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An Attempt To Classify Common Plant Pigments, With Some Observations On The Meaning Of Colour In Plants

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MEETING OF THE SOCIETY,

Thursday, April 9, 1896.

Dr. AITKEN, President, in the Chair.

Mr. TAGG exhibited from the Museum of the Royal Botanic Garden a glass jar containing a dissection of the inflorescence of *Juglans regia*, to illustrate a method of preparation of Museum jars by the cementing together of plates of glass. The process is briefly this:—Pieces of glass to make the sides of the vessel are prepared, the edge being carefully smoothed, and then these are smeared with a cement made in the following way:—1 oz. Nelson's amber gelatine is soaked in water for twelve hours, the water not absorbed by the gelatine is then poured off and the softened gelatine melted over hot water. To the melted gelatine is now added 0·5 grammes of bichromate of potassium and 10 drops of glycerine. The whole is well stirred together. The cement is applied hot.

In cementing the plates of glass care must be taken to have the edges even, any unevenness of surface is likely to lead to the cracking of the glass when the cement hardens.

Mr. Tagg also explained that for fixing to glass for exhibition, in spirit, gelatine is preferable to photoxylin in the case of bulky and heavy subjects. The difficulty in applying the gelatine consequent on its rapid setting in spirit being got over by the use of a pipette with a hot-water jacket.

Professor BAYLEY BALFOUR informed the Society that the magnificent collection of species of *Masdevallia* brought together at Newbattle by the Marquess of Lothian had been acquired for the Royal Botanic Garden. A number of specimens from the collection were exhibited. A large series of species and hybrids of *Primula* in flower from the Royal Botanic Garden were also exhibited.

The following papers were read:—

AN ATTEMPT TO CLASSIFY COMMON PLANT PIGMENTS, WITH SOME OBSERVATIONS ON THE MEANING OF COLOUR IN PLANTS. By Miss M. J. NEWBIGIN, B.Sc. (Lond.).

It must be obvious to all who have followed the contemporary literature of pigments, that although there has been in many cases a lavish amount of experimentation, there is as yet little definiteness of knowledge. This is especially true in the case of the pigments of plants, where, for example, we find that the literature of chlorophyll is, even to a German eye, "Sehr umfangreiche," and yet exact results are few. Apart, however, from the general tendency to assume that all plant pigments are derivatives of chlorophyll, and most animal ones derivatives of hæmoglobin, there has undoubtedly been accumulated a mass of facts on the subject of chromatology. The present paper is an attempt to collect and systematise the known facts as to the pigments of plants, and to view them from the standpoint of the biologist rather than from that of the botanist proper.

We will take up first the lipochrome pigments, not on account of their functional importance, for of this nothing is known, but because they are common, readily recognised, and form a convenient starting-point. The lipochromes, or fat pigments, are known through the researches of Kühne (10), Krukenberg (9), and others, and are characterised by the combination of the following characters:—They are insoluble in water, but are soluble in alcohol, ether, chloroform, benzol, turpentine, carbon disulphide, etc. In the dry condition they are coloured blue by the addition of concentrated sulphuric or nitric acid. They are very readily destroyed by light, yielding cholesterin or some similar body. Their colour varies from yellow to red; they contain only carbon, oxygen, and hydrogen, and are usually found associated with fats. They usually give a spectrum of one, two, or three bands in the blue or violet. Although the above characters enable us to recognise the lipochromes very readily, their chemical nature is as yet

very insufficiently known, and the ten years which have elapsed since Krukenberg's work have not advanced our knowledge much. They are probably of fatty nature. Good examples of lipochromes are the colouring matters of the feathers of the ibis or cardinal bird, and of the common carrot. As compared with animal lipochromes, those of plants appear to be characterised by a greater readiness to crystallise. Thus Krukenberg never succeeded in crystallising tetronerythrin, the animal lipochrome mentioned above, with which he worked much; and among the vast number of animal lipochromes, that of the yolk of hens' eggs seems to have been almost the only one crystallised. Among plant lipochromes, on the other hand, that of the carrot occurs naturally in crystals, while almost every worker with chlorophyll has obtained one of the associated lipochromes in a crystalline condition. This result is, perhaps, due to the fact that the lipochromes of plants occur in a purer condition than those of animals, but comparative investigations are few. Another peculiarity which may probably one day prove of service in the classification of lipochromes, is the reaction with iodine in potassium iodide. With this reagent some, but not all the lipochromes, are coloured a bluish green, the reaction being certainly much commoner with vegetable lipochromes than with animal. Thus Mr. Cunningham and Dr. MacMunn (3) who investigated the lipochromes of twenty-two species of fishes, distributed in fifteen genera, did not succeed in a single case in obtaining a definite colouration with iodine, although in some cases they obtained the reaction with Schultze's fluid. On the other hand, in the case of the lipochromes of Fungi, a greenish colour with iodine is usually readily obtained (see Zopf in Schenk's *Handbuch der Botanik*, vol. iv.), and this is generally true of plant lipochromes. It is possible that this again is an indication that the lipochromes of plants usually occur in a purer condition than those of animals.

