

RESEARCH BULLETIN NO. 9.

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

**CAROTIN—THE PRINCIPAL NATURAL YELLOW
PIGMENT OF MILK FAT—PART I**

**Review of the Literature Concerning the Yellow
Plant and Animal Pigments**

COLUMBIA, MISSOURI
April, 1914

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FOREWORD.

The investigational work upon which this bulletin is based was conducted in co-operation with the Dairy Division of the United States Department of Agriculture. The bulletin herewith presented is Part I of a series of four bulletins reporting a detailed investigation of carotin, the principal natural yellow pigment of milk fat—its sources, its relation to feed consumed and milk produced, factors which are influenced by it as well as the factors by which it is influenced. Part I is prefatory in nature; the investigation proper is reported in parts II, III, and IV.

F. B. MUMFORD,
Director.

CAROTIN—THE PRINCIPAL NATURAL YELLOW PIGMENT OF MILK FAT.

Its Relations to Plant Carotin and the Carotin of the Blood Serum, Body Fat and Corpus Luteum.

LEROY S. PALMER AND C. H. ECKLES.

The investigations dealing with the natural yellow pigment of milk fat will appear as a series of four bulletins as follows:

Part. I. A Review of the Literature Concerning the Yellow Plant and Animal Pigments. Missouri Agricultural Experiment Station Research Bulletin No. 9.

Part II. The Chemical and Physiological Relation of the Pigments of Milk Fat to the Carotin and Xanthophylls of Green Plants. Missouri Agricultural Experiment Station Research Bulletin No. 10.

Part. III. The Pigments of the Body Fat, Corpus Luteum and Skin Secretions of the Cow. Missouri Agricultural Experiment Station Research Bulletin No. 11.

Part IV. (A). The Yellow Lipochrome of Blood Serum. (B.) The Fate of Plant Carotin and Xanthophylls During Digestion. (C). The Pigments of Human Milk Fat. Missouri Agricultural Experiment Station Research Bulletin No. 12.

The present paper is the first of the series. As indicated it will be confined entirely to a review of the extensive literature in regard to the yellow plant and animal pigments.

Part II will be a report of the chemical identification of the milk fat pigment. It will also include a number of investigations showing the relation between the amount of pigment in the milk fat and the character of the ration and the breed of the cow.

Part III will consist of the data showing the chemical identification of the pigments mentioned. Data will also be presented showing the relation between the color of the body fat and the character of the

ration and the breed of the cow. A brief experiment will also be reported showing the absence of these pigments in the body of a new-born Jersey calf.

Part IV (A) will report the chemical identification of the blood serum pigment. It will show how blood carries the pigment and what effect the character of the ration has upon the amount of pigment carried by the blood and the amount secreted in the milk at the same time. A brief study of the cause of the high color of colostrum milk will also be reported. (B) This will consist of the report of a few investigations relative to the fate of the carotin and xanthophylls of plants during their passage through the cow's body. (C) The experiments reported here will show the character of the pigments of human milk fat.

A REVIEW OF THE LITERATURE CONCERNING THE YELLOW PLANT AND ANIMAL PIGMENTS.

It has been the custom for generations to judge the quality of dairy products to a large extent by their yellow color. This has been carried to such an extent that the manufacturer of butter, whether it be on a large or small scale, finds it impossible to market butter that does not have a standard yellow color. The consumer of milk or cream as a rule looks upon a yellow color as indicating the richness and quality of the product. Although it is well known that the color has no relation to the food value of milk or cream, the popular prejudice is so strong that the producer of market milk has to take it into account and try to supply a product with as much natural yellow color as possible.

During part of the year, namely during the spring and early summer and usually also in the early fall, the fresh green feeds which the cows receive give the shade of yellow to the milk fat which the consumer demands. During the winter months, or in summer if the pastures become dry, this yellow color is wholly or in part absent from the milk fat, and the butter manufacturer is then forced to color the butter artificially, in order to maintain the required standard.

It is generally accepted as a fact that the breed of the cow has a pronounced relation to the color of the milk fat and that the Guernsey and Jersey breeds rank first in this respect. The breeders of this class of cattle have emphasized this characteristic as one of the strong points of their respective breeds. This characteristic of Guernsey and Jersey breeds, as compared with the Holstein and Ayrshire breeds, has been generally attributed to physiological differences. According to this view, Guernsey and Jersey cattle are able to produce a higher colored fat due to some inherent quality, just as they are able to produce a higher percentage of fat in their milk. It is a well-known fact that the skin and the secretions of the skin of Guernsey and Jersey cattle have a higher yellow color than other breeds, and this characteristic is looked upon by cattle breeders as an indication of the ability of animals of these breeds to produce highly colored milk fat.

The body fat of Guernsey and Jersey cattle is also characterized by a high yellow color and for this reason beef from these animals is often looked upon with disfavor by the butcher and the consumer.

That the yellow color of butter has a relation to its market value is shown by the fact that "color" has a place on the standard butter score cards with a value of fifteen out of one hundred points. The

oleomargarin manufacturers have also recognized the value of color and, so far as the law has permitted, have made a practice of coloring oleomargarin in imitation of butter. When the law placed a tax on artificially colored oleomargarin, or in some cases prohibited it entirely, the manufacturers began using only the highest colored beef fats that could be bought or mixed the oleomargarin with butter having a high natural color, in order to produce the color they sought.

The Pigment of the Butter Fat as a Factor in the Coloration of Milk.

The more or less yellow color of cows' milk which is especially evident in the cream and butter has not been attributed in all cases to the same pigment. On the one hand a few authors have stated that the pigment of butter is manifested in the familiar yellow color of milk whey. This view originated with Blyth¹ who called the whey pigment lactochrome and the view has found its way into a number of texts. On the other hand a larger number of authors have ignored the whey pigment and considered the lipochrome-like pigment of the milk fat to be the only factor causing the yellow color of cream and butter.

