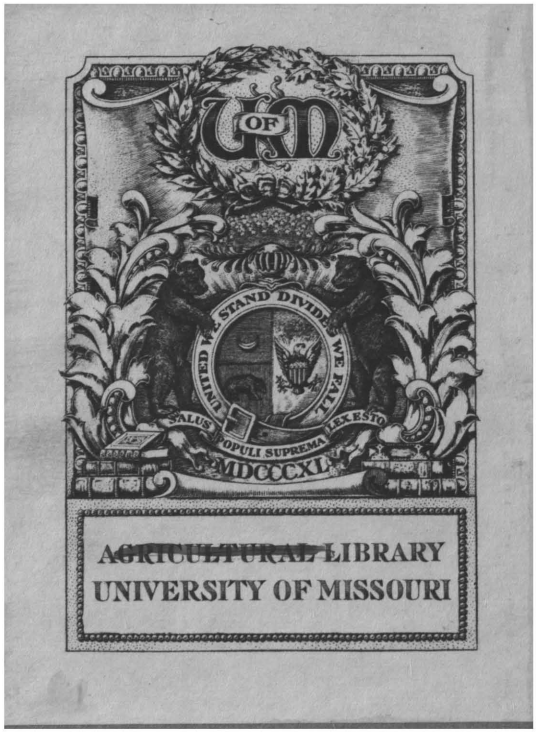

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# A STUDY OF THE COLORING MATTER IN MILK SERUM

by

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# A STUDY OF THF COLORING MATTER IN MILK SERUM.

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# INTRODUCTION

During the course of an investigation of the natural pigments of milk carried on in the Research Laboratories of the Dairy Department, at the University of Missouri, it became evident that more than one pigment existed in the milk; namely that the pigment which remained in the whey after the removal of casein and fat was not identical with the pigment found in the fat. The investigation reported here deals only with the whey pigment.

The color of milk serum was observed as long ag o as 1784 when Schoeff, in a very learned paper (1), containing full references of the work of his predecessors noticed the yellow color of the whey- "Liquidem colore diluti citrinum".(2) Since that time several investigators have taken up the study of this pigment, but with widely varying results.

As far as could be ascertained no work has been done toward determining the factors which influence the amount of the pig-ment present in the milk serum.

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(1) "Schoepff, Ludevicus Augustus,- Specimen Inaugurale Chemico- medicum de Varus Lactis Buli Salibus alusque Substantuis in ejusdem parte Aquosa Contentis, etc."

(2) Blyth, A. W., - Foods; Their Composition and Analysis.

The fact that large quantities of milk are required to obtain a small amount of pigment, and that the operation of the isolation is extremely tedious, probably accounts in a large measure for the lack of knowledge upon this phase of the chemistry of milk.

The object of this investigation was to repeat the investigations upon the chemical nature of this pig-ment; to add any possible points; to coordinate the varying results which have been obtained and to determine the factors which influence the varying amount of the pigment and if possible assign a reason for its presence in the serum.

Pigments and Milk Constituents Studied.

Lactochrome-- In 1864, E. Millon and Commaille [3] after coagulating and separating the casein and albumen, obtained a precipitate from the yellow whey of milk by means of a solution of mercuric nitrate. This precipitate was white, amorphous, and became slightly red on drying; it was insoluble in water, alcohol, and ether. This precipitate was washed with water, then with alcohol, and finally with ether. To this body they ascribed the following formula - $C_{30}H_{31}N_5O_{18}$, Hg O Hg O, $NO_3$, and gave it the name Lacto- proteine (2). In 1879 A. W. Blyth [4] in "A Study of the Composition of Cow's Milk in Health and Disease", isolated from this Lacto- proteine, galactin and a pigment to which he gives the name Lactochrome. He was able to separate these compounds from the milk whey by precipitating with phospho-tungstic and phospho-molybdic acids, and by precipitating with nitrate of mercury. The first two methods had to be abandoned because of decomposition in washing the precipitate. The last method i.e. the precipitation with nitrate of mercury, gave the so-called Lacto-proteine of Millon and Commaille. Blyth showed

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(3) "M. M. E. Millon et Commaille,- Comptes Rendus, 59, p. 301, 1864."

(4) Blyth, A. W., - Journal of the Chemical Society of London, 1879.

that this precipitate was not a simple substance but was a mixture of galactin, lactochrome, and any albumen which may have remained in the solution. This precipitate after being separated by decantation, and filtration, was well washed and decomposed with hydrogen sulphide. Upon filtering and removal of the excess of hydrogen sulphide, the filtrate yielded a lead salt with lead acetate. To this salt Mr. Blyth gave the name galactin. It was a fawn colored salt which was obtained perfectly white by decomposing andreprecipitating. It was noncrystaline, soluble in water, insoluble in strong alcohol, and precipitated by the general alkaloidal reagents of Sonnenschein. Blyth gave it the formula $(Pb\ O_{23})\ C_{54}\ N_{78}\ N_4\ O_{45}$.

The filtrate from which the galactin had been separated was found by Blyth to contain a substance which after any excess of lead had been removed, gave a precipitate with mercuric nitrate. This he describes as an alkaloidal coloring matter and gives it the name lactochrome. He ascribed to it the formula $Hg\ O\ C_6\ H_{18}\ N\ O_6$. He obtained the pigment in a free state by decomposing the mercury compound, and evaporating its solution. "As obtained in this way it was in the form of a bright red-orange, resin-like mass, softening at 100° C., very soluble in hot alcohol but partially separating out o-n cooling. It was freely soluble

in water. The concentrated solution gives a simple spectrum allowing most of the red and yellow rays to pass through, and no bands were observed". (4)

Lactochrome is thought by several authors and investigators to be the cause of color in butter. Oliver(5) says: "Lactochrome is probably derived from the haematin of the blood, is of a resinous character, of a bright orange color, and is soluble in water. This causes the color of butter and of whey. It is in very small proportions and varies much in that respect according to the action of the alveolus cells." Blyth(4) says- "Milk fat under the form of butter is constantly tinted more or less yellow from dissolved lactochrome, but it may by the use of suitable solvents, be obtained almost colorless."

Orotic Acid (Orotsäure)

Biscaro and Belloni(6) in 1905, discovered a new constituent in milk, which, because its method of isolation was similar to that of lactochrome, was studied in connection with this problem. It was found however, to have no connection with the color of serum. The investigators isolated it as follows:- 1 litre of milk was precipitated with a few drops of rehnet, filtered through a linen cloth and neutralized with sodium carbonate to a weak acid reaction. It was filtered,

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(5) Oliver,- Milk, Cheese, and Butter, Page 44.

(6) Biscaro and Belloni,- Chem. Central. 1905, vol. 2, p. 63.

