RESEARCH BULLETIN NO. 11.

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

CAROTIN—THE PRINCIPAL NATURAL YELLOW PIGMENT OF MILK FAT—PART III

The Pigments of fhe Body Fat, Corpus Luteum and Skin Secretions of the Cow

COLUMBIA, MISSOURI
April, 1914

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

Agricultural Experiment Station

TABLE OF CONTENTS

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

The Pigments of the Body Fat, Corpus Luteum and Skin Secretions of the Cow.

LEROY S. PALMER AND C. H. ECKLES

Recent investigations in regard to yellow animal pigments have shown that some of them are closely related chemically or identical with yellow pigments of plant origin. Willstätter and Escher [1] have found that the pigment of egg yolk is isomeric with a crystalline xanthophyll of green plants, and Escher [2] has found the pigment of the corpus luteum to be identical with the widely distributed hydrocarbon pigment, the carotin of fruits, flowers and green plants.

In a study of the commonly observed yellow lipochrome of butter fat we have found [3] that it is composed principally of a pigment identical with carotin, with one or more minor constituents which are evidently identical with the xanthophyll pigments. We have furthermore shown by an unbroken chain of evidence that these pigments are present in the milk fat as a result of feeding a ration containing an abundant amount of these pigments. The presence of these pigments in milk fat is therefore not due to any synthetic powers which the animal possesses, but merely to the fact that the organism absorbs the pigments along with the products of food digestion and subsequently secretes them dissolved in the milk fat. We were accordingly able to vary the amount of pigment in the milk fat by simply choosing the proper feeds, i. e., either deficient in carotin and xanthophylls or very rich in these pigments. The above relations between the carotin and xanthophylls of milk fat and the carotin and xanthophylls of feeds were found to hold good for all breeds of dairy cows.

1. Zeit. f. Physiol. Chem. 76, pp. 214-225 (1912).
2. Zeit. f. Physiol. Chem. 83, p. 198 (1913.
3. Research Bulletin No. 10 Missouri Agr. Exp. Sta; Jour. Biol. Chem. 17, p. 191 (1914).

*See Foreword, Part I, for statement of co-operation with Dairy Division, U. S. Department of Agriculture.

The establishment of the chemical identity of the pigment of milk fat and of its simple physiological relation to the carotin and xanthophylls of green plants at once opened the question of a similar relation of the pigment which is so often observed in the body fat of cows, especially those of certain breeds, such as the Jersey and Guernsey. The question is also raised as to the presence of xanthophylls in the corpus luteum pigment. In addition an interesting question is opened as to whether the yellow skin secretions of certain breeds of dairy cows, which is often interpreted as indicating the ability of these animals to secrete yellow milk fat, is also due to the same pigments that characterize the butter fat.

The present investigation was undertaken for the purpose of studying these questions. In addition some information was gathered relative to the relation of the breed of the cow to the amount of color found in the body fat.

METHODS OF IDENTIFICATION.

The general methods of studying and identifying the pigments of the body fat, corpus luteum and skin secretions of the cow were the same as were used in the study of the pigment of milk fat. A detailed account of these methods may be found in the preceding paper of this series, which deals with the milk fat pigment.

These methods were a study of what we have called the spectroscopic, solubility, and adsorption properties of the pigments. The methods were confined to characteristic, physical and chemical properties of the pigments for the same reasons that they were used for the study of the milk fat pigment, namely because, in the case of the body fat at least, the very large amount of fat with which the pigments are associated precludes their isolation in sufficient quantity for chemical analysis. In the case of the pigments of the corpus luteum and skin secretions not enough material was available for isolating any great quantity of pigment.[1]

The Spectroscopic Properties.—It was found that carotin and xanthophylls isolated from green alfalfa hay, carrots or other plants rich in these pigments showed characteristic absorption bands when viewed in a spectroscope of narrow dispersion. When the spectroscope was set at an arbitrarily chosen standard, each class of pigments exhibited bands in characteristic position, especially in carbon bisulphide solution, and could then be readily identified. The arbitrary

1. Escher succeeded in isolating less than 0.5 gram of impure crystals of corpus luteum pigment from 10,000 cows' ovaries.

standard was obtained by fixing the arbitrary scale attached to the spectroscope at a constant figure which was furnished by a sodium flame, the spectrometer slit being closed to furnish the narrowest possible line. This standard did not of course give absolute measurements of absorption bands but merely a means of comparing the position of bands of various solutions, which was the desired end in view. Before measuring the bands of an unknown pigment, the strength of its solution was adjusted to give bands of as nearly the same intensity and clearness as the bands whose arbitrary measurements furnished the standard. The arbitrary standards for the absorption bands of carotin and xanthophylls, which were adopted, are given in Table I.

TABLE 1.—SPECTROSCOPIC STANDARDS OF CAROTIN AND XANTHOPHYLLS.

Pigment	Solvent	Measurement of absorption bands.		
		Band I	Band II	Band III
Carotin	CS_2	225—242	261—278	301—319
Carotin	$C_2H_5(OH)$	257—275	303—318	345—364
Xanthophyll	CS_2	233—253	272—291	312—330
Xanthophyll	$C_2H_5(OH)$	263—280	305—325	355—. . .

