"The Xanthophyll Group of Yellow Colouring Matters." By C. A. SCHUNCK. Communicated by HORACE T. BROWN, F.R.S. Received April 17,—Read May 14, 1903.

[PLATES 6 AND 7.]

The Xanthophyll Group of Yellow Colouring Matters.

The group under consideration comprises those colouring matters occurring in flowers, leaves, fruits, etc., which are insoluble in water but soluble in alcohol, ether, carbon-bisulphide and other organic solvents, to which collectively the name of xanthophyll has been applied. It is to these colouring matters that the characteristic yellow colour of flowers, autumnal leaves and fruits in the majority of cases is due. There are, however, present, varying in amount but generally in considerably less relative quantity, other yellow colouring matters which are soluble in water and alcohol, sparingly soluble in ether but insoluble in carbon-bisulphide; they, however, absorb the violet and ultra-violet rays without giving any absorption bands, whereas the members of the xanthophyll group transmit the ultra-violet rays and give definite absorption bands in the less refrangible region of the spectrum, whereby they may be distinguished from one another, and which is their chief characteristic feature. The present memoir is mainly a spectroscopic investigation of this group, whereby I have endeavoured to distinguish the different yellow colouring matters present in various flowers, leaves and fruits, and to show their spectroscopic relationship one to another, and which may, perhaps, be an aid to elucidate their chemical constitution, of which so little, if anything, is known so far.

The spectroscopic observations, as in a former investigation,* were made by means of photography, an Iceland-spar prism, and quartz lenses being used, the source of light being a Welsbach incandescent gas mantle.

Flowers.

In all, twenty common yellow flowers were examined. The method of procedure was to obtain an alcoholic extract of the pigment contained in the petals, boiling for a short time being generally necessary to extract the whole. On cooling the hot filtered extract a fatty waxy deposit forms, in most cases, which carries down a certain quantity of the pigment with which it is deeply impregnated. The extract, filtered from this deposit, is now agitated with carbon-bisulphide which takes up the greater portion of the xanthophyll, leaving in the alcohol those colouring matters which obscure the violet

* 'Roy. Soc. Proc.,' vol. 63, p. 391.

and ultra-violet region of the spectrum and which occur along with the xanthophyll in greater or less quantity in all flowers. In some flowers they are almost absent, while in others (as, for instance, the yellow calceolaria) they are present in a large quantity compared to the xanthophyll, and in the yellow dahlia the pigment is entirely comprised of them. These colouring matters are soluble in boiling water and alcohol, sparingly soluble in ether, but appear to be insoluble in carbon-bisulphide, and towards alkalies behave in a different manner to the xanthophylls, the alcoholic extracts becoming deeper yellow, in some cases orange, and in others (as from the yellow dahlia) brick-red, on addition of ammonia, and crimson with sodium hydrate, the original yellow colour being reproduced on neutralising with acid. On the other hand, alkalies appear to have no action upon the members of the xanthophyll group and saponifying does not appear to alter them.

It is essential for the spectroscopic investigation of the xanthophylls that the above-mentioned colouring matters be first got rid of. For if not, the bands of the former are obscured by the general absorption of the latter, and I find the separation by means of carbon-bisulphide preferable to extracting the petals first with boiling water, whereby they can also be removed, as this has a tendency to affect certain of the xanthophylls, due perhaps to the acid contained in the cell-sap.

The separation by carbon-bisulphide is effected by agitating the extract several times with equal quantities of the solvent until no more pigment is taken up; the several carbon-bisulphide portions or fractions, which vary from three to four in the case of flowers, are allowed to spontaneously evaporate and the pigment taken up again with alcohol, and then each is examined spectroscopically together with the original alcoholic extract for comparison, as well as the pigment contained in the fatty deposit and that remaining in the alcoholic extract after the carbon-bisulphide separation.