(4) Cit.

the filtrate and the lead precipitate precipitated with basic lead acetate, decomposed with hydrogen sulphide and again filtered. The filtrate was evaporated to dryness o-n the water bath, taken up with water and filtered through animal charcoal. Potassium hydroxide was then added to a weak acid reaction. The crystalline residue, found upon evaporation was let stand 48 hours with 55 % alcohol to free it of chlorides. It was then let stand 24 hours with more alcohol and finally allowed to crystallize from hot water.

Biscaro and Belloni gave this acid the formula $C_5 H_4 O_4 N_2, H_2 O$. It was crystalline and decomposed at 260° C. It was slightly soluble inwater, slightly soluble or insoluble in organic solvents. The potassium, silver, barium, and many other salts were made and described by the authors.

Urobilin.-- Desmonliere and Gautrelet[7] in 1903 isolated a pigment from milk, which from their investigations they felt justified in calling Urobilin. Their proceedure of isolation was as follows:- several litres of milk were coagulated and filtered through a cloth and then through a paper. In this manner a slightly cloudy liquid with a greenish yellow color and a slight greenish flourescence was obtained. After acidifying with $H_2 S O_4$ this solution was saturated with ammonium sulphate and allowed to stand for several hours, then filtered. The filtrate was entirely colorless. The precipitate upon the filter paper was washed with a

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(7) Desmonliere and Gantrelet,- The Constant Presence of Urobilin in Cow's Milk.
Compt. Rend. Soc. Biol. 55, 1903, P. 632.

saturated solution of ammonium sulphate and extracted with 90 % alcohol. The alcohol took up the pigment and had a yellow color with a greenish fluorescence.

This alcoholic solution was acidified, and upon examination gave the absorption band of urobilin. They also found the greenish fluorescence of this solution was destroyed by acids; that the dicroism reappeared by the action of ammonia and that there was a distinct increase of fluorescence by adding ammoniacal zinc chloride. This ammoniacal zinc chloride solution still showed the absorption bands of urobilin.

In order to precipitate the pigments from the remaining alcoholic solution, they evaporated to dryness at a low temperature, took up the residue with ammonia, acidified with $H_2 S O_4$ and treated with an excess of ammonium sulphate. The precipitate formed was in rusty colored flakes. These were with difficulty soluble in water, but readily soluble in water made slightly alkaline. The pigment did not show the characteristic reactions of lipochrome, not giving the blue coloration with concentrated sulphuric acid, or the green coloration with concentrated nitric acid.

In order to show that the pigment exists in the milk as isolated, and is not converted into urobilin during the process of isolation, the investigators saturated a volume of milk with ammonium sulphate and added one half its volume of 95 % alcohol. The solution

was shaken and let stand until a clear separation was obtained. The alcoholic layer was colored a greenish yellow which was all the more pronounced because of the lack of color in the remainder of the solution.

Desmouliere and Gantrelet found the pigment to exist in all milk examined in the same proportion and because of this hoped to find a simple method of detecting adulterated milk.

Urobolin is thought by a number of investigators (8) to be identical with hydrobilirubin, a pigment of the liver. Although at one time thought to be the principal pigment of the urine, urobolin is now known to exit in the freshly voided urine only as the chromogen-urobilinogen, which by the action of light is converted into urobilin.

Urobilin according to Hammarsten(9) may have slightly different properties according to the method of isolation and the nature of the urine used. As isolated by the different investigators it is known as normal, fibrile, physiological, and pathological urobilin . It is characterized by its greenish fluorescence and absorption spectra. It varies in color from brown, reddish brown, red, to reddish yellow. It is easily soluble in alcohol, amyl alcohol, and chloroform; with difficulty soluble in ether, acetic ether and water; easily soluble in water made slightly alkaline. It is completely

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(8) Hawk,- Practical Physiological Chemistry.

(9) Hammarsten,- Text Books of Physiolog ical Chemistry, 6th English Edition, 1911, p. 704.

precipitated from the urine by saturation with ammonium sulphate. It is very soluble in alkalies and may be precipitated from alkaline solutions by the use of acid. Neutral alcoholic solutions in strong concentration are brownish yellow. In greater dilutions they are rose colored or yellow. Upon addition of zinc chloride solution to the ammoniacal solution of the pigment, it becomes red and shows an increase in the green fluorescence. This solution in the spectroscope shows a strong band between E and F.

Urochrome. Urochrome is the principal pigment which imparts the color to urine. It is closely related to urobilin, according to Garrod (10) giving a urobilin like pigment by the action of impure aldehyde. This reaction is used by Garrod as a test for urochrome. It is also claimed that urobilin yields a pigment similar to urochrome by the oxidizing action of permanganate.

Urochrome according to Garrod and Hammarsten(9) has the following properties:- brown, amorphous, readily soluble in water and ordinary alcohol, but less soluble in absolute alcohol. It dissolves slightly in acetic ether, amyl alcohol, and acetone but is insoluble in ether, chloroform, and benzene. It is precipitated by silver nitrate, lead acetate, mercuric acetate, and

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(10) Garrod,- Journal of Physiology, Vol. 21 and 29.
(9) Cit.

phospho-molybdic and phospho-tungstic acids. "When its solution is saturated with ammonium sulphate the greatest part of the pig ment remains in solution" (9). Urochrome shows no absorption bands, does not fluoresce with zinc chloride, and is easily decolorized by the action of acids.

## Experiments to Determine the Chemical Nature of the Pigments in Milk Serum.

### A Study of the Pigment obtained from Milk by the Lactochrome Method.

Lactochrome for these experiments was isolated from milk according to Blyth's method as follows:- 1000 cubic centimeters of separated milk was divided into three portions. One of these portions was diluted with water to about four times its original volume and acidified with dilute acetic acid until the casein coagulated. The whey was then syphoned off into a second portion of the milk, and more acid if necessary added. The resulting whey was in its turn syphoned into the third portion of the milk. More acid if necessary was added and the casein filtered out. This solution, free from casein, was now raised to the boiling point and gently boiled for several minutes. The albumen coagulated and was easily separated. The whey was then precipitated with acid nitrate of mercury solution.* This dense precipitate formed was washed by decantation and suspended in a small amount of water. It was then

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*"100 grams of pure mercury is dissolved in a litre of nitric acid; a further quantity of acid added until no red fumes are evolved; the solution is evaporated to a syrup, and after adding enough nitric acid to prevent the formation of a basic salt, is made up with distilled water to exactly 1400 cubic centimeters.

decomposed with hydrogen sulphide in an acid solution using either nitric or hydrochloric acids. The mercuric sulphide was filtered off, the hydrogen sulphide removed with the addition of heat. An excess of acetate of lead was added, forming a dirty white precipitate of galactin. This was filtered from the solution, and the excess of lead acetate in the filtrate decomposed with hydrogen-sulphide. The lead sulphide was filtered off, the lactochrome remaining in solution. This lactochrome in solution was purified by reprecipitating several times with mercuric nitrate, decomposing the final mercury salt, evaporating the aqueous solution to dryness, and dissolving the residue in hot absolute alcohol. This alcoholic solution was considered to be practically pure lactochrome.

