

The Absorption-band-raising Power of Solvents.

The identification of the individual colouring-matters has been effected by means of their spectra, sometimes as seen by examining the plants themselves, but generally when dissolved in various liquids, with or without the addition of appropriate reagents; but any other peculiarities were taken advantage of, according to the circumstances of the case. For further particulars connected with the general method I refer to my former papers*.

* Proc. Roy. Soc. 1867, vol. xv. p. 433. Quart. Journ. of Microscopical Science,

Here, however, I would remark that the exact position of the absorption-bands given by particular substances varies considerably, according to the nature of the solvent, even though it is quite neutral and exerts no chemical action on the colouring-matter. Thus, for example, the bands may lie much nearer to the red end when dissolved in bisulphide of carbon than when dissolved in benzole or absolute alcohol. This kind of fact has been often noticed by others, but its bearing on some important questions has generally been overlooked. Apparently, as a rule, the bands are more raised towards the blue when the colouring-matter is dissolved than when in a solid state, and this power of raising them varies with the solvent; but the extent also depends very much on each particular substance. I have carefully determined the order in which various liquids thus raise bands, and compared it with the order of their specific gravity, and of their refractive and dispersive power, but have hitherto failed to recognize any simple connexion between what may be called the *band-raising power* and any other physical property. In some cases the position of the bands given by the solid substance appears to vary according as it is in a colloid or a crystalline state. Though at first this difference in position may seem to be an objection in the use of the spectrum method, yet it only shows the need of comparing together our specimens in exactly the same condition, and it may be taken advantage of as a means for distinguishing closely allied substances which may be influenced in a very different manner and for enabling us to ascertain the condition of a colouring-matter as it exists in an animal or plant. It is thus possible to determine whether it is in a free state or dissolved in an oil or wax, and in the latter case whether it is liquid or solid. In this manner I have been able to detect a difference in the relative quantity of oil in different parts of the same leaf. Various forms of spectrum-microscopes were employed, according to the requirements of the case; and the amount of material at command would often have been too small for examination with any other kind of instrument; so that I think I may fairly say that comparative vegetable chromatology is the first great subject which has resulted from the use of the spectrum-microscope.

Separation of Colouring-matters by chemical means.

On the present occasion I propose to consider almost exclusively those colouring-matters which are soluble in bisulphide of carbon and in fixed oils, but insoluble in water. These appear to be by far the most important in connexion with plant life, or at all events are more uniformly distributed. Those soluble in water and insoluble in bisulphide of

carbon or fixed oils are much more numerous, and often very partially and, as it were, accidentally present: those of the former class may often be separated from one another by means of bisulphide of carbon, alcohol, and water, mixed in proper proportions. As an illustration I take the very simple case of olive *Algæ*, like *Fucus* or *Laminaria*. They should be well crushed, slightly dried, and heated in spirits of wine of the usual strength. Absolute alcohol must not be used, since it dissolves out the chlorophyll in such a manner that it is not carried down as usual, when agitated with bisulphide of carbon. When cold the solution should be agitated in a test-tube with so much excess of the bisulphide that a considerable quantity subsides to the bottom, carrying with it the whole of the orange xanthophyll* and the greater part of the blue chlorophyll, leaving some of the latter in the alcohol along with nearly all the fucoxanthine and chlorofucine. This solution should then be removed by a pipette, and agitated with a fresh quantity of bisulphide, the process being repeated until it subsides with only a very slight tinge of green. The alcoholic solution, on being evaporated and treated with bisulphide, yields approximately pure fucoxanthine. Uniting the different lots of bisulphide containing chlorophyll, and agitating them over and over again with fresh spirit, taking care that a small excess of bisulphide is always present, the whole of the chlorophyll may be removed, and the orange xanthophyll left in the final residue. On adding a little water to the washings containing the chlorophyll, the dissolved bisulphide is precipitated, carrying down all the chlorophyll. By proceeding in a similar manner, with or without the addition of weak acids or alkalies, various other colouring-matters found in plants may be more or less perfectly separated. All these processes should be carried on without exposure to strong daylight, or else some of the constituents might be in great measure destroyed.

Photochemical Analysis.

There are many cases in which certain colouring-matters cannot be separated in a satisfactory manner by merely chemical methods, and then fortunately we are able to resort to what I propose to call *photochemical analysis*. The action of light on the coloured constituents of plants was made the subject of an excellent memoir by Herschel, published in the Philosophical Transactions for 1842, p. 181; but the object he had in view and the methods he employed were altogether different to mine. He adopted no means for separating the different constituents of the plants, and some of his solutions probably contained as many as ten different coloured substances; and, moreover, at that time no attention had been paid to their spectra. He exposed paper coloured with such mixtures to the solar spectrum, so as to learn the effect of each portion; and his results are of great interest, as showing that the decomposition

* The exact use of all these terms will be explained in the sequel.

is due to the luminous rays, which act with variable intensity in different parts of the spectrum, according to the nature of the substance. On the contrary, my method of photochemical analysis consists in using either the whole or particular rays of the sunlight passing through coloured glasses as reagents to decompose some of the constituents of a mixed solution, and leave others in a state of approximate purity. In employing light for such a practical purpose, it would be almost impossible to use a spectrum. Without this method the study of the colouring-matters of plants could not be carried out in a successful manner. I have sometimes studied a substance for several weeks before I was able to prove that it was a mixture by ordinary chemical methods, whereas I afterwards found that this could be easily proved by simply exposing the solution to the sun. I, however, now give only such examples as have an important bearing on the subject before me. For instance, in the case of some *Algæ* it is impossible to separate the xanthophyll from the orange xanthophyll, and the spectrum of the mixed solution in bisulphide of carbon gives absorption-bands in an intermediate position, not agreeing with those characteristic of either one or the other. It might thus be imagined that these *Algæ* differed entirely from allied species, in containing a single and peculiar kind of xanthophyll instead of the usual two, thus making an apparent complete break in what is really perfect continuity; but by exposing the solution to the open sun the orange xanthophyll is so much more rapidly destroyed than the xanthophyll, that in a while the absorption-bands of the latter are seen in their normal position; and knowing this fact, and comparing the spectrum with that of the original, it is easy to see that at first there must have been a mixture of the two kinds. Of course in all such experiments care must be used to prevent overexposure, and the specimens should be examined from time to time. In most cases it is also extremely difficult to separate yellow xanthophyll from lichnoxanthine; but by exposure to the sun this xanthophyll is soon decomposed, its absorption-bands disappear, and the uniform absorption of the lichnoxanthine remains. Solutions which originally give very different spectra can thus be proved to be merely variable mixtures of two or more substances, each well known in a pure state. There are, however, cases in which both constituents would be destroyed by exposure to white light; but one is changed chiefly by one kind of rays, and the other by another. For example, when the mixed solution in bisulphide of carbon of phycoxanthine and orange lichnoxanthine, obtained from certain *Algæ* and lichens, is exposed to the open sun, the phycoxanthine is soon destroyed, but at the same time some of the orange lichnoxanthine, and it is almost impossible to cease exposing at the proper point; when kept under deep green glass, however, it is easy to decompose nearly all the phycoxanthine without materially diminishing the amount of the orange lichnoxanthine. Other

applications of this method will be given when describing special cases.

General Action of Light.

Independent of its value in such practical analyses, the study of the action of light on vegetable colouring-matters is of great interest in connexion with changes that occur in the plants themselves; but since so much still remains to be learned, I will now only give a general summary of what I have already ascertained. As a general rule, when solutions of such substances are exposed to the open sun, their colour is destroyed without the formation of any intermediate coloured product; but in a few cases entirely different coloured compounds are generated, and afterwards themselves destroyed on further exposure. This bleaching effect depends upon the combined presence of light and air; for if kept in the dark, or if sealed up in tubes quite free from air, and then exposed to the sun, very little or no such alteration occurs. Other things being equal, the rate of change varies enormously according to the nature of the substance, and, with the same substance, varies much according to the solvent.

Sensitizers.

The presence of minute quantities of certain oils very greatly increases the rate of change in a solution exposed to the light, and it is convenient to call this their *sensitizing* action. Probably in many cases it depends upon their *ozonizing* influence. Such substances occur in certain plants, and the result is that the effect of an equal amount of light is sometimes increased threefold or even more. If this substance could be procured pure in moderate quantity it would be a most valuable reagent, since it has very little or no effect on colouring-matters unless exposed to the light. In the mean time I have found turpentine a very useful sensitizer, the chief objection being that it sometimes acts as an oxidizer, even in the dark, and is too energetic, so that very little should be used. Oil of citronelle has the most powerful reverse action of any that I have examined, and greatly reduces the effect of light. I therefore often use it to protect from change.

Correlation of Optical and Chemical Characters.

We might naturally expect that those rays of light which are absorbed most strongly would be most instrumental in effecting decomposition. If this be a law, it is subject to modifying conditions, not yet apparent; and since many of the changes do not take place without the presence of air, they are due not to light itself alone, but to oxidization intensified by its presence. At all events it is not merely the blue end of the spectrum (the so called chemical rays) that act, for some substances are rapidly destroyed in red light, as was shown by Herschel and subsequently by Gerland*. I have met with so many cases where a number of substances

* Archives Néerlandaises, 1872, vol. vii. p. 1 *et seq.*

having many properties in common, but differing in the position of their absorption-bands, are decomposed when exposed to the light in the order in which the bands lie towards the red end, that I cannot but think there is some connexion between these facts. When the spectra are quite unlike (for example when one has two and the other three bands, or two at a different interval, or no bands at all) there is no such connexion. Those which seem to approach most closely and uniformly to the law belong to simple groups, and give spectra more or less closely analogous, as it were, to the same musical chord in different keys; and this leads me to think it probable that, if we had such a perfect series of substances, the rate at which they would be decomposed by the action of light would vary directly as the length of the waves absorbed, or as would express the facts equally well in many cases, as though the power of decomposition varied directly as the width of the spectrum over which the absorption extends, *i.e.* as the number of different rays absorbed. This would also, in great measure, correspond to what occurs when the same substance is dissolved in liquids which cause the absorption to extend more and more towards the red end. Whether this be a general law or only a common fact, the results are of very great importance in connexion with my present subject. The effect of this difference in the rate of change will be better understood by means of the following rough illustration. Suppose that there is a mixture of three analogous colouring-matters, *a*, *b*, and *c*, the absorption-bands of *a* being nearest to the red and those of *c* to the blue end of the spectrum, and suppose that on exposure to the sun, in the time required to destroy one part of *c*, two parts of *b* and three of *a* are decomposed, it is obvious that the original proportion between the different substances would soon be very materially changed. This would alter the ratio in which they would be decomposed by further action until a sort of equilibrium was established; so that, whatever might have been the original proportion, there would at length be a sort of ultimate vanishing ratio, depending on the nature of the substance and of the solvent and on the kind of light and its intensity. I find this fluxional manner of viewing such facts often very useful, and cannot but think that it might be introduced with advantage into similar subjects. However, for the sake of simplicity, supposing that the assumed rate of decomposition remained constant, and adopting arbitrary units, which only express the ratios for each substance, we should have some such result as the following:—

| | | |
|--|-------|-------------------|
| Original state | | $10a + 10b + 10c$ |
| Successive states, after more and more exposure to the sun. | { | $7a + 8b + 9c$ |
| | | $4a + 6b + 8c$ |
| | | $a + 4b + 7c$ |
| | | $2b + 6c$ |
| | | $5c$ |

If instead of an analogous substance (*a*) we had an entirely different

(A), very slightly affected by light, the result might be expressed thus:—

$$\begin{array}{l} \text{Original state} \dots\dots\dots 10A + 10b + 10c \\ \text{Subsequent states} \dots\dots\dots \left\{ \begin{array}{l} 9A \quad + \quad 5c \\ 8A \end{array} \right. \end{array}$$

Comparative Quantitative Analysis.