The investigations which were carried on in this laboratory have been the first to point out that the whey pigment and the butter fat pigment are not identical but are distinct substances; and that both are of importance in causing the yellow color of milk. The pigment of the butterfat is the more important of the two, however. The pigment of the whey is of secondary importance, and is of an entirely different nature. Its probable identity with urochrome, the specific urinary pigment, has recently been shown by one of us.²

Object of the Present Investigations.

The present investigations were undertaken primarily to study the chemical nature of the yellow butterfat pigment and to classify it from a scientific standpoint. At the same time information was gathered with the hope of ascertaining to what extent the generally accepted views concerning the color of milk fat are correct in order to establish a scientific basis for the subject which would be of value to those interested in the handling of dairy products in a commercial way.

In the principal part of the investigation it was sought; (1) to show the chemical and if possible the physiological relation of the butter fat pigment to similar animal pigments such as the

1. A. W. Blyth, "Foods. Their Composition and Analysis" Text, 4th Edition 1896, p. 239.

2. Lactochrome: The Yellow Pigment of Milk Whey, etc., by Leroy S. Palmer and Leslie H. Cooleedge. Missouri Agricultural Experiment Station Research Bulletin No. 13;; Jour. Biol. Chem. XVII, p. 251 (1914).

corpus luteum pigment, the body fat pigment, and the blood serum pigment; and (2) to show the chemical and physiological relation of the butterfat pigment to the carotin and xanthophylls of green plants.

In the secondary part of the investigation it was sought to study the influence of certain factors which have both practical and scientific bearing upon the color of the butterfat, among which are the breed of the animal and the character of the ration, the latter in connection with the chemical and physiological studies indicated above.

THE PIGMENTS OF PLANT ORIGIN.

The earliest researches on plant pigments dealt with the green pigments. Caventon first called them chlorophyll in 1817. His work, however, was preceded by the pioneers in this field, among which the names of Grew, whose work is dated 1682, and Rouelle, Meyer, Fourcroy, Berthelot, Senebier, Proust and Vanquelin are of historical interest.

The Carotins.

The Pigment of the Carrot. The yellow pigment of the cultivated carrot (*Daucus Carota*) has long been of interest to botanists and chemists, the investigations of this body having extended over almost one hundred years.

Wachenroder¹ was the first investigator of the carrot pigment. He isolated it and called it Karotin. The work of Vanquelin and Bouchardat² soon followed and a little later Zeise³ took up the study. He obtained the first crystals and assigned to them the chemical formula $C_5 H_{10}$ or $10 (C_5 H_8)$.

Husemann⁴ was the next investigator. He found six per cent of oxygen in his pure preparation and gave the pigment the formula $C_{18} H_{24} O$. A secondary pigment which he thought always accompanied the carotin in small amounts, he named hydrocarotin and gave it the formula $C_{18} H_{30} O$.

It is to Arnaud⁵ however that we are indebted for the first thorough research in regard to the carrot pigment carotin. The crystals which he obtained were flat, rhombic-shaped crystals, red orange by transmitted light, and greenish blue by reflected light. They melted at $168^\circ C$. He showed beyond a doubt that the pigment was simply

1. *Dissertatio de Anthelminticis* Göttingen 1826; also *Geigers Magaz. Pharm.* 33 p. 144 (1831); also *Berzelius Jahresber.* 12 p. 277 (1833).

2. *Schweizg. Jour. Chem.* 58, p. 95 (1830).

3. *Lieb. Ann.* 62 p. 380 (1847); *Annal. Chem. Phys.* (3) 20, p. 125 (1847).

4. *Lieb. Annal.* 117 p. 200 (1860).

5. *Compt. Rend.* 102 p. 1119 (1886), p. 1319 (1887); *Jour. Pharm. Chim.* 14 p. 149 (1886).

an unsaturated hydrocarbon. He gave it the formula $C_{26} H_{38}$ and the iodine derivative the formula $C_{26} H_{38} I_2$.

Eüler and Nordenson¹ report the most recent investigations in regard to the carrot pigment. They found their crystalline preparation to be mixed with crystals of xanthophyll; they also showed that the belief often advanced that carotin is chemically related to cholesterol, is unfounded.

The Carotin of Green Plants.—Arnaud² was one of the first investigators to show that the carrot carotin is identical in properties with a yellow constituent of chlorophyll, although the existence of this yellow constituent of chlorophyll had long been the subject of investigation.

Berzelius³ first sought to isolate a yellow pigment from autumn leaves by extracting with alcohol. He called it "Blattgelb" or xanthophyll, and expressed the belief that the pigment pre-existed along with the green coloring matter of the leaf.

The subject subsequently received the attention of many investigators. Fremy,⁴ Michels, Millardet, Müller, Tinisnsseff, Gerland, Rannenhoff, Askennasy, Stokes, Sorby,⁵ Tschirch,⁶ Kraus,⁷ Filhol,⁸ Hansen,⁹ Conrad,¹⁰ Wiesner,¹¹ and many others took up the investigation.