Lactochrome as obtained by the above method was amorphous, softening at 100° C. and had a peculiar odor. It conformed to the preparation obtained by Blyth. The maximum yield obtained by this method was .04 gram per liter of milk. Some of the pigment was lost with each purification. About .5 gram in all was obtained for the study of the pigment.

The solution of lactochrome in absolute alcohol which was judged to be approximately pure was evaporated and the residue taken up with water. The aqueous solution was divided; one portion was saturated with pure ammonium sulphate. A scant brown precipitate

was thrown down. It was filtered off, the filtrate apparently retaining all of it's former color. One half its volume of absolute alcohol was added and the solution well shaken. On standing the clear alcoholic layer which separated had extracted practically all of the color. The alcoholic layer was removed and evaporated to a low volume. Absolute alcohol was then added until no more precipitate of ammonium sulphate came down. After filteration, the clear yellow filterate was concentrated to about 100 c.c. The following tests were made upon this solution:- Mercuric nitrate readily precipitated the coloring matter as did also silver nitrate, both precipitates having a yellowish color. Lead acetate also threw down the coloring matter from the alcoholic solution but the precipitate was readily soluble in an excess of lead acetate. A portion of the alcoholic solution was evaporated to dryness, the residue was brown in color. It was readily soluble in ordinary alcohol. Chloroform took up a mere trace of color from the dried residue. A drop of concentrated ammonium hydroxide seemed to increase the color. Alcoholic zinc chloride when added, however caused no marked green fluorescence. Amyl alcohol seemed to extract a slight amount of color from the dried residue. The amount of color absorbed seemed to increase on standing. This was probably due to the traces of water in the amyl alcohol and also to the absorption of water from the air, -- the pigment being

very soluble in water. The amyl alcohol solution when acid with hydrochloric acid (which showed a good yellow color when viewed through a 5 c. cm. layer) showed no absorption bands when examined in the spectroscope.

Tests on the Aqueous Solution of Lactochrome.

The following tests were made upon the aqueous portion which had not been saturated with ammonium sulphate. Mercuric nitrate readily precipitated the pigment from this solution as did also silver nitrate, both precipitates being brown in color. Lead acetate when carefully added to the aqueous solution threw down all of the color but the precipitate was readily soluble in an excess. It was found that the lead salt and also the mercury salt of lactochrome was soluble in ammonium acetate solution. Very little of the ammonium acetate was necessary to prevent the precipitation with lead acetate; it required more however to prevent the precipitation of the mercury salt.

Since in the preparation of the lactochrome lead acetate does not precipitate the pigment, an explanation was sought of the apparent difference between the isolated lactochrome and the pigment in the milk serum. As the solution to which lead acetate is added, for the separation of the so-called galactin and

lactochrome, contains considerable free nitric or hydrochloric acid whose presence was necessary in the decomposition of the mercury salt by hydrogen sulphide, the solubility of the lead salt of lactochrome in nitric and hydrochloric acids was tried. It was found to be soluble in both. The lead salt was not soluble, however, in acetic acid.

The lactochrome was found to be very soluble in milk serum, a very small portion of it greatly increasing the color of the serum, indicating that the pigment is a very intense one and that only a very small amount is necessary to give the normal milk serum its coloration.

A Study of the Pigment obtained from Milk by the Urobilin Method.

About 6 liters of milk from cow 305 were taken for this experiment. The fat was separated by means of a hand separator, and the casein precipitated from the skim milk by acidifying with acetic acid, and warming to about 50° C. After filtering off the casein, the slightly cloudy serum had a good yellow color. This color by by boiling a small portion, filtering and then clearing up with ammonium hydroxide, gave a coloration of 4.5 units of yellow in the 10 c. cm. cell, using the Lovibond Tintometer.

The bulk of the precipitate was acidified with sulphuric acid, and saturated with ammonium sulphate. After standing for some time the heavy precipitate which was formed was filtered off, washed carefully with a saturated solution of ammonium sulphate, dried in a steam oven, and extracted with 90 % alcohol. A greenish yellow extract was obtained. It was evaporated until all of the alcohol had been driven off, leaving the pigment dissolved in aqueous ammonium sulphate solution. Some impurities which had formed were filtered, and the filtrate saturated again with ammonium sulphate, after acidifying with sulphuric acid. The precipitate which formed was filtered off but it was noticed that most of the coloring matter was in the filtrate. The precipitate which had formed was washed with a saturated solution of ammonium sulphate, dried in a steam oven, and extracted with hot absolute alcohol. The alcoholic extract had a slightly greenish color. It was carefully evaporated to dryness and taken up with cold absolute alcohol, in which it was readily soluble. A golden yellow solution was obtained which had a dark greenish fluorescence. When acidified with hrdrochloric acid it showed no absorption bands in the spectroscope. A small portion evaporated to dryness gave a brownish residue which was only slightly soluble in amyl alcohol. Mercuric nitrate threw down a yellow precipitate from the slightly colored aqueous solution.

A similar residue was also readily soluble in chloroform giving a yellow solution with a slight green fluorescense. The addition of ammoniacal alcoholic zinc chloride to the chloroform solution gave no indicitation of the bright green fluorescence of the Wiramy's test for urobilin, and the solution showed no absorption bands. Mercuric nitrate and silver nitrate threw down the color from the alcoholic solution.

The filtrate from which this coloring matter was precipitated with ammonium sulphate, and which as noted above contained the bulk of the pigment which had been precipitated from the original milk serum by means of ammonium sulphate was treated as follows:- one half its volume of absolute alcohol was added. The alcoholic layer which formed on standing, had extracted all of the color. The alcoholic solution was greenish yellow. It was evaporated almost to dryness in a vacum oven, and the residue taken up with hot absolute alcohol, in which it was much more soluble than in the cold. The alcohol was filtered off and tested as follows:- the alcoholic solution had a beautiful green fluorescence. Muric nitrate precipitated the color from this solution as did also silver nitrate. The precipitates had a brownish color. Lead acetate also threw down a heavy precipitate, readily soluble in an excess of the ~~pigment~~ reagent.