By applying the above-described general methods to the study of the mixed coloured solutions obtained from plants, there is usually no serious difficulty in carrying out a *qualitative* analysis, even though, as often happens, half a dozen or more coloured substances are present. It is, however, often very desirable to determine them *quantitatively*; not, indeed, their relative *weights* in one case, but the relative amount of each particular colouring-matter in different plants or in the same when grown in different conditions—a kind of *comparative quantitative* analysis. In some cases this can be done without difficulty. The various coloured constituents can be separated with sufficient accuracy, and the relative volumes of equally strong solution determined. The most convenient plan is to have test-tubes of the same internal diameter, and to dilute the solution in one or in both until the intensity of the colour is exactly the same, or, still better, until the characteristic absorption is equal, as seen in the spectroscope, with equal illumination. The relative length of the column in the tubes then, of course, gives the relative amount of the particular colouring-matter. We may also thus ascertain the relative quantity of two or even more in the same solution, by making the respective bands equal, first in the case of one and then in that of the other. I intend to construct a form of spectrum-microscope and prepare apparatus specially suited for accurate comparisons, which require perfectly equal illumination; but even those analyses which I have been able to make by means of my present instruments are sufficient to show the value of such quantitative methods. I have hitherto chiefly compared the different specimens together, but for the future I shall prepare suitable standard solutions, sealed up in tubes with as little air as possible, protected by oil of citronelle; and if it turn out that they remain unaltered by long keeping, it will thus be possible to determine the relative amount of the various colouring-matters in plants growing at different seasons of the year.

Fundamental Colouring-matters.

Trusting that the above account will be sufficient to indicate the general characters of the methods I have employed, I now proceed to the consideration of the individual colouring-matters. The total number met with in plants is very great, possibly some hundreds; but a large proportion occur only in the petals, fruits, seeds, and roots. The study of these will no doubt throw much light on particular questions, but

they are of far less importance in connexion with my present subject than those which occur in the leaves of the higher or in the fronds of the lower classes of plants. I purpose, therefore, to confine my remarks almost exclusively to these. Even then it is very necessary to distinguish between those which appear to be essential for the healthy growth of each class of plants and those which have the character of accidental products, formed under particular conditions, and often absent, without apparently interfering with the normal development of the plant. There is exactly the same difference in the case of animals. The hæmoglobin of the blood and the coloured substances in bile are of the utmost physiological importance, whereas those in the hair and feathers are either of no importance whatever, or are of advantage to the animal only in some very indirect manner. Similarly, the colouring-matters belonging to the chlorophyll and xanthophyll groups appear to be essential, whereas those of the erythrophyll group have all the characters of products varying with accidental circumstances; and if they are of any value to the plant, it is only indirectly, as, for instance, in attracting to the petals insects instrumental in causing fertilization. These accidental and unessential colouring-matters are often so much more conspicuous than those of real importance, that they might easily lead any one to conclude that the study of the colouring of plants could not lead to satisfactory results, since very often the colour is not of value even in distinguishing different species. It is, however, not the mere *colour* that is of importance, but the nature and relative amount of what may be called the *fundamental colouring-matters*, of which there are altogether somewhere between a dozen and a score. For example, we often have varieties of plants which have red leaves instead of green: this is owing to the unusual amount of erythrophyll; and, independent of this, the fundamental constituents of the higher classes of plants are present in their normal quantity, or their equilibrium only slightly modified by a corresponding difference in conditions. In those classes of plants where conspicuous accidental colouring-matters are seldom developed (as, for instance, in the different groups of marine *Algæ*), the importance of the colour is universally admitted; and I find that when studied in accordance with the principles described in this paper, the presence or absence of the different kinds of colouring-matters, or their relative amount in different classes of plants, is in some way or other connected with very important differences in organization and vital energy.

Description of the Groups of Colouring-matters.

In accordance with these principles, I now propose to describe those colouring-matters which appear to be of the greatest physiological importance, dividing them into various groups, and in the case of each group arranging them in the order of their optical characters—*i.e.* commencing with those in which the absorption extends nearest to the red

end of the spectrum, and following the order in which it retreats towards the blue. This is, moreover, the order in which they are decomposed by light, and also often that in which they vary in other important peculiarities. I have chosen specific names which indicate the generic relations of the separate substances.

Stokes's Researches.

In the Proceedings of the Royal Society for 1864* there is a paper by Professor Stokes, rather more than a page long, on the supposed identity of biliverdin with chlorophyll, with remarks on the constitution of chlorophyll, which, in my opinion, in the space of a few sentences contains more valuable information on that subject than all other subsequent papers put together. In this he says, "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. . . . Green sea-weeds agree with land-plants, except as to the relative proportions of the substances present; but in olive-coloured sea-weeds the second green substance is replaced by a third green substance, and the first yellow substance by a third yellow substance, to the presence of which the dull colour of those plants is due." No particulars were given respecting the manner in which these different colouring-matters had been separated, nor any description of their individual characteristics, and this is probably the reason why these statements have had so little influence on subsequent researches. If I may judge from my own experience, an experimenter might well have paid a great amount of attention to the subject without being able to discover the nature of the evidence which had led the author to adopt such conclusions; and it would require a very considerable amount of independent discovery to enable any one to appreciate their real merit. As for myself, I investigated the subject quite independently, and it was not until after I had satisfied myself as to the real facts of the case that I compared my results with those described by Stokes in the above-named paper, and then saw that they corresponded in many of the most important particulars, though I have no doubt that I was led to them by studying a very different class of facts. The principal green substance of the author is manifestly my blue chlorophyll; his second green substance my yellow chlorophyll, and his third green substance my chlorofucine, whilst his third yellow substance, found in olive sea-weeds, must be my fucoxanthine. His other two yellow substances must, in some way or other, represent the four yellow substances described by me as orange xanthophyll, xanthophyll, yellow xanthophyll, and lichnoxanthine, with perhaps a little of my orange lichnoxanthine. It will thus be seen that my conclusions fully confirm what he has said respecting the compound

* Vol. xiii. p. 144.

nature of chlorophyll (I mean of the green constituent of leaves, independent of the yellow colouring-matters), though, as far as I am aware, no one else has hitherto adopted that opinion. Having said this much, I now proceed to give an account of my own experiments, and of the conclusions which seem to explain the facts in a satisfactory manner.

Chlorophyll Group.

This comprises three perfectly distinct substances, soluble in bisulphide of carbon and insoluble in water, which agree in having absorption at both ends of the spectrum, in having well-marked absorption-bands at the red end, to the principal of which the strong red fluorescence is related, and in being decomposed with greater or less facility by weak acids into new products, giving somewhat analogous, but yet quite distinct spectra to those characteristic of them in their natural state. All are rapidly decomposed in strong sunlight, when air is also present, but not otherwise.

Blue Chlorophyll.

I was first led to distinguish the compound nature of the chlorophyll in ordinary green leaves by studying that found in *Oscillatoria*, as named in former papers. This kind is that which occurs throughout the whole vegetable world, and the difference consists in the usual but not universal presence of another along with it, which second kind differs in the highest and in the very lowest classes of plants. Blue chlorophyll can be best obtained in an approximately pure state from olive *Alga*, like *Fucus* or *Laminaria*, by the method already described. Its spectrum shows three absorption-bands at the red end, that nearest the extreme red being by far the most intense. Unless the solution be very strong, the whole of the green and a considerable portion of the blue are transmitted, so that the colour is such a blue-green that the term *blue chlorophyll* appears to be very appropriate. Any decided band in the green is proof of the presence of a small quantity of the well-known product of the action of the acids, which very readily decompose it. This change may be avoided by crushing up acid plants with a little bicarbonate of ammonia before dissolving the chlorophyll; but it is far better to make use of those whose juice is more neutral. Mere boiling in water, as adopted by some experimenters, is not sufficient, since the alteration will take place even when the chlorophyll is not in solution. The absorption at the blue end is due to a broad band between the blue and the violet, which can be seen only in a moderately pure solution, with excellent daylight. Besides the product of the action of acids just alluded to, several others can be prepared from blue chlorophyll by decomposing it with acids or alkalis; but they are of little interest in connexion with living plants, except as showing that it is in a state of very unstable equilibrium.

Yellow Chlorophyll.

This substance has not been obtained in a pure state, but yet suffi-

ciently so to enable me to determine many of its peculiarities. The best method of obtaining it is to boil *Ulva latissima* first in water and then in spirit. If this plant cannot be procured, the small green *Algæ* so common on damp wood and stones may be used with advantage, and need not be boiled in water. The deep green solution in alcohol should be agitated with bisulphide of carbon, and a few drops of water added, if requisite, to cause nearly all the green colouring-matter to be carried down, and nearly all the xanthophylls to remain in solution. On removing this and agitating the bisulphide with fresh spirit of wine of the usual strength, taking care to have a considerable excess of the bisulphide present, a large portion of the yellow chlorophyll and some of the blue are dissolved. Removing this solution to another tube, adding a little water, and separating the diluted alcohol, the precipitated bisulphide should be evaporated to dryness at a gentle heat. On redissolving in benzole and examining the spectrum of a properly diluted solution, it will be seen that it differs in many important particulars from that of pure blue chlorophyll. Besides bands exactly corresponding with those characteristic of that substance, it has an independent dark and narrow band in the red, and another much broader in the middle of the blue, as though the solution were a mixture of blue chlorophyll with some other substance*—a conclusion completely borne out by further examination, since the same blue kind as that found in olive *Algæ* occurs almost free from this second substance in the residue left after repeated agitation with spirit. Taking, then, a solution in benzole of blue chlorophyll, of such a strength that the principal absorption-band in the red may exactly correspond with that seen in the above-named mixture, and comparing together the spectra side by side, it is easy to see what absorption-bands are due to the other substance. Again, since blue chlorophyll is far more easily decomposed by weak acids than the yellow, and yields a product very slowly decomposed by light, we may take a mixed solution in which the blue has undergone this change, and, by treating it in the manner described above, may obtain a mixture of yellow chlorophyll and the product of the action of acids on the blue. Dissolving this in benzole, and taking a solution of the product of the action of acids on the pure blue, of such a strength as to give the corresponding absorption-bands equal when seen side by side, those due to the yellow chlorophyll can easily be distinguished; and on exposing for a while to the direct rays of the sun, these bands disappear, whilst those due to the product of the action of acids on the blue kind remain just as in the specimen used for comparison. By these means I have been able to prove that the spectrum of yellow chlorophyll dissolved in benzole has a very dark and well-defined narrow band in the red, further from the red end than that due to blue chlorophyll, and probably another much fainter in the orange. There is also a broad and strong

* See my paper "On Mixed Colouring-matters," Monthly Microscopical Journal, vol. vi. p. 124.

band in the middle of the blue; the whole of the blue is absorbed, and not, as in the case of blue chlorophyll, a considerable portion transmitted. The general colour is therefore such a yellow-green that the term *yellow chlorophyll* seems very appropriate. When dissolved in alcohol the principal band in the red is made about twice as broad, and half as dark, as when in benzole or bisulphide of carbon, and can no longer be seen quite detached and separate from that due to blue chlorophyll, especially if the relative amount of the latter be considerable, and merely causes an unsymmetrical shading on the side towards the orange. It is to this circumstance in great measure that must be attributed the fact of the compound nature of chlorophyll having been overlooked, alcohol having been so generally used as the solvent.

