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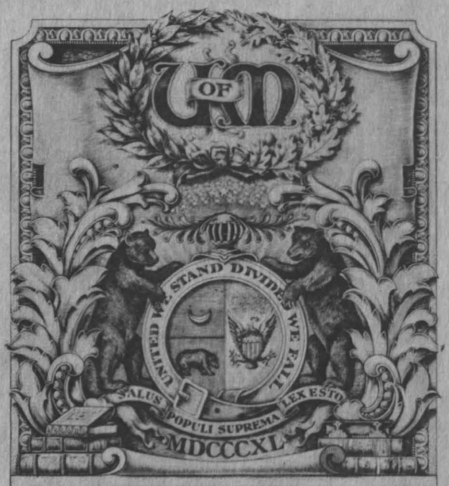

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Dear Dr. Miller :

The thesis of Mr. Roy B. Davis submitted for review seems to be written in good smooth English, arranged in logical order, and in so far as I can tell full up to the standard required. Indeed, it seems to be a mighty good piece of work.

Very respectfully,
WJ Calvert

WJC-MB.

May 16, 1913.

Dr. W. J. Calvert,

3 Hospital.

Dear Dr. Calvert:

It is customary for the Graduate Committee to refer dissertations, submitted by candidates for the degree of Master of Arts, to some member of the Group who is not connected with the Department in which the candidate's work has been done. I am sending you herewith a dissertation which has been submitted by Roy Davis.
I shall be greatly obliged if you will kindly examine the same at your earliest convenience and report to us for the Graduate Committee whether in your opinion the dissertation meets the general standard which has been established in this University for the Master's dissertation.

Very truly yours,

Walter Miller

Chairman, Graduate Committee.

THE FATTY ACIDS PRESENT IN
HUMAN MILK

by

enton
Roy B. Davis, B. S.

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SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS
in the

GRADUATE SCHOOL

of the

UNIVERSITY OF MISSOURI.

Approved
S. Calvert

1913

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INTRODUCTION.

In beginning this thesis an effort was made to find all the available references on the subject of the chemistry of human milk fat in order that a bibliography might be made which would cover all the work done on the subject up to the present time. In order to investigate the methods for separating the fatty acids in butter fat it was found necessary to look up the work that has been done on cow's butter and in most cases to follow the same methods in this work.

Compared with what has been done on the butter fat of cow's milk, very little has been done on human milk fat. The fatty acids of cow's butter have been fairly well analysed both qualitatively and quantitatively, and the physical and chemical constants have been determined several times under varying conditions. These determinations have been worked out for various abnormalities due to disease, the feeding of different kinds of food, various degrees of starvation and thirst, etc., etc.

The work which has been done on human milk fat includes a few determinations on the physical and chemical constants, a few qualitative determinations, and one or two quantitative results for some of the fatty acids. Almost all of the results differ greatly even among determinations made by the same investigator. The causes for the varying results are taken up under HISTORY.

In writing a thesis of this kind one is bound to come across various difficulties in every determination he

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attempts, owing to the fact that our methods for separating the fatty acids at the present time are very crude. It is very difficult, if not impossible, to make a complete separation of only two fatty acids but where there is a mixture of perhaps eight or ten different acids the best that can be done is to make only approximate determinations for the quantitative part, and in a measure this is true for the qualitative part as well. For this reason and for the reason that various other factors, depending on the temperament and bodily constitution of the woman, the nature of the food she eats, the period of lactation at which the milk is drawn, and the race of the woman, have to be considered it is not at all surprising that the results of investigators differ so widely.

Another difficulty that every one who works on this subject finds is getting material in large enough amounts to work with. Perhaps the best source is from colored women but their prices are so exorbitant as to be almost prohibitive.

Since the physical and chemical constants of human milk fat were worked out very thoroughly by Mr. Harry G. Bristow in his thesis submitted in partial fulfillment of the requirements for the degree of Master of Arts at this University in 1911, I confined my work to the qualitative determinations, and made a few quantitative determinations where it was possible. These determinations were made only on the fatty acids present as saturated and unsaturated acids.