Fremy designated the yellow pigment Phylloxanthin. Filhol noticed that by treating crude alcoholic chlorophyll solutions with animal charcoal it was possible to remove the green constituent of the mixture leaving a yellow colored solution, the color of which he believed was due to a pre-existing pigment or pigments associated with the green one. Kraus confirmed the observations of Filhol, and was the first to notice that when an alcoholic solution of chlorophyll is shaken with benzoline (petroleum ether) the alcohol retains the yellow coloring matter, the benzoline taking up the green constituent. Kraus' investigation was also the first to show that the ordinary chlorophyll spectrum was due partly to the green and partly to the yellow constituent, which he called xanthophyll. Kraus' xanthophyll gave a

1. Zeit. f. Physiol. Chem. 56, p. 223 (1908).
2. Compt. Rend. 100, p. 751 (1885); 104 p. 1293 (1887).
3. Ann. d. Chem. 21, p. 257 (1837).
4. Ann. Sc. Nat. 13, p. 45 (1860); Compt. Rend. 41, p. 189 (1865).
5. Proc. Roy. Soc. 21, p. 456 (1875).
6. Botan. Zeitung. 42, p. 817 (1884).
7. Flora, p. 155 (1875).
8. Compt. Rend. 39, pp. 9-184; 50, pp. 545 and 1182.
9. Sitz. ber. d. phys. Med. Ges. Würzburg (1883); and Arbeiten d. Botan. Gessel. Würzburg, 3, p. 127 (1884) and "Die Farbstoff des Chlorphylls" (1889).
10. Flora, Vol. 25 (1872).
11. Flora, Vol. (1874); Sitz. der. Wein. Akad. 89, 1. abts. p. 325.

dark blue coloration with concentrated H_2SO_4 , and bleached very quickly in the sunlight.

Sorby, using carbon bisulphide as the separator in place of benzoline, was the first to show that there is more than one yellow pigment associated with chlorophyll.

Hansen's method of isolating the yellow pigments was still different. He treated the alcoholic extracts with caustic alkali, evaporated the liquor to dryness and extracted the yellow pigment from the residue with ether, the spectroscopic study of which led him to believe that it exhibited three absorption bands. He believed also that it was identical with the pigment of the carrot.

E. Schunck¹ obtained from all crude alcoholic chlorophyll extracts minute sparkling red crystals which deposited on standing, and which he considered identical with the crystals which Bougarel² had called erythrophyll, and which Hartsen³ has called crysophyll. This pigment showed two absorption bands.

Tschirch⁴ using Hansen's method, found two yellow coloring matters, to which he gave the name xantho-carotin, showing three bands, and xanthophyll proper which showed no bands.

Returning now to Arnaud's⁵ work, we find that he identified the red orange crystalline pigment which he obtained from spinach leaves with the carotin of the carrot, both as regards to crystalline form, melting point and chlorine derivatives.

We are indebted to Immendorff⁶ for the confirmation of Arnaud's results indicating that the carotin of green plants is identical with the carotin of the carrot. Immendorff gave the pigment the formula which Arnaud found for carotin, namely $C_{26}H_{38}$. He states, however, that the percentage composition of the pure pigment corresponded best with Zeise's formula, C_5H_8 . Immendorff believed that carotin was the only yellow pigment accompanying chlorophyll in the green leaf.

One of the most extensive publications in regard to carotin is that by F. G. Kohl⁷. This author also gives one of the best and most voluminous compilations of the carotin literature that is to be found, besides a large amount of experimental data. The literature is also excellently reviewed by Tammes.⁸ Kohl gave carotin the formula

1. Proc. Roy. Soc. 44, p. 449.
2. Ber. Chem. Gessel, 10, p. 1173 (1877).
3. Arch. Pharm. 207, p. 166 (1875).
4. Botan. Zeitung. 42, p. 817 (1884); Ber. der Deutsch. Botan. Ges. 14, pt. 2, p. 76 (1896).
5. Compt. Rend. 100, p. 751 (1885).
6. Landwirtschaftliche Jahrbücher 18, p. 507 (1889).
7. Untersuch. Über d. Karotin, Leipzig. 1902.
8. Flora, p. 205 (1900).

$C_{26}H_{38}$, and the iodine derivative $C_{26}H_{38}I_2$. He also gave a detailed description of the spectroscopic absorption of carotin. In ether and carbon-bisulphide he measured three bands:

	<i>In ether</i>	<i>In carbon bisulphide</i>
I	490-475 λ	I 510-485 λ
II	455-445 λ	II 470-458 λ
III	430-418 λ	III 437-425 λ

Carotin is lævorotatory, according to Kohl, α_D at 15° in chloroform being -30.17° .

Schunck¹ in his spectroscopic study of the yellow pigments of leaves and flowers, described the properties of carotin. Schunck also photographed the absorption bands of crysophyll (carotin) from the daffodil leaf, from spinach, from the carrot and from grass, in alcoholic solution. All of the carotin preparations showed the same three pronounced bands situated between F and H the first band of which lay almost directly upon the F line.

The most recent detailed investigation of the carotin of green plants is that of Willstätter² and Meig, and a study of their data shows that their results are to be accepted as the final proof of the chemical constitution and properties of this pigment.

Willstätter and Meig describe the properties of carotin as follows: Its crystals are copper colored plates of almost quadratic form, and melt at 167.5° to 168° C. Its crystals are soluble with great difficulty in hot ethyl alcohol and almost insoluble in cold ethyl alcohol, and in methyl alcohol they are still less soluble; one gram of the crystals requires 1.5 liters of petroleum ether (b. p. $30-50^\circ$ C.) for solution and about 900 c.cm. of hot ethyl ether; the crystals are difficultly soluble in acetone, easily soluble in benzol, very easily soluble in chloroform and instantly soluble in carbon bisulphide; the crystals are soluble in concentrated sulphuric acid with an indigo blue color and are precipitated as green flakes on dilution with water.

The carotin obtained by Willstätter and Meig crystallized from its deep red carbon bisulphide solution on addition of absolute alcohol, but analysis showed that the crystals contained $\frac{1}{2}$ to $\frac{2}{3}$ of a molecule of alcohol of crystallization. The carotin showed the composition of a pure hydrocarbon only after crystallization from low boiling point petroleum ether. From this solvent the preparation of Willstätter and Meig showed the composition $C_{26}H_{38}$. A preparation of carotin which the same authors obtained from the carrot showed the same

1. Proc. Roy. Soc. 72, p. 170 (1903).
2. Ann. der Chemie, 355, p. 1 (1907).

composition. A molecular weight determination of both the carotin from the carrot and from the Brennessel leaves, by the ebullioscopic method in carbon bisulphide gave an average of 533 which corresponds exactly with $8(C_5 H_7)$ or $C_{40} H_{56}$. This shows that Arnaud's formula of $C_{26} H_{38}$ is not quite correct. The same difference is brought out by the analysis of the iodine derivative which Willstätter and Meig also prepared.