First, as to the distribution of the lipochromes of plants. Lipochromes are associated with true chlorophyll in the chlorophyll bodies of the green Algæ, the higher cryptogams, and phanerogams. Phycoxanthin, an apparently well-defined lipochrome, occurs in the *Phycochromaceæ*,

the Diatomaceæ, and the Phæophyceæ. Chlororufin, which is apparently of lipochrome nature, is found in the Chlorophyllaceæ. All these lipochromes occur in close association with chlorophyll, but without this direct association we have many of the pigments of Fungi, the carotin of the carrot, and the red and yellow colouring matters of many fruits and flowers. In almost all these instances, except in Fungi, the lipochrome either occurs in crystals, or associated with differentiated portions of protoplasm, the chlorophyll bodies, or chloroplasts. The lipochromes of Fungi occur as oily drops in the protoplasm. Recently Schrötter-Kristelli (16) has announced that in the aril of *Afzelia cuanzensis* a lipochrome, which he regards as identical with that of the carrot, occurs dissolved in a fatty oil, and not in connection with chromoleucites. In addition to the above, lipochromes are the cause of the colour of many autumnal leaves. The origin of such lipochromes is apparently always from the lipochromes of the chlorophyll corpuscles, but the lipochrome colour may be masked by other pigments.

In consequence of the frequent association of lipochromes with the chlorophyll corpuscles of plants, there has been a general tendency to regard the former as derivatives of true chlorophyll, but of this there is no direct evidence. There seems no doubt that chlorophyll contains nitrogen, while all the lipochromes are non-nitrogenous. Again, lipochromes occur in abundance in Fungi, and in animals, where an origin from chlorophyll is impossible. On the other hand, whatever future research may disclose as to the nature or origin of chlorophyll, it is at present a false simplification to assume that because it is usually found in association with a fatty pigment, it necessarily arises from this pigment.

So far, we have dealt with more or less well-established facts, but it is when we attempt to discriminate between the various lipochromes of phanerogams, that we enter upon controversial matter. Since Hansen first performed the experiment, now repeated in every laboratory, of separating the lipochrome of green leaves from the chlorophyll proper, the association of lipochrome and

chlorophyll has been almost universally admitted. The point of dispute is whether there is only one or several such lipochromes. Hansen's experiment was to extract green leaves with alcohol, and shake up the extract with benzol. If the experiment be performed with care, a separation into two layers takes place, the benzol taking up the chlorophyll-green, and leaving the yellow lipochrome in the alcohol. To this lipochrome the name of chlorophyll-yellow, or xanthophyll, has been given. As Dr. Schunck (14) points out, however, it is impossible by such means to produce a chemically pure product, so that conclusions based only upon the results of the investigation of this alcoholic solution are not absolutely reliable. Be this as it may, the beauty and simplicity of the experiment have determined its persistence, and xanthophyll has found its way into all the more recent text-books. The probable existence of another lipochrome in connection with the chlorophyll of leaves, is shown by the following experiment, which is almost as simple. Boil green leaves with strong alcohol and filter hot, on standing in the dark the filtrate deposits a dark greenish precipitate, consisting of fats, impurities, a lipochrome, and a greater or less amount of (modified) chlorophyll-green, according to the time the solution is allowed to stand, and the degree of its concentration. The lipochrome frequently occurs in the form of reddish crystals. As might be assumed from its method of precipitation, it is little soluble in cold alcohol, but is readily soluble in chloroform, and can be easily obtained in pure crystalline condition. This experiment has been performed by many investigators, notably by Macchiati and Schunck, but usually only as part of the method of chlorophyll purification. Macchiati (11) washed his leaves with alcohol and ether before extracting with alcohol, and thus obtained on cooling a precipitate of nearly pure lipochrome (mixed with some modified chlorophyll-green?). He then removed the lipochrome by filtration, and succeeded in obtaining crystals of so-called "pure chlorophyll" by concentrating the filtrate. These crystals, when dissolved in alcohol, give Hansen's reaction with benzol, and undoubtedly consisted of a