The results of the spectroscopic observations of the various flowers experimented with, show the presence of three yellow colouring matters, each giving, like chrysophyll, the crystalline substance obtained from alcoholic extracts of the green leaf, three pronounced absorption bands in the violet region of the spectrum, and are further characterised and distinguishable from one another by giving a different spectroscopic reaction with acid which, in the case of two, is very definite and sensitive. Though spectroscopically there appears to be good evidence of the existence of three distinct colouring matters, yet trying various means I failed to obtain them in the crystalline form or in sufficient state of purity, free from the accompanying fats, etc., to be able to examine their chemical properties, pending which it will, I think, be sufficient to term them for the present L. B. and Y. xanthophyll. They are distributed as follows in the flowers examined:—

L. xanthophyll.	Calceolaria, Nasturtium (*Tropæolum*).
B. xanthophyll.	*Doronicum* (two species), *Chrysanthemum*, Charlock (*Raphanus raphanistrum*), Buttercup (*Ranunculus acer*), Sunflower (*Helianthus annuus*), Dandelion (*Taraxacum officinale*), Musk (*Mimulus moschatus*), Laburnum (*Cytisus laburnum*), Coltsfoot (*Tussilago farfara*), Marigold (*Calendula officinalis*).
L. and B. xanthophyll.	Wallflower (*Cheiranthus cheiri*).
Y. xanthophyll.	Tulip, Pansy (*Viola tricolor*), Gorse (*Ulex europæus*).
L. Y. xanthophyll.	African Marigold (*Tagetes erecta*), Daffodil (*Narcissus pseudo-narcissus*).

Thus, in the above flowers the B. xanthophyll greatly preponderates. In certain varieties of the above, as the dark-coloured calceolaria and wallflower and the various shades of nasturtiums and the red tulip, the xanthophyll is present as usual but is masked by the presence of these additional pigments, which in each case can be removed by extracting with hot water in which they are easily soluble. The depth of colour of the flowers appears to depend upon the amount of pigment present and not upon the particular xanthophyll; thus in the case of the chrysanthemum three different varieties were examined, a very rich yellow, a medium coloured and a very pale one, but in each the pigment consisted of B. xanthophyll. Of the African marigold, three shades of flowers were also examined, varying from a rich orange to a very pale yellow, but the majority of the pigment in each consisted of L. xanthophyll.

The spectra of these three xanthophylls each consist of three bands situated between the solar lines F and H, and compared to chrysophyll and to one another the bands exhibit a gradual shifting towards the violet, those of chrysophyll being the least refrangible and those of Y. xanthophyll the most (Plate 6, figs. 1—5). They form a very similar and closely connected series of spectra, which points to a close relationship between these colouring matters. There is generally an indication in each of a fourth more refrangible band, but this is not apparent in the fresh extracts and I believe is due to spontaneous change which takes place after a time. The photographic plates of their spectra, as well as of chrysophyll, show that the ultra-violet rays are transmitted to a considerable extent, though this visibility is somewhat lost in the reproduction. Though the bands of each occupy different positions in the spectrum, it is by the change brought about in their spectra by the action of acid that these three xanthophylls can be definitely distinguished from one another. In the case of L. xanthophyll HCl has no immediate effect, the bands fading after a time

with an indication of a fourth more refrangible, the solution becoming by degrees paler and assuming a slight green tinge before becoming colourless. HNO_3 on the other hand rapidly affects the colouring matter, a fourth more refrangible pronounced band is formed, the first and second by degrees disappear and the third becomes faint, the solution in a short time assumes a green tinge and finally becomes colourless (Plate 6, figs. 10—14). The same reaction takes place with H_2SO_4, H_2O_2, and nascent H, but with these reagents the action is slower. On the other hand HCl produces an immediate and striking effect upon the spectra of B. and Y. xanthophyll, their solutions at once became paler yellow in colour; in the former the first band disappears the resulting spectrum consisting of three distinct bands, the first two a little less refrangible in position than the original second and third with an indication of a fourth in the ultra-violet at M, while in the latter three pronounced bands are formed, removed considerably towards the violet and ultra-violet with an indication of a fourth at N (Plate 6, figs. 6—9).