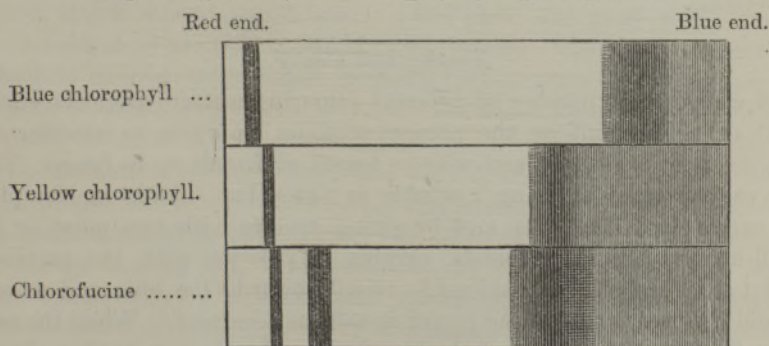
Chlorofucine.

It is some time since I was first led to believe that a third kind of colouring-matter belonging to the chlorophyll group existed in some freshwater *Algæ*. I first obtained it separate from blue chlorophyll in examining a complicated mixture of that and other substances obtained by Mr. Charles Horner from an *Actinia*, viz. *Anthea cereus*, var. *smaragdina*. The special bands due to this other substance had attracted his attention, and led him to think that the chlorophyll, as a whole, was different to that in plants. I have since found that this third member of the group is one of the fundamental constituents of *Fucus* and other olive *Algæ*, and have therefore named it *chlorofucine*. In order to obtain it approximately pure, the fresh fronds should be crushed in a mortar and slightly dried, so that on adding ordinary spirit of wine too much water may not be present. For the same reason the solution should be kept covered up at a gentle heat, and not boiled. On agitating with repeated quantities of bisulphide of carbon, the whole of the blue chlorophyll may be removed, and the alcoholic solution then contains much fucoxanthine and the chlorofucine, showing two well-marked absorption-bands, one between the red and the orange and the other in the yellow. On adding water and agitating with more bisulphide, both these substances are carried down; and on evaporating the mixed solution, the chlorofucine is often partially lost by decomposition in some unknown way. To prepare it free from fucoxanthine, the above-named alcoholic solution should be diluted with an equal bulk of water, a drop or two of ammonia added, and agitated over and over again with fresh bisulphide, until it subsides to the bottom almost colourless. By thus adding ammonia the chlorofucine is retained in solution, and the whole of the fucoxanthine is carried down in the bisulphide. The dilute alcoholic solution is then of a pale green colour, and contains chlorofucine mixed with a yellow substance soluble in water. On evaporating to small bulk at a gentle heat, the loss of alcohol causes impure chlorofucine to be deposited; and after transferring to a narrow tube, allowing it to subside, and removing the yellow aqueous solution, almost pure chlorofucine

can be dissolved out from the deposit by means of absolute alcohol: at all events it is free from chlorophyll, and perhaps only contains a small quantity of the pale yellow substance. The colour of this solution is a yellow-green, very much like that of a crude solution from green leaves. The spectrum shows two dark absorption-bands, one between the red and the orange, and another between the orange and the yellow. The whole of the green is transmitted, but the whole of the blue cut off; and when the solution is dilute, a broad and somewhat obscure band may be seen in the centre of the blue, if excellent daylight is used. The addition of a little ammonia slightly increases the intensity of the band nearest the red end. When thus modified by dilute alkalies, chlorofucine is very sparingly, if at all, soluble in bisulphide of carbon. Excess of hydrochloric acid causes immediate decomposition into a new compound, with a single absorption-band in the orange. Weak acids slowly produce the same effect; and it is important to bear this in mind, since this product of the action of acids, and not the original substance, is met with in studying certain plants.

The difference between the spectra of the above-named three green substances will be better understood by means of the following figure, which represents the absorption-bands as seen in solutions diluted so as to show those at the blue end, and only the darkest and most characteristic of those in the red.

Fig. 1. *Spectra of the chlorophyll group compared.*



Fluorescence of the Chlorophyll Group.

The position of the narrow bright red bands seen in the spectra of the light of fluorescence of these three members of the chlorophyll group differs even more than that of the absorption-bands, since in the case of chlorofucine the bright orange-red band almost coincides with the dark band of absorption, whereas in the case of both blue and yellow chlorophyll the more red bands lie just on the red side of the centre of their absorption-bands. The result is that the three different narrow red bands characteristic of the light of fluorescence of the three different

substances have their centres nearly at equal intervals. All three kinds are so rapidly decomposed by strong direct sunlight, that the study of their fluorescence is a matter of some difficulty. Whilst thus alluding to this subject, I would take the opportunity of describing a method which I have found extremely convenient in deciding whether a substance possesses genuine fluorescence, or whether it is spurious and due to the presence of solid particles so minute that the solution may appear quite clear and transparent. I illuminate by means of light which has passed through a solution of sulphate of didymium. If, then, there be only a spurious fluorescence, the spectrum shows all the absorption-bands of the didymium—in fact we obtain merely a spectrum of that body modified by reflection; whereas if it be a true and pure fluorescence, no trace of the bands of didymium can be seen. If there be a genuine fluorescence along with some reflected light, a more or less faint spectrum of didymium will be visible due to this spurious light, with a brighter part due to the genuine fluorescence.

Action of Acids on the Chlorophyll Group.

As previously mentioned, all the above-named members of the chlorophyll group are decomposed with greater or less facility by acids. Each gives rise to an independent product; so that, though they have many generic peculiarities in common, they must be regarded as well-marked species, and not in any way as mere accidental modifications of a single substance.

Xanthophyll Group.

A considerable number of different colouring-matters might be classed with this group, but on the present occasion I purpose to consider only the six most commonly met with in leaves or fronds or in fungi. They are characterized by being insoluble in water, but soluble in bisulphide of carbon and fixed oils, and by giving spectra with two more or less well-marked absorption-bands, varying in position with the particular substance, and very considerably raised towards the blue end when a liquid of great band-raising power is used as a solvent. When the solution is strong, the whole of the blue end is absorbed up to these bands. Sometimes three absorption-bands may be seen; but in that case it is generally easy to prove, either by chemical or photochemical methods, that the third band is due to a second substance. Weak acids or alkalis have no immediate effect in altering the position of the bands; but when dissolved in absolute alcohol all the members of the group are slowly decomposed by a little hydrochloric acid, gradually fading to a colourless solution, even in the dark. The rate at which this takes place and the production of intermediate coloured products depend to some small extent on the presence of air, but still more on the presence of minute quantities of sensitizing substances. The nature of this action is still

rather obscure; but a further study of it seems likely to throw light on the cause of the production of some of the colouring-matters found in particular plants. When such a sensitizing substance is absent, so that the alteration takes place too slowly, a little turpentine is an excellent substitute. The changes which occur vary according to the particular species of the group, and thus furnish a valuable means for distinguishing them, or for recognizing small quantities of some when mixed with others.

Phycoxanthine.

This name was first proposed by Kraus* for a substance he obtained from *Oscillatoria*; but there is no doubt whatever that his preparation was a mixture of two or three distinct kinds of colouring-matters, which can easily be separated, and do occur separately in other plants. That for which I adopt the name may be obtained in the most pure state from the lichen *Peltigera canina* when growing in a very damp and shady place, or from some species of *Oscillatoria* when growing quite open to the sun. The various coloured substances should be dissolved out by hot spirit, and the solution when cold diluted, if requisite, with a little water, and agitated over and over again with fresh quantities of bisulphide of carbon until it subsides tinged only a pale pink. By this means almost the whole of the other colouring-matters are carried down, with the exception of the lichnoxanthines. On evaporating the alcoholic solution and redissolving in bisulphide of carbon, a red solution is obtained, which, when more dilute, is a fine pink, and gives a spectrum with two excellent absorption-bands in the green. When it is dissolved in absolute alcohol these bands lie much further from the red end, and the colour is sufficiently yellow to justify the name *phycoxanthine*, or, at all events, to make it undesirable to introduce another. On adding a little hydrochloric acid and turpentine, the colour gradually fades, without the production of any well-marked intermediate coloured substance. I am much inclined to believe that this phycoxanthine may be formed from orange xanthophyll, both artificially and naturally; but since this change occurs only in very rare and special cases, it probably depends on the presence of some sensitizing substance, only occasionally present or under conditions not yet understood.

Peziza Xanthine.

This is the name I propose for an orange-yellow colouring-matter found in *Peziza aurantia* and some other fungi. It resembles phycoxanthine in many particulars, but differs in the position of the absorption-bands, which lie further from the red end.

Orange Xanthophyll.

This is one of the most universally distributed of all vegetable colour-

* Chlorophyllfarbstoffe, p. 109.

ing-matters, since it occurs in greater or less quantity in all classes of plants, including fungi. It may be obtained in its most pure state from the orange-coloured antherozoids of *Fucus serratus* in spring, which exude from the antheridia when the fronds are kept damp. These may be washed off the fronds into water and separated by filtering, after any coarser particles have subsided. The colouring-matters should then be dissolved out in hot strong alcohol, and the clear solution agitated with bisulphide of carbon. This carries down the orange xanthophyll, which may be somewhat further purified by repeated agitation with fresh alcohol. It may also be obtained from *Peltigera canina* or from *Oscillatoria* grown in bright light by a similar process; but in that case, in order to separate the chlorophyll, it is requisite to agitate very many times with fresh spirit and repeated additions of bisulphide of carbon, so as always to leave only a small quantity undissolved. The success of this method depends upon the fact that, unless the relative amount of the orange xanthophyll be too great, the whole is carried down in the bisulphide of carbon, when agitated with spirit of the usual strength. The amount present in the leaves of the higher classes of plants is smaller than in those just named; but by following the same process a final residue of orange-colour may be obtained, which, however, is seldom free from impurities. When pure and dissolved in bisulphide of carbon, the colour is a slightly pink-orange, and the spectrum shows two obscure absorption-bands in the blue end of the green and green end of the blue. These lie much further from the red end when it is dissolved in absolute alcohol; and on adding a little hydrochloric acid and turpentine it gradually becomes colourless, sometimes turning slightly green; but this is probably due to the presence of another substance—generally to a trace of yellow xanthophyll.

Xanthophyll.