The absorption bands of carotin were measured by Willstätter and Meig and they coincided almost exactly with those given for it by Tschirch¹ and Monteverde². They did not attempt to measure the third band in the violet which they considered to be end absorption, but measured only the two bands in the blue and indigo blue.

Willstätter and Meig (alcohol sol.)	Tschirch (alcohol sol.)	Monteverde (petroleum ether sol.)
I 488-470 λ	I 487-470 λ	I 491-472 λ
II 456-438 λ	II 457-439 λ	II 461-444 λ

Carotin in Flowers, Fruits and Seeds. According to Czapek³ carotins have been identified in many flowers by Hansen,⁴ Immendorff,⁵ Kohl,⁶ Tammes,⁷ Hilger,⁸ and his pupils, Wirth,⁹ Pabst,¹⁰ Kirchner,¹¹ Ehrung¹² and Schuler.¹³

Among the fruits, Arnaud,¹⁴ Passerini,¹⁵ Kohl,¹⁶ Schunck¹⁷ and Montanari¹⁸ have investigated the tomato pigment and believed it to be a carotin. Its identity as a truly isomeric carotin has recently been proved by Willstätter and Escher.¹⁹ Schrötter²⁰ has shown that the pigment of the pumpkin is in all probability a carotin and Desmoliere²¹ has identified carotin in the apricot.

1. Ber. d. deut. botan. Ges. 14, 76 (1896); 22, 414 (1904).
2. Acta Horti. Petropolitani XIII Nr. 9, 123 and 150 (1893).
3. Bichemie der Pflanzen, vol. I, p. 172, etc.
- 4, 5, 6, 7. Loc. cit.
8. Botan. Centr. 57, p. 335 (1894).
9. Dissert. Erlangen (1891).
10. Arch. Pharm. 230, p. 108 (1892).
11. Dissert. Erlangen 1892.
12. Botan. Cent. 69, p. 154 (1897).
13. Dissert. Erlangen. 1899.
14. Compt. Rend. 102, p. 1119 (1886).
15. Compt. Rend. 100, p. 875 (1885).
16. Loc. Cit.
17. Proc. Roy. Soc. 72, p. 172 (1903).
18. Le Staz. sp. agra. ital. 37, p. 909 (1904).
19. Zeit. Physiol. Chem. 64, p. 74 (1910).
20. Vehr. Zool. bot. Gessel. 44, 298 (1895).
21. Chem. Centr. 2, p. 1001, 1902.

Among the seeds, Schunck¹ has found the annatto pigment to be a carotin.

The Xanthophylls.

It was mentioned above that it has been found that a second class of pigments usually accompanies carotin. Investigations of this class of pigments, now called xanthophylls, has not been as extended as that of carotin but the constitution and properties of the xanthophylls are nevertheless at present established.

Sorby² differentiated the pigments accompanying chlorophyll as xanthophyll, orange xanthophyll, and yellow xanthophyll, all with spectroscopic properties. J. Borodin³ observed that besides carotin, a second crystallizable yellow substance exists in leaves which is much more soluble in alcohol than carotin and insoluble in benzine. Imendorff⁴ denied the existence of more than one pigment as was noted above. Monteverde⁵ confirmed Borodin's observations. Tschirch⁶ in 1896, showed that green leaves contain a second yellow pigment which, however, showed no absorption bands. Tschirch called the second pigment xanthophyll. The name, however, was a misnomer, for Schunck⁷ later showed that Tschirch was dealing with a group of water and alcohol soluble pigments probably identical with the lichenoxanthine described by Sorby.⁸ Tschirch⁹ later recognized the existence of a true second yellow crystallizable pigment.

Molisch¹⁰ in his critical study of the yellow pigments left the question of their plurality an open one, and Tammes¹¹ also left the question undecided.

Schunck¹² in his widely known spectroscopic study of the yellow pigments of plants and flowers, demonstrated beyond a doubt that a second great group of pigments, which he designates the xanthophylls, accompanies the crysophyll. He differentiated three different xanthophylls and designated them L. B. and Y. xanthophyll, respectively.

He found that the xanthophylls were all characterized by giving the same color reactions in the dry state as crysophyll and three

1. Proc. Roy. Soc. 72, 1903.
2. Proc. Roy. Soc. 21, p. 457 (1875).
3. Melanges Biol. tir. d. bull d. L'Acad. Imp. d. St. Petersb. 11, p. 512 (1883).
4. Loc. cit.
5. Loc. cit. p. 148 (1903).
6. Ber. d. d. Botan. Gessel, 14, p. 76 (1896).
7. Proc. Roy. Soc. 72 (1903).
8. Loc. cit.
9. Ber. d. d. Botan. Gessel. 22, p. 414 (1904).
10. "Die Krystallization und der Nachweis des Xanthophylls (carotins) in Blatte" (Ber. d. Deut. Botan. Ges. 14, p. 18 (1896).
11. Loc. cit. (1900).
12. Proc. Roy. Soc. 65 (1899); 68 (1901); 72 (1903.)

similar absorption bands in the violet region of the spectrum. The bands of the xanthophylls, however, were all shifted somewhat towards the blue with respect to the bands of crysophyll, the amount of shifting depending on the xanthophyll, L xanthophyll being shifted the least and Y xanthophyll the most. Schunck found the absorption bands of the different xanthophylls especially characterized by the action of their alcoholic solutions in the presence of HCl and HNO₃, the details of which are given in his latest paper.¹

Schunck also made the very interesting discovery that the yellow pigment of egg yolk and fowl serum shows the identical properties of L xanthophyll both with respect to the position of the original absorption spectra and also the action of acids upon the spectra.