The Solubility Properties.—The relative solubility properties of carotin and xanthophylls are based on the fact that organic compounds are best soluble in solvents of similar composition. Accordingly carotin, which is a hydrocarbon, is much more soluble in hydrocarbons like petroleum ether than in the alcohols. Similarly the xanthophylls are much more soluble in the alcohols than in a hydrocarbon like petroleum ether. These phenomena, as stated in the preceding paper of this series, were discovered and elaborated by Tswett[1] and by Willstätter and Mieg.[2] At this station they were found to be very characteristic of carotin and xanthophylls and in addition very useful for separating and differentiating the carotin and xanthophyll constituents, not only of plants but also of the milk fat pigment. Thus the xanthophyll constituents of a mixed pigment could be readily separated by shaking the petroleum ether solution of the

1. Ber. d. Deut. Botan. Gessel. 24, pp. 316, 384 (1906); 29, p. 630 (1911).

2. Ann. d. Chemie. 355, p. 1 (1907).

mixed pigment with eighty to ninety per cent alcohol. Or if an eighty to ninety per cent alcoholic solution of the mixed pigments was shaken with petroleum ether, the latter solvent would completely extract the carotin, leaving the xanthophylls in the alcohol. By the second method especially, it was possible to show the presence of xanthophyll pigments in butter fat which could not be extracted from their alcoholic solution by petroleum ether.

The Adsorption Properties.—These properties were discovered by Tswett.[1] They are based on the fact that carotin and the xanthophylls show a great difference in regard to the ease with which they enter into combination with certain finely divided organic and inorganic compounds, such as Inulin, Saccharose or $CaCO_3$ For instance, carotin is not adsorbed at all by $CaCO_3$ from its perfectly anhydrous carbon bisulphide or petroleum ether solutions, while the xanthophylls are adsorbed to a greater or less extent. Briefly then, it has been found that if a carbon bisulphide solution (in which the pigments have an unusually brilliant color) of the mixed pigments is filtered slowly through a column of $CaCO_3$, previously moistened with the solvent, the pigments will be differentiated into zone-like rings as they pass through the column, depending on their adsorption affinity towards the $CaCO_3$. Carotin being unadsorbed will pass through first as a rose or red orange colored zone, with the various xanthophylls distributed above as zones of different shades of yellow or orange. The xanthophylls which are completely adsorbed by the $CaCO_3$ can be washed out afterwards by a stream of petroleum ether containing ten per cent absolute alcohol.

THE PIGMENTS OF THE BODY FAT.

The pigment of the body fat of the cow has never been subjected to a critical examination. Newbigin[2] reports the only attempt to identify it. He extracted the pigment from a sample of bright yellow body fat and compared its properties with those of a yellow pigment which he isolated from the salmon, which pigment, he says, belongs to a widely distributed group of animal pigments commonly confounded with the lipochrome pigments. He found the body fat pigment very similar in properties to the yellow non-lipochrome pigment. It did not give the lipochrome properties and was very little soluble in methyl alcohol. Newbigin also compared the body

1. Ber. d. Deut. Botan. Gessel. 24, pp. 316, 384 (1906).
2. D. Noel Patton, Report of Inv. on Life History of Salmon (1898), Article XN, p. 159.

fat pigment with the pigment from maize, with the result that, "The maize pigment gave the lipochrome reaction faintly with H_2SO_4, distinctly with HNO_3 while the fat pigment gave no lipochrome reaction. In other respects, in tint, solubility, etc., the pigments closely resembled each other." The experience of this station in studying the lipochrome properties of the milk fat pigment seems to indicate that Newbigin's results were due to the fact that he was working with decomposed pigment. Nevertheless particular attention was paid to the lipochrome properties of the body fat pigment.

Method of Isolation.

The method was the same as that used for isolating the milk fat pigment. It consisted in careful saponification of the fat with alcoholic potash (2 c.c. of 20% solution per gram of fat) and subsequent extraction of the unsaponifiable matter from the diluted soap (3 volumes of water to one of soap) with ether. The ether extract was sometimes purified by re-saponification and re-extraction with ether, and sometimes was freed from cholesterol with digitonin. Only small amounts of fat were used for each test, i. e., 25 to 30 grams, as the studies of the milk fat pigment showed that the best results could thus be obtained.

Identification of Pigments.

Only two typical experiments showing the character of the body fat pigments will be reported.

Experiment I.

Twenty-five to thirty grams of pure rendered kidney fat from a Jersey cow was used for this experiment. The fat had a high yellow color testing in the one-inch tintometer cell 54 yellow, 2.3 red. The unsaponifiable matter of the fat had a golden yellow color in ether solution and a blood red color in carbon bisulphide solution. The carbon bisulphide solution left no adsorbed zone in the $CaCO_3$ when analyzed chromotographically, but passed through unadsorbed as an orange-red or rose colored zone. A very small amount of pigment was left in the column, however, which was readily washed out by a stream of alcoholic petroleum ether.