A portion of the alcoholic solution examined in the spectroscope showed no absorption bands. A portion evaporated to dryness was very sparingly soluble in hot chloroform. Alcoholic zinc chloride had no effect upon this solution. The dried residue was somewhat soluble in amyl alcohol. Silver nitrate threw down the color from an aqueous solution of this pigment.

As the serum from which Lectochrome is obtained is first boiled to remove the albumen it was thought that the heating might have changed the pigment to such an extent that its properties might be rendered somewhat different, and that a portion of it might no longer be precipitated by saturating with ammonium sulphate. To study this point the proceedure was as follows:- Five to six liters of milk from a cow giving a high colored serum was run through a cream separator, the skim milk warmed to 50° C. and the casein precipitated by 10 % acetic acid, using 70 c.c. per liter. A slightly cloudy, brightly colored, yellow serum was obtained. About half of this was then boiled to remove the albumen, which was filtered off. The filtrate was still slightly cloudy but had a high color and a green fluorescence. The color and the fluorescence of this solution were unusually brilliant especially in the sunlight. The solution was neutralized with ammonium hydroxide and concentrated on the steam bath

to about 600 c.c. Theprecipitate which had formed was filtered off and the filtrate combined with 150 c.c. of a similar solution obtained in a similar way from the milk of another cow. The combined solutions were acidified with sulphuric acid and saturated with ammonium sulphate. This solution was allowed to stand for several hours, and the precipitate which had formed filtered off, and the precipitate on the filter washed with a saturated solution of ammonium sulphate. It may be noted in passing, that the filtrate, which for convenience may be labeled Solution M, was perfectly clear, had a beautiful golden yellow color, and a strong green fluorescence. It apparently had retained all of its former coloring matter.

The paper containing the precipitate which had been washed with ammonium sulphate solution was allowed to drain until thoroughly dry, and the paper and precipitate let stand under 90 % alcohol. The alcohol slowly extracted considerable yellow color. After several hours of such extraction, the alcohol was removed by filtration and concentrated to 15 c.c. This solution was allowed to stand for one hour in cold brine and the precipitate of ammonium sulphate which formed filtered off. The filtrate had a beautiful golden yellow color and a green fluorescence. A small portion carefully acidified with hyrochloric acid showed no absorption bands in the spectroscope. The remainder was evaporated to dryness in the vacuum oven and

the residue extracted with absolute alcohol as noted before. A small portion was made ammoniacal and a small amount of alcoholic zinc chloride added. There was no increase of fluorescence. Mûric nitrate as also silver nitrate threw down a yellow precipitate from the alcoholic solution leaving the solution colorless. Lead acetate also threw down a precipitate from the alcoholic solution when carefully added, but the precipitate was readily redissolved upon addition of a little more lead acetate. The remainder of the alcoholic solution was evaporated to dryness, The residue, of brownish color was only with difficulty soluble in water.

The result of the tests upon this pigment indicate that boiling the serum has no effect upon the pigment which is precipitated by saturating with ammonium sulphate.

A Study of the Pigment Isolated by the Urochrome Method.

Hammarsten (9) states that on saturation of urine with ammonium sulphate a great part of the urochrome remains in solution. It was found that on saturating the casein free milk serum and also the albumen free milk serum, with ammonium sulphate, the precipitates which formed yielding the pigments noted in the preceeding paragraphs, that by far the greatest part of the color remained in the filtrate. This

(9) Cit.

led to the isolation of the unprecipitated pigment with a view of comparing its properties with those of urochrome.

Although no special difference was anticipated the pigment was isolated separately, from the serum which had been boiled to remove the albumen, and then saturated with ammonium sulphate, i. e. solution M, noted above; and also from the filtrate which resulted from saturating the unboiled but casein free serum, which solution will be designated solution A.

As the method of isolation of the pigment from both solutions is identical, only that from solution A will be recorded.

The volume of solution A was 760 c.c. Following the method of Desmouliere and Gautrelet (7), one half of this volume of 90 % alcohol was added and thoroughly mixed. On standing the alcoholic layer which separated ~~on standing~~, contained the bulk of the pigment. The alcohol was decanted and the remainder extracted again using 250 c.c. of 90 % alcohol. The aqueous layer was now practically free from coloring matter. The two alcoholic portions were combined, filtered to remove suspended ammonium sulphate, and concentrated on the steam bath to 150 c.c., and the hot golden colored solution poured on to quite a bulk of solid ammonium sulphate. After thorough shaking, two layers formed, the ammonium sulphate having removed most of the water from

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(7) Cit.

the dilute alcoholic solution, leaving the pigment concentrated in the small alcoholic layer. The aqueous layer had taken up considerable of the color, so the layers were thoroughly mixed again and the solution let stand over night. The aqueous layer was then removed by means of a separatory funnel and about an equal quantity of absolute alcohol added to it. The alcoholic layer whcih separatedhad now removed practically all of the coloring matter. As it, however, had taken up considerable ammonium sulphate it was decanted and treated with solid ammonium sulphate as before which removed the bulk of the water and ammonium sulphate. The alcoholic layer which separated was then added to the other alcoholic solution and the combined solutions evaporated in a vacuum. During the evaporation a slight brownish precipitate came down ~~which~~ and was filtered off.

Garrod (11) states that when fairly pure concentrated alcoholic solutions of urochrome are poured into rather more than their own bulk of ether much of the pigment is precipitated in an amorphous state. Following this proceedure the above concentrated alcoholic solution was poured into a bulk of ether. No precipitation took place, probably the solution contained a little water, very

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(11) Garrod,- Proceedings of the Royal Society, vol. 55, 1894.

little of which will prevent the precipitation according to Garrod.

The ether was evaporated off and the residue taken to dryness in a vacuum. It was let stand for some time under hot absolute alcohol, the resulting extract having a golden yellow color, leaving considerable brown-red color with the undissolved residue. This residue was very soluble in water in which it was taken up. This aqueous solution was saturated with ammonium sulphate and absolute alcohol added. The alcohol extracted considerable color. It was decanted and the extraction repeated. The combined alcoholic extracts were evaporated to dryness on the steam bath and the residue extracted with absolute alcohol. The golden yellow colored extract which resulted was filtered off, and added to the similar extract obtained above after evaporation in vacuum. The study of the solution, which may still be distinguished solution M, and a similar solution obtained in practically the same manner from solution A now follows. Both of these solutions had a rich, warm brown color with a dark green fluorescence which was especially noticeable in the sunlight. Upon dilution these solutions passed through various shades of yellow to greenish yellow.