mixture of (modified) chlorophyll-green and xanthophyll. Schunck (15) allowed the hot alcoholic extract to stand until both the lipochrome and chlorophyll-green were precipitated, and then rejected the pale filtrate with its contained xanthophyll, and proceeded to separate the lipochrome and chlorophyll-green. This he did by treating the precipitate with a solution of caustic soda in boiling alcohol, which he says dissolves only the chlorophyll-green. The remaining lipochrome was purified by successive crystallisation from chloroform, and by treatment with hot glacial acetic acid to remove fatty impurities. This substance is the erythrophyll or chrysophyll of many authors, is said by Arnaud (1) to be identical with carotin, and when pure forms crystals of "orange-red colour and golden lustre" (Schunck). It is very readily destroyed by light, is much more readily dissolved by benzol or chloroform than by alcohol, and gives a spectrum of two bands in the blue region. Although this substance can be so readily obtained from green leaves, there is some doubt as to whether it actually exists as such in the leaves, or whether it is not formed during the process of extraction. Schunck (14) opposes the theory of its existence on account of the absence of its bands in the ordinary spectrum of chlorophyll. For a converse reason he considers that it is present in some autumnal leaves. As, however, the spectrum of the same lipochrome frequently differs considerably according to the solvent used, conclusions based on isolated spectroscopic observations are not of very great value.

The lipochromes of autumnal leaves are apparently the same as those of green leaves, and do not therefore require separate consideration. The removal or destruction of the true chlorophyll in such leaves allows the lipochromes to become visible, and their slow modification gives rise to many of the brilliant tints of autumn.

FLOWERS AND FRUITS.

The lipochromes of flowers and fruits arrange themselves in two series, which apparently correspond to the xanthophyll and erythrophyll of leaves. The red and yellow

pigments, in the case of flowers and fruits, are called respectively anthoxanthin and xanthin, though their distinct nature must be regarded as doubtful. They are always associated with chromoplasts, which, as is now well-known, frequently originate from modified chlorophyll corpuscles.

Looking generally at the lipochromes of phanerogams, we may say that, while little is known of individual pigments as a whole, they resolve themselves into two series. The one includes orange to orange-red pigments, which are little soluble in cold alcohol, but are readily soluble in chloroform and benzol. Of these, carotin is the type. The other series includes yellow pigments, which, according to Courchet (2) are not readily crystallisable, and which are readily soluble in cold alcohol, but less soluble in benzol and chloroform. Of these, xanthophyll, if it be a distinct pigment and not a mixture, is the type. As to the relations of the two series it is not yet possible to say much, but it is not improbable that further investigation will show that the members of one series can be readily transformed into members of the other, and that this occurs both in the living tissues, and as a result of chemical reagents. How far the deepening of colour, frequently observable when yellow lipochromes are treated with solutions of caustic soda or potash, corresponds to such a change, must also be left to be decided by further investigation. In this connection it may be noted that Courchet (2) separates the two lipochrome series named above very widely, on the ground that the orange series frequently occurs naturally in a crystalline condition, and can be readily crystallised artificially, whereas the yellow series is always amorphous. From his figures, however, there seems some reason to believe that his orange crystals were in part cholesterin, coloured with unaltered lipochrome, and until we have extended and exact investigations on the subject it seems better to regard the lipochromes as a natural group.

As to the meaning of the lipochromes in metabolism, and their direct origin, we unfortunately, as yet, know nothing. Recently, Schrötter-Kristelli (16) has put forward a hypothesis upon the subject, which has been adopted and elaborated by Simroth (17). Schrötter-

Kristelli proposes the name of Lipoxanthin for the lipochromes of plants, and takes carotin as the type of these; the necessity for a new term for plant lipochromes, while their relation to those of animals has not been worked out, can hardly, however, be said to be quite apparent. Further, he puts forward the view that while lipochromes (lipoxanthins), which occur in oily drops in the protoplasm, function as reserves, those associated with chromoleucites are of extreme importance as carriers of oxygen, the process not being accompanied by any destruction of the pigment. Simroth adds to this the suggestion that chlorophyll is merely an oxidised lipochrome, and that the colours of flowers and fruits are due to a deoxidation of chlorophyll. The last suggestion is just the common one of the connection between chlorophyll and lipochrome, and of the first we can only say that it seems to be premature as yet.