I propose to restrict this name, as applied to an individual substance, to the principal constituent of the mixture which has usually been so called. This generally, if not always, must have been mixed with my yellow xanthophyll and lichnoxanthine. Xanthophyll, as the term is applied by me, may be obtained in the most pure state from *Porphyra vulgaris*, by heating in spirit, removing the blue chlorophyll by agitation with bisulphide of carbon, and then adding water to the alcoholic solution, which precipitates the bisulphide along with the xanthophyll. The solution in bisulphide of carbon is of a slightly orange-yellow colour, and gives a spectrum with two absorption-bands between the green and the blue. When dissolved in absolute alcohol they lie at the green end of the blue; and on adding a little hydrochloric acid and turpentine the colour gradually disappears, without the production of any new intermediate coloured substance, or only such a faint tinge of green as is probably due to impurity. Xanthophyll may also be procured from dark-coloured varieties of Wallflowers (*Cheiranthus cheiri*), and with more

difficulty from green or yellow leaves; but being then mixed with yellow xanthophyll, it is almost impossible to obtain it pure, and it therefore turns green when treated with hydrochloric acid and turpentine. If no sensitizer be present, such mixtures may change so slowly that the blue product may fade as fast as it is formed, and never be very visible.

Yellow Xanthophyll.

This may be obtained in its most pure state from different kinds of bright yellow flowers, as, for instance, the yellow *Chrysanthemum*. When dissolved in bisulphide of carbon it is of a clear lemon-yellow colour, differing most decidedly from that of xanthophyll properly so called. The spectrum shows two very distinct absorption-bands in the green end of the blue: these lie still further within the blue when absolute alcohol is used as the solvent, and, on adding a little hydrochloric acid, the yellow xanthophyll is decomposed, first into a new and paler yellow substance*, with the absorption-bands at a different interval and more towards the blue end, and afterwards into a fine deep blue colouring-matter, which will be more fully described further on. Yellow xanthophyll may be obtained in an impure state from ordinary green leaves, by first removing the chlorophyll and then adding successive small quantities of water, agitating with bisulphide of carbon after each addition, and keeping separate each quantity thus carried down. The first contains much xanthophyll and a little yellow xanthophyll, and each subsequent quantity contains less of one and more of the other, as may be proved by both chemical and photochemical methods, or by the position of the absorption-bands, until, towards the last, the yellow xanthophyll greatly preponderates, mixed, however, with so much lichnoxanthine that the absorption-bands seen in its spectrum are far less distinct than when it is prepared from the petals of such flowers as I have named. On treating the solution in absolute alcohol with hydrochloric acid, though it turns to a blue-green, a residual yellow colour (lichnoxanthine) remains when the blue has faded, which is not the case when pure. If the leaves have an acid juice, the last residue consists, not of yellow xanthophyll, but of the above-named yellow product of the action upon it of weak acids.

General relations of the Xanthophyll Group.

These five different kinds of colouring-matters belonging to the xanthophyll group are related to one another in a very interesting manner. Making use of same scale of measurement and notation as described in my former paper †, the centres of the absorption-bands of

* This fact has also been noticed by Stokes (Journal of Chem. Soc. vol. ii. 1864, p. 309).

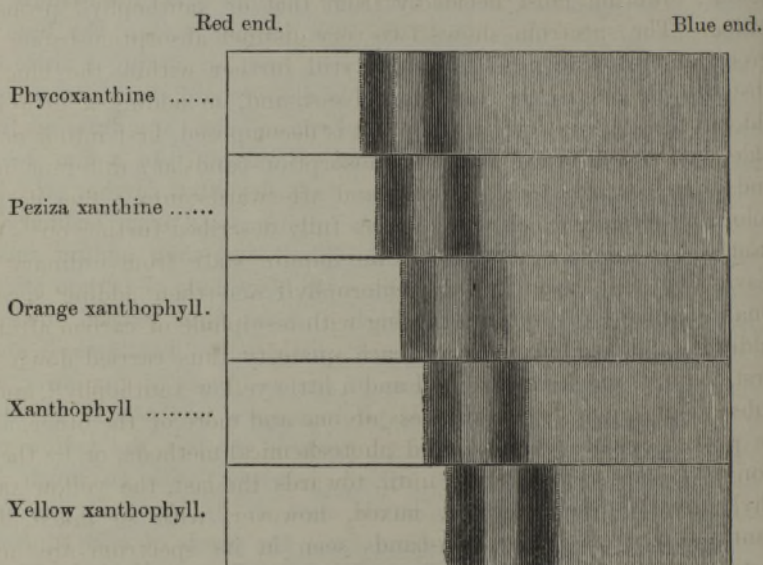
† Proceedings of Roy. Soc. 1867, vol. xv. p. 434.

these various substances, when dissolved in bisulphide of carbon, are very closely as follows ($D=3\frac{1}{2}$, $F=7\frac{1}{2}$):—

| | | | |
|------------------------------|----------------|----------------|----------------|
| Phycoxanthine | 5 | $6\frac{1}{2}$ | |
| Peziza xanthine | $5\frac{1}{2}$ | 7 | |
| Orange xanthophyll | 6 | $7\frac{1}{2}$ | |
| Xanthophyll | | $6\frac{1}{2}$ | 8 |
| Yellow xanthophyll | | 7 | $8\frac{1}{2}$ |

These relations will be better understood by means of the following figure:—

Fig. 2. *Spectra of the xanthophyll group compared.*



When these various substances are dissolved in benzole, their absorption-bands are all equally raised towards the blue end, so that we appear to have a remarkable series of very closely related substances.

Action of Light on the Xanthophyll Group.

The behaviour of these substances when exposed to light is very interesting. Taking a mixture of any two in bisulphide of carbon, and exposing to the open sun, that which has its absorption extending over the wider space, and has its absorption-bands the nearer to the red end, is destroyed more rapidly than the one of which the absorption extends over a less space, and the absorption-bands lie nearer to the blue end. If any contiguous two species be mixed, the rate at which they are decomposed differs so little that very much of both must be destroyed before the one whose absorption lies the nearer to the blue end is left

almost free from the other. On the contrary, when we have a mixture of two more extreme, a very considerable quantity of one is left when the other has disappeared.

I fear it will be difficult to find any other such series of very similar colouring-matters; but if, on further investigation, such a connexion between the rate of decomposition and the absorption of light turn out to be a general law, it will be an additional proof of some kind of connexion between chemical and optical characters. The most striking modification of this rule that I have so far met with, is in the case of phycoxanthine and orange xanthophyll when mixed with chlorophyll in solution. When alone, dissolved in a mixture of alcohol and bisulphide of carbon, the phycoxanthine is so much more rapidly decomposed by bright sunlight than the orange xanthophyll that the relative amount of the latter is soon increased to double the original, as determined by separating them from one another; whereas if a considerable quantity of blue chlorophyll be present, the very opposite result occurs, as proved by comparative analysis. Here, then, we have a most striking effect of the presence of the chlorophyll, which itself was more rapidly decomposed than either of the other substances mixed with it. Having only very recently established this fact, I have not yet been able to decide whether it is due to the loss of the energy in decomposing the chlorophyll of those rays of light which, in its absence, would have been instrumental in decomposing the phycoxanthine, or whether it depends upon some chemical action connected with the presence of the changing chlorophyll; but it seems to me that we have in such a case the first example of a new branch of inquiry, which can hardly fail to be of value in explaining the influence of chlorophyll in the growth of plants. Many facts seem to prove that one substance may protect another by absorbing the active rays or by consuming the oxygen, which, in its absence, would decompose the other.

Fucoxanthine.

This is the name I propose for the principal colouring-matter of *Fuci* and other olive *Algæ*. It may be obtained in the manner already described, only that in order to separate it from the chlorofucine, after separation of all the chlorophyll, a few drops of ammonia should be added to the alcoholic solution, and the whole diluted with an equal bulk of water. The bisulphide of carbon is then precipitated with almost all the fucoxanthine, whilst nearly the whole of the chlorofucine remains in the dilute alcohol. As thus purified, fucoxanthine dissolved in bisulphide of carbon is of a beautiful amber colour, and its spectrum shows two obscure absorption-bands, the position being intermediate between those of orange xanthophyll and xanthophyll, so that a mixture of these gives nearly the same spectrum. The difference, however, is completely proved by other facts. The bands of fucoxanthine are much less raised by alcohol and other liquids of high band-raising power than the bands of

those two kinds of xanthophyll, and it resists the action of light far more than they do. A mixture of pure orange xanthophyll and xanthophyll in absolute alcohol treated with hydrochloric acid would, at the most, give only a pale green, whereas fucoxanthine is changed into a splendid blue substance, and subsequently into a sort of claret-coloured, before finally and slowly fading. Though the spectra of fucoxanthine and yellow xanthophyll are essentially different, yet this blue product is the same. It absorbs the whole of the red end of the spectrum, not transmitting even the extreme red; and on adding an excess of ammonia this absorption is entirely removed, and the colour is changed to a bright yellow. The spectrum then shows a well-marked absorption-band at the violet end of the blue. On adding excess of hydrochloric acid, the original blue colour is restored; and hence this substance has the unusual peculiarity of being made blue by acids and yellow by alkalies. Hitherto I have never met with it in plants themselves. Taking every thing into consideration, we must look upon fucoxanthine as closely related to xanthophyll; but at the same time the different effect of solvents in raising the absorption-bands, and the greater permanence when exposed to light, may perhaps make it desirable to class it in a subgroup. The dull olive colour of those *Algæ* in which it occurs so abundantly (the *Melanospermæ*) is apparently mainly due to it in a *free state*, not dissolved in any oil. On comparing the spectrum of the light transmitted by a frond in its natural condition with that of the light transmitted by a portion which has been boiled for a short time in water until the colour has changed to green, it may be seen that the absorption due to the fucoxanthine is considerably raised, just as if at that high temperature it were attacked and dissolved by the oil present in the plant.

Lichnoxanthine Group.

This is a name which I propose for a number of colouring-matters found in a great variety of plants. Two are more especially abundant in fungi; but since they occur in a more marked manner in lichens than in any other plants having true leaves or fronds, it appears to me desirable to adopt the name *lichnoxanthine* rather than *fungoxanthine*, which perhaps may be found convenient for some of the substances found more especially in fungi. So far I have met with three different kinds of lichnoxanthine in the leaves and fronds of plants. They are all insoluble in water, and soluble in bisulphide of carbon, but have a far greater tendency to dissolve in alcohol; and in some cases easily pass into a modification which is insoluble in the bisulphide, and yet readily dissolved by absolute alcohol. The spectra do not show any well-marked absorption-bands, but merely a general absorption, extending more or less from the blue end, according to the particular species, and very little if at all modified by the addition of weak alkalies or acids. All are of a much more permanent character than the different kinds of xanthophyll,

resisting the action of light and acids so well that they are very often obtained as residues when all the colouring-matters of that group have been decomposed. They, however, do ultimately fade. It is not yet certain whether these substances are essential for the growth even of lichens, or only constant products.

Orange Lichnoxanthine.

This particular species occurs in many fungi, but I have more especially studied it as obtained from lichens. *Peltigera canina*, growing well exposed to the sun, should be crushed and heated in spirit. After separating the whole of the chlorophyll in the manner already described, the alcoholic solution should be evaporated to dryness, and the lichnoxanthine and the phycoxanthine dissolved out by bisulphide of carbon. On exposing this to the sun under green glass until the pink tinge is lost and the absorption-bands of the phycoxanthine have disappeared, the lichnoxanthine remains of an orange colour. Its spectrum, when the solution is sufficiently strong, shows absorption of the whole of the blue and green, gradually shading off to about the end of the yellow. The modification soluble only in alcohol may be obtained from *Platysma glaucum* and many other lichens by agitating the alcoholic solution with bisulphide of carbon, and then diluting the alcohol with water, so that all the substances soluble in the bisulphide may be carried down in it, all soluble in water remain dissolved, and the modified orange lichnoxanthine may be deposited in a free state, and, after separation, dissolved in absolute alcohol.