It was found that on evaporation of small portions of these solutions that their residues on heating emitted a strong odor of acetic acid and the fumes turned blue litmus paper red. The aqueous solutions from these residues although having the same yellow color of the alcoholic solutions, and passing through the same shades of color on dilution did not give precipitation tests with mercuric nitrate, silver nitrate, and lead acetate. Both the alcoholic and aqueous solutions when made strongly alkaline, and heated, gave off ammonia. Ammonium acetate is soluble in alcohol. It was found on studying lactochrome as will be rembered, that the mercury, silver, and lead salts were soluble in a small amount of ammonium acetate which also prevented the precipitation of the salts from the milk serum. These facts were taken to show conclusively that solutions A and M were contaminated with ammonium acetate which was preventing the precipitation of the metallic salts of the pigment. While its presence seems hardly probable, yet the method of isolation from the milk in which both acetic acid and ammonium hydroxide were used renders its presence highly possible, especially since ammonium acetate is soluble in alcohol. An attempt was made to rid the solutions of ammonium acetate. They were evaporated to dryness and heated strongly at the temperature of boiling water,

and the residues taken up again in absolute alcohol. The evaporation and the heating rendered part of the pigment insoluble. Garrod noticed this in his preparation of Urochrome. The solutions however still contained some ammonium acetate. It was found necessary to decompose it with dilute sodium hydroxide before the mercuric nitrate, silver nitrate, and lead nitrate would throw down the coloring matter. The silver precipitate of course consisted partly of the brown silver oxide $Ag_2O$, but as the solution was left cclorless it was concluded that the pigment was also precipitated.

Ammoniacal solutions, when alcoholic zinc chloride was added, showed no marked green fluorescence, as in the Wirsmy's test for urobilin. Amyl alcohol took up some color from the dried residues of the pigment (due to water perhaps). When acidified the amyl alcohol solution showed no absorption bands in the spectroscope. Chloroform extracted practically no color from the dried residues of the pigment. The lead salts of the pigment as in all other pigments studied was soluble in an excess of the reagent, and as in the case of lactachrome, also in nitric and hydrochloric acids. It was not soluble in acetic acid.

## Study of the Pigment Urochrome.

For the sake of comparison a brief study

was made of urochrome.

For this a fairly pure alcoholic solution of urochrome from human urine was used. The urochrome was obtained as follows:- about 150 c.c. of fresh urine were allowed to stand in the sunlight to transform whatever urobilogen might be present into urobilin, and the urine saturated with ammonium sulphate. After standing for some time the precipitate which was formed was filtered off. Half its volume of 95 % alcohol was then added to the filtrate. The alcoholic layer had a g ood yellow color. It was decanted and the extraction of the aqueous solution repeated and the two alcoholic portions combined. They were then evaporated to a low volume, filtered and absolute alcohol added, precipitating the dissolved ammonium sulphate. The process of evaporation, filtration, and addition of absolute alcohol was continued until the solution was practically free from ammonium sulphate. The final rather crude alcoholic solution had a golden yellow color. Silver nitrate, mercuric nitrate and lead acetate, all threw down a yellow precipitate from the alcoholic solution. A portion was evaporated to dryness and taken up in water, in hwich it is very soluble. Silver nitrate, mercuric nitrate, and lead acetate threw down yellow precipitates from this solution. The first two containing the bulk of the color, the last apparently all. The lead salt was soluble in an excess of the

reagent.The alcoholic solution of urochrome ~~showed no absorption~~ showed no absorption bands in the spectroscope when acidified with hydrochloric acid. The solution showed no green fluorescence. Garrod (10) claims that urochrome in alcoholic solutions is readily transformed into urobilin by action of impure acetaldehyde.

When a small amount of this active aldehyde is added to a dilute alcoholic solution of urochrome, and the solution warmed on the water bath, according to Garrod, in a very short time the solution changes from a pale yellow, to a rich orange tint, and exhibits an absorption band in the identical position of that of urobilin. The solution at this stage will exhibit a brilliamt fluorescence upon the addition of ammoniacal zinc chloride, and the band becomes narrower and shifts toward the red. If the action of the aldehyde is allowed to proceed the color of the liquid changes to a dark red-brown, and a second absorption band appears in the violet, which will become equally intense as the primary band. This is considered by Garrod to be the characteristic test for urochrome. He states that it is so delicate that a solution of one part of urochrome is 30000 will yield the reaction, even in crude solutions of urochrome.

An attempt was made to obtain an active aldehyde, its activity to be tested upon a crude urochrome solution. The aldehyde used was obtained in the ordinary way by distilling together ethyl alcohol and a dilute sulphuric acid solution of potassium.dichromate. Although when the alcoholic urochrome was heated ~~until~~ with some of this aldehyde, the color of the solution passed through the color changes described by Garrod, the absorption bands could not be distinguished in the spectroscope. As Garrod gives no way of distinguishing between active and non active aldehyde other than its action on urochrome, and as lack of time made it impossible to experiment with other solutions of acet-aldehyde, it was found necessary to abandon this test for urochrome for the present investigation. Experiments are being conducted however with a view of finding an active aldehyde, and applying it to urochrome, and the similar pigment obtained from milk serum.

## Experiments to Determine Factors Influencing the Color of Milk Serum.

### Method for Determining the Relative Amount of Color Present in the Milk Serum.

In order to compare the amount of pigment in different samples of milk, the proceedure was as follows. The milk was collected at the barn in gallon bottles. It was usually taken in the morning and run through a cream separator within a few hours. The skim milk was warmed to about 35° C. and precipitated with the smallest possible amount of 10 % acetate acid. A portion of this cloudy yellow serum was then boiled for a few minutes to coagulate the albumen. After filtering a yellow serum was obtained which in some instances was cloudy. To get a clear solution in every case these solutions were neutralized with ammonium hydroxide, heated and filtered at once. In this way a perfectly clear yellow solution was obtained.

This yellow serum was then placed in a 100 centimeter cell and compared with the standard colors in the Lovibond tintometer. The colors were readily matched. In some cases a few tenths of red were found necessary.

### Effect of Breed upon the Color of Milk Serum.

While working with milk serum taken from different animals, it was noticed that the serum taken

from the Ayrshires and Jerseys almost invariably had a higher color that that of the Holsteins and Shorthorns. In order to find what relation existed the colorimetric tests in table I were made. The procedure which has already been described for taking tintometer readings was used.

The University herd now in milk is composed of 4 Ayrshires, 20 Jerseys, 15 Holsteins, and 4 Shorthorns. All of these animals are pure bred dairy cows. They are giving milk varying from 23.7 to 2.1 pounds per milking; have been in milk for periods varying from 13 to 1 month, and vary in age from 15 to 3 years.

The average tintometer reading for the Ayrshires was found to be 4.78 units of yellow; for the Jerseys 3.59 units of yellow; for the Holsteins 3.41 units of yellow, and for the Shorthorns 2.15 units of yellow.

