termination, but that would follow an observation as to whether resins and gums would poison the electrode or have any buffer action upon the solutions.

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PLANT COLORING MATTERS.

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Recently there appeared in THIS JOURNAL (1) an interesting contribution by Wurdack dealing principally with the distribution, general characteristics, and possible functions in plants of natural coloring matters. While the subject matter of this paper is still fresh in our minds, it seemed desirable to present at this time a number of additional facts of interest which relate to these coloring substances.

It is necessary to mention again only briefly a few salient points concerning the nature of the pigments themselves in view of the well-prepared résumé published. The brief references will be made solely to recall the compounds about which further discussion is to be had.

PLASTID PIGMENTS: CHLOROPHYLLS AND CAROTINOIDS.

That the chlorophyll molecule is exceedingly complex has already been indicated by Wurdack. Endeavors to isolate the pure pigment appeared for many years hopeless because of its instability, its chemical indifference, and on account of the many impurities which accompany it. A knowledge of its structure was finally gained when Willstätter and his collaborators deduced its constitution from a study of the decomposition products formed from it by hydrolysis with acids and alkalies. When the preparation of pure chlorophyll was ultimately accomplished in 1911, the facts learned from its analysis fully corroborated the former deductions as to its chemical makeup. As a result of the work on the decomposition products of chlorophyll, it became possible to compare the green pigment in over two hundred plants from numerous genera of cryptogams and phanerogams. This work demonstrated that leaf-green of all the plants examined consists of two components, chlorophyll a ($C_{55}H_{72}O_5N_4Mg$), a blue-black powder, and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$), a dark green powder. It was also observed that with the exception of the *Phæophyceæ*, or brown algæ, all of the plants investigated contained approximately three molecules of chlorophyll a to one of chlorophyll b. In the case of the brown algæ, the green matter proved to be mostly chlorophyll a with but a small percentage of the other component.

It was early recognized that yellow substances accompany the green pigments in the plastid. This mutual occurrence of the coloring matters suggested a significant physiological rôle of the yellow pigments, consequently their separation became an important problem. Ultimately, it was shown that there are present in green leaves two easily crystallizable, nitrogen-free pigments. The members of this group, which are hydrocarbons or their derivatives, are designated carotinoids, but the exact nature of their basic structure or of their function is still unknown. The yellow pigment, carotin, is found not only accompanying chloro-

phyll in leaves but also in carrots (very little green pigment present), in nearly all yellow flowers and many fruits. Lycopin, an isomer of carotin, is the red pigment which occurs in the flesh of tomato and watermelon and in the skin of peppers. In the flesh of the tomato, which is an instance where carotin is not confined to the pastids, the coloring matter is distributed throughout the cells in the form of crystals or semi-crystalline granules. Other carotinoid pigments, for example, xanthophyll in leaves and carrots, and fucoxanthin in brown algæ, are oxides of carotin. These pigments as a group are very unstable and easily auto-oxidizable, therefore, in their preparation considerable care is necessary with regard to the temperature to which they are exposed and also in protecting them against the action of oxygen from the air.

### SOLUBLE SAP PIGMENTS: FLAVONE AND ANTHOCYANIDIN DERIVATIVES.

In contrast to the chlorophylls and carotinoids, which are plastid coloring matters, derivatives of flavone and anthocyanidin pigments occur as soluble cell-sap substances. In all more than twenty different yellow flavones (and flavonols) have been isolated from a large number of plants. A great many investigators have been concerned with the chemistry and distribution of these colors and as a result of their researches it is known that they are widely distributed in nature and differ from each other in the number and position of hydroxyl groups and other radicles which are joined to the basic nucleus. An easy way to demonstrate the presence of flavone is to treat almost any white flower, which is not an albino, with ammonia vapor. Under these conditions, the flower will turn yellow because of the presence of a member of the flavone group.