HISTORY.

The first work that was done on the butter fat of human milk was to determine its percentage in the milk. Simon, working in Berlin, in 1838 made several determinations and his results show that the percentage may vary from 2.53 to 3.88 per cent. Clemm and Scherer (Handworterb. d. Physiol., Vol. 2, p. 464.) investigated the percentage of butter fat with reference to the time after delivery and found on the fourth day 4.29 per cent, on the ninth day 3.53 per cent, and on the twelfth day 3.34 per cent. Chevallier and Henry (Journ. de Pharm. T. 25, pp. 333 and 401.) made similar investigations and found that the percentage of butter fat is greatest the first few days after delivery. Other investigators have done similar work on the butter fat of cows, asses, bitches, etc., and have found the same to hold true with them also.

L'Heritier (Traite de Chimie pathologique, Paris, 1842, p. 638.) investigated the percentage of butter fat in the milk of blonde and brunette mothers and found that the milk of the former contained from 3.5 per cent to 4.0 per cent of butter fat, while that of the latter contained from 5.6 per cent to 6.5 per cent. The mothers from whom the milk was taken were each twenty-two years old.

Vernois and Becquerel, working in Paris, in 1853 found that the age of the woman affects the amount of fat present and that the milk taken from a young woman will be richer in butter fat than that taken from a woman twenty-five years of age or over. They also found that the periods of

menstruation greatly influenced the percentage of fat present, and that the amount also depends somewhat upon the number of children the mother has borne previously.

Decaisne (Hoppe-Seyler, l. c., 739.) investigated the milk of nursing women during the siege of Paris and found that insufficient food would cause a decrease in the amount of fat present in the milk and Soxhlet, working later, found that a diet rich in fats would cause a very large increase in the percentage of fat. A modern investigator (E. Merkel, Pharm. Zentralhalle, 53, pp. 495-496.) found that the fatty acids from the milk of one woman agreed very closely with the fatty acids of margarine and upon investigation he found that she had been eating a diet composed very largely of that fat.

Sourdat (Compt. rend., Vol. 71.) and Brunner (Pfluger's Arch., Vol. 7.) found that the two breasts of the same woman yield different percentages of fat. It is considered that the milk from the left breast is richer in butter fat than that from the right one. They also found that different parts of the same milking have various percentages of fat, the last portions being richer than the first ones. But another investigator found that when the milk was taken after comparatively short periods, every two hours for instance, that there was very little perceptible difference in the amount of fat found.

Ruppel (Zeitschr. f. Biologie, Vol. 31.) determined the melting and solidifying points of butter fat and qualitatively determined some of the acids. He found that the volatile acids were a very small per cent of the total acids present, that almost half of the non-volatile acids was oleic,

and that myristic, palmitic, and stearic acids were present in large quantities but he made no quantitative determinations of the amounts present. Later, Ruppel and Laves (Zeitschr. f. Physiol. Chem., Vol. 19, 1894.) by a method of crystallizing the barium salts of the acids found that the volatile acids were 1.4 per cent of the total acids, that 1.9 per cent of the acids were water soluble, and that the unsaturated acids were 49.4 per cent of the total acids. They also found that myristic and palmitic acids were present in larger amounts than stearic acid and claimed that the volatile acids were butyric, caprylic, caproic, and capric.

Edelfelsen (Muchener med. Wochenschrift, 1901, Vol. 48, p. 7.) also found that the percentage of volatile acids was very small and that oleic acid was present in larger quantities than the saturated acids.