The main pigment was now examined with respect to its solubility relations toward petroleum ether and 80 per cent alcohol. A very minor constituent was thus obtained more soluble in 80 per cent alcohol than in petroleum ether (b. p. 30-50). This constituent, when combined with the pigment adsorbed by the $CaCO_3$ in the chromotogramm, did not show sufficiently sharp absorption bands for accurate measurements.

The main petroleum ether soluble pigment was transferred to carbon bisulphide in which it showed two strong bands and a third faint one, the measurements of which are given in Table 2. The residue from this solution gave a greenish-blue color with concentrated sulphuric acid.

Experiment II.

An unusually high colored fat taken from the back of a Jersey cow was used for this experiment. The pure rendered fat tested in the one-inch tintometer cell 80 yellow, 2.7 red. Thirty grams of this fat was saponified with 300 c. c. of 5 per cent alcoholic potash by dissolving the fat in the hot alkaline solution, letting stand for 24 hours in the cold with frequent shaking and finally boiling on the steam bath for one half hour. By using this procedure not a trace of foreign pigment was developed. One extraction with ether rendered the diluted soap colorless. The golden yellow ether extract, after purification, was concentrated into 50 c. c. of absolute alcohol. On careful analysis of this solution it was found possible to separate its pigment into a major pigment which was readily extracted by petroleum ether and a minor pigment which could not be extracted by petroleum ether. The main petroleum ether soluble pigment was readily soluble in carbon bisulphide with a blood red color, and in this solvent showed two strong absorption bands and a third faint one, the measurements of which are given in Table 2. Analyzed chromotographically, the carbon bisulphide solution passed through as an unadsorbed beautiful rose colored zone. There was no differentiation. The residue from the solution gave a deep blue color with concentrated sulphuric acid.

The alcohol soluble pigment, which probably comprised several per cent of the total, was transferred to ether by diluting the alcoholic solution with much water in a separatory funnel. Petroleum ether was added, precipitating some water, and the ethereal solution washed with water until clear. The solution was now evaporated and the yellow residue dissolved in carbon bisulphide, giving a yellow-orange solution which showed two fine absorption bands, and a third fainter one, the measurements of which are given in Table 2. The bands seem to be shifted more toward the blue than the usual xanthophyll bands.

The orange-yellow carbon bisulphide solution was now analyzed chromotographically. Only one pigment was present, which passed through the column very slowly as a narrow orange zone, leaving no pigment in the $CaCO_3$ which could be washed out with alcoholic petroleum ether.

TABLE 2.—ABSORPTION BANDS OF CAROTIN AND XANTHOPHYLLS OF BODY FAT.

Experiment No.	Measurements of absorption bands			
	Carotin		Xanthophylls	
1.	Band I Band II Band III	223—242 259—279 300—319	....	
2.	Band I Band II Band III	224—243 262—288 302—322	Band I Band II Band III	235—252 278—302 315—335

It must necessarily be concluded from these experiments that Newbigin's "inert" class of lipochromes does not exist in the body fat of the cow, but, on the other hand, the pigment of this fat is, like the butter fat pigment, composed of a major carotin and one or more minor xanthophyll constituents, all of which also show the properties of lipochromes. It is to be noted also that the number of xanthophylls in the body fat varies, as was found to be the case in the butter fat studies.

The Relation Between the Color of the Body Fat and the Food of the Cow.

Numerous feeding experiments in connection with the study of the pigment of milk, reported in the preceding paper of this series, showed that the carotin and xanthophylls which were found to characterize the milk fat were present there on account of the fact that the food contained these pigments. Since the pigment of the body fat is also composed of carotin and xanthophylls it is natural to suppose that it is, like the pigment of milk fat, derived from the food, the carotin and xanthophylls being carried to the fat depots and fat synthesizing body cells in the same manner that they are carried to the milk glands.

In order to obtain evidence of this fact, however, the following experiment was undertaken. Two barren and dry Jersey cows in moderate flesh were fed wheat straw alone for sixty days or until the animals had lost as much fat as was considered necessary for the second part of the experiment. The daily ration of cow No. 25 was then changed to 9 pounds of yellow corn and 20 pounds of green alfalfa hay, which was rich in carotin and xanthophylls. Cow No. 21 was given a daily ration averaging 11.4 pounds of white corn and 14 pounds of bleached clover hay, very deficient in carotin and xanthophylls. Cow No. 25 was slaughtered at the end of 81 days. Her gain in weight during this period was 160 pounds. Cow No. 21 was slaughtered at the end of 95 days. She had gained materially in condition during her "fattening" period although the scales showed little gain in weight. This was probably due to a much greater "fill" when receiving wheat straw. Samples of fat from various parts of the body were taken from each cow at slaughtering and used for color readings. The results are given in Table 3. The colorimetric readings in this and subsequent tables were made on the rendered, melted fat, measured by the Lovibond Tintometer. A complete description of this instrument may be found in the preceding paper of this series.

TABLE 3.—THE RELATION OF FEED TO COLOR OF BODY FAT.