The reaction in both cases is a most delicate one, a very small quantity of acid being required, otherwise the reaction takes place too rapidly to be observed. It is evidently due to the formation of two other yellow colouring matters from the B. and Y. xanthophylls, which are, however, unstable in the presence of the acid and are rapidly destroyed, the solution becoming a greenish-blue and finally colourless in a short time. A similar change takes place in these two xanthophylls on standing for a time, the above two colouring matters being formed spontaneously, but under these circumstances they appear to be more or less stable and their alcoholic solutions can be kept for a considerable time without further change taking place.

In the case of some flowers (as coltsfoot and marigold) the extracted pigment appears to be a mixture of B. xanthophyll and the colouring matter formed from it by the action of acid or spontaneous change. Sorby* has already noticed the formation of this colouring matter from his "yellow xanthophyll" by the action of acids, but the change that the Y. xanthophyll undergoes has not before been noticed I think. H_2SO_4 and HNO_3 produces in both the same effect as HCl, the action being more energetic especially with the latter.

In the separation by carbon-bisulphide the greater portion of the pigment is taken up in the first fraction, the subsequent ones containing less and less and by examining together the spectra of each and the action of HCl one can readily determine which xanthophyll is present. In cases of mixtures of L. and B. and L. and Y., which occur in the wallflower, daffodil and African marigold, the L. being more soluble in carbon-bisulphide than the B. and Y., the latter will be found more or less free from the L. in the subsequent fractions and the action

* 'Roy. Soc. Proc.,' vol. 21, p. 459.

of HCl will confirm their presence, and so sensitive is the reaction that the admixture of very small quantities of B. and Y. with L. can be easily detected. The alcoholic extract, after the carbon-bisulphide separation, besides containing the yellow colouring matters which are soluble in water and absorb the violet and ultra-violet rays will also contain some xanthophyll, but the bands of the latter are generally so obscured by the former, that only in cases where there is but little obscuration is an examination possible, but a certain amount of clearing up of the spectrum may be effected by adding water and ether, the xanthophylls being more soluble in ether than these colouring matters. If the fatty waxy deposit which, as mentioned before, forms on cooling from the extracts, and is deeply impregnated with pigment be dissolved in alcohol and examined, it will be found to contain the xanthophyll group only and in cases of mixtures to contain the L. xanthophyll free from the B. and Y. which points to the fact that in the presence of fats the former is less soluble in alcohol than the latter.

In contradistinction to the B. and Y. the L. xanthophyll is more or less stable, its alcoholic solutions showing but little change even after the lapse of several weeks when kept away from the light, and is in fact, more stable than chrysophyll under the same conditions. Towards acids, H_2O_2 and nascent H both appear to behave in a very similar manner though chrysophyll withstands the action of HCl to a greater extent. This similarity together with the close resemblance of their spectra, the slight shift in the bands being the only difference, indicates but a slight difference in their chemical constitution, and though in none of the flowers experimented with was any chrysophyll obtained, yet under certain conditions it may be that chrysophyll is elaborated from the L. xanthophyll.

With HCl no colour reaction is produced in alcoholic solutions of chrysophyll and L. xanthophyll, but with the B. and Y. a striking effect is produced. Taking fairly concentrated solutions and adding a little concentrated HCl, after a short time a deep green coloration is produced which changes to peacock blue, purple and then gradually fades, the solutions becoming colourless in a day or two, the reaction being more rapid in the case of the Y. and the colour effect being slightly more brilliant. On the addition of ammonia the original yellow colour though less intense is produced, the blue colour reappearing on acidifying and *vice versâ*. Sorby* mentions this reaction in connection with his "Yellow Xanthophyll," and is, I believe, the first to have noticed it. If the blue solutions be examined spectroscopically, but a faint indication of bands is discernible, but after the addition of alkali the spectra produced by the action of acid upon the B. and Y. xanthophylls are exhibited, so that it appears the action of acid first produces these two yellow colouring matters from the B. and

* *Loc. cit.*

Y. which under the action of the acid give rise to a blue pigment which is reconverted into them by the addition of alkali and *vice versâ*. The same reaction takes place with H_2SO_4 and HNO_3 but the colour effects are not so brilliant. In the dry state both chrysophyll and L. xanthophyll as well as the B. and Y. turn a Prussian-blue colour with a drop of concentrated HNO_3 which is evanescent, an indigo-blue with concentrated H_2SO_4 which is more lasting, and the two latter assume a green colour with concentrated HCl, there being no alteration in colour the two former in this case.