Lichnoxanthine.

This is the most common member of the group, and is so widely distributed that it occurs in almost all plants, though sometimes in very small quantity and not easily detected. It is best obtained from the yellow fungus, *Clavaria fusiformis*. In the absence of that plant, it may be prepared from the root of the carrot by dissolving out the colour in spirit, and agitating with bisulphide of carbon to remove the orange xanthophyll and the greater part of the xanthophyll. On evaporating the alcoholic solution to dryness bisulphide of carbon dissolves the lichnoxanthine along with a good deal of xanthophyll and yellow xanthophyll; but by exposing the solution to the sun, those two colouring-matters may be destroyed, and the lichnoxanthine left alone. When pure and dissolved in bisulphide of carbon it is of fine, somewhat orange-yellow colour, and the spectrum shows a very uniform absorption, extending over the whole of the blue, and ending suddenly about the centre of the green. It is so difficult to separate this lichnoxanthine, properly so called, from orange lichnoxanthine, that I shall frequently call the mixture the *lichnoxanthines*, without attempting to give them alone.

Yellow Lichnoxanthine.

I have adopted this name for a substance very similar to that just described, but giving a spectrum in which the absorption does not extend so far from the blue end, so that the colour is a more pure yellow. It is best obtained from *Parmelia (Physcia) parietina* by the same process as that just described.

Phycocyan Group.

I have described the two principal species of this group in former papers*, and will therefore only briefly allude to a few characters. *Phycocyan* gives a spectrum with a well-marked absorption-band in the orange, and has a very intense red fluorescence, due to a narrow red band. *Pink phycocyan* gives a similar kind of spectrum, only the principal absorption-band lies between the yellow and the green. It, too, is highly fluorescent, but the light of fluorescence is orange, due to a much broader band. These two mixed together constitute Cohn's phycocyan. Both are soluble in water, but exist in the plants in a solid form or concentrated solution, as shown by the position of the absorption-bands, which lie considerably nearer to the red end than when the corresponding substances are dissolved in water.

Phycoerythrine Group.

This is the name I propose to adopt for two colouring-matters found in *Algae*, described by me in a former paper†. One is pink, giving a spectrum with an absorption-band at the yellow end of the green, and the other is red, the spectrum showing a band at the blue end of the green. I therefore call one *pink phycoerythrine* and the other *red phycoerythrine*. Neither are fluorescent, and both are soluble in water.

Erythrophyll Group.

This is the name I have applied to a large number of colouring-matters, soluble in water and insoluble in bisulphide of carbon, found in the leaves and petals of plants. I shall not now attempt to describe them in detail, but refer to my former papers for a further but yet very imperfect account of their peculiarities‡. They do not appear to be essential constituents of leaves, but rather products formed under particular conditions. Much remains to be learned respecting the different kinds; for though an accidental constituent, there are important differences between those formed under similar circumstances in the leaves or fronds of different

* Monthly Microscopical Journal, 1870, vol. iii. p. 229, and 1871, vol. vi. p. 124.

† Monthly Microscopical Journal, 1871, vol. vi. p. 124.

‡ Proc. Roy. Soc. 1867, vol. xv. p. 433. Quart. Journ. of Science, 1871, vol. i. p. 64. Quart. Journ. of Microscop. Science, 1871, vol. xi. p. 215.

classes of plants. On the whole they are very slowly changed by the action of light.

Chryso-tannin Group.

I have also described this in the above-named papers. The various substances included in the group are all soluble in water, and of a more or less pale yellow colour; they are very slowly changed by the action of light. The connexion between them and the different classes of plants is still very obscure. They are interesting chiefly in connexion with the production of the tints of autumnal foliage, some of which are due to their oxidization.

Comparative Chromatology.

Having thus described the principal groups of colouring-matters, and those particular species which appear to be of the greatest importance in connexion with the present subject, I now proceed to the consideration of their distribution. The individual species are the elementary coloured constituents which exist in some and are absent from other plants, or occur in a very different proportion, according to the kind of plant or the conditions in which it grows. In my opinion it is only by a careful qualitative and quantitative determination of the coloured constituents that we can arrive at any satisfactory conclusions respecting them. Mere general colour is altogether deceptive: it might lead us to conclude that there was a complete difference in the colouring-matter, when there is merely a difference in the relative amount of the same substances; whilst, at the same time, it might appear to indicate that two cases were alike, when they really differ in most essential particulars. On the contrary, by such a method of qualitative and quantitative analysis the exact differences and the exact character of the connexion may be seen at a glance; and I cannot but think that by carrying out such principles our knowledge of certain questions connected with plants will be very greatly extended. The whole subject, however, is in its infancy; very much remains to be learned respecting nearly every thing connected with it; and what I now describe must be looked upon as very little more than a few general conclusions, which appear to follow from what is already known. We may fully expect that further research will much modify the explanations which now seem the most plausible; but it appears to me undesirable not to point out the general bearings of what is known, merely because they may be modified, or even disproved, by what is still unknown, since a mass of unconnected facts would be of very little interest.

The subject of comparative vegetable chromatology may be divided into two parts, viz.—first, that in which different specimens of the same plant growing in different conditions are compared, in order to learn the effects due to external agencies; and, secondly, that in which different plants growing in similar conditions are compared, in order to ascertain the effects due to internal organization.

Comparison of the same Plants growing in different conditions.

There are many facts connected with the colouring of plants which appear to be almost or quite unintelligible, if we suppose that the colouring-matters when once formed undergo no immediate further change: for example, if we suppose that the chlorophyll is formed in the young leaves in spring, and remains permanent until they fade in autumn. If, on the contrary, we assume as an hypothesis that the various colouring-matters (or at least some of them) are in a state of constant change, we can readily explain many striking facts. Whatever may be the ultimate conclusion, I shall therefore discuss my subject in accordance with the hypothesis that the colouring-matters are being more or less constantly formed by the internal constructive energy of the plants, and decomposed by the destructive action of the oxygen of the air, intensified by the presence of light; and that the condition of each particular part of a plant, at all seasons of the year and under different circumstances, depends upon and expresses the temporary or more permanent equilibrium between these two opposing forces, both of which may vary very considerably. By means of such an hypothesis it is possible to employ accurate physical methods in the study of vegetable physiology; and since the laws which regulate the destructive force may be determined by experimenting with dead matter, we may learn what does and what does not depend on life, and thus be in a better position to understand its essential nature.

According, then, to these principles we may expect to find in plants variations depending on a change in the constructive force, as well as others due to variations in the amount of light to which they are exposed.

Action of Light on Chlorophyll.

When a solution of chlorophyll is exposed to the sun and air it is decomposed, the rate of change and the character of the result varying much according to the solvent, and also depending on the presence of minute quantities of sensitizing or protecting oils. When dissolved in benzole or absolute alcohol, and protected by oil of citronelle, the chlorophyll slowly disappears, and very little of a red colouring-matter is formed, or, if it be formed, it is destroyed nearly as quickly, not accumulating so as to become conspicuous. On the contrary, when the chlorophyll is dissolved in bisulphide of carbon, the change is far more rapid, and several different crimson and red substances are formed, the production of each particular kind depending on a variety of conditions not yet completely understood; and this makes it still uncertain whether some are exclusively or only mainly due to the blue, and some to the yellow chlorophyll. Since these red substances are destroyed by the action of the blue rays, they are obtained to greater advantage by exposing to red light, which acts upon the chlorophyll, but not on these pro-

ducts. It thus appears that chlorophyll is in the first instance decomposed by light into various coloured compounds, which become colourless on further exposure, and that these may be developed so as to be very conspicuous or scarcely visible, according to particular conditions.

Production of Red Leaves.

The red substances thus formed artificially differ from those met with plants in being soluble in bisulphide of carbon, and more rapidly destroyed by the action of light; but yet it appears to me extremely probable that those often found in leaves are products formed by the action of light on chlorophyll, under conditions which have not been reproduced artificially. At all events, by adopting this hypothesis, the changes that occur in many leaves at different seasons of the year admit of very simple explanation. I have constantly noticed that the leaves of some plants are more especially subject to this disappearance of chlorophyll and formation of erythrophyll when the twig on which they grow is partially broken, or when the leaf is actually severed from the plant, but remains in a damp place, so as not to actually wither, especially when the underside is exposed to the light. The change is also often dependent on the presence of parasitic fungi or insects, and often takes place along the dying edge of a leaf, where it is not actually dead, but, so to speak, only half alive; and hence there seems to be no doubt that one essential condition is that the leaf should be in a state of low vitality. It also depends upon the presence of light; for if a leaf that seems likely to change be partially covered with some opaque substance, that part will remain green when the rest has turned red. Such rough natural photographs are often found where one leaf has partially shaded another. This explanation of the facts agrees very well with what occurs at different seasons of the year. The leaves of some plants are almost perfectly green in summer, but as winter approaches they turn more or less red, and on the return of spring these self-same leaves lose their red colour and become green. From this I should draw the very simple and probable conclusion, that as the temperature falls the equilibrium between the constructive and destructive agencies is so much modified by the reduction of vital activity, that the amount of chlorophyll formed is relatively less than that of the red substance, whereas at a more favourable season of the year more of the chlorophyll and less of the red are produced, and the leaves again become green. On this principle it is also easy to understand the reason of the distribution of red colour in different parts of the same plant—as, for instance, only in the tips of the leaves or in the leaf-stalks, and in those parts well exposed to the sun. According to these views of the subject, permanent red varieties of plants are due to a similar difference in the equilibrium, sometimes greatly modified by the conditions in which they grow, the development of the erythrophyll being also generally dependent on exposure to light.

The fact of some kinds of leaves never turning red is analogous to what occurs in the case of particular solutions of chlorophyll. It is not at all improbable that in some cases certain kinds of erythrophyll may be formed independent of chlorophyll.

Waste and supply of the Colouring-matters in Plants.