Considerable interest has been manifested in the relation which appears to exist between yellow flavone glucosides and the innumerable shades of blue, violet and nearly all reds which are collectively termed anthocyanins (anthocyanidin glucosides). The flavone glucosides are regarded as the chromogens of anthocyanins since the available evidence tends to show that the latter pigments are formed in the plant from preexisting flavone glucosides. This view is indirectly supported by genetical investigations and by the chemical transition from flavone to anthocyanidin through reduction in acid solution. A more direct evidence has been found by Everest (2) who has shown the coexistence in purple-black pansies of the closely related pair, the flavone myricetin ($C_{15}H_{10}O_8$) and the anthocyanidin delphinidin ($C_{15}H_{10}O_7$). Reliable information on the simple chemical relationship between the flavone and anthocyanidin series of pigments was obtained when Willstätter and his students isolated and characterized over twenty different anthocyanins. A list included in Wurdack's compilation shows the plant sources from which the various anthocyanins have been obtained. It is interesting to note that the coloring matter from the beet root (3) has been established as the first member of the anthocyanin series which contains nitrogen in the molecule. The anthocyanins yield on hydrolysis anthocyanidins which differ from each other, just as the flavones do, in the number and position of the hydroxyl groups and occasionally in the replacement of an hydroxyl group by the methoxyl radicle.

IMPORTANCE OF CHLOROPHYLL AND CAROTINOIDS.

Chlorophyll is by far the most important and interesting coloring matter in the vegetable kingdom because it is regarded as the active medium in the

photosynthesis of glucose from carbon dioxide and water. The photosynthetic process is one of such great complexity that contributions of many botanists and chemists concerning it have remained largely in the hypothetical and speculative stage. On the other hand, our knowledge of the chemistry of the substances involved has been greatly extended. Willstätter's elucidation of the chlorophyll molecule is an outstanding example of such progress. As a tentative explanation of the building up of sugars from carbon dioxide and water, the hypothesis which has received the greatest recognition and which still continues to hold the field is the formaldehyde or Bayer theory. The reactions in their simplest forms which this theory suggests are as follows:

$$CO_2 + H_2O = H-CHO + O_2$$
$$6 \ H-CHO \longrightarrow C_6H_{12}O_6$$
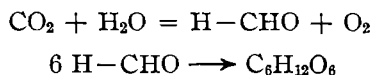
Much doubt has been felt that formaldehyde ($H-CHO$) is produced in the plant, but Willstätter's researches tend to strengthen the supposition that this is actually the case. The experiments of Baly, Heilbron, and Barker (4) confirm the fact that it is possible to obtain formaldehyde from an aqueous solution of carbon dioxide under certain conditions. In their work, the energy repsonsible for the reaction was supplied by the action of ultra-violet light. Of course, this does not definitely prove that the compound in question is actually formed or exists as such in the plant life processes. It does suggest interesting possibilities.

Taking it all in all, the evidence which we now possess seems convincing in showing that sugars are produced in the plant from carbon dioxide and water and that this reaction depends on the formation and subsequent polymerization of formaldehyde. While the presence of free formaldehyde in plants has not been indubitably proved, Baly and his co-workers attempt to explain any doubt by assuming the formation of a very active formaldehyde which is immediately converted into other substances. According to Willstätter a chlorophyll-carbonic acid complex is formed and by virtue of this combination the carbon dioxide is reduced to formaldehyde. It is not at all certain, if these changes take place, how the leaf manages to accomplish them in sunlight, although it has been suggested that chlorophyll acts as a photocatalyst in the process. In this instance, the photocatalyst appears to be a substance which is capable of absorbing light of longer wave-length than 350 millimicrons and subsequently radiating energy at infra-red frequencies. This energy is then reabsorbed by carbon dioxide or its complex whereby the carbon dioxide undergoes transition to formaldehyde and then to carbohydrates. Willstätter's work on chlorophyll and the recent investigations of Baly and his collaborators (5) which led to their conception of a photocatalytic mechanism of photosynthesis have suggested a multitude of fundamentally important problems which are in need of investigation. The question of energetics which plays such an important part in these reactions is in need of thorough study. The subject has hardly been touched. The suggestion of Dixon and his associates (6) that a source of energy may be the action of light displacing electrons within the chlorophyll molecule opens up an interesting and fruitful field for research.