Even in the cloudy yellow serum after removal of casein, it was easy to distinguish by mere observation the Ayrshires and Jerseys from the Holstein and Shorthorns, and in some cases even between the Ayrshires and Jerseys, the forms showing such a high color in the serum.

The fact that such a marked difference exists between the average of the readings for the different breeds, each breed representing widely different conditions of milk production and period of lactation,

indicate that the coloration of the milk serum is primarily a breed characteristic. Within a narrower limit it is also an individual characteristic. This is emphasized by the fact that all of the animals were receiving practically the same feed. Some data on the effect of feed will also be given.

TABLE I.

| Breed | Herd number | Units of yellow |
|---|---|---|
| Airshire | 305 | 4.5 |
| " | 306 | 4. |
| " | 307 | 5. |
| " | 301 | 5.5 |
| | Average | 4.75 |
| Jersey | 57 | 2.9 |
| " | 23 | 3. |
| " | 11 | 2.7 |
| " | 41 | 3.1 |
| " | 53 | 3.5 |
| " | 16 | 3.5 |
| " | 124 | 5. |
| " | 10 | 3.5 |
| " | 3 | 3. |
| " | 30 | 2.7 |
| " | 54 | 3. |
| " | 50 | 3.1 |
| " | 8 | 3.3 |
| " | 2 | 2.7 |
| " | 13 | 3.2 |
| " | 317 | 5.3 |
| " | 27 | 4.5 |
| " | 22 | 5.2 |
| " | 59 | 4.5 |
| Jersey | 14 | 4.2 |
| | Average | 3.59 |
| Holstein | 223 | 1.5 |
| " | 217 | 1.7 |
| " | 208 | 2.2 |
| " | 213 | 2.2 |
| " | 209 | 3. |
| " | 204 | 3. |
| " | 219 | 2.2 |
| " | 222 | 3.6 |
| " | 227 | 2.7 |
| " | 224 | 2.6 |
| " | 220 | 1.3 |
| " | 210 | 2.2 |
| " | 215 | 3. |
| " | 226 | 2.3 |
| " | 211 | 2.7 |
| | Average | 2.41 |
| Shorthorn | 400 | 2.6 |
| " | 403 | 3. |
| " | 404 | 1.6 |
| " | 406 | 1.4 |
| | Average | 2.15 |

## Effect of the Period of Lactation upon the Color of the Milk Serum.

In table II the cows are arranged according to their period of lactation. This table indicates that the cows of any of the breeds milked less than five months have less color in their milk serum than those of the same breed which have been milked for a longer period. A calculation from the table shows that 78.5 % of the cows which had been in milk for less than five months gave a tintometer reading below the average of the particular breed to which the cows belonged. However the difference is not pronounced enough to overbalance the possibilities of other factors entering in. For this reason definite conclusions can not be drawn.

TABLE II

| Cow No. | Period of Lact. | Tintometer Reading | Units of Yellow Comp. with Av. for Br'd | Cow No. | Period of Lac. | Tintometer Reading | Units of Yellow Comp. with Av. for Br'd |
|---|---|---|---|---|---|---|---|
| 317 | 13 | 5.3 | Above | 217 | 7 | 1.7 | Below |
| 27 | 13 | 4.5 | Above | 227 | 7 | 2.7 | Above |
| 23 | 12 | 3. | Below | 3 | 6 | 3. | Below |
| 41 | 12 | 3.1 | Below | 53 | 5 | 3.5 | |
| 22 | 12 | 5.2 | Above | 124 | 5 | 5. | Above |
| 54 | 11 | 3. | Below | 10 | 4 | 3.5 | |
| 50 | 11 | 3.1 | Below | 213 | 4 | 2.2 | Below |
| 222 | 11 | 3.6 | Above | 220 | 4 | 1.3 | Below |
| 301 | 10 | 5.5 | Above | 219 | 4 | 2.2 | Below |
| 30 | 10 | 2.7 | Below | 226 | 4 | 2.3 | Below |
| 223 | 10 | 1.5 | Below | 305 | 3 | 4.5 | Below |
| 57 | 8 | 2.9 | Below | 306 | 3 | 4. | Below |
| 209 | 8 | 3. | Above | 8 | 3 | 3.3 | Below |
| 224 | 8 | 2.6 | Above | 208 | 3 | 2.2 | Below |
| 215 | 8 | 3. | Above | 204 | 2 | 3. | Above |
| 403 | 8 | 3. | Above | 210 | 2 | 2.2 | Below |
| 211 | 8 | 2.7 | Above | 307 | 1 | 5. | Above |
| 406 | 7 | 1.4 | Below | 2 | 1 | 2.7 | Below |
| 16 | 7 | 3.5 | | 59 | 1 | 4.5 | Above |
| 14 | 7 | 4.2 | Above | 404 | 1 | 1.6 | Below |

## Effect of Milk Production upon the Color of the Milk Serum.

The results obtained in table III point to no definite connection between the milk production and the color of the serum.

The fact that milk serum from fresh cows seemed to have less color might be thought to indicate that cows producing the most milk would give a low color. This however does not seem to be the case.

The fact that the pigment is an intense one might lead also to the supposition that the low average color shown by the Holsteins would be explained on the ground that they are the heavy milk producers. The data does not show this to be true. For instance cow 204 a Holstein producing 23 lbs. of milk per milking gave a tintometer reading 3. while cow 224 also a Holstein but giving only 6 lbs. of milk per milking gave a lower tintometer reading i.e. 2.6. A similar example can easily be found in the Jersey breed. Cow 134 producing 18 lbs. of milk per milking gave a tintometer reading of 5. while cow 30 produced only 4.2 lbs. per milking and gave almost the lowest reading for the breed i.e. 3.7. Many other examples could be given as the table will show.