As is well known, when plants grow in the dark very little or no chlorophyll and other colouring-matters are formed; they are, as it is said, *blanched*. Hence it appears that the production of such substances is some direct function of the action of light. At the same time chlorophyll when in solution is very rapidly decomposed by light, and the other colouring-matters are similarly affected, but in less and varying degree. The power with which they resist decomposition when in living plants is very remarkable, and may in part be due to their not being in solution, and to the unknown force which I have called constructive energy; but it appears to me very difficult or impossible to explain many facts, unless we suppose that light does to some extent exert this decomposing action on the colouring-matters, even when they exist in the leaves. Perhaps, indeed, it may be one of the most important changes that take place in them, and quite essential to plant life. It may seem strange to suppose that any thing can be formed by the action of light and also decomposed by it, but this corresponds with what does certainly occur in artificial experiments. The red substances formed from chlorophyll by the action of light are themselves afterwards decomposed by it, as I have already described; and if we suppose that chlorophyll and other colouring-matters are in some way or other formed through the agency of light from other constituents of the plants, and are afterwards decomposed by further action, we can easily explain a number of remarkable facts. It appears to me that their development is some direct function of light, until a certain quantity of each has been formed and a sort of equilibrium established, varying in its character according to the particular plant, but that at the same time the amount of each decomposed continues to increase directly as the intensity of the light; so that the equilibrium between the different substances is not the same for light of different intensities, but, after having reached a maximum, the quantity of each more or less decreases with increased light. This view of the subject seems justified by such an experiment as the following:—I selected two perfectly similar and contiguous leaves, nearly a year old, growing in the month of May on the outside of an *Acuba japonica*, where much exposed to the sun, and covered part of one with black cloth. After only a week the part of the leaf thus covered up and protected from the light had become most decidedly greener, and after three weeks it was a moderately deep green; whereas the part of the same leaf exposed to the light was still, as at first, the same very yellow-green as the whole of the other leaf which had not been covered up, the line of junction

being sharply defined. I then took exactly equal quantities of the part thus covered up and of the corresponding leaf which had remained the whole time exposed to the light, and determined the relative quantities of the chlorophylls, of the xanthophylls, and of a yellow colouring-matter soluble in water, by the quantitative methods already explained. Taking the amount of each at 100 for the leaf left in its natural exposed state, the relative quantities in that kept shaded were as follows:—

| | Chlorophylls. | Xanthophylls. | Soluble in aq. |
|--------------------------------|---------------|---------------|----------------|
| Natural state | 100 | 100 | 100 |
| Shaded for three weeks | 150 | 75 | 200 |

Here, then, we see that the chlorophyll had increased 50 per cent.; which is an increase of above 2 per cent. per diem; and this, be it remembered, in a leaf nearly a year old. The increase in the yellow colour soluble in water was still greater, whereas the xanthophyll had diminished. This alteration may be greater than common, because I selected the plant on account of its being unusually sensitive to the action of light. Shading a leaf in the manner described above is of course quite a different thing to so smothering up an entire plant as to cause it to become sickly and fade, or to preventing the development of young leaves by keeping them in the dark. It seems to me that such a striking difference, about which there could be no doubt, must prove either that the absence of light is favourable to the development of chlorophyll, which would scarcely agree with other well-known facts, or else that the increase in its amount does in some measure indicate the quantity formed also in the other, but decomposed in the same interval by exposure to the sun. At all events the facts seem to prove that the equilibrium between the different constituents of leaves can soon be changed by altered conditions, as though, like the bodies of animals, the apparent permanence were only an approximately uniform equilibrium between construction and decomposition, the actual chemical changes being of course entirely different in the two cases. We may very well believe that the old and effete colouring-matters are thus destroyed, and that the result of this constant change is that the endochrome in the leaves is always in a young and vigorous condition. I use this term *endochrome* to signify any kind of mixed colouring-matter found in the cells.

Various comparative quantitative analyses.

This change of equilibrium is fully established by a comparison of leaves grown under different circumstances. The exterior leaves of *Acuba japonica*, on the south side, where fully exposed to the sun, are usually very much dappled with yellow patches almost free from chlorophyll, and even the green parts themselves are pale; whereas the leaves in the interior of the plant, where much shaded, have very few or none of the yellow patches, and are of a dark green. There is a similar differ-

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ence in the general colour of the young leaves. I selected for comparative analysis leaves nearly a year old, representing a fair average of those on the outside and in the interior, and, by methods similar to those already described, I separated the colouring-matters into the mixed blue and yellow chlorophyll, orange xanthophyll, and the mixed xanthophyll, yellow xanthophyll, and lichnoxanthine, and obtained the following results:—

| | Chlorophylls. | Orange xanthophyll. | Mixed xanthophylls &c. |
|------------------------|---------------|---------------------|------------------------|
| In the shade | 100 | 100 | 100 |
| In the sun | 31 | 90 | 100 |

I must here explain that in this and similar analyses the numbers in each column have no connexion with those in the other columns. For example, the 100 parts of chlorophyll have no relation to the 100 parts of xanthophyll; they do not indicate equal weights or equality in any other respect. If it were desirable to compare them together, it should, I think, not be by weight, but according to the total quantity of light which they absorb. I take 100 to express the maximum for each colouring-matter, and calculate out the relative amount of the same substance in the other specimen accordingly—that is to say, in this case the amount of chlorophyll in the leaves exposed to the sun is only 31 per cent. of that in those grown in the shade. The orange xanthophyll is in a similar manner reduced to 90 per cent., whereas the mixed xanthophylls &c. are as nearly the same in both as could be ascertained. This great reduction in the quantity of chlorophyll in the leaves exposed to the sun corresponds of course to the increase found in the case of the one covered up and protected from the light, but due to the permanent and normal difference in the amount of exposure to light of different parts of the plant. The effect of this in the case of *Acuba* is unusually great, and apparent at once on comparing the leaves; but I found that there was an analogous difference in the leaves of a deep green common holly (*Ilex aquifolium*), though it would scarcely have been noticed without special examination. I found the relative quantities to be as follows:—

| | Chlorophylls. | Orange xanthophyll. | Mixed xanthophylls &c. |
|------------------------|---------------|---------------------|------------------------|
| In the shade | 100 | 100 | 100 |
| In the sun | 62 | 86 | 94 |

In the case of a lichen (*Platysma glaucum*) I found:—

| | Chlorophylls. | Xanthophylls. | Lichnoxanthines. |
|------------------------|---------------|---------------|------------------|
| In the shade | 100 | 100 | 100 |
| In the sun | 75 | 77 | 100 |

In this case I did not compare equal weights of the fronds, but calculated out the results of the analyses on the supposition that the amount of the lichnoxanthines was the same in both.

For equal weights of the fronds of *Fucus serratus*, in spring, I found :—

| | Blue chlorophyll. | Chlorofucine. | Orange xanthophyll. | Fucoxanthine. |
|-----------------|----------------------|---------------|------------------------|---------------|
| In the shade .. | 100 | 100 | 100 | 98 |
| In the sun | 79 | 87 | 90 | 100 |

Change in Equilibrium due to the Action of Light.

The principal conclusions to be drawn from the above facts are, that leaves exposed to the sun contain nearly (or, at all events, more nearly) the same quantity of the colouring-matters least changed by the action of light as those grown in the shade, but that when grown in the sun those substances which are more and more readily decomposed by the action of light are more and more reduced in amount in the same order. This difference in the equilibrium could be imitated artificially by so contriving that a certain quantity of each colouring-matter should be added at a uniform rate to a solution exposed to the sun. In that case the relative proportion in which they would remain would depend on the rate at which they were introduced and the intensity of the action of the light. Thus, making use of the same sort of rough illustration as that already adopted, we might have :—

| | |
|--|-------------------|
| Added in a given time | $10a + 10b + 10c$ |
| Decomposed in the same time by a moderate light | $3a + 2b + c$ |
| Equilibrium for a moderate light | $7a + 8b + 9c$ |
| Decomposed by equal extra light | $3a + 2b + c$ |
| Equilibrium for much light | $4a + 6b + 8c$ |

In the above illustration the amount supposed to be added at a uniform rate of course represents the quantity of each colouring-matter formed in the leaves in the time required to produce the corresponding decomposition. The results correspond so well that it seems to me a good provisional hypothesis; and the facts are of much interest, as showing that very important changes occur in living plants in accordance with the same laws as regulate the changes in the same colouring-matters when treated as simple dead compounds in experiment tubes.

Changes in Peltigera canina.

I now proceed to a more complicated case. The fronds of *Peltigera canina* contain at least seven different colouring-matters; but in my analyses I did not attempt to distinguish the two kinds of lichnoxanthine, and therefore give them as united. The apothecia are coloured by these latter substances, with little or no trace of any others. The results of

my analyses are calculated out, so as to compare the different specimens, on the supposition that the amount of orange xanthophyll is constant. I examined five examples of the plant, growing in very different situations, viz. :—

I. Where very damp and shady, so that no fructification was developed.

II. Where open to the sun, but slightly shaded by the grass amongst which it grew, and having fructification developed to great perfection, which of course was separated.

III. Rather more exposed to the sun, with no fructification.

IV. Still more exposed to the sun.

V. Very much exposed to the sun, growing on a bare rock, and being of much browner colour than I., II., and III., which were of a most decided green.

| | Blue chlorophyll. | Chlorofucine. | Phyc-xanthine. | Orange xanthophyll. | Lichnoxanthines. |
|------|-------------------|---------------|----------------|---------------------|------------------|
| I. | 61 | 100 | 65 | 100 | 40 |
| II. | 100 | 99 | 100 | 100 | 95 |
| III. | 86 | 73 | 79 | 100 | 100 |
| IV. | 43 | 54 | 76 | 100 | 71 |
| V. | 32 | 51 | 64 | 100 | 67 |

On comparing these together it will be seen that the specimen with such abundant fructification (II.) contained the maximum, or nearly the maximum, quantity of all the different colouring-matters, as though the amount of light were just that best suited for its growth. The specimen in extreme shade (I.) shows a remarkable falling off in some of the constituents, as if the plant were in a sickly state; and the small amount of the lichnoxanthines is particularly interesting, because they are the special colouring-matters of the fructification, which in this specimen was not developed, perhaps for that very reason. The difference in the proportion of the other substances in the specimens more and more exposed to the sun (III., IV. & V.) is in strict accordance with the laws already explained, and the reduction in the amount of chlorophyll is most remarkable. The lichnoxanthines, however, do not follow that law, but seem more connected with the presence or absence of fructification. In reference to this question I may mention that, as before named, the antherozoids of *Fucus serratus* are exclusively, or almost exclusively, coloured by orange xanthophyll, which occurs only in small quantity in the fronds themselves. Taking two portions of the plant, one from the lower part of a frond bearing many well-developed antheridia, and the other from a similar part of a frond of the same specimen with no antheridia, I found that the amount of orange xanthophyll was about 40 per cent. less in the former, as though this substance had been to a considerable extent drawn off from the frond to supply the antherozoids.

There is a decided indication of the same fact in the case of the lichno-xanthine in *Peltigera*, but scarcely sufficient to prove it conclusively.

The above examples must be looked upon merely as indications of what may be done; but they will, I trust, be sufficient to show that the study of the relative amount of the various colouring-matters found in the same plant, under different conditions, is likely to throw much light on certain questions connected with vegetable physiology.

Comparison of different Plants.

Though I have examined a good many of the principal classes of plants, yet there are many that I have not yet been able to procure in a proper state for examination. It is also desirable to investigate more fully certain of the lower classes of animals, especially the sponges and polyps, and to ascertain the laws regulating the distribution of some of the colourless constituents of plants, though that would require entirely different methods of study. Residing, as I do, inland has increased my difficulties, and I have been obliged to rely on what I could learn during a temporary residence in Devonshire, and from material subsequently sent to me by Dr. Baker of Dawlish, Mr. Charles Horner of Mortlake, and other kind friends. At the same time, being able to spend several hours every day in the open country, at all seasons of the year, has been of great value in studying other parts of my subject.

In the present state of the question there is some difficulty in deciding what general principles should be adopted in attempting to arrange the various classes in a natural order, in accordance with the nature or relative amount of the colouring-matters. For example, whether they should be arranged in a single linear series, or in two or more parallel or divergent groups. This difficulty is partly due to the existence of sudden gaps, not yet filled up by intermediate examples, so that the true connexion is not quite apparent. I have constructed a table, representing what is so far known; but I think it better not to publish it until more complete—only to describe some of the principal conclusions deduced from it.

Connexion of the different Groups of Algæ.