Leaves.

From the concentrated alcoholic extracts of healthy green leaves, as is well known, minute sparkling red crystals having a metallic lustre form in the course of a day or so, and in cases where deposits form from the hot extracts on cooling, the crystals will also be found embedded in it. This substance which appears to be universally present in all green leaves but varying in amount, is the Chrysophyll of Hartsen and Schunck*, and according to Arnaud† is identical with Carotin, the crystalline substance obtained from the carrot root and which name he applies to it. These minute crystals from the green leaf extracts can be freed from chlorophyll and the other colouring matters present by washing with cold alcohol in which they are almost insoluble and recrystallising from ether in which they are very soluble, and from which this substance crystallises in the form of beautiful red metallic leaflets on slow evaporation. Chrysophyll is also soluble in boiling alcohol and glacial acetic acid, readily soluble in carbon-bisulphide, but insoluble in alkalies. No very definite chemical reactions of this substance so far are known, it is not a stable body and in contact with the air soon decomposes, it can, however, be preserved in an atmosphere of hydrogen without change. HCl has no effect upon the crystals, they dissolve, however, in concentrated H_2SO_4 producing a deep Prussian-blue solution which soon changes to purple and then brown, and HNO_3 decomposes them at once. The colour reactions the crystals assume as mentioned above with a drop of concentrated HNO_3 and H_2SO_4 appear to be a general reaction of this group of colouring matters. According to Tschirch‡ who applies to it the name Xantho-Carotin, this substance forms with iodine a green derivative.

The absorption spectrum of three pronounced bands situated between F. and H (Plate 7. fig. 1) appears to be the most characteristic and distinguishable property of chrysophyll which on the addition of a small quantity of HNO_3 to the alcoholic solution undergoes similar changes to that which L. xanthophyll passes through the bands fading

* 'Roy. Soc. Proc.,' vol. 44, p. 449.

† 'Compt. Rend.,' vol. 102, p. 1119, and vol. 104, p. 1293.

‡ 'Berichte der Deutschen Botanischen Gesellschaft,' vol. 14, Part II, p. 84.

without change in position and a fourth more refrangible one being formed, but with Chrysophyll, it is not so pronounced, the solution assumes a greenish tinge and in a short time becomes colourless. The action of H_2SO_4, H_2O_2 and nascent H appears to be the same, but the action is slower. HCl has a very feeble action, the solution gradually becoming paler in colour without any green tinge, and colourless in a week or two, the three bands gradually fading without the formation of any additional ones. Alkalies have no effect upon the spectrum.

The means whereby the yellow colouring matters accompanying chlorophyll in the alcoholic green-leaf extracts can be separated from the latter, and their subsequent separation by carbon-bisulphide has been the subject of a previous investigation.* In the light of the results obtained with the flowers it appears now that besides chrysophyll the other xanthophylls present are the L. and B. and the colouring matter formed from the latter by the action of acid, and in addition there are present those yellow colouring matters that cause obscuration in the violet and ultra-violet, and as in flowers vary in amount with the particular plant. In those cases where a fourth band is visible in the spectrum this is due to the acid derivative of B. xanthophyll, the proportion of these two colouring matters varying in different leaves, and the preponderance of one or the other can be decided by the aspect of the spectrum and the action of acid thereon. In the above investigation I considered that the interpretation of the series of spectra obtained by the carbon-bisulphide separation was that the crude xanthophyll extract is a mixture of chrysophyll and what now turns out to be B. xanthophyll and its acid derivative, but in the light of the experiments upon flowers, and further experiments upon the xanthophyll of the leaf taken in comparison, I am of the belief that L. xanthophyll must also be present in order to fully satisfy the spectroscopic observations. The xanthophyll of all leaves appears to be composed of these same components, and where the flower xanthophyll varies, as in the daffodil, wallflower, charlock and tulip, yet their leaf xanthophyll is the same as in other green leaves.