Coming now to the possible function of carotinoids, we must admit that the subject has always been wrapped in mystery. Responsibility for this may be

traced to a lack of definite information concerning the exact constitution of these pigments. While a number of interesting suggestions have been offered as to possible roles which the carotinoids play, the writer believes a promising field for research lies in the direction which assumes a relation of these pigments to photosynthesis. In the assimilation process it is admitted and it seems probable, that chlorophyll b may be formed by the action of chlorophyll a upon carbon dioxide. The two atoms of oxygen which are required in this reaction could be supplied during the conversion of a molecule of carbonic acid to formaldehyde. If chlorophyll b is formed in this way then it follows that reduction to the a component must take place before a transformation of carbon dioxide to formaldehyde can again proceed. It has been suggested that carotin is the reducing agent in this reaction and that it is oxidized to xanthophyll at the same time. A reconversion of xanthophyll to carotin must also follow.

It will be seen from the above that the function of carotinoids is a complex one and that the steps which have been brought forward really belong to the realm of speculation. However, it would really be a decided step forward, as the author of these suggestions has pointed out, if it could be proved that carotin could be converted into xanthophyll and *vice versa*. During a recent trip to this country Professor Baly stated in an address at Washington that xanthophyll had been obtained directly from carotin by careful oxidation.

While we have been in doubt concerning the use of carotinoids to the plant, this has not prevented our taking advantage in a practical way of certain information which was gained through studies of these compounds. Palmer (7) cites a use which was made of such information in connection with the detection of camouflaged foliage by the American Army during the war. It is well known that carotinoids in leaves reflect red light and chlorophyll green light. Owing to a predominance of chlorophyll in leaves the yellow pigments are masked to such an extent as to cause the leaves to appear green. If filters are so constructed as to transmit only light from a wide band in the red region and a little light from a narrow band in the green part of the spectrum, natural green foliage when viewed through these filters will appear red. Camouflaged foliage, however, will appear green because of the fact that green paint contains no carotinoids.

Blakeslee and Warner (8) found a correlation to exist between yellow carotinoid color in the visible skin parts of certain breeds of hens and their egg laying capacity. During laying, the yellow pigment consumed with their food selectively accumulates in the ova, while after the laying season, or in hens which have not been productive the pigment is excreted by way of the skin parts. Even before the correlation was scientifically proved, some practical poultry men recognized the fact that during the laying season, pale shanks, ear lobes, and beaks, indicated a large egg production, as a result they were able to cull out those unprofitable hens which had colored skin parts.

THE VALUE OF NATURAL COLORING MATTERS AS DYESTUFFS.

Since the introduction of synthetic dyestuffs, the use of natural dyeing materials has gradually become more and more restricted until at the present time the few vegetable dyestuffs which are still in use may be looked upon as the "survival of the fittest." During the war, when difficulty was experienced in obtaining

fast dyes, the use of natural coloring matters received a stimulus, but this was only temporary. The following list represents the most important natural dyes in use at the present time in this country.

ANNATTO is an orange-yellow dye which is used to color oils, butter, cheese, and margarine. It is prepared from the seed sheels of *Bixia arellana* which is native to Central America, East and West Indies, India and Guiana. The fruit of this shrub is a miter-shaped capsule containing 30 to 40 seeds which possess a waxy coating containing the dye.

ARCHIL and CUDBEAR are purple dyes prepared from certain lichens such as *Roccella tinctoria, R. fuciformia* and *Ochrolechia tartocea.* Oxidation in alkaline solution of colorless phenols occurring in the lichens gives rise to the coloring matter which is chiefly dihydroxy methylbenzene. Archil and cudbear were used during the war on ladies' woolen dress goods to produce bluish red colors.

CHLOROPHYLL EXTRACT, the green coloring matter of leaves, is used for coloring fats, oils, alcoholic liquids, soaps, ointments and for confectionery and preserves. Lately a product has appeared on the market under the name of "phyllosan," an active chlorophyll product, which is supposed to improve the condition of the blood by increasing hemoglobin content.

CUTCH is a noteworthy and largely used dye prepared from the wood of *Acacia catechu* and from wood of allied varieties. The *Acacia catechu* is a crooked shrubby tree growing in India, East Indies and Hindustan. Cutch is famous for its brown colors. It is used principally in dyeing and textile printing, as a tanning material, and in medicine as an astringent.