Zopf (21 and 22), who of late has worked much at the pigments of plants, also inclines to the opinion that the lipochromes function as reserves, and instances the case of the disappearance of the lipochrome and the associated fat during the germination of the spores and gemmæ of various species of *Pilobolus*. The fact is of the same nature as that of the disappearance of fat and pigment from the muscles of the fasting salmon, and cannot be regarded as a conclusive proof that the lipochromes are in themselves really reserves,—indeed, speculation as to their function is perhaps better postponed until their chemical composition is known.

As to the classification of the lipochromes, Zopf, who prefers the term carotin, proposes the following:—

1. EUCAROTINS, hydrocarbons which do not form compounds with the alkalies or alkaline earths, and which in alcohol, ether, and petrol-ether form yellow solutions.

2. CAROTININES, oxygen-containing bodies which form compounds with the alkalies and alkaline earths, and which in alcohol, ether, and petrol-ether form red solutions.

It will be observed that this classification agrees with that proposed by Courchet in separating the two series very widely, in this case on the ground of different chemical composition. In working at the pigments of the Norway lobster, however, I have found that the red pigment of the

skin gives a reddish orange solution in alcohol, and a pure yellow one in petrol-ether, while it undoubtedly forms a sodium compound. It thus falls into neither of Zopf's classes. So far as my own experience goes, the question as to the formation of sodium compounds is much more complicated and more dependent upon the method employed than is apparent from Zopf's statements, and, in consequence, should not be too rashly employed for purposes of classification. For purposes of convenient reference the following scheme of the common lipochromes of plants may be suggested; it is chiefly based upon the solubilities in the various solvents as described above, and, therefore, can be readily refuted or confirmed in practice.

CLASSIFICATION OF PLANT LIPOCHROMES

All give in dry state blue colouration with H_2SO_4	1ST SERIES. Colour yellow. All readily soluble in benzol and chloroform. Not readily crystallised.	Xanthophyll. Xanthin. Phycoxanthin.
	2ND SERIES. Colour orange to red. All insoluble in cold alcohol, but very soluble in ben- zol and chloroform. Readily crystallised.	Carotin. Erythrophyll(chrysophyll) Anthoxanthin. Chlororufin.

The next great group of pigments which we shall consider includes these often known as the anthocyan series, which are soluble in water, and frequently produce very conspicuous colouration. On account of their solubility they occur in the cell-sap and not in chromatophores like the lipochromes, which are often called *fixed* colours as opposed to these *fluid* ones. Anthocyan, or the series of pigments included under this name, occurs as a red, blue, or violet colouring-matter, and gives their colour to many flowers and fruits, to some autumnal leaves (*e.g.* vine and Virginian creeper), to the purple or "copper" varieties of forest trees (*e.g.* beech), and to many plants or parts of plants which, either habitually or as a variation, show a red colour (*e.g.* "runners" of strawberry plants, shoots of rose, purple cabbage, etc.). The distinguishing characteristic of these pigments, besides their solubility, is that they vary in colour according to the reaction of the solvent.

Thus red anthocyan can be converted into blue by the addition of a small amount of an alkali, while the blue can be converted into red by the addition of acids. The addition even of a small amount of a strong alkali like caustic potash or soda produces a greenish colour, which becomes yellow on further additions of alkali, and finally becomes colourless. The cautious addition of a salt like disodium hydrogen phosphate, on the other hand, to red anthocyan, produces first a violet and finally a blue colour (Hansen). These reactions can be very readily observed with anthocyan obtained from a rosy apple. A pretty and familiar illustration of the same fact is seen in nature in the change of colour in the flowers of many Boraginaceæ from pink to blue, which is associated with a corresponding change in the reaction of the cell-sap.

It is curious to note that, while there are practically no suggestions as to the functions of the lipochromes, those as to the meaning of the anthocyan pigments are as numerous as could be desired. As, however, in most cases these suggestions have been offered in relation to particular cases, it may be well first to give a complete list of the conditions under which anthocyan is found to occur. Frank in his *Lehrbuch der Botanik* (Bd. I. s. 646) classifies the organs in which anthocyan occurs under the following heads:—First, in flowers and fruits; Second, in vegetative organs. In vegetative organs in the following conditions:—(a) In many young growing shoots in spring; (b) In many autumnal leaves; (c) In many organs when exposed to cold, whether in spring or autumn; (d) In many stems, petioles, etc., on the side which is exposed to the sun; (e) In many leaves and fruits at spots where injury has taken place; (f) In many parts of plants as a variation. To this list may be added the case of Alpine plants, which exhibit a general tendency to develop anthocyan.