Perhaps I cannot choose a better illustration than that furnished by the different groups of *Algæ*—the olive, the red, and the green. They contain at least twelve different colouring-matters, distributed very differently, in such a manner as to connect, and yet to distinguish, the different groups very characteristically. I have not yet made any accurate quantitative analyses, and therefore express the relative amount of the various substances by the following signs:—

| | |
|--|---|
| A relatively large quantity | * |
| A relatively moderate quantity | + |
| A relatively small quantity | . |

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| | Olive. | Red. | Green. |
|------------------------------|--------|------|--------|
| Blue chlorophyll | + | + | * |
| Yellow chlorophyll | | | + |
| Chlorofucine | + | . | |
| Orange xanthophyll | + | + | + |
| Xanthophyll | | + | + |
| Yellow xanthophyll | | | + |
| Fucoxanthine | * | . | |
| Lichnoxanthines | . | . | + |
| Phycocyan | | + | |
| Pink phycocyan | | * | |
| Red phycoerythrine | | * | |

On inspecting this Table it will be seen that the olive *Algæ* are characterized by the relatively large amount of chlorofucine and fucoxanthine, and the total absence of yellow chlorophyll, of xanthophyll, and of yellow xanthophyll. The red are especially distinguished by the colouring-matters of the phycocyan and phycoerythrine groups, but also differ from the olive in containing xanthophyll and very little chlorofucine and fucoxanthine. The green are characterized by the presence of yellow chlorophyll and yellow xanthophyll, as well as by the absence of chlorofucine, fucoxanthine, and the substances soluble in water, so characteristic of the red group. Blue chlorophyll, orange xanthophyll, and the lichnoxanthines are common to all. It will also be seen that the red group is intermediate between the olive and the green, and, independent of the red colouring-matters, it differs from each of the other groups far less than they do from one another. It is also still more closely connected with each by other examples. My endeavour has been to extend such a method of comparison to all the leading classes of plants and to some of the lower classes of animals, and to ascertain the order in which they should be arranged, so as, in like manner, to show the most gradual and unbroken passage from one to the other.

Connexion between the lowest classes of Animals and Plants.

Comparing these various groups of *Algæ* with other classes of plants, and with such low classes of animals as *Actinæ*, I found that the whole of the colouring-matters present in green *Algæ* are those most characteristic of all the higher plants, the only difference being that in certain circumstances these latter contain in addition various more or less accidental and unessential substances, belonging to the erythrophyll and chryso-tannin groups, some kinds of which, nevertheless, do to some extent appear characteristic of particular classes. As far as their constituent colouring-matters are concerned, the green *Algæ* are therefore perfectly typical plants. On the contrary the olive *Algæ* differ in a very marked manner; they contain no yellow chlorophyll, nor either of the two kinds of

xanthophyll, all so characteristic of the most perfect plants, but contain chlorofucine and fucoxanthine, both of which occur in certain species of *Actiniæ*, like *Anthea cereus*, var. *smaragdina*. The presence of such colouring-matters, therefore, connects the olive *Algæ* with the lower classes of animals, in the same manner that the presence of blue chlorophyll connects some animals with plants. Such substances, though essential to the growth of plants, are not constant in closely allied species of animals, as though they were of no more importance for the life of animals than the accidental vegetable colouring-matters are for the life of plants. The value of these connexions between plants and animals remains to be determined, but in any case such definite facts must, I think, have some very important signification. If, then, according to these principles, the olive *Algæ* be looked upon as a link connecting the lowest classes of plants with some of the lowest classes of animals, there is a perfect and simple continuity; whereas if they were to be considered intermediate between green *Algæ* and the higher *Cryptogamia*, there would be two great breaks of chromatological continuity.

Changes occurring in Oscillatoria.

The olive *Algæ* are also connected in another manner with lichens, through *Oscillatoria*. These latter plants are extremely interesting, since they are subject to most remarkable changes, depending on the conditions in which they grow. I have made a series of quantitative analyses, which show this in a striking manner. I may here say that the chief difficulty in the analyses was the determination of the amount of the lichnoxanthines in presence of chlorofucine and fucoxanthine, and therefore the quantities given must be looked upon as only approximate, derived from several different methods, none of which were perfectly satisfactory, though they all agreed in leading to the same general conclusions. In discussing the results of the analyses, it was requisite to take the amount of blue chlorophyll as uniform, since it was the only constituent occurring in any considerable quantity throughout the whole series. To have taken equal weights of the plants themselves would have been almost impossible, and would often have made those which really correspond very closely appear to differ extremely, since the constitution of the endochrome is the important question. Of course by thus calculating the results as if the amount of chlorophyll were the same in all, there appears to be an increase in some of the other constituents in the specimens exposed to the sun, due, however, in reality to a reduction in the relative quantity of chlorophyll.

For comparison I give the following:—

- I. *Fucus serratus* grown in the shade.
- II. The same plant grown in the sun.
- III. *Oscillatoria* grown under water, in a cold spring, in a very shady place.

IV. The same plant, in the same spring, where more exposed to light.

V. The same plant, growing in and on the surface of water, where fully exposed to direct sun.

VI. Probably a different species of *Oscillatoria*, growing on a damp wall, completely exposed to the sun.

VII. *Peltigera canina*, slightly shaded and having much fructification.

VIII. The same plant, where much exposed to the sun.

| | | Blue chloro-phyll. | Chloro-fucine. | Phyc-xanthine. | Orange xantho-phyll. | Fuco-xanthine. | Lichno-xanthines. |
|----------------------|-------|--------------------|----------------|----------------|----------------------|----------------|-------------------|
| <i>Fucus.</i> | I. | 100 | 90 | 0 | 3 | 77 | 11 |
| | II. | 100 | 100 | 0 | 3½ | 100 | 14 |
| <i>Oscillatoria.</i> | III. | 100 | 13 | 0 | 1 | 51 | 6 |
| | IV. | 100 | 19 | 36 | 3 | 55 | 10 |
| | V. | 100 | trace | 67 | 25 | 11 | 9 |
| | VI. | 100 | „ | 100 | 77 | 25 | 23 |
| <i>Peltigera.</i> | VII. | 100 | „ | 27 | 32 | 0 | 32 |
| | VIII. | 100 | „ | 54 | 100 | 0 | 100 |

In this Table are compared together the same or very similar plants growing in different conditions, as connected by the brackets, and also plants belonging even to different classes. On comparing together the amount of the different constituents of the same plants grown in less or more light, it will be seen that some of the differences are in perfect agreement with those already described; but the differences in the *Oscillatoria* are evidently not a mere change in equilibrium, due to the decomposing action of the light, and point unmistakably to a great difference in the constructive force of the plant, depending on increased light. There is a remarkable development of phycocyanine and orange xanthophyll, and a great decrease in the amount of chlorofucine and fucoxanthine, and the result is that we have a change almost from the type of olive *Algæ* to that of certain lichens. When growing in a very shady place the colouring-matters soluble in bisulphide of carbon are all identical with those in *Fucus* and other olive *Algæ*, whereas when grown exposed to much sun there is a great reduction in the amount of those substances which are so characteristic of that group, and at the same time a great development of others which are almost or altogether absent from it, but occur in large quantity in, and are very characteristic of, such lichens as *Peltigera canina*. The olive *Algæ* are, however, distinguished from those *Oscillatoria* which approach them most closely by the absence of the phycocyanins; and though these occur in *Peltigera*, it

is distinguished by the absence of fucoxanthine from those *Oscillatoria* which in other respects agree with it. We may also draw another important conclusion from the above facts. *Oscillatoria* approach most closely to the olive *Algæ* when their vegetative energy is the weakest, when so little light is present that they can only just keep alive. This seems to show that the colouring of olive *Algæ*, in some way or other, belongs to a lower type than that of the green *Algæ*, as indicated by other facts previously described.

General connexion of different classes of Plants.

The olive *Algæ* are thus connected with the lowest green plants by means of two different groups of the red *Algæ*, one leading gradually to the green *Algæ* through *Porphyra*, and the other to lichens through *Peltigera*. There is the same sudden break in both, where the phycocyan and phycoerythrine colours cease and yellow chlorophyll and yellow xanthophyll make their appearance—at least I have hitherto met with no good connecting links containing a small quantity of both instead of a normal amount of one or of the other; and if this be really a universal fact, it would seem to show that, in some way or other, the presence of the phycocyan excludes yellow chlorophyll and yellow xanthophyll. Curiously enough this break does not occur between one great natural class and another, but in passing from those red *Algæ* which are so closely related to the green series as *Porphyra*, and from *Peltigera* to other lichens. So much remains to be learned of the details that it would be premature to put forward any general scheme with the expectation of its being finally adopted; but at the same time it may perhaps be well to express what is already known, if only as a guide for further research. Of course I refer simply to the distribution of the colouring-matters; and this could hardly be expected to depend upon, or accurately follow, the difference in the development of the reproductive organs; but, on the contrary, it seems to represent something special in the constitution of the plants, for which no name has hitherto been adopted, but which I have called *constructive energy*. If such be really the case, an arrangement founded on chromatological characters alone would by no means necessarily agree in every particular with a natural system founded on structural peculiarities. Taking into consideration the various facts described above, the following arrangement expresses every thing so far known respecting the distribution of the different colouring-matters:—

Actiniæ.

Anthea cereus, var. *smaragdina*.

Olive group of *Algæ*.

Red *Algæ*.

Oscillatoria.

Porphyra.

Peltigera.

Green *Algæ*.

Lichens.

Higher Cryptogamia.

Highest classes of plants.

The colouring-matters found in *Actiniae* are very various, and it is only particular species that contain those found in *Algæ*. Lichens, as a whole, are characterized by a number of what may be called accidental constituents—such, at least, as occur in one species and not in another closely allied to it. Many of these are almost or quite colourless substances, which easily give rise to colouring-matters when treated with various reagents. This fact, combined with their partial distribution, is taken advantage of in studying lichens as a means for distinguishing closely connected species. Their more constant and apparently fundamental colouring-matters correspond with those found in the higher classes of plants, but differ considerably in relative proportion, the lichnoxanthines usually being relatively more abundant.

Relation of Fungi to other Plants.

Fungi cannot be arranged in a direct order in any part of the series shown in the Table given above. Their most common colouring-matters exactly correspond with those found in the apothecia of lichens, and their more accidental constituents are also quite analogous to those occasionally found in the apothecia of particular lichens—for example in those of *Cladonia cornucopioides*.

According to the principles adopted in this paper, fungi ought then to be looked upon, not as fronds, but as the fructification of a low type of plants; and I think that the fact of the colouring-matters alone leading to such a satisfactory conclusion, shows that they must have some important physiological signification.

Connexion between the Colouring-matters of Flowers and those in the Leaves.