FUSTIC, the yellow dyewood from the tree, *Chlorophora tinctoria* which grows in the West Indies, Mexico, Central and South America, contains two coloring matters, the principal one belonging to the flavone group and the other known as maclurin. Fustic is widely used in wool dyeing for producing yellow to olive colors with varying mordants and still other shades by combination with other natural dyes. "Young fustic" is a totally different product from fustic. The former is derived from *Rhus cotinus* which is a tough little sumac found in Southern Europe and West Indies. The product from this source is no longer in use. During the war fustic furnished the dyestuffs for our doughboys' uniforms.

INDIGO is the dye produced from different species of *Indigoferae.* At the present time most of the indigo which is used so extensively for printing and dyeing cotton goods is synthetically produced from components of coal tar. However, there still exists a preference to use the natural product in dyeing wool by the fermentation vat and for this purpose it is cultivated in China, Japan, Philippines, Africa, Brazil and Guatemala.

LITMUS is prepared through air oxidation of a colorless product occurring in lichens which grow abundantly in Scandinavia and the French Pyrenees. It is similar in character to archil and is employed mostly as an indicator in chemistry, although use has been made of it for coloring wine and vinegar.

LOGWOOD or Campeche wood is one of the most important present-day dyestuffs. It is used almost exclusively as a black dye on such materials as silk, wool, leather, cotton, fur and straw, etc. It is particularly useful in connection with the dyeing of silk since over 200% of extract is absorbed during the process, thus acting as a weighting agent and as an aid in increasing strength. Crude logwood is the heartwood of a tall tree, *Haematoxylon campechianum,* native to Central and South America and West Indies. It is imported into this country in large quantities in the form of rough logs, and later reduced to small chips or raspings and extracted with hot water which is concentrated to 51° Tw. From logwood extract other products are made by oxidizing the coloring matter hæmatoxylin to a compound known as "hematine." The latter substance may be obtained in either the liquid or solid form.

OSAGE ORANGE or bow wood is obtained from the native born North American trees known botanically as *Maclura pomiferum.* It grows extensively in Oklahoma, Texas and Arkansas. The coloring matters present in osage orange are the same substances which occur in fustic, consequently osage orange extracts have displaced fustic to some extent.

PERSIAN BERRIES are the dried half-ripe fruit of *Rhamnus* sp. They are used principally in cotton printing for yellow, orange, and olive-green shades. The chief coloring matter is the yellow flavonol, rhamnetin, which occurs in the berries in the form of its glucoside.

QUERCITRON is a domestic dyestuff consisting of the ground inner bark of *Quercus velutina* or black oak which grows in the Eastern United States. The coloring matter quercitrin, a flavonol rhamnoside, is obtainable either as the powdered bark or in the form of concentrated preparations known as "flavine." Quercitron and flavine are very important dyestuffs.

As far as the writer is aware there are now no anthocyanin preparations which have assumed any commercial importance. Even though the natural anthocyanins possess brilliant shades which leave nothing to be desired and cannot readily be matched by means of artificial dyes, yet the shades produced, while being very fast to light, are fugitive with respect to alkalies and acids and to washing. Nevertheless, interest is being attached to these pigments because they represent a chemical makeup which indicates high tinctorial power.

PIGMENTATION IN CONNECTION WITH PROBLEMS OF INHERITANCE.

From the standpoint of fundamental research, flavone glucosides and antho-cyanins are important in connection with problems of inheritance. Anthocyanin colors have attracted geneticists because they have afforded an almost unlimited supply of material for Mendelian research, consequently a great deal has been learned concerning the genetical constitution of certain plants with respect to pigmentation. A combination of chemical and genetical lines of endeavor should make it possible to give a chemical interpretation to Mendelian color factors. To make this clear a simple illustration may be cited. A sweet pea plant bearing purple flowers may be obtained by crossing two white flowered strains of certain genetical constitutions. From this it is evident that the two white flowered parents have inherited, between them, the ability to produce chemical substances neces-sary for the production of anthocyanin. It has been suggested in a general way by Miss Wheldale (9) that one parent contains a chromogen (flavonal glucoside) and the other an enzyme which acts upon this chromogen to form anthocyanin. The object of research is to isolate and identify these substances and to determine the actual process involved in the production of anthocyanin.