As to the meaning of the anthocyan the commonest suggestion, based especially on conditions *c* and *d*, is of course that it is a direct adaptation destined to prevent the decomposition of chlorophyll by strong light. Detmer especially supports this theory in his *Pflanzenphysiologie*, pointing to the reddening of some evergreen leaves in winter, and to an experiment by Kerner, who transplanted

some valley plants to a mountainous district, and found that only those which became red flourished.

Hassack (7), studying especially the case of coloured leaves, also regards the colour as an adaptation, but adds that it is also a direct result of the action of light. Wigand with greater caution says that the favouring conditions are feeble or suppressed assimilation and strong light. Pick (13), on experimental grounds of very doubtful validity, holds that the pigment is of direct importance in the transport of starch, and believes that the conditions of formation are sunlight and low temperature—two somewhat opposed factors. The anthocyanins of flowers and fruits are of course usually ascribed to the continued selection of insects and birds.

In attempting to reach a decision as to the meaning of anthocyan, the most important point is first to obtain a definite statement as to the effect produced by its presence in the cell-sap of leaves. Although the question involves considerable technical difficulty, there seems little doubt that careful experiments prove that leaves, such as those of the copper beech, which contain abundant anthocyan assimilate more slowly than ordinary green leaves. See for example Jumelle (8). It might, however, be said that although such examples as the copper beech are dependent upon man for their persistence, yet, under certain circumstances, the diminished assimilation of red leaves and shoots may be temporarily compensated by the preservation of the chlorophyll, and that in this way the reddening of conifer leaves in autumn is an advantage. This explanation hardly, however, applies to the reddening of growing shoots in spring. We can hardly suppose that the light is then strong enough to lead to the decomposition of the chlorophyll, especially in view of the fact that the red colour is frequently lost when such parts have made their growth. Further, the parts of the plants which frequently redden on exposure to strong light are not such as to make us suppose that the preservation of their chlorophyll is of great importance, being usually stems, petioles, veins of the leaf, etc. As far as the effect of external conditions is concerned, we must note that the apparent contradiction between the two usually assigned causes for the production

of anthocyan (*i.e.* strong sunlight and diminished temperature), make it appear very doubtful whether we have not to do with indirect, rather than direct environmental effects; while the hypothesis of universal adaptation is negatived by such facts as that fruits, *e.g.* apples, redden only on the side exposed to the sun. We shall return to this subject after considering the origin of anthocyan; but it may be noted meantime that the evidence points to the conclusion that anthocyan arises primarily as a consequence of particular states of metabolism, and that any function which it may possess is secondary.

As to the origin of the anthocyan series, there is a general consensus of opinion that it arises from tannin or a related substance. Gautier (4), who analysed the colouring matters of the vine, calls these tannic acids. Wigand (19) says that the anthocyan colours arise from a colourless chromogen which is directly derived from tannin, and that the disappearance of the colours is associated with the reformation of tannin. Pick (13) confirms this, adding the interesting remark that the colours are notably absent from plants with little or no tannin. Hansen, as quoted by Krukenberg (9), found a colourless chromogen in the leaves of the aloe, which took up water and oxygen, and became converted into a pigment resembling the anthocyan series; but no opinion is expressed as to the nature of the chromogen. An origin from tannin seems, on the whole, to be fairly well established, and is of great interest, because it connects these pigments with many other plant pigments. Thus the phlobaphenes, the colouring matters of bark and of many injured tissues, *e.g.* cut apples, are almost certainly produced by the direct oxidation of tannins. The yellow or brown colours of some autumnal leaves, *e.g.* oak and beech, are produced in the same way. See Sorby (18). Again, according to Vines, the colouring matters of woods, such as hæmatoxylin, santalin, brasilin, etc., are probably derived from tannins. Further, since most tannins are glucosides, it seems not unnatural to associate with the chromogens mentioned above, those well-known glucosides which, outside the organism, act the part of chromogens. Thus there is reason to suppose that the process by which