Part of body.	Color of fat.					
	Cow No. 25.			Cow No. 21.		
	Yellow	Red	Light	Yellow	Red	Light
Rib plate	50	2.3	1.0	1.4	0.1	0
Caul	47	2.1	1.0	3.6	0.5	0
Thoracic cavity	29	1.3	1.0	8.0	1.0	0
Around ovaries and uterus	49	2.3	1.0	2.5	0.3	0
Attached to fourth stomach	33	1.6	1.0	24.0	1.7	1.0
In pelvic cavity	50	2.3	1.0	47.0	2.1	1.0
Kidney	54	1.6	1.0	50.0	2.1	1.0
Crops	50	2.3	1.0	47.0	1.9	1.0
Over last rib	47	2.3	1.0	50.0	2.1	1.0
Over outside chuck	47	2.0	1.0	47.0	1.8	1.0

The results of this experiment are even more striking when the amount of fat on the various parts of the bodies of the two cows is taken into consideration. Aside from the kidney fat and pelvic cavity fat, which were probably not disturbed to any extent during the starvation period, and which furthermore were of equal color in the two animals, the largest proportion of the entire fat of the two cows was represented by the caul fat. The caul apron of Cow 25 had a net weight of 12,132 grams; that of Cow 21 a net weight of 7,364 grams. It is here that the great difference in color is noticeable. In fact, if all the fat on the body of Cow 21 had been rendered and its color compared with the same from Cow 25, the difference in color would have been very marked.

There can be no doubt that the above data is conclusive as to the effect of feeding a non-pigmented ration to a fattening cow whose fat had been largely eliminated by starvation previous to the feeding of the colorless ration. In other words, it is apparent that the body fat of the Jersey cow is colored primarily because the food is rich in carotin and xanthophylls during the time the fat is on. The blood serum of both cows contained carotin at the time of slaughtering. Unfortunately no comparison was made of the amount in the serum of the two cows. No doubt there was a much smaller amount in the blood serum of Cow 21, than in the serum of Cow 25. In both cows there was no known path of elimination of the blood pigment during the starvation period, the cows being both dry and barren. During the fattening period of Cow 25, the demands made on the blood store by the body fat producing cells, was replenished by the food. In the case of Cow 21, however, there was no replenishing source, and the amount in the serum must have greatly diminished.

The data resulting from this experiment have some significance aside from the relation of the body fat to the food of the cow. The fact that the outside fats of the two cows were of equal color, and the inside fats, especially the caul and rib plate fat, were of such widely different colors, would seem to indicate what fats are drawn upon first in starvation in this class of animals, and what fats are laid on first during fattening.

Relation Between Color of Body Fat and Breed of Cow.

Considerable attention was given in connection with the study of the milk fat pigment, to a study of the relation of the breed to the amount of pigment in the fat. This study showed that the relation of breed to milk fat coloration is a relative one, Holstein and Ayrshire cows producing well-colored butter fat as well as Jerseys and Guernseys under proper feeding conditions. It was also shown that under certain conditions there was no difference in milk fat coloration among the different breeds. These results naturally raised the question whether a similar study of the body fat pigmentation would lead to the same conclusions. It is not uncommon to find the body fat of Jersey and Guernsey cows with a high yellow color. This has led to a general belief that this phenomenon is a characteristic of only these breeds of cows. As a matter of fact butchers and also the consumer look with disfavor upon beef from these animals on account of this high color of the fat. Although Table 3, above, shows very clearly that the color of the body fat of Jersey cows is as much dependent upon the feed as the color of the milk fat, it was nevertheless important to study the coloration of the body fat, of the different breeds, which had accumulated under ordinary conditions. In this way the normal breed relation could be determined.

Only a few animals were available for this study. Besides the data for the two Jersey cows given in Table 2, we have the colorimetric study of the body fat of one Jersey and 3 Holstein cows. The data from these animals is given in Table 4.

TABLE 4.—RELATION OF BREED TO COLOR OF BODY FAT.

Part of body	Cow No. 2, Jersey		Cow No. 207, Holstein		Cow No. 226, Holstein		Cow No. 221 Holstein	
	Yellow	Red	Yellow	Red	Yellow	Red	Yellow	Red
Rib plate	54	1.5	47	1.7	..	..	15.0	1.2
Crops	63	1.8	14	1.2	..	..	21.0	1.2
Thoracic cavity	17	1.0	36	1.5	9	1.0	6.0	1.2
Caul	50	1.7	54	1.7	18	1.1	12.0	1.2
Pelvic cavity	47	1.5	61	1.8	..	..	10.0	1.2
Over last rib	63	1.8	17	1.3	..	..	23.0	1.2
Ovaries, uterus	..	..	62	1.8	..	..	..	..
Chuck	54	1.7	14	1.0	..	..	22.	1.2
Kidney	47	1.5	64	1.8	..	..	20.	1.2
Stomach	..	..	..	..	24	1.0	11.	1.2

The most important points presented in this table are the wide difference between the color of the fats of Holstein Cow No. 207 and the other two Holstein cows; and the wide difference between the color of the inside and outside fats of Holstein Cow No. 207. The first point is possibly due to an individual characteristic of Cow No. 207, although it is not known under what conditions the fat was formed. It should be stated in connection with the data of Cow No. 226 that the animal died in parturition, and the reason so few samples of fat are recorded is due to the fact that the animal had no fat on the body at those particular places. In regard to the data on Cow No. 207, it is to be noticed that the inside fat all had a color equal or greater than the corresponding fat of Jersey Cow No. 2, while the outside fats were uniformly much lighter in color. In explanation of this result it may be said that milk cows are known to lay on fat first on the inside of their body, and we have data to show, not only that this particular Holstein cow normally produced high-colored milk fat under favorable feeding conditions, but also that the laying on of most of the fat, whose color is shown in the table, was during the summer when her ration was largely fresh green grass.