One of the most interesting and important studies of chlorophyll and its accompanying yellow pigments was made by Tswett² who discovered and thoroughly investigated the adsorption properties of these pigments. He was able to demonstrate the presence of at least four different xanthophylls which he designates as xanthophylls *A*, *A'*, *A''* and *B*. A more detailed review of this work will be given in connection with a report of the present investigations. It is of interest here especially on account of its historical position with respect to the establishment of the chemical constitution of the xanthophylls.

It was Willstätter and Meig³ who isolated and identified the crystalline xanthophyll pigment accompanying the carotin in green plants and leaves, and, as noted above, Eüler and Nordenson⁴ have recently found xanthophyll crystals in their extracts from the carrot, thus indicating a more general distribution of the xanthophylls in connection with carotin than has been believed.

The results of the study of the crystalline xanthophyll show that it is composed of carbon, hydrogen and oxygen in the proportion C₄₀H₅₆O₂ and is thus merely carotin dioxide.⁵ The pigment is further distinguished from carotin by the color and shape of its crystals, which are yellow or orange trapezium plates sometimes spear or wedge-shaped which are characterized by a steel blue reflection. The pigment exhibits an entirely different solubility toward petroleum ether and absolute alcohol than carotin, being insoluble in the former and readily soluble in the latter solvent. According to these authors, the pure

1. Proc. Roy. Soc. 72 (1903).

2. Ber. Botan. Gessel, 24, pp. 316 and 384 (1906).

3. Ann. der. Chemie, 355, p. 1 (1907).

4. Loc. cit.

5. Willstätter and Meig point out the probable identity of xanthophyll with the hitherto unexplained hydrocarotin found by Husemann.

crystals have a melting point of 172° C. (corrected) which is slightly higher than the melting point of the carotin crystals; and the absorption bands of the pigment are slightly shifted toward the violet from the corresponding bands of carotin, as was also shown by Schunck¹ for the xanthophylls which he differentiated.

It might be readily assumed that xanthophyll is formed directly from carotin in the plant. In fact Tschirch² has claimed that carrot carotin goes over to xanthophyll in the air. Euler and Nordenson³ do not credit this statement and state that, "One may well suppose that in the plant, xanthophyll normally is formed from the carotin, but outside of the plant it has not been possible to imitate this transformation, the most skillful oxidation always leading to a much higher oxidized product." Willstätter and Meig believed in this connection that xanthophyll although carotin dioxide is not the end product of the oxygen absorption of carotin in the plant. Monteverde⁴ and Lyubimenko have recently claimed that chlorophyll and xanthophyll originate from the same colorless substance, carotin being a complimentary product generated during the formation of chlorophyll, but not necessarily from the xanthophyll.

The Pigments of Animal Origin.

The Luteins.—We will now direct our attention to a review of the literature bearing upon the yellow pigments of so-called animal origin. Thudichum⁵ was one of the first to investigate the yellow animal pigments and he classified a great many of them together with the yellow pigments of plants under the name lutein, the name being taken from the pigment of the corpus luteum. He states, "Various parts of animals and plants contain a yellow crystallizable substance which has hitherto not been defined, and which I call lutein. It occurs in the corpora lutea of the ovaries of animals, the serum of the blood, the cells of adipose tissue, in butter, in the yolks of eggs of oviparous animals, in seeds such as maize, in husks and pulps of fruits such as annatto, in roots such as carrots, in leaves such as those of coleus, and in the stamens and petals of a great many flowers."

It is unfortunate that none of the above statements are supported by experimental evidence, for it can hardly be accepted that Thudichum was able to obtain crystals of lutein from all the bodies in which he

1. Loc. cit.
2. Ber. Botan. Gessel. 22, p. 414 (1904).
3. Loc. cit.
4. Bull. Acad. Imper. Sc. St. Petersb. 30, p. 609 (1912).
5. Proc. Roy. Soc. 17, p. 253 (1869).

claims to have found it, or was able to show all the properties which he describes for the crystals which he evidently did obtain.

Crystalline animal pigments were apparently obtained before Thudichum's claims in this regard. According to Krukenberg,¹ Wittich² obtained crystals of a red pigment from *Euglenia Sanguirubo*, and Piccolo and Lieben³ found a crystalline animal pigment. Pouchet⁴ a little later obtained a yellow crystalline pigment from lobsters.

The Chromophanes.—The early workers in the field of animal pigments laid great emphasis upon the so-called color reactions, one of which, the blue reaction which concentrated HNO_3 , was mentioned by Thudichum. That a similar reaction is given by concentrated H_2SO_4 was first noticed by Wittich in 1863, and Buchholz also noticed it with a fat pigment from a Ganglion cell of an invertebrate. Piccolo and Lieben had also noticed the blue reaction with concentrated H_2SO_4 . Besides Thudichum, Filhol⁵ and Städeler⁶ noticed the blue reaction with concentrated HNO_3 . Städeler attempted to isolate the egg yolk pigment. He failed to do so, however, but attempted to establish the difference between it and Bilirubin with which it had been considered identical. A little later a third reaction of the luteins was discovered by Schwalbe,⁷ namely a blue-green color with a solution of iodine in potassium iodide. Schwalbe first noticed the reaction with the conglobules of the retinas of birds and lizard's eyes. The red globules gave a beautiful blue to blue-black color, and the yellow oil globules a green to blue-green to blue. The pigments thus characterized were called chromophanes by Schwalbe and the existence of these pigments was a little later considerably extended by Capranica⁸ who also made use of the iodine reaction.