An experiment was made with the yellow pigment of the etiolated leaf of the daffodil compared to the normal green leaf of the same plant, and the xanthophyll in each was found to be the same, save that from the etiolated leaf no chrysophyll crystals were obtained, which were plentiful in the extract from the normal leaf in which the chlorophyll had formed after the etiolated plant had been subjected to the action of sunlight. Lastly, as regards the xanthophyll of the autumnal leaf spectroscopic observation shows the presence of L. xanthophyll, and a great preponderance of the acid derivative of B. xanthophyll over the normal B. xanthophyll, which causes the spectrum to be four-banded in the majority of cases, the chlorophyll no longer being

* 'Roy. Soc. Proc.,' vol. 68, p. 474.

formed at this season and disappearing, leaving the accompanying xanthophylls which gives the leaf its characteristic autumnal colour.

From these few observations it seems that the formation of chrysophyll within the leaf depends on similar conditions to the elaboration of the chlorophyll, but whether it is formed independently or from one of the xanthophylls present is still a problem to be solved.

Fruit, etc.

Several varieties of the orange were examined, and here a considerable amount of the pigment of the rind is soluble in water and causes the great amount of obscuration in the violet and ultra-violet observed in the alcoholic extracts of the pigment. The redness of the rind which is present in many (as the Blood, Seville and Tangerine) appears to be due to these colouring matters, which can be removed by boiling water leaving the rind the normal orange colour. By treating the rind with boiling alcohol a rich orange-coloured extract of the pigment is obtained, from which on cooling a deep orange deposit forms. If this deposit be dissolved in a little absolute alcohol chrysophyll crystals form in a small quantity on slow evaporation. The spectrum of the alcoholic solution of the deposit, which as in the deposits from flowers exhibits no obscuration in the violet and ultra-violet, indicates that besides chrysophyll there are also present the acid derivatives of B. and Y. xanthophyll. The mother liquor of the deposit which contains the majority of the pigment spectroscopically does not show the presence of chrysophyll, indicating that this substance is present in but small quantity, the absorption bands visible after the separation by carbon-bisulphide appearing to be due principally to the acid derivatives of B. and Y. xanthophyll. In the lemon the yellow pigment of the rind consists principally of the colouring matters producing obscuration together with the above acid derivatives.

The crystalline substance obtained from the pigment of the carrot root (*Daucus Carota*) and which is termed Carotin, has been the subject of investigation by Arnaud,* who considers it a hydrocarbon of the formula $C_{26}H_{38}$, though Husemann who has also examined the substance applies that of $C_{18}H_{24}O$. I have compared this substance with chrysophyll and find, as with Arnaud, that they have the same properties and that their spectroscopic properties, which he did not examine, are identical save that the bands of carotin appear to be very slightly moved towards the violet as compared to those of chrysophyll (Plate 7, figs. 1 and 2). Towards acids the spectroscopic reaction is identical in each. It may be the slight difference in the positions of the bands is merely due to spontaneous change or oxidation that has

* 'Compt. Rend.,' vol. 100, p. 751; vol. 102, pp. 1119, 1319; vol. 104, p. 1293; vol. 109, p. 911.

taken place in the carotin while within the root. From the extract of the pigment prepared by boiling the grated root with alcohol, the carotin crystallises out on cooling, but a larger yield is obtained from the juice of the grated root, which contains the majority of the carotin in suspension, from which on drying it can be dissolved in ether and recrystallised in the same form of crystals as chrysophyll. There is a certain amount of those colouring matters present which obscure the violet and ultra-violet, but the majority of the total pigment appears to consist of carotin alone.