This does not hold true for Holstein Cow No. 221, for much of her fat was also laid on while on grass. It should be added too that the milk fat of this cow was never known to have a very high color. This was brought out especially in a carrot-feeding experiment with this animal which was reported in the preceding paper of this series. The maximum color obtained in that experiment was practically the same as the maximum color found in her body fat. There seems to be a breed characteristic evident here, but owing to the high color readings obtained from Holstein Cow No. 207, it may be due to the individual rather than to the breed.

Perhaps the most important point brought out by this data is that the color of the body fat of any individual, regardless of breed, laid on under given feeding conditions is practically the same as the color of the milk fat under the same conditions.

Another point which should be mentioned here, but which will be more readily understood in the light of the results which will be given in a subsequent paper, is that this difference between individuals is not due to lack of carotin in the blood. The amount of carotin in the blood of Cow No. 221 at the time of slaughtering was as great as is found in the blood of a Jersey cow receiving the same feed.

Our data is not sufficiently extensive to warrant any conclusions as to the normal difference in body fat pigmentation between the different breeds. Very probably it is considerably greater than the normal difference between the milk fat pigmentation of the different breeds. The reason for this is not evident from our present knowledge of the physiology of pigmentation. The fact that such a wide difference often does exist is of considerable importance, however, in explaining why the milk fat of Jersey and Guernsey cows often has a higher color than can be explained by the character of the ration. Reference was made to this in connection with the feeding experiments reported in the preceding paper on the milk fat pigment.

It was there shown that changing the ration of a Jersey cow from one rich in carotin and xanthophylls to an unpalatable one very poor in these pigments did not result in an immediate lowering of the color of the butter fat, but resulted rather in a gradual reduction in color, extending over a considerable period of time. The animal at the same time usually lost weight. This fact taken in connection with the normal high color of the body fat of Jersey cows which was brought out in the present experiments, gives a clear explanation of the entire phenomenon. The pigments of the body fat were being drawn upon, or rather the utilization of the body fat for energy liberated pigments which furnished a partial temporary supply for the milk fat. The milk fat of cows, whose body fat lacked this high color would, therefore, under similar conditions lose color very much faster. The high color of the milk fat of Jersey cows on a nonpigmented ration is, therefore, due to the fact that their body fat has a normal high yellow color.

THE PIGMENTS OF THE CORPUS LUTEUM.

Viewed in the light of the foregoing investigations it is not surprising that Escher[1] has found that the corpus luteum pigment belongs to the carotin group, thus establishing its identity with the principal milk fat and body fat pigments. In view of the plurality that has been established for both the milk and body fat pigments it became at once important to study the corpus luteum pigment in this connection also. Only a few corpora lutea were available for the study, in fact the ovaries of only six cows at different times were available for examination and in three cases only were well-developed corpora lutea found. Of Jersey Cows No. 21 and No. 25 slaughtered at the same time, only Cow No. 25 had a corpus luteum of any development. Jersey Cow No. 8 and a Hereford cow were slaughtered at the same time but only the beef bred cow had a well-developed corpus luteum. Cow No. 207 slaughtered at another time had no well-developed corpus luteum but there were the remains of a number of former corpora lutea and one just developing. Holstein Cow No. 221 slaughtered some time later, had a well-developed corpus luteum.

The investigations of the corpora lutea of the Jersey cows, Nos. 21 and 25, were carried out on the combined pigments previous to the discovery of the xanthophyll constituent of butter fat pigment, but the data obtained is nevertheless very instructive.

1. Loc. cit.

The corpora lutea were carefully cut away from the surrounding tissue, ground up with sand, and extracted with ether. In this solvent and in alcohol, the pigment showed two absorption bands in the blue part of the spectrum. Solubility tests on the alcohol solution showed that petroleum ether and carbon bisulphide extracted *almost* all the pigment. That which was not extracted was treated with hot alcoholic potash and the soap extracted with ether in which the pigment all readily went. The saponified pigment was transferred to alcohol and freed from cholesterol with digitonin. After concentrating, the cholesterol-free filtrate was extracted with carbon bisulphide. Not all the pigment was extracted even with two extractions, and petroleum ether extracted no color from the remaining light-yellow alcoholic solution. The experiments with the secondary pigment were not carried farther at this time as the significance of its presence was not appreciated, but viewing the data in the light of the results of the milk fat and body fat investigations it is evident that a secondary xanthophyll pigment was present here. This has been emphasized because the results of the investigations subsequently conducted were unfortunately vitiated because of unexpected aldehyde resin colorations which developed during saponification. It was shown by a special study that these reddish yellow bodies when present in considerable quantity are extracted from the diluted alkaline solutions by ether, but are not readily extracted from alcohol by petroleum ether. Consequently they interfere with a proper study of the pure pigments. Such a result was obtained in the study of the corpora lutea pigments of Cow No. 8 and the Hereford cow. The only noteworthy result of that investigation was to obtain a beautiful rose colored unadsorbed zone in a chromotogramm of a carbon bisulphide solution of the combined pigment. This solution showed three absorption bands, the measurements of which are given in Table 5.