indican and ruberythric acid give rise respectively to indigo blue and alizarin, is paralleled by the process by which within the organism another glucoside (?) gives rise to the colour of the rose. These facts are of more importance than is apparent at first sight. They surely suggest that whatever part the selection of insects has played in the final development of the colour of the rose, this is primarily due to the chemical properties of the tannin which ontogenetically precedes it. Now tannin is almost certainly a waste product, a result of destructive metabolism. As such it probably tends to occur wherever assimilation is feeble or suppressed. What the conditions are which determine the conversion of the tannin into the chromogen of anthocyan, rather than into the chromogen of other pigments, such as the phlobaphenes, is of course unknown, but it is probable that they are purely chemical. It may of course be said that this is the point at which selection intervenes, favouring the production of the useful (*i.e.* attractive) anthocyan rather than of the useless dull-coloured phlobaphenes. This, however, is doubtful on the following grounds. When tissues are injured as by gall-producing insects, pigment is usually produced, but this may be either one of the phlobaphenes or anthocyan. As it is difficult to see how such colouration can be subject to selection, this furnishes a strong presumption that the particular pigment produced is determined primarily by chemical agencies. Again such a vigorous selection as the statement would imply does not seem to be borne out by facts. Thus, observations on our fruit-eating birds do not lead one to suppose that the result of their preference would be to favour the dissemination of red varieties of apples rather than of the "russet" ones, supposing the size of the apples to be such that they could be readily carried off. In this case, the fact that "russet" apples, when cut, become discoloured far more rapidly than red ones, suggests that the russet colour is the consequence of the marked tendency of the tannins of the apple to become converted into phlobaphenes, and is, therefore, simply a consequence of the chemical composition of the cell contents.

Although so little is known as to the conditions upon which the presence of anthocyan is dependent, yet the

following observations by Molisch seem to shed a little light on the problem. Molisch (12) found that in many cases, *e.g.* brightly coloured leaves of *Coleus*, the anthocyan was so rapidly destroyed on boiling with water, that it was impossible to obtain a solution. This rapid destruction, he believes, apparently with good reason, to be due to alkaline substances contained in the protoplasm of the cells. While the cells are living, the cell-sap with its contained anthocyan is separated from the alkaline protoplasm; in dying cells, a mingling takes place, and in some cases the alkalinity of the protoplasm is sufficient to destroy the anthocyan (*e.g.* effect of strong alkalis as detailed above). Molisch finds that this destruction of anthocyan is especially liable to take place in cells which contain chlorophyll. As this cannot easily be a direct effect, he concludes that, in chlorophyll-containing cells, the conditions for the production of alkaline substance must be especially favourable. Further, he found that leaves, whose anthocyan did not change at death, were especially remarkable for the acidity of their cell-sap.

Now, although Molisch does not himself make the suggestion, it seems hardly possible to doubt that the alkalinity of the protoplasm must have some effect upon the anthocyan of the cell-sap during life; there must surely be a constant process of diffusion going on between protoplasm and cell-sap. If this be so we can readily understand how it is that anthocyan is so frequently associated with diminishing chlorophyll. The particular (alkaline) condition of the protoplasm, which is associated with the development of chlorophyll, is unfavourable to the production of anthocyan tending to decolourise it; the process being probably the "reconversion into tannin" of Wigand. Conversely, conditions which are unfavourable to the development of chlorophyll are directly favourable to the development of anthocyan. This is probably the physiological reason for that association of anthocyan with diminishing powers of assimilation which we have already noticed as a fact of experience.

The following table is intended to illustrate the suggested relations between the various chromogens of glucoside nature found in flowering-plants:—

CLASSIFICATION OF THE CHROMOGENS WHICH ARE OF GLUCOSIDE NATURE.

Chromogens = tannins or other glucosides.	1. Usually remain in plant in condition of chromogens.	i. Tannins of many tissues.	When oxidised.	[= Brown colouring matters of injured tissues.
		ii. Glucosides of many tissues, <i>e.g.</i>		[= Phlobaphenes.
		Indican.		[= Indigo blue.
		Ruberythric acid.		[= Alizarin.
		iii. Hansen's chromogen (= tannin ?)		[= Anthocyan pigment.
	2. Give rise to pigments within the plant.	a. Tannins of young shoots, of young floral leaves, of unripe fruits, etc.	When oxidised (?)	[= Anthocyan pigments.
		b. Tannins (?) of wood.		[= Hæmatoxylin, Brasilin, etc.
		c. Tannins of bark.		[= Phlobaphenes.