The pigment of the tomato (*Lycopersicum esculentum*) is interesting in so much as it consists of a crystalline substance not before described, I believe, and which gives a very characteristic spectrum of similar character to the other xanthophylls and from its reactions appears to be of similar constitution. The tomatoes were first boiled with water which extracted a little yellow colouring matter, the watery extract filtered off and the pulp and skin washed with cold alcohol and the red pigment extracted with boiling alcohol, in which it is not easily soluble and which takes some time to extract the whole. A deep orange-coloured extract is obtained, from which on cooling a rich red deposit forms which is sparingly soluble in alcohol. In ether the deposit dissolves readily, and on slow evaporation deep-red crystalline needles form which can be purified by recrystallising from ether. If the pulp and skin be first dried and the pigment extracted with ether in the cold, which takes some days, the same substance crystallises out on slow evaporation. The crystals are with difficulty soluble in boiling alcohol from which they crystallise out on standing, they are not so soluble in ether as chrysophyll neither have they the metallic lustre of the latter; in alcohol and ether their solutions are orange, but in carbon-bisulphide, in which they are easily soluble, the colour is reddish-pink, due to the shifting of the bands in this solvent considerably towards the red. They dissolve in hot glacial acetic acid giving pale yellow solutions, but spectroscopic examination shows in contra-distinction to chrysophyll that this substance is acted upon by the acid during solution, producing another colouring matter similar to that formed by the action of strong acids upon its alcoholic solutions. In alkalies the crystals are insoluble. Like chrysophyll they dissolve in concentrated H_2SO_4, producing a deep Prussian-blue solution which soon changes to purple and then brown. HNO_3 causes immediate decomposition and HCl has no action. With a drop of concentrated H_2SO_4 and HNO_3 the same colour reactions are produced as with chrysophyll. It is not a stable substance, and exposed to the air in the absence of light it is decomposed in the course of a few weeks.

The absorption spectrum is a very distinctive and definite one of three bands considerably less refrangible in position than those of chrysophyll, the first one being situated in the green (Plate 7,

figs. 3 and 4). The first two bands are very intense, the third fainter, there is also a pronounced band a little less refrangible than the solar line N, which is absent in the chrysophyll spectrum and which appears to be less intense in the ethereal solution, though the other bands are of the same intensity and occupy the same positions in the two solvents. As with chrysophylls and the other xanthophylls the ultra-violet rays are transmitted. Spectroscopic observation also shows that this substance constitutes nearly the whole of the pigment present in the tomato, there being evidence of another in small quantities which can be formed from it by the action of acids, but those that obscure the violet and ultra-violet are almost absent. Though its ethereal solutions are fairly stable, a change soon takes place in the alcoholic solutions, the colour changing from orange to yellow and gradually becoming paler, at the same time the bands become fainter and are replaced by three faint ones in the same positions as those of B. xanthophyll. With HCl, H_2SO_4 and HNO_3 a similar change in the spectrum takes place, its alcoholic solutions gradually assuming a pale yellow colour without any decided green tinge and finally colourless. The spectrum produced by the action of acid upon B. xanthophyll is not assumed, thus one cannot say whether the yellow colouring matter which it undoubtedly gives rise to and the gradual formation of which can be traced in the spectrum under the influence of acid, is B. xanthophyll or not. A similar reaction appears to take place with nascent H. Believing that this substance has not been isolated before, or if it has, has been mistaken for carotin, I venture to apply to it the name *Lycopin*.

From the seeds of the annatto (*Bixa Orellana*) Bixin, to which the formula $C_{28}H_{34}O_5$ has been given, can be obtained by dissolving the annatto of commerce in boiling alcohol, which gives rise to a reddish-brown crystalline mass on evaporation. If this be washed with a little alcohol, dried, and dissolved in boiling glacial acetic acid, the bixin crystallises on cooling and standing in the form of brownish-red leaflets, which are soluble in alcohol, ether and carbon-bisulphide and also in ammonia and sodium hydrate, but insoluble in water. It resembles chrysophyll and lycopin in many respects, dissolving in concentrated H_2SO_4 with a very intense blue colour, which soon changes to purple and finally becomes reddish-brown. HNO_3 likewise decomposes it and HCl has no action even on boiling. The crystals with a drop of H_2SO_4 and HNO_3 turn a brilliant blue with the former and the evanescent Prussian-blue coloration with the latter. Its spectrum resembles chrysophyll save that the three bands which are of the same intensity in each are less refrangible in position, being situated between those of chrysophyll and lycopin, there is no band at N as in the latter, and the ultra-violet rays are transmitted. The alcoholic solution is a rich orange-yellow which fades on the addition of HNO_3

without assuming a green tinge, the bands, however, shift slightly towards the red and gradually fade without the formation of any additional ones; the addition of ammonia causes the bands to shift a little towards the violet. It thus appears that bixin is an allied substance to chrysophyll and the other xanthophylls, and as it is stable and more easy to prepare than the other crystallisable members of the group, it may with advantage form a starting point to study the chemical constitution and relationship of the xanthophylls.