The next investigation was with the corpora lutea of Holstein Cow No. 207. As stated above there was no well-developed corpus luteum, the largest part of the pigment obtained being from the remains of several former corpora lutea which were present as small red colored patches about the size of a pin head. These were carefully cut out and macerated with a little sand and $CaSO_4$ and extracted with carbon bisulphide for several hours. The solution, of about 25-50 c. c. volume, had a deep orange-red color, which showed three beautiful bands, the third band being considerably fainter than the first two. The measurements are given in Table 5.

A chromotogramm of this solution showed only one rose colored zone which passed rapidly through the $CaCO_3$ column. A little of the solution was evaporated into absolute alcohol and after making the alcohol eighty per cent, the pigment was studied in regard to its solubility properties toward petroleum ether (b. p. 30-50° C) and carbon bisulphide respectively. In both cases the alcohol was left absolutely colorless. In this case then, where the pigment was chiefly from the remains of former corpora lutea, carotin was the only pigment present.

TABLE 5.—ABSORPTION BANDS OF CORPUS LUTEUM CAROTIN IN CARBON BISULPHIDE.

Bands	From Hereford Cow No. 8. Good corpus luteum.	From Holstein Cow No. 207. Remains of corpus luteum.,
Band I	225—242	225—242
Band II	262—282	262—285
Band III	305—320	305—320

The final investigation was with the well-developed corpus luteum from Holstein Cow No. 221. Both ovaries of the cow were ground up with sand and plaster of paris and the mass extracted with petroleum ether (b. p. 30-50° C.) until the extract was colorless. The pigment thus extracted was carefully differentiated between the petroleum ether and 85 per cent alcohol. No pigment whatever was extracted by the alcohol. The pigment was then submitted to saponification with KOH after transferring to alcohol. No aldehyde resin pigments formed during saponification. The pigment was extracted from the soap with ether. The ether extract was thoroughly washed with water as usual and then concentrated at a low temperature with the constant addition of petroleum ether so that the pigment finally remained in petroleum ether solution. This solution was then shaken with 85 per cent alcohol. The alcohol extracted no pigment whatever. In this case then, although a normal corpus luteum was used, and the entire pigmented extract submitted to saponification, carotin was the only pigment present.

The result of this study was to confirm the results of Escher[1] that the corpus luteum pigment is identical in properties with carotin. In addition we have shown that this pigment, like the principal pigments of milk fat and body fat, may have associated with

1. Loc. cit.

it small quantities of xanthophyll pigment. It is possible, however, that these xanthophylls are present in the fat which may be extracted along with the carotin of the corpus luteum.

THE PIGMENTS OF THE WAXY SECRETIONS IN THE EARS AND ON THE SKIN OF JERSEY COWS.

It was stated in the introduction that the secretions of the skin of Jersey and Guernsey cows is often considered as indicating the ability of these breeds to secrete yellow milk fat. It was accordingly thought that a brief investigation of this pigment would be of interest and possibly of some scientific value.

The yellow skin secretion of Jersey cows is especially abundant in the ears. A few grams of the yellow waxy matter was accordingly scraped from the ears of several pure bred Jersey cows and the wax macerated with ether, which readily dissolved away the pigment and some fatty matter, giving a bright yellow solution. The ether solution was concentrated to low volume and diluted with about 100 c. c. of 2 per cent alcoholic potash and the solution boiled on the steam bath for 30 minutes. The pigment was extracted from the soap solution with ether in the usual way. The extraction of the pigment was easy and complete. The ether solution was freed from alkali as usual and then diluted with some petroleum ether. The slightly cloudy solution which resulted was washed with water until clear and evaporated into absolute alcohol. The alcohol was now diluted with petroleum ether (b. p. 30 to 50° C) and water added sufficient to cause separation. Several extractions with petroleum ether resulted in the division of the original pigment into a major petroleum ether soluble pigment and a minor pigment which could not be extracted from 80-90 per cent alcohol with petroleum ether.

The petroleum ether pigment gave a red orange carbon bisulphide solution showing the carotin absorption bands:

I. 224—243
II. 263—287
III. 303—320

and a beautiful rose colored unadsorbed zone in the $CaCO_3$ chromotogramm.

The 80 per cent alcohol soluble pigment which amounted to two or three per cent of the entire pigment, gave a yellow-orange carbon bisulphide solution. The solution showed only one absorption band however, the other bands being obscure.

I. 232—254

It is thus seen that the yellow pigment of the skin secretions of the Jersey cow is identical with the other yellow lipochromes of the body and like them belongs chiefly to the carotin group of pigments.