TABLE III

| Cow No. | Milk Production | Tinto-meter Reading | Units of yellow compared with average for br'd. | Cow number | Milk production | Tinto-meter reading | Units of yellow compared with average for breed. |
|---|---|---|---|---|---|---|---|
| 210 | 23.7 | 2.2 | Below | 220 | 11.5 | 1.3 | Below |
| 204 | 23 | 3. | Above | 227 | 10.4 | 2.7 | Above |
| 211 | 20.8 | 2.7 | Above | 301 | 9.7 | 5.5 | Above |
| 213 | 20.4 | 2.2 | Below | 54 | 9.5 | 3. | Below |
| 209 | 19.2 | 3. | Above | 53 | 9.2 | 3.5 | Below |
| 124 | 18. | 5. | Above | 41 | 7.5 | 3.1 | Below |
| 219 | 17.1 | 2.2 | Below | 14 | 7.1 | 4.2 | Above |
| 59 | 16.7 | 4.5 | Above | 215 | 7. | 3. | Above |
| 305 | 16. | 4.5 | Below | 2 | 6.8 | 2.7 | Below |
| 307 | 15.4 | 5. | Above | 317J | 6.7 | 5.3 | Above |
| 208 | 15. | 2.2 | Below | 50 | 6.5 | 3.1 | Below |
| 223 | 14.8 | 1.5 | Below | 8 | 6.2 | 3.3 | Below |
| 217 | 14.4 | 1.7 | Below | 27 | 6.2 | 4.5 | Above |
| 10 | 14. | 3.5 | Below | 224 | 6. | 2.6 | Above |
| 404 | 13.6 | 1.6 | Below | 406 | 5.6 | 1.4 | Below |
| 16 | 13.6 | 3.5 | Below | 57 | 5.4 | 2.9 | Below |
| 226 | 13.6 | 2.3 | Below | 11 | 5. | 2.7 | Below |
| 403 | 13. | 3. | Above | 30 | 4.2 | 2.7 | Below |
| 306 | 12.5 | 4. | Below | 222 | 4. | 3.6 | Above |
| 400 | 12. | 2.6 | Above | 22 | 4. | 5.2 | Above |
| 3 | 11.8 | 3. | Below | 13 | 2.1 | 3.2 | Below |

The Effect of Age upon the Color of the Serum.

An examination of table IV shows that age apparently has something to do with the color of milk serum. All of the cows over seven years of age, are above the average for the particular breed to which the animal belongs. Of the cows under seven years of age 67.7% are below the average. Although the indications are that as a cow gets older the amount of color in her milk serum increases, these results would need further corroboration before one could draw positive conclusions.

TABLE IV

| Cow number | Age | Tint. read. | Units of yellow comp. with av.for brd | Cow number | Age | Tint. read. | Units of yellow comp. with av.for brd. |
|---|---|---|---|---|---|---|---|
| 124 | 15 | 5. | Above | 208 | 5 | 2.2 | Below |
| 317 | 15 | 5.3 | Above | 215 | 5 | 3. | Above |
| 16 | 13 | 3.5 | Average | 305 | 5 | 4.5 | Below |
| 204 | 12 | 3. | Above | 306 | 4 | 4. | Below |
| 27 | 10 | 4.5 | Above | 307 | 4 | 5. | Above |
| 209 | 10 | 3. | Above | 23 | 4 | 3. | Below |
| 403 | 10 | 3. | Above | 8 | 4 | 3.3 | Below |
| 301 | 9 | 5.5 | Above | 2 | 4 | 2.7 | Below |
| 211 | 7 | 2.7 | Above | 223 | 4 | 1.5 | Below |
| 210 | 7 | 2.2 | Below | 219 | 4 | 2.2 | Below |
| 10 | 7 | 3.5 | Average | 222 | 4 | 3.6 | Above |
| 57 | 6 | 2.9 | Below | 220 | 4 | 1.3 | Below |
| 41 | 6 | 3.1 | Below | 404 | 4 | 1.6 | Below |
| 53 | 6 | 3.5 | Average | 30 | 3 | 2.7 | Below |
| 54 | 6 | 3. | Below | 22 | 3 | 5.2 | Above |
| 50 | 6 | 3.1 | Below | 14 | 3 | 4.2 | Above |
| 59 | 6 | 4.5 | Above | 227 | 3 | 2.7 | Above |
| 213 | 6 | 2.2 | Below | 224 | 3 | 2.6 | Above |
| 3 | 5 | 3. | Below | 226 | 3 | 2.3 | Below |
| 217 | 5 | 1.7 | Below | 406 | 3 | 1.4 | Below |

Effect of Feed Upon the Color of Milk Serum.

A Jersey cow which had been fed for 24 days upon clover hay and white corn was tested in the usual way and gave a reading of 3.5 units of yellow. The butter fat was tested at the same time and gave a reading of 8. units of yellow in a one inch cell. The same animal was then fed for 12 days upon green alfalfa hay and yellow corn. At the end of this time the serum gave a reading of 3.5 units yellow while the fat reading in the one inch cell had increased to 45 units of yellow.

This data will not conclusive, certainly shows that feeds which have marked effects upon the color of the butter fat have no effect on the color of the milk serum. This will be further discussed in the following paragraphs.

## Discussion of Results.

The properties of urobilin, urochrome, and the pigments isolated from the milk serum are tabulated in table V.

Pigment I is Blyth 's Lactochrome. Pigment II is the pigment isolated from milk by the urochrome method. Pigment III is the pigment which remains in solution on saturating with ammonium sulphate an aqueous solution of the pigment precipitated by saturating the unboiled serum with ammonium sulphate. Pigment IV is the pigment which was precipitated from the aqueous solution of pigment III, whose properties were identical with the pigment obtained from the boiled serum by the urobilin method.

TABLE V (Part 1)

| | Urobilin | Urochrome | Pigment I |
|---|---|---|---|
| Solubility in:- | | | |
| ord. alcohol | slightly sol. | readily sol. | readily sol. |
| absolute " | readily sol. | readily sol. | readily sol. |
| amyl " | readily sol. | slightly sol. | slightly soluble |
| chloroform | readily sol. | insoluble | very sli. sol. |
| Result of saturating aqu. sol. with ammn. sulphate | completely precipitated | slightly precipitated | slightly precipitated |
| Action of precipitants | | | |
| lead acet. | precipitated | precipitated | precipitated (sol. in excess) |
| mercuric nitrate | not precipitated | precipitated | precipitated |
| silver nitrate | | precipitated | precipitated |
| phos. tung. acid | precipitated | precipitated | precipitated |
| phospho moly. acid | | precipitated | precipitated |
| Color of alcoholic solution | brownish yellow when con. Dil. sol's yellow or rose col'rd. | alcoholic sol are golden yellow when concentrated | alcoholic solutions are golden yellow |
| Fluoresc. neutral alcoholic solutions | strong green | none | slight |
| Aqueous solutions | | none | none |
| Absorption bands Acid alc'hl Acid amyl alc'hl sol's | one absorp'n band between green & blue | none | none |