Of the other plant pigments not yet mentioned, chlorophyll and etiolin are, of course, by far the most important. From the present point of view, however, there is little to say of either, for, in spite of numerous investigations, little is known of their constitution or origin. The two are probably very nearly allied in composition, and differ from most other plant pigments in containing nitrogen. That many plants, when grown in normal conditions, contain chlorophyll, is at present an observed fact beyond which it is impossible to go.

We have already seen that, normally, chlorophyll seems to be always associated with lipochromes, and this is probably also true of etiolin. The lipochromes are not, however, the only pigments thus associated; many cryptogams have their chlorophyll masked by other well-defined colouring-matters. Thus the Red Algæ owe their apparent colour to a reddish pigment which is soluble in water, forming a fluorescent solution, and which, according to Krukenberg, resembles very closely a red pigment found in some sponges. Its presence in the Floridæ appears to be

associated with some modification of the chlorophyll. Again, both the Phæophyceæ and the Cyanophyceæ have associated with their chlorophyll both a lipochrome and another pigment soluble in water. Phycophæin, the pigment of the former, is of a brownish colour, while phycocyan, that of the latter, forms a beautifully blue solution with red fluorescence.

The only other pigments which need to be mentioned here are the soluble yellows, which are liable to be confused with the lipochromes. The rind of the lemon, the florets of the dahlia, and some other plant tissues, contain yellow colouring-matters, soluble in both hot and cold water, which do not give the lipochrome reactions; of their chemistry little is known.

In concluding this discussion of the common pigments of plants, it seems not out of place to consider the bearing of the facts mentioned upon current theories as to the origin of colour. In spite of criticism from various quarters, it is still the custom to regard the colours of fruits and flowers as entirely the result of the selective action of other organisms. The colours of these structures are, as we have seen, generally speaking, due to lipochromes or to anthocyan pigments, or to a combination of the two. Now we know nothing of the origin of the lipochromes, it may be that they are simply the outward expression of a well-nourished condition of the organism; but the colours of autumnal leaves, and of the variegated leaves of cultivated plants, show that the predominance of the lipochrome pigments is simply evidence of diminishing powers of assimilation. It is, therefore, eminently natural that these pigments should become increasingly prominent, as the parts of the flower, including the carpellary leaves, diverge more and more from the leaf type, and become increasingly incapable of assimilation. (For an essentially similar line of argument, see a paper by Mr. Philip Sewell (17).) This fact of observation is quite independent of theories such as that of Simroth, which assume that the lipochromes are directly produced by the alteration of chlorophyll. Again, the anthocyan arise in certain conditions from tannins, which tend to occur naturally in parts of the plant which are growing actively or assimilating feebly. Thus we see that the

presence of anthocyan, or of other derivatives of the tannins, in flowers and fruits is essentially the same phenomenon as their appearance in young growing shoots, and in injured tissues. Whatever part birds and insects have played in the development of the colours of flowers and fruits, it can only have been the acceleration of the movement of organisms along lines of development upon which their own inherent tendencies have led them. It is most probable that the effect of the choice of birds and insects has been to convert a merely indifferent property into one important for the maintenance of the species. The colours of the rose and tulip were primarily of no more importance to them than the indican of *Indigofera* is to it now, and all are still facts of the same nature. The result of the preference of the bee has been to render the colour of the rose of some importance to the species, but the colour is independent of this preference, and is no more likely to disappear, if the bee's preference were to disappear, than is the hæmatoxylon of *Hæmatoxylon campechianum* likely to disappear if man's preference for it faded.

It may be said that the occurrence of white flowers is contrary to this reasoning, because here there is no pigment at all, and yet the æsthetic effect is well-marked. On the other hand, a very similar appearance of whiteness occurs in many variegated leaves. See Hassack (7). Here there is no doubt that the albinism is due to degeneration, and is probably a consequence of the artificial conditions of cultivation. It is again an indication of diminishing capacity for assimilation, and may therefore be naturally expected in floral leaves.

On the whole, a consideration of the pigments of plants certainly seems to support the view, now rendered familiar by Mr. Bateson's work, that conspicuous variations are determinate rather than indefinite, are primarily dependent upon the physical and chemical conditions existing within the organism, rather than upon the result of the selective action of other organisms working upon minute fortuitous variations, as Weismann and his followers would maintain.

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