THE BODY FAT AND BLOOD SERUM PIGMENTS OF THE NEW-BORN CALF.

Carotin and xanthophylls having been found to be normal constituents of the body fat of cows which had been fed green feeds or other feeds containing an abundant amount of these pigments, an interesting question was raised as to whether these pigments are present in the body of the new-born calf. If these pigments should be found to be entirely absent from the new-born calf, additional proof would therefore be offered that these pigments were the result of subsequent feeding. The presence of carotin and xanthophylls in the new-born calf, however, would not be proof that these pigments cannot arise from the food, but would merely indicate that they were able to traverse the placental barrier from the mother whose body is normally rich in these pigments. In this connection the question would be especially interesting in view of the fact that Mendel and Daniels [1] have recently found that fat soluble dyes, such as Sudan III, do not traverse the placental barrier of small animals such as cats and rats, whose milk fat and body fat, however, is readily tinted as the result of feeding the dyes.

In order to study this question, the following experiment was carried out:

A new-born pure bred Jersey calf weighing 50 pounds was not allowed to suckle its mother but was slaughtered a few hours after its birth.

Five hundred c. c. of the blood was caught in a cylinder and allowed to clot. After standing 48 hours, 250 c. c. of serum was obtained. The proteins were precipitated from the serum with alcohol and were filtered off on a Bücher funnel with suction. They had a reddish gray color. They were rubbed up to a paste with absolute alcohol in a mortar and then extracted with boiling absolute alcohol. The extract was absolutely colorless. The alcoholic filtrate from the precipitated proteins had a greenish-yellow color. It was concentrated to 50 c. c. and absolute alcohol added, precipitating a little protein. The filtrate had a beautiful greenish-yellow color but the pigment was not extracted by carbon bisulphide, petroleum

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

ether, or ether, but seemed to be partly thrown down by lead acetate and by saturation of a dilute alcoholic solution with $(NH_4)_2SO_4$. Acid mercuric nitrate solution decolorized the alcoholic solution at the same time throwing down a white precipitate.

The only conclusion that can be drawn from this experiment is that the blood serum of the new-born calf is free from carotin. A small amount of an unknown pigment was present which was readily soluble only in alcohol, and insoluble in water, ether, carbon bisulphide, and petroleum ether.

There was practically no fat on the body except a little around the kidneys and in the tissue of the caul apron. In the body the latter tissue had a slight brownish color. All the fat tissue that could be obtained was ground up, rendered and filtered. About 40 grams in all was obtained. The rendered fat had a slight yellow color giving a tintometer reading in 1-inch layer of 4 yellow and .3 red. When solid the fat had a greenish-yellow tint. Thirty grams of the fat was saponified with alcoholic potash and the soap extracted with ether. It was possible to differentiate the small amount of pigment thus obtained so that it was about equally divided between petroleum ether and 80 per cent alcohol. In carbon bisulphide solution these portions showed their relation to carotin and xanthophyll both in the spectroscope and chromotogramm. Both portions showed two beautiful bands which measured as follows:

Body Fat Carotin (In CS_2)		Body Fat Xanthophyll (In CS_2)	
Band I	225—244	Band I	235—250
Band II	263—280	Band II	270—285

The results of this experiment show that a small amount of carotin and xanthophyll are present in the body fat of the new-born Jersey calf. The results present the apparent anomaly, however, of the presence of the pigments in the body fat and their absence in the blood. In explanation of this it may be said that the body fat of the new-born calf, the amount of which is very small indeed, probably arises from the fat of the mother, being transferred to the foetus a very small quantity at a time. The small quantity which would be present in the blood stream under these conditions, i. e., dissolved in fat, would not have been detectable by the method used for the investigation of the pigments of the blood serum. It is absolutely certain that there were none of these pigments present in the blood serum in the way in which they are normally found in the mature animal.

The results of the investigation are of further value in indicating that under proper feeding conditions, it might be possible to raise even a Jersey cow with practically none of the characteristic carotin and xanthophyll pigments in her body.

DISCUSSION OF RESULTS.

The results of the experiments reported in this paper are in perfect accord with those of the preceding paper. The discovery of the carotin and xanthophyll nature of the milk fat pigment would lead quite naturally to the supposition that the other lipochrome pigments of the body of the cow are of the same character. This supposition was fully borne out by the result of experimental study. The yellow lipochromes of the body fat, of the corpus luteum and of the skin secretions were found to be composed principally of carotin with one or more minor xanthophyll constituents.

In addition to the establishment of the chemical relation of these pigments to the carotin and xanthophyl of green plants in the case of the body fat at least it has been possible to show that the pigments are derived from the food in a manner identical with pigments of the milk fat. The carotin and xanthophylls of the corpus luteum and skin secretions must therefore be derived from the same source.