It goes without saying that the train of reactions responsible for the formation of chromogen and anthocyanin in such cases must be exceedingly complex. In fact, the available evidence would seem to indicate that cases of extreme simplicity are not often to be expected. We have, for example, a more complex situation (10) in the case of Emerson's series of colors in maize in which six main color types have been established as follows: (*a*) purple, (*b*) sun-red, (*c*) dilute purple, (*d*) di-lute sun-red, (*e*) brown, and (*f*) green. The genetical relations of these six types have been established. There can be little doubt that the phenomena observed in the course of the genetical studies have a discoverable chemical basis. It is the problem of the chemist to disentangle and isolate the substances whose combina-tion is responsible for the observed phenomena. Thus far no series of color typed of known genetical constitution has been thoroughly examined chemically, although an excellent beginning in this work was made by Wheldale and Bassett (11) in con-nection with the isolation of flavones from two yellow varieties of *Antirrhinum*. Investigations of this kind, which have in view a chemical interpretation of Men-delian character, would be helpful in an understanding of interacting factors in heredity.

Pigmentation in plants is not only of fundamental importance to the genet-

icist but it has considerable value to the practical plant breeder who is constantly attempting to intensify desirable qualities in fruits and vegetables and to eliminate objectionable ones. Many correlations occur between color and other plant characters which sometimes enable the breeder to use color as a valuable index in selection. In a number of cases pigmentation seems to bear a relation to flavor, acidity, sweetness, and astringency. Beach (12) gives the following statement concerning the relation of color in apples to quality:

"The quality of the fruit corresponds in a general way with the development of the color of both its skin and its flesh. It has already been observed in speaking of the color of the flesh that under certain conditions it is not normally developed. When the color of either the skin or the flesh is not properly developed, there is a corresponding lack of development of the quality of the fruit. This statement may be easily verified, as already noticed, by testing highly colored Northern Spy apples in comparison with poorly colored fruit of the same variety. It will be found that the poorly colored fruit, even though it may have been produced on the same tree as that which bore the highly colored fruit, is decidedly inferior in quality."

Analyses from various sources (13) show that white currants are sweeter and contain less acid than red currants. In the case of the beet we find the white varieties containing more sugar than the red ones. Sufficient data are lacking in other cases to justify definite conclusions, but the impression seems to exist that white-fleshed watermelons, yellow raspberries, yellow table beets and white carrots, are sweeter than the corresponding red varieties. According to Caldwell (verbal communication) a relation seems to exist between soluble red color in grapes and astringency. The more astringent grapes appear to be the darker colored ones.

A correlation has been discovered by Hedrick (14) to exist in the peach between the inside color of the calyx cup and the flesh color. The observations, which were made on two trees of each of three hundred and seven varieties, showed that flowers whose calyx cups were colored green inside produced white-fleshed peaches, while those having calyx cups of an orange color developed into yellow-fleshed fruits. This correlation has proved of practical value in peach breeding because of the time saved in a predetermination of flesh color. Ordinarily, the first crop of blossom seldom sets fruit, and the correlations have made it possible to know the flesh color a year or two in advance of fruiting.

Another correlation in the case of the peach which has practical application is the relation which has been found between the time of blossoming and the bark color of the branches. The time of swelling of peach buds in early spring is very important because of the danger of frost injury, a delay in blossoming for several days being sometimes sufficient to prevent the loss of the crop. Whitten (15) has shown that varieties possessing green branches blossom later than those having purple-colored branches. Even on the same tree the branches showing less purple color will produce flowers later than more highly colored ones. The cause of this phenomenon is due to the fact that different coloring matters absorb heat rays in varying degrees. It is well known that green leaves in general register lower temperatures than red ones and this is also true in the case of green and purple peach twigs. The effect of higher temperatures on peach buds is to cause them to advance more rapidly. One would be expected to conclude from these results that peach varieties with green branches would be less susceptible to injury from early frosts

and would be more desirable than trees bearing purple-colored branches. Unfortunately, other considerations such as character and size of fruit have prevented the selection of these hardier varieties for commercial purposes. Perhaps as a result of breeding it might be possible to discover green-stemmed varieties which will at the same time bear desirable fruit. It has been suggested that whitewashing peach trees might serve as a protection against disastrous injury from frost. Such a procedure has been found to delay blossoming from two to six days.