Kühne⁹ took up the study of the chromophanes of the conglobules of bird retinas, and separated three pigments which he designated Rhodophan, Chlorophan and Xanthophan, respectively, according to the color of their solutions.

Kühne also studied the absorption spectra and color reactions of the pigment of the egg yolk and the corpus luteum and compared them

1. Grundzuge einer vergleichenden Physiologie der Farbstoff und der Farben; 1884.

2. Arch. f. Path. Anat. 27, p. 573 (1863).

3. Giornals d. Scienze Naturali et. Economich. Palermo 2, p. 258 (1866).

4. Jour. d. L'Anat. et. Physiol. 12, p. 12 (1876).

5. Compt. Rend. T. 39, p. 184, T. 50, pp. 545 and 1182.

6. Jour. f. Pract. Chem. 100, p. 149 (1867).

7. Hand D. Ges. Augenheilkunde von Graefe u. Saemisch I, p. 414 (1874).

8. Arch. f. Anat. Physiol. p. 283 (1877).

9. Untersuch. des Physiol. Universität Heidelberg I, 4th Heft, p. 341 (1878); IV, p. 169 (1882); Jour. Physiol. 1, p. 109 (1878).

with these properties of the retinal pigments. A study of their spectroscopic absorption properties led him to believe that the pigments were not identical.

Kühne in his celebrated work on "Optochemie" occupied himself somewhat again with the egg yolk pigment and called it Ontochrin or Lecithochrin. He did not try to isolate it free from nitrogen, but he did succeed in observing crystals. He again was careful to distinguish between the egg yolk pigment and the corpus luteum pigment, which he at this time considered as extraordinarily closely related to carotin.

The Lipochromes. Basing his work on the researches of Kühne, Krukenberg commenced a series of researches which extended from 1879 to 1886, the most important of which appeared in his "Vergleichende Physiologische Studien" ¹ and especially in the paper, "Grundzüge einer vergleichenden Physiologie der Farbstoff und der Farben" which appeared in 1884. Krukenberg made an exhaustive study of what had been done on animal pigmentation and included under one head all those pigments which had previously been known as luteins, carotin, zoonerythrin (tetronerythrin) and Kühne's chromophanes, and called them lipochromes.

Krukenberg believed that carotin, the pigment of the carrot, was the best representative of the lipochrome coloring matters, and accepted Husemann's formula for carotin ($C_{18}H_{24}O$) as representing the chemical composition of the lipochromes.

In regard to the origin of lipochromes Krukenberg believed, "It is probable that in most cases they originate from fatty substances, for frequently, if not without exception, they occur in company with fat and allow themselves to easily go over into cholesterin-like bodies."

In 1885 Krukenberg ² isolated a yellow lipochrome from the blood serum of the ox by extracting the serum with amyl alcohol. The solution showed two absorption bands, one enclosing the line F and the other lying between F and G. A year later Halliburton ³ reported that he extracted a yellow lipochrome from the blood serum of the pigeon, hen, dove and tortoise by means of alcohol. Halliburton reported an identical pigment in the body fat of these same animals.

MacMunn ⁴ was the next investigator of animal pigments, and like Krukenberg, he extended the classification lipochrome to include

1. Zoonerythrin (Tetronerythrin):—Central, f. d. Medic. Wiss. 1879. Vergl. Physiol. Studien I Reihe, II Abth. s. 67-71; III Abth. s. 114-115; IV Abth. s. 30-35; V Abth. s. 87-94; II Reihe, I Abth. s. 165-167; III Abth. s. 135).

2. Sitz. ber. d. Jen. Gessel. f. Med. 1885.

3. Jour. Physiol. 7, p. 324 (1886).

4. Philos Trans. Roy. Soc. 177, p. 247 (1886).

the yellow constituent of chlorophyll or Hansen's "Chlorophyll Yellow." He believed that the lipochromes were chemically closely related to chlorophyll.¹

MacMunn's greatest contribution to animal chromatology was in 1889.² The pigments of a great many marine animals, Crustacea, worms and sponges were examined and classified. Lipochromes were found abundantly, MacMunn drawing a distinction as to whether the lipochrome was a rhodophan or a chlorophan-like lipochrome.

In regard to some of the properties of the lipochromes MacMunn states, as did Krukenberg, that they are sensitive to light, both in the solid state and in solution, and yield in many cases cholesterin-like substances. He believed that many of the plant lipochromes were identical with the animal lipochromes.

It will be remembered that for a long time there were many followers of the view that a close relationship existed between carotin and cholesterol and that this view was only finally discredited by a study of the pure crystalline pigment.

Cotte³ recently carried out an investigation in which he sought and claims to have shown that the lipochromes, both animal and vegetable are intimately associated with cholesterol. Cotte's results have been thoroughly disproved by Henze.⁴

Since the early work of Pouchet⁵ and Maly⁶ who distinguished between yellow and red crustacean lipochromes many investigators have classified the lipochromes according to their red or yellow color. Newbiggin⁷ in a recent investigation of the pigments of the skin, muscle and ovaries of the salmon, reports that he found two pigments present, a red and a yellow, which he claims he was able to separate from each other. Newbiggin concluded from the color reactions of the pigments that the red pigment was a true lipochrome while the yellow pigment was not.

In regard to the yellow pigment, Newbiggin says that, "It belongs to a group of pigments that are apparently exceedingly widely distributed in the animal kingdom, but which have been little investigated. They have been commonly confounded with the lipochrome pigments."

He extracted the pigment from the bright yellow body fat of a cow and found it to have properties identical with the yellow pigment.