Viewing the results from a physiological standpoint, it is seen that the establishment of such a source for these pigments and the ease with which they are therefore increased and decreased,[1] throws great doubt upon any physiological significance which these pigments have been supposed to exert in the animal body. In the case of the corpus luteum for instance, the accumulation of the carotin during the formation of this body is merely a phenomenon incidental to the rupture of the Graafian follicle and the subsequent formation of the cellular tissue around the central blood clot, and to the fact that the blood serum is normally very rich in carotin, as will be shown in the following paper. This, of course, does not explain the mechanism of the accumulation of the cărŏtiñ-containing cells around the ruptured Graafian follicle. The chemical combination of the carotin in the blood serum is no doubt of importance in this connection.

The popular opinion that the body fat of Jersey cows is normally characterized by a higher yellow color than Holstein cows has been at least partially confirmed by experimental study, although it

1. This is especially true of the milk fat and, as will be shown in the succeeding paper, the blood serum.

was found that Holstein cows may also possess high-colored body fat. At least there seems to be more breed characteristic in this respect, than in the case of the pigmentation of the milk fat. There is no foundation, however, for the belief that beef has a lower value because its fat has a high color. If this pigment is the same as is demanded by the consumer for butter, why should not beef with high-colored fat also be more desirable? It is recognized of course that some of the unfavorable attitude toward beef with highly colored fat arises partially from the fact that it indicates that the beef probably came from a dairy cow. The two ideas are nevertheless very closely associated.

The normally high color of the body fat of Jersey cows and also of those of the Guernsey breed, explains why cows of these breeds often appear to be producing well-colored butter on a ration deficient in carotin and xanthophylls. Several statements in regard to this have already been made. This will bear repetition, however, because the subject is an important one. Briefly, it may be said that when cows whose body fat has a high yellow color are put upon a ration deficient in carotin and xanthophylls and also, as is usually the case with such rations, deficient in food value, the body fat is called upon to furnish energy value for the animal and also in many cases to supplement the food digestion products in the production of milk fat. It is readily seen that in such cases an important source is opened up for pigments for the milk fat. Just how important this source could be would depend upon the amount of highly colored body fat available for the needs of the body, and upon the rapidity with which it would be used up. If our experimental data are correct showing that the inside fats, such as the caul fat and rib plate fat, are the first drawn upon in starvation of this class of animals, then the amount of available highly colored fat would be rather large. Dairy cows usually have a fairly abundant amount of these fats, especially the caul fat. It is thus readily seen that a continuous drawing upon these inside fats for a long period of time would result in a very slow and gradual reduction of the color of the milk fat. The deduction that the animal was actually producing colored milk fat on a carotin-xanthophyll-free ration would, therefore, be quite natural but nevertheless entirely false.

In a similar manner it is readily seen why the breeders of Jersey and Guernsey cattle have been led to believe that the yellow skin secretions of these breeds are indicative of their ability to produce yellow milk fat. It is interesting to find that the yellow pigments of these secretions are carotin and xanthophylls. It should be clearly borne in mind, however, that the only indication that a cow will secrete yellow milk fat is that the food contains an abundance of carotin and xanthophylls.

SUMMARY.

1. The yellow lipochrome of the body fat, corpus luteum and skin secretions of the cow, like the lipochrome of butter fat, is composed principally of a pigment whose physical and chemical properties are identical with the carotin of green plants. The same pigment may have associated with it one or more minor constituents whose physical and chemical properties are identical with the xanthophylls of green plants.

2. The carotin and xanthophyll pigments of the body fat are derived from the food of the cow. The body fat of Jersey cows formed on a ration deficient in carotin and xanthophylls, is devoid of color.

3. The body fat of Jersey and Guernsey cows is usually characterized by a higher yellow color than cows of other breeds. This is of great importance in explaining why cows of these breeds may sometimes show a much slower elimination of the pigment from milk fat on a non-pigmented ration, as during the winter months. In these cases the body fat furnishes a supplementary source of pigments for the milk fat.

4. The yellow body fat of Jersey and Guernsey cows should not be a point against the use of these animals for beef. The pigments here are the same as those for which the consumer will pay a higher price when present in butter.

5. The breeders of Jersey and Guernsey cattle are probably correct in their belief that the yellow skin and skin secretions of these animals are characteristic of the breeds. It is not correct, however, that this characteristic is indicative of the ability of these animals to secrete yellow milk fat under all conditions. The only indication of this is whether the food contains an abundance of carotin and xanthophylls.

6. The blood serum of the new-born Jersey calf is free from carotin and xanthophylls. The small amount of fat on the body is tinted very faintly with these pigments.

BIBLIOGRAPHY.

1. Escher: Zeit. f. Physiol. Chem. 83, p. 198 (1913).
2. Mendel and Daniels: Jour. Biol. Chem. 13, No. 1, p. 72 (1912).
Salmon" 1898, Article XV, p. 159.
3. Newbigin: D. Nöel Paton, "Report of Inves. on Life History of
4. Tswett: Ber. d. Deut. Botan. Gessel. 24, pp. 316, 384 (1906).
5. Tswett: Ber. d. Deut. Botan. Gessel. 29, p. 630 (1911).
6. Willstätter & Escher: Zeit. f. Physiol. Chem. 76, pp. 214-225 (1912).
7. Willstätter and Mieg: Ann. d. Chem. 355, p. 1 (1907).