Yellow Pigment of the Egg Yolk and Fowl Serum.

The above yellow pigment together with that of the serum of other animals, of fats, butter, of the *corpus luteum* of the ovary has been the subject of investigation by Thudichum, Hammarsten, Malay, Krukenberg, MacMunn, Halliburton and others. Thudichum was the first to examine the pigment of the *corpus luteum* and the name Lutein was given to it, which name was extended to the whole group. Krukenberg's word, Lipochrome has, however, generally been adopted by physiologists. As they are characterised by giving bands towards the violet region of the spectrum, and also by the same colour reactions with H_2SO_4 and HNO_3 in the dry state as exhibited by the xanthophylls, a comparison was made between the yellow pigment of the egg-yolk and serum of the fowl and the xanthophyll of flowers. The pigment of the egg-yolk can be extracted by treating the yolk freed from the white with alcohol in excess, which precipitates the proteids which take up the pigment leaving the filtrate colourless. If the precipitate be now treated with hot absolute alcohol, the yellow pigment is dissolved and the separation by carbon-bisulphide applied. In the case of the serum the blood-clot is allowed to stand twenty-four hours, broken up and the serum filtered off; it is of a deep yellow colour but more or less masked by the red corpuscles held in suspension. Alcohol is now added in excess, and the yellow pigment which is carried down by the precipitated proteids is extracted with hot absolute alcohol and the separation by carbon-bisulphide is performed. They both form bright yellow alcoholic solutions and the spectrum of each is identical with that of L. xanthophyll, and from the examination of the crude extracts and the carbon-bisulphide fractions the pigment appears to consist of this colouring matter only, with an almost total absence of those colouring matters that obscure the violet and ultra-violet (Plate 7, figs. 5—7). The action of acids upon the spectra is identical with the action upon L. xanthophyll (Plate 7, figs. 8—13), and the colour reactions in the dry state with HCl, H_2SO_4 and HNO_3 are also the same, the blue coloration being not quite so brilliant, which may be accounted for perhaps by the presence of fats. In the case of the egg-yolk an attempt was made to get rid of the fats by saponifica-

tion, after which the pigment was taken up with ether, and on slow evaporation a few vermillion-coloured crystals formed, which gave the same spectrum as before, but the quantity was so small that it could not be definitely decided whether these crystals represented the pigment or were some other substance coloured by it.

Whether the lipochromes from other sources will also prove to consist of the same colouring matter opportunity of investigation has not so far been afforded, but from the spectroscopic properties of the lipochrome in the above cases it appears to be identical with L. xanthophyll, and as it thus appears to be present along with both chlorophyll and hæmoglobin an interesting speculation is presented whether this colouring matter, too, is of physiological importance.

EXPLANATION OF THE PLATES.

(The solvent in each case is alcohol.)

Plate 6.

Spectra.

1 and 5. Chrysophyll from the Daffodil leaf.
2. L. xanthophyll from Wallflower.
3. B. „ „ Doronicum.
4. Y. „ „ Tulip.

6. B. „ „ Doronicum.
7. B. „ „ „ + HCl.
8. Y. „ „ Tulip + HCl.
9. Y. „ „ „

10. L. „ „ Wallflower.
14. L. „ „ „ (stronger solution).

13, 12, 11. Action of HNO_3 upon L. xanthophyll of 14, after successive intervals.

Plate 7.

Spectra.

1. Chrysophyll from Spinach.
2. Carotin from the Carrot.

3. Lycopin, the colouring matter of the Tomato.
4. Chrysophyll from Grass.

5. L. xanthophyll from the Nasturtium.
6. Colouring matter of the Egg-Yolk.
7. „ „ „ Fowl serum.

11, 12, 13. Action of HNO_3 upon 5, 6, 7, respectively.
8, 9, 10. „ „ „ „ after a further interval.