An example of the association of color with qualities undesirable from the standpoint of canning is found in peas. Nearly all peas from vines bearing colored flowers blacken on cooking and this dark color is imparted to the liquor in the can making a discolored product which is very objectionable to the consumer. From the viewpoint of the canner the best peas bear white flowers. In 1920, a blackening of canned peas caused considerable loss. The condition was caused by the admixture of a field pea, known as Bengalia, with an Alaska pea. The former bears pink flowers in the field, while the latter has white flowers. The two varieties are not related genetically, hence the mixture was a purely mechanical one.

One of the aims of the plant breeder is to develop highly colored varieties of fruits and vegetables. For example, canning factories desire a tomato richer and brighter in color than the ones we now have. This desire for more color in the natural product is mainly to offset for the reduction of carotinoid pigment which occurs during processing because of the ease with which it oxidizes. Without a means for the quantitative determination of color, the breeder must depend on the eye to indicate whether or not proper crosses or selections are being made. If judged by eye alone, as much as a ten per cent increase in color could easily be overlooked. The chemist's part is concerned with development of methods for pigment determination. Such methods would be useful also in determining any change in color content of a certain variety from year to year, and in the same variety grown at different localities.

POSSIBLE RELATION OF PLANT PIGMENTS TO DISEASE RESISTANCE.

One of the recent developments in connection with the study of pigments is their possible relation to disease resistance in some plants. Such a relation has been suggested by Walker (16) in the case of the onion, in which he showed that white varieties are susceptible to smudge (Colletotrichum circinans) while colored varieties, with bulbs containing flavones and anthocyanins, show a high degree of resistance.

In addition to the onion a number of cases have been noted where a correlation seems to exist between resistance and pigmentation. Fromme and Wingard (17) state that all varieties of beans with solid red or red-mottled seed which were examined, were resistant to rust (Uromyces appendiculatus), while white-seeded varieties were more susceptible as a class than those of any other color. Purple-skinned varieties of sweet potatoes seem to show more resistance to the soil rot than white or yellow-skinned varieties. White carnation plants are much more subject to stem rot than are the colored types. In the case of apple scald, which is a storage disease not attributable to any organism, we have a striking illustration of a greater liability to injury in the less colored parts. The red areas of the skin seem to remain freer from browning or scald effects than other portions of the

same fruit. It may be interesting to point out here the possible relation (18) of flavones to browning of scalded apples. A flavonol is undoubtedly the parent substance of the anthocyanidin or red pigment of the apple. It appears, therefore, that those areas which contain the unchanged flavonol are more subject to scald than the areas in which the flavonol has been converted into anthocyanidin. Proof that flavonols are capable of giving rise to brown oxidation products has been supplied by the investigations of Nagai (19) which show that brown or reddish brown pigments are formed from flavonols when treated with oxidases. Since a flavonol and oxidizing enzymes are normally present in the apple, a possible connection between flavonol and scald browning becomes more apparent. It must be borne in mind, however, that other substances such as tannins are also capable of giving rise to brown oxidation products under certain conditions.

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CINCHONINE AS A TANNIN PRECIPI-
TANT, WITH SPECIAL REFERENCE
TO THE ANALYSIS OF CUTCH
AND GAMBIER.

BY DAVID HOOPER.

The cinchonine method of A. Chaston Chapman (*J. Inst. Brewing,* 1907, 13, 646–657; 1909, 15, 360–374), elaborated by him for the determination of tannin in hops, has been extended with concordant results to a number of other tannin-containing materials. The method has been found of special value for the determination of tannin in cutch and gambier, since cinchonine does not precipitate catechin. Hide-powder, on the other hand, absorbs catechin.