1. Jour. Physiol. 9, p. 1 (1888).
2. Quart. Jour. Micros. Sc. 30, p. 15 (1889).
3. Compt. Rend. Soc. Biol. 55, p. 812 (1903).
4. Zeit. Physiol. Chem. 41, p. 109 (1904).
5. Jour. d. l'Anat. de la Physiol. 1, 12, 10 (1876).
6. Sitz. d. k. Akad. d. Wiss. zu. Wein. 83 (1831).
7. D. Noël Patton—Report of Inv. on Life Hist. of Salmon (1898), Article XV.

of the salmon with the exception that it was very little soluble in methyl alcohol, but dissolved readily in ether.

General Properties of the Lipochromes. It will not be out of place to give a brief summary here of the general characteristics and properties of the lipochrome pigments as found up to this time.

Lipochromes⁷ may be classed as salve-like, yellow or red or orange colored residues, which have been obtained in needles or rhombic plates, where they have been crystallized. They are soluble in alcohol, ether, benzol, petroleum ether, amyl alcohol, chloroform, carbon bisulphide, ethereal oils and fats with a yellow or yellow-orange color. They are insoluble in cold and hot water and alkalies and dilute acids, but are soluble in alcoholic alkaline solutions and are unchanged when these solvents are heated. In alcohol or other solvents they are unstable, and readily bleach, as do the residues from these solutions. The bleach product is unknown, but it is certainly not identical with cholesterol. On addition of concentrated H_2SO_4 or HNO_3 , the lipochromes give a color change of blue-green-violet to brown. The color reactions are often interfered with by the presence of a small amount of foreign substance. The lipochromes generally give a blue-green coloration with a solution of iodine in potassium iodide. Spectroscopically the lipochrome solutions show two bands and sometimes three in the blue part of the spectrum, and again they sometimes show no bands at all.

The lipochromes may be extracted from the fresh or dried tissues in which they are found, by organic solvents, best by hot or cold alcohol, ether, petroleum ether, carbon bisulphide or chloroform, the choice of the solvent resting with whether some foreign pigment is present. When fat is present, the pigment may be heated with alcoholic alkali which will not saponify the lipochromes. The lipochromes can be extracted from the soap with ether, petroleum ether, or chloroform, either directly or after acidifying, or the lipochromes can be salted out of their alkaline soap solutions with sodium chloride, and the lipochromes obtained by extracting the precipitated soap with alcohol or ether.

The Lipochromes of Algae, Fungi, and Bacteria. While a wide distribution of the lipochromes has already been mentioned, a review of their literature would not be complete without mentioning their distribution in algae, fungi and bacteria.

1. Summarized from "Lipochromes" by Franz Samuely. Alderhalden's Biochemisches Handlexikon vol. 6, and Handbuch der Biochemischen Arbeitsmethoden, vol. 2.

Hansen¹ first showed the presence of lipochromes in algae and Tammes² has lately shown their presence in a large number of these plants. Zopf³ has investigated the lipochromes of fungi and especially of bacteria, the first lipochrome-producing bacteria being pointed out by him.

The Lipochrome or Lutein of Egg Yolk. It will be readily agreed that while some order has been attained in classifying the widely distributed animal pigments, by means of the convenient and flexible classification "lipochromes," our knowledge of the animal pigments is far from being satisfactory when compared with the status of the orange and yellow plant pigments, the carotins and xanthophylls. The science of animal chromatology should accordingly be exceedingly grateful for the recent work of Willstätter⁴ and Escher, on the lutein of egg yolk, the result of which has been to throw new light upon the constitution of the lipochromes of the higher animals and upon their relations to the carotins and the xanthophylls.

The main pigment of the yolk of hen's eggs was isolated in crystalline form by these investigators, and when in approximately pure condition showed sufficiently close agreement with the constitution of xanthophyll that the authors claim that the egg lutein on account of its melting point (195-196° C. corrected) is a true isomer of the crystalline xanthophyll of green plants. In all its other properties including its spectroscopic absorption bands, the egg lutein was identical with the crystalline plant xanthophyll.

It is worthy of note also that during the isolation of lutein a minor constituent was noticed which gave every indication of being closely related to carotin; but as it was present in very small amount compared with the xanthophyll it was disregarded.

In concluding the review of this investigation it will be important to mention that the authors state that one of them, i. e., Escher, is at present investigating the pigment of the corpus luteum which they state has been found to belong to the hydrocarbon or carotin group of pigments.⁵

1. Arbeit. Botan. Inst. Würzburg 3, 296 (1883).

2. Loc. cit.

3. Ber. Botan. Gessel. 9, 27 (1891).

4. Zeit. Physiol. Chem. 76, pp. 214-225 (1912).

5. Note—Since writing the above, Dr. Escher has published his investigations which show that the corpus luteum pigment is in every respect identical with the carotin of the carrot and of green plants. Zeit. f. Physiol. Chem. 83, p. 198 (1913).

The Physiological Relation Between Plant and Animal Lipochromes.

With the review of the chemical side of this problem complete, it yet remains to consider what has been shown in regard to relations other than chemical, between the animal and plant pigments whose properties are so nearly related and in many cases identical.

The literature has been found to be very brief on this point. Newbigin¹ gives a rather extensive consideration of this subject and attempts to explain the presence of the red and yellow pigments found by him in the salmon organism. While he considered the most obvious explanation to be that they were derived directly from the food, he found a number of difficulties in the way of the acceptance of such an explanation, the most important of which was that he was able to show the presence of but a trace of only the yellow pigment in the usual food of the salmon.

As to the possibility of transference of yellow pigments from one organism to another, Newbigin points out what he believes to be some evidence apart from the case of the salmon. He says, "Poulton² has shown by experiment that certain caterpillars derive their pigments from their food. Again it is not uncommon to find fat of sheep and cows dyed a deep yellow color. According to some authorities this occurs quite sporadically without known cause, while according to others, special foods, notably maize, are the important agents." Newbigin says in this connection, "I have examined the yellow pigment of maize, and compared it with the pigment from yellow fat. The maize pigment gives the lipochrome reaction faintly with H_2SO_4 distinctly with HNO_3 , while the fat pigment gives no lipochrome reaction. In other respects, in tint, solubility, etc., the pigments closely resemble each other." Newbigin did not feel warranted to conclude from these experiments that all yellow pigments of animals are derived from their food, for with such a conclusion, he states, "It would be difficult to understand why such colored fat should not be universal in herbivorous animals, for all green parts of plants contain also a certain amount of yellow pigment."