TABLE V (Part 2)

| | Pigment II | Pigment III | Pigment IV |
|---|---|---|---|
| Solubility in water | readily soluble | readily solub. | slightly sol. |
| ordinary alc'h'l | readily soluble | readily solub. | readily sol. |
| absolute " | not. read. sol. | not read. sol. | readily sol. |
| amyl " | slightly sol. | slightly sol. | readily sol. |
| chloroform | very slightly s. | sparingly sol. | readily sol. (did not give Wirsmy's test) |
| Results of saturating aqueous solutions with ammonium phosphate | slightly precip. | not precipita. | not precip. |
| Action of precipitants | | | |
| lead acetate | (sol. in excess) | precipitated (sol. in exc.) | precipitated (sol in exc.) |
| mercuric nitrate | precipitated | precipitated | precipitated. |
| silver nitrate | precipitated | precipitated | precipitated |
| phospho-tungst. | precipitated | | |
| phospho-molyh. | precipitated | | |
| Color of alcoholic solution | golden yellow | greenish yel. | golden yellow |
| Fluorescence of neutral alcoholic solution | strong green | beautiful gr. | green fluoresc |
| Aqueous solution | strong green | | |
| Absorption bands acid alcoholic and acid amyl alcoholic solutions | none | none | none |

It will be noticed that as far as studied the properties of the pigment which Blyth called lactochrome are identical with the properties of the pigment which for the most part remain in solution on saturating the milk serum with ammonium sulphate, to which the milk serum undoubtedly owes its color. It will also be noticed that these properties are practically identical with the properties of the pigment to which urine largely owes its color, namely urochrome. The only distinguishing property so far discovered is the strong green fluorescence of the milk pigment, both in aqueous and alcoholic solutions. This property was not so noticeable however with Blyth's Lactochrome. This is probably dde to the method of isolation as all of the other properties are identical. That the color of the milk serum is not due to urobilin as Desmoulere and Gantrelet state, is shown conclusively by the fact that the milk pigment in no stage in it's isolation showed characteristic absorption bands of urobilin. Repeated attempts to make the properties of the serum pigment conform with the properties of urobilin were unsuccessful. Very few of these attempts are recorded in this paper. The fact that only a very small portion of the milk serum pigment was precipitated on saturating with ammonium sulphate in itself disproves the work of

Desmoulere and Gantrelet who claimed that the filtrate after this treatment was entirely colorless.

The claim of Blyth that the butter fat owes its color to the pigment which he called lactochrome is groundless, as the pigment is a water soluble one, which property has never been ascribed to the fat pigments. No further proof is needed of this fact. The French authors claim that the milk owes its coloration to the pigment which they call urobilin and one would infer from their statement that the pigment which they isolated did not give the reaction of a fat pigment, that no fat pigment was present in the milk. This conclusion was too hasty.

That a urobilin-like pigment is present in the milk in a very small quantity is indicated by the reactions of pigment IV in table V. It was however not conclusively shown to be urobilin for it lacked the spectroscopic property of that pigment. It was also present in the milk in a very small proportion in comparison to the one to which the serum owes its color. It should be noted that a small portion of what may be called the urochrome-like pigment was precipitated on saturating the unboiled serum with the ammonium sulphate. This is shown by the fact that pigment III showed the properties of the urochrome-like pigment. It is interesting to note also that boiling the serum evidently renders the urochrome-like pigment non-precipitable on saturating

with ammonium sulphate. Until the milk pigment can be shown to give Garrod's urochrome reaction with active acetaldehyde, the conclusion that the pigment is urochrome would not be warranted. The only safe conclusions at present are these:- first, that Blyth was correct in ascribing the coloration of the milk serum to the body which he called Lactochrome, and, second, that the pigment which is largely responsible for the color in the serum conforms closely to the properties of the pigment Urochrome.

A study of the factors which influence the color of milk serum reveal very interesting facts, the chief one of which is that this property of milk is undoubtedly a breed characteristic, at least as far as the breeds studied are concerned.

The grouping of the breeds according to the color of the milk serum results in the surprising fact that the Ayrshires, which have always been ranked barely above the Holsteins as color producers, rank ahead of the Jerseys, the color producing breed, and far ahead of the Shorthorns which have always been ranked just below the Jerseys. In fact the color of the Ayrshire serum is sometimes so intense, and the same may be said of a great many Jerseys, that the yellow color of the milk is undoubtedly in a large measure due to it. This is emphasized by the fact that Holstein milk, often called blue, sometimes shows practically no color in the serum. The conclusion may well be drawn

that the milk of some breeds owes its color only in part to the color of the suspended fat g lobules, the factor before considered entirely responsible for the color.

The arrangement of the data according to the period of lactation, milk production and age of the animal reveals several additional interesting facts which however are not as conclusive as those just discussed. The indications are, however, that a cow does not produce the maximum color for the breed until she has been in milk for several months, and that when she has reached the age of seven years she reaches a maximum which is above the average for her breed. The data also indicates that the milk production plays no part whatever in the amount of color in the serum, and also that the feed has no effect. The latter result perhaps needs further corroboration since the feed is known to play an important part in the color of the milk fat. As noted in the single experiment recorded the animal was receiving such widely different feeds, when samples were taken for study of the serum coloration that one must conclude that at least these two feeds have no effect upon the coloration of the milk serum. It should be mentioned also that the clover hay and white corn produced practically no color in the milk fat while the green alfalfa hay and yellow corn produced a highly colored fat. It was in reality this difference of the milk fat coloration which warranted

the conclusion thatthe feed had no effect upon the color of the serum. It is possible that feeds might be found which would affect the color of the serum, but this is not likely.

If the pigment to which the serum owes its color is urochrome, the question at once arises as to the explanation of its presence in the milk. Oliver said that the lactochrome of Blyth is probably derived from the haematin of the blood. This explanation is the only one which experiments noted in the literature would justify. Mac Munn (12) prepared a pigment by the oxidation of haematin which was apparently identical with urinary urobilin, and Riva (10) claims to have obtained a body similar to urochrome by the oxidation of urobilin. While these oxidations were made with laboratory reagents the conclusions would not be justifiedthat they could not be easily carried out in Nature's laboratory in the body of the cow.

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(12) Mac Munn,- Proceedings of the Royal Society, Vol. 31.
(10) Cit.

## SUMMARY

I. The color of milk serum free from fat and casein is due to a pigment with properties which closely resemble those of urochrome, the principal pigment of urine, and not to the pigment urobilin.

II. A very small amount of the color may be due to a urobilin-like pigment which was isolated by saturating the serum with the ammonium sulphate, which precipitated the pigment along with the proteins. The filtrate however retained the major pigment, i. e. the urochrome-like pigment.

III. The urochrome-like pigment is identical with the pigment to which Blyth gave the name Lactochrome.

IV. The serum pigment is in no way related to the pigment which causes the color of milk fat.

V. The color of milk serum is due primarily to the breed of cow, the four breeds studied ranking as follows:- Ayrshire, first; Jersey, second; Holstein, third; and Shorthorn, fourth. The latter two are practically the same.

VI. The period of lactation and age of the cow slightly influence the color of the serum when considered with respect to the breed.

VII. The milk production and the feed of the animal have no influence upon the color of the serum.

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