Since a comparison between the colouring-matters found in the petals of flowers with those in the foliage has many points of interest in connexion with the relationships of fungi, it seems best now to describe some of the principal facts. The coloured substances in the petals are in many cases exactly the same as those in the foliage from which chlorophyll has disappeared; so that the petals are often exactly like leaves which have turned yellow or red in autumn, or the very yellow or red leaves of early spring, but in other cases special coloured substances are developed which do not occur in leaves. Many yellow flowers are coloured by a very variable amount and variable mixture of lichnoxanthine and the three kinds of xanthophyll, but orange-coloured flowers sometimes contain in addition other substances analogous to peziza xanthine and phycoxanthine. The colour of many crimson, pink, and red flowers is due to the development of substances belonging to the erythrophyll group, and not unfrequently to exactly the same kind as that so often found in leaves. The number of different species is very considerable, and it would make this paper far too long to describe them; and, besides this, it would be requisite to explain methods of study

differing entirely from those given above. Some of the particular colouring-matters occur in very different natural orders; whereas I have found others only in one particular order, or even only in a single species of plant. Much remains to be learned in connexion with this branch of the subject; but many facts seem to indicate that these various substances may be due to an alteration of the normal constituents of leaves, some being probably formed from chlorophyll, others from the xanthophylls, and perhaps some from other constituents. So far as I have been able to ascertain, their development seems as if related to extra oxidization, modified by light and other varying conditions not yet understood; and it will be an interesting field for inquiry to determine how far they are due to simple changes in the normal constituents of leaves—in fact how far the petals of flowers are modified leaves, in colour as well as in structure.

Effects of Light on Flowers.

I have made very few experiments with the view of ascertaining the effect of a different amount of light on the petals of flowers, but what I have thus learned in the case of a dark variety of the common wallflower (*Cheiranthus cheiri*) clearly indicates that very important facts could be learned in this manner. By diminishing the exposure to light, a complete alteration was produced in the relative amount of the colouring-matters. No erythrophyll was developed, which was abundant in those flowers exposed to the sun, and, for an equal quantity of petals, only about one third the amount of xanthophyll, and yet about the same quantity of chlorophyll and lichnoxanthine were formed. The result was that I had produced a temporary alteration in the proportion of the colouring-matters, which corresponded closely with what is the permanent condition of a different natural variety or even of a different but closely allied species. Judging from certain facts which have lately attracted my attention, I think it extremely probable that in some cases natural varieties may be imitated in a similar temporary manner by reducing the constructive energy of the plants by other means. The extension of such inquiries will probably throw much light on the cause of the production of different coloured varieties of the flowers of the same species of plant, and show that some, at all events, may be due to permanent arrested or increased development of a particular kind.

Connexion between Fungi and Lichens.

Such, then, being the relation between the organs of reproduction and the foliage, it is to some extent possible to understand the connexion between parasitic plants like fungi, which do not derive their support from the constructive energy of their own fronds, and those which are self-supporting and possess true fronds. In the highest classes of plants the flowers are connected with the leaves more especially by means of xanthophyll and yellow xanthophyll; whereas in the case of lichens the

apothecia contain very little, if any, of those substances, but a large amount of the lichnoxanthines so characteristic of the class. Looking upon fungi from this chromatological point of view, they bear something like the same relation to lichens that the petals of a leafless parasitic plant would bear to the foliage of one of normal character—that is to say, they are, as it were, the coloured organs of reproduction of parasitic plants of a type closely approaching that of lichens, which of course is in very close, if not in absolute agreement with the conclusions drawn by botanists from entirely different data.

Yellow varieties of Leaves.

One of the most interesting questions connected with the higher classes of plants is the production of yellow varieties of leaves. Some, instead of being of the usual deep green, are far more yellow, or have special leaves or parts of leaves containing a very little chlorophyll generally diffused. Such leaves must be distinguished from those of normal green character with very bright patches free from chlorophyll, like *Croton variegatum*. Now I find that in many, if not all, such cases there is not only a great change in the total amount, but also a most important difference in the relative quantity of the blue and the yellow chlorophyll. This may be seen by examining the spectra of the leaves themselves, as illuminated by such bright, direct, concentrated sunlight as will penetrate through so many thicknesses of the leaves, pressed together by a strong compressorium, and show the spectrum to advantage. If in this manner, for example, ten thicknesses of very yellow leaves be compared with a single green one, it may be seen that the absorption-bands in the red due to the chlorophylls differ most materially. By proper management the band in the red due to yellow chlorophyll can always be seen in the spectrum of normal green leaves, but no trace is visible in the case of these very yellow leaves—only that due to blue chlorophyll. I have confirmed this conclusion by examining the chlorophyll when in solution in benzole, and find that, though some of the yellow kind is present, the relative amount is much less than normal. The method of study which I have adopted is as follows:—I crush the leaves, and heat them in spirit until all the chlorophyll is dissolved. When cold I agitate with bisulphide of carbon, adding so much water that the whole of the chlorophylls may be carried down; and after evaporating the bisulphide at a gentle heat, I redissolve in benzole. Taking two tubes containing a solution of such a strength that the spectrum shows the absorption-band in the red of the yellow chlorophyll to the greatest advantage, I add benzole to one tube until the band in the red due to blue chlorophyll is of the same intensity as that due to the yellow chlorophyll in the other tube, as seen by comparing the spectra together side by side with equally bright illumination, and carefully measure how much the original

solution had to be diluted in order to obtain that equality. This being a constant point of comparison, it is possible to determine the relative quantity of yellow chlorophyll in different kinds of leaves. So far I have not worked out the question in full detail, because the present form of instrument and apparatus is not suited for very accurate determinations, and therefore the following Table must be looked upon as a mere first attempt. Great care must be taken not to expose such dilute solutions to much light, since they are so rapidly changed, even by gaslight, that serious errors might easily result from this cause. In all cases I have taken the amount of blue chlorophyll as 100, and expressed the relative quantity of yellow chlorophyll in percentages, in accordance with the above named principle. I also give some other cases for comparison.

| | Blue chlorophyll. | Yellow chlorophyll. |
|---|-------------------|---------------------|
| Olive and red <i>Algae</i> | 100 | 0 |
| Leaves grown nearly in the dark | 100 | 5 or 6 |
| Normally yellow leaves grown in the light . . | 100 | 5 or 6 |
| Healthy green leaves | 100 | 13 to 17 |
| Green leaves faded to yellow | 100 | 25 or 30 |
| Final equilibrium in solutions exposed to the sun | 100 | near 100 |

The normal relative amount of yellow chlorophyll in green leaves certainly varies, and there seems reason to believe that this to some extent, if not mainly, depends on the length of time to which they have been exposed to the sun. When healthy green leaves fade and turn yellow the relative amount of yellow chlorophyll is increased, in accordance with what is known respecting the greater ease with which blue chlorophyll is decomposed, but the relative amount does not appear to become so great as in the case of a mixed *solution* exposed to the sun. On the contrary, when leaves are very yellow, owing to having been grown almost in the dark, the relative amount of yellow chlorophyll is much less than normal, as though under such unfavourable conditions the blue chlorophyll were more readily formed than the yellow. It might therefore be said that when the constructive energy is weak, the initial proportion of the yellow is about one third of the normal, but that when the leaves fade, the vanishing ratio is about double the normal. The blue chlorophyll is therefore, as it were, more readily formed and more readily decomposed than the yellow. The relatively small amount of yellow chlorophyll found in those leaves which are normally very yellow cannot therefore be due to the decomposing effect of light, but must rather be attributed to weak constructive energy, like that in leaves abnormally yellow on account of having been grown almost in the dark. This great reduction in the relative quantity of yellow chlorophyll causes them to approach towards the normal type of those low orders of plants from which it is altogether absent. The determination of the relative amounts of the two

kinds of xanthophyll is often rather difficult; but in some cases, at all events, the relative quantity of the yellow xanthophyll is greatly reduced in yellow varieties of leaves, so that, as in other circumstances, it seems to vary in the same manner as yellow chlorophyll. This question, however, requires further examination. The very great relative amount of the xanthophylls is readily explained by supposing that they are formed when the constructive energy is too low to give rise to chlorophyll, and that they may remain in faded leaves when the energy is too weak to prevent its decomposition.

Condition of Chlorophyll in Leaves.

By carefully studying the position of the principal absorption-band in the red, seen in the spectra of the leaves themselves, by means of a compound prism of considerable dispersive power, and a bright dot, seen by reflection from the upper surface, made to move over the spectrum by a micrometer-screw, as proposed by Mr. Browning and modified by myself, I find that there is every reason to believe that in normal healthy green leaves the chlorophyll is chiefly in a free state. On boiling for a short time in water, the oils or wax present in the leaf combine with the chlorophyll, and raise the band somewhat further from the red end, especially if the relative amount of chlorophyll is small. Of course I here refer to leaves which have little or no free acid in the juice to decompose the chlorophyll into the well-known product of the action of acids. When dissolved in the common fixed oils the band is still further raised. The small quantity of chlorophyll in very yellow leaves appears to be chiefly combined naturally with some oil, fat, or wax, so that the spectrum resembles that of a green leaf that has been boiled. This may perhaps be due in part to the total amount being so small in comparison to that of the oil or wax, but at the same time it is interesting to find that a relatively large quantity is thus combined in olive *Algæ*; and it will be a subject for further inquiry to ascertain whether, when chlorophyll occurs in animals, it is not similarly combined, or even dissolved, in a fat or oil.

Taking, then, every thing into account, leaves which are normally very yellow appear to be characterized by a very low constructive energy—so low, in fact, that a plant could not live if all its leaves were of the extreme condition of that type; and this, like what was described in connexion with *Oscillatoria*, seems to point to the conclusion that those classes of plants (like the red and olive *Algæ*) which more or less closely resemble these yellow leaves in certain important particulars are lower in the scale as regards some peculiarity on which the production of the colouring-matters depends, which, for want of a better name, I have called constructive energy. We might thus speak of constructive energy as being weak or strong in the same plant in different conditions, or as being of a low or high type in different classes of plants; and such

cases as those just named seem to show that a weak constructive energy has a great tendency to give rise to results analogous to those due to one of low type. If this conclusion should be confirmed by further research, it would be an important fact in connexion with the theory of evolution, since it would show that, as in the organization of animals, where development is arrested, there is a more or less permanent continuance of a lower type of structure, so in plants there is a permanent continuance of a lower type of colouring.

Conclusion.

Such, then, is a general account of the present state of what I have named comparative vegetable chromatology. I cannot but feel that it is very incomplete, both in compass and in detail, and that much remains to be learned respecting nearly every thing connected with it. Not only are the natural objects requiring careful study very numerous and often difficult to obtain in a proper condition, but, as will have been seen, several new questions are most intimately connected with my subject—questions of great interest in connexion with optics and chemistry, which would probably never have been raised if it had not been for their relation to the facts I have described. There are indications of other general questions which have not yet been fairly examined, and, in fact, the whole subject must be looked upon as being quite in its infancy. At the same time I trust that what I have described will be sufficient to show that the nature and relative proportion of the various colouring-matters in plants must have some very important signification, and that a more complete knowledge of comparative vegetable chromatology may be expected to throw much light on the development of plants, and enable us to examine some of the most fundamental questions in biology from a new and independent point of view. The subject is also interesting in other respects. The storing up of the energy of the sun's rays in the various compounds formed by plants is probably so intimately connected with the optical and chemical properties of some of their coloured constituents, that the further extension of such inquiries as I have described may possibly assist in clearing up this very difficult and yet most important problem.

XXIII. "On the Action of Electricity on Gases.—No. II. On the Electric Decomposition of Carbonic-Acid Gas." By Sir B. C. BRODIE, Bart., D.C.L., F.R.S., late Waynflete Professor of Chemistry in the University of Oxford. Received June 19, 1873.

(Abstract.)

In my previous experiments the maximum amount of ozone obtained by the action of electricity upon pure oxygen passed through the induction-tube of W. Siemens was about 20 per cent., an amount which, under