It seemed to Newbigin, however, that a reasonable explanation for salmon, domesticated cattle and caterpillars would be to suppose that when they ingest a moderate amount of colored fat in their food, that they could utilize or eliminate the pigment, and so deposit colorless fat in the tissues; but when the ingestion of colored fat is in excess of the actual requirements as it so often is, especially with domes-

1. Loc. cit.

2. Proc. Roy. Soc. 54, p. 417; Nat. Sci. 8, p. 98.

ticated cattle, an elimination or utilization of the pigmented fat is impossible and fat colored with the pigment in a more or less modified condition is thus stored up.

There is abundant proof in this literature aside from the above speculations that animals are able to lay up fat soluble dyes in the organism and even eliminate them in the milk. Only recently Mendel and Daniels¹ have shown that Sudan III and other fat soluble dyes may be deposited in the organism in adipose tissue and bone marrow when introduced into the organism either dissolved in fat or when fed alone. When fed with fat or when fat was present in the alimentary tract the dyes entered the organism through the lymphatics in solution in fat, but when fat was absent, through the portal circulation dissolved in bile in which they are nearly all soluble. In the latter case the pigments did not pass beyond the liver unless fat was present to transport them, in which case only they were subsequently found in the blood. When fat stained food was fed to small animals (cats, rats, guinea pigs, etc.) in lactation, and in one case with a goat, the dye appeared in the milk shortly after the first feeding of the dye. The same authors feeding fifteen grams of Sudan III to a Holstein cow for three successive days were unable to detect the dye in the milk. The authors also made the interesting observation that stained fat does not traverse the placental barrier; the blood and foetus and fat of the young born of Sudan-stained female cats and rats were free from the dye.

Gogitidse² fed hog fat (100 grams per day) colored with Sudan III to a bitch and after two days found the dye in the milk. The body fat did not show this coloration so soon and then not so clearly, in fact only after long continued feeding of the stained fat.

Backhaus³ studying the "Influence of Feed and Individuality on the Taste and Healthfulness of Milk," says that a number of plants influence the color of milk and butter. The same author conducted several pigment feeding experiments with cows. Negative results were obtained with respect to the milk when feeding Fuchsin, Bismark brown, and curcuma powder, although the feces showed the pigments abundantly. When feeding sodium fluorescin the urine was affected but not the milk. When feeding methyl violet, however, the author was able to show that this pigment was carried into the milk fat in a reduced condition so that on contact with the air and with the aid

1. Jour. Biol. Chem. 13, No. 1, p. 72 (1912).

2. Zeit. f. Biol. 45, 353 (1904).

3. Berichte, Landwirt. Inst. U. Königsberg 5 (1900).

of heat, the milk fat showed an intense blue coloration. The feces, however, showed the pigment in an unchanged condition.

Summary.

The foregoing review of the literature has shown that the great number of pigments that exist throughout the entire plant and animal kingdoms have long been of interest from a scientific standpoint. The pigments of botanical origin have been thoroughly and exhaustively investigated. This is especially true of the yellow pigments carotin and xanthophylls, and their chemical constitution and properties are now established.

The yellow and orange pigments of plants were at first classified in one group, and were called carotins, the name being derived from the pigment of the carrot, which was the first one investigated. A great many different names were given to this pigment as it was independently discovered in various plants but the identity of these pigments with the carrot pigment has now been established. It was eventually discovered that the carotins are always accompanied, especially in green plants, by a second great class of pigments which have been called xanthophylls, whose relation to carotin has but recently been established.

As the work on plant pigmentation developed, it was recognized that the general properties of a great many yellow pigments found in animals were similar to the so-called carotins. The first investigators classified these animal pigments under the name lutein, the name being derived from the pigment of the corpus luteum, which was the first one investigated. The name lutein was extended by the animal chromatologists to include the carotins of plants and its related pigments. Later, when the animal luteins had become generally recognized by their association with fat, the name lutein was changed to lipochrome and this designation was also extended to include all similar pigments of both plants and animals.

The classification of the plant and animal pigments which is at present generally accepted is to restrict the names carotin and xanthophylls to the two great classes of yellow plant pigments, and to include under the name lutein or lipochrome only those yellow pigments which are considered to be of animal origin.

The most recent work in the field of animal chromatology has shown that the luteins can also be subdivided into carotin and xanthophyll groups depending on their chemical relation to the carotin or xanthophylls of plant origin. Accordingly Schunck¹ has shown the

1. Loc. cit.

spectroscopic identity of the egg yolk pigment with a xanthophyll which he isolated from the yellow daffodil, the nasturtium, and green leaves. Willstätter and Escher¹ have confirmed this with a chemical analysis of the egg yolk pigment, showing it to be a true isomer of the crystalline xanthophyll of green plants; they have called it xanthophyll B. Escher² has recently published his investigation showing that the pigment of the corpus luteum is identical in chemical composition and properties with the carotin of green plants.

These recent discoveries have opened the way for an extension of such investigations to other yellow animal pigments whose isolation is rendered much more difficult by their association with very large quantities of fat and other substances. These discoveries have also raised the question whether any relation other than chemical exists between the yellow animal and plant pigments. This question has never been investigated. The investigations which will be reported in the succeeding papers are the first to show that there is a definite relation other than chemical between the yellow plant and animal pigments.

1. *Loc. cit.*

2. *Loc. cit.*

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