W. Arnold (Zeitschrift f. Untersuchung d. Nahr. u. Genussmittel, Vol. 23, May, 1912.) worked out most of the physical and chemical constants and then from the molecular weights he found for the various fractions he worked out a few of the acids that must be present. For instance, the acids which were obtained when the Polenske number was taken (the volatile, insoluble acids) had a molecular weight of 193.7. The acids supposed to be present in the Polenske number determination are capric, lauric, and myristic, with molecular weights of 172, 200, and 228 respectively. Since the molecular weight found for the mixture was lower than that of either lauric or myristic he decided that it was capric which lowered the molecular weight of the mixture. He did no quantitative determinations for the individual acids but found

the saponification number in the fat from the milk of two women to be 206.08 and 205.0; the Polenske number to be 1.65 and 1.45; the Reichert-Meisl number to be 2.65 and 1.5; the iodine number to be 46.25 and 45.65. One strange fact about his determinations is that he failed to identify stearic acid.

In summing up, the principal work that has been done on human milk fat up to the present time consists of several determinations of the physical and chemical constants of the fat and a few qualitative determinations of the fatty acids. Quantitative determinations have not been done for all of the fatty acids and the percentage of oleic acid seems to be the only one that is very well settled, and even it varies considerably. One fact that has caused a great deal of comment among investigators is the fact that the iodine number is always too high for the percentage of oleic found to be present. The high iodine number would lead one to believe that there are other unsaturated acids present besides oleic but no one has been able to identify them up to the present time.

The acids determined qualitatively so far are butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic. Those determined quantitatively are oleic, and the palmitic-stearic-myristic group.

PHYSICAL CHARACTERISTICS

The butter fat from human milk is an opaque mass, very light yellow in color when solid, but when melted it becomes very transparent and the color changes to a much darker shade of yellow. The fat has an odor a great deal like that of castor oil and this odor seems to be due to the volatile acids and not to the non-volatile acids as only the former have it after the separation. The non-volatile acids have a peculiar, not unpleasant fatty odor. When solid they are almost perfectly white, but when melted they have a faint tinge of yellow and are very transparent. As they cool, the entire mass does not solidify at once but masses of crystals form which are embedded in an amorphous mass.

THE ANALYSIS.

Preliminary Experiments.

The various difficulties found in trying to free the butter fat of human milk and the methods for freeing it have been written by Mr. Bristow in his thesis mentioned above and need not be further explained here.

After the butter had been churned and separated from the milk as well as possible, it was well dried by means of filter paper and then melted and poured into a tight, glass-stoppered bottle. The bottle was kept in a cooler at a temperature of from one to three degrees Centigrade until the fat could be analysed.

The usual method of separating the acids from the esters by means of saponification with alcoholic potassium hydroxide solution and then hydrolysis of the resulting soaps with dilute sulphuric acid was the method used to get the free acids. A few preliminary experiments were made with small amounts of fat to find out whether or not there were any peculiarities and to see how the method would work with this particular fat.

A small amount of fat, eight to ten grams, was placed in a flask and treated with twenty cubic centimeters of fifty per cent alcoholic potassium hydroxide solution and about ten cubic centimeters of water. As soon as the potassium hydroxide solution was added the mixture turned a very dark brown, almost a black, and became a thick, gelatinous mass. It was thought that perhaps the heat of reaction

had scorched the fat but the mixture did not have a burnt odor and the heat developed was not very great. The experiment was completed however, and the various groups were separated. A few qualitative tests were made, and it was found that volatile acids were present.

Separation and Analysis of the Groups.

Fifty grams of butter fat were placed in a round bottom flask of about five hundred cubic centimeters capacity, and fifty cubic centimeters of fifty per cent potassium hydroxide solution in water were added. To this were added one hundred cubic centimeters of alcohol. The potassium hydroxide solution was added very slowly in order to find out whether or not it was the heat of reaction that caused the mixture to turn brown in the preliminary experiment. The brown, pasty mass formed just the same and remained throughout the saponification.

To complete the saponification the flask was fitted to a reflux condenser and kept at a little higher than a simmering temperature on the water bath for about fifteen minutes, then raised to a slightly higher temperature and kept boiling for thirty minutes more. The condenser was then removed and the flask again heated on the water bath for about fifteen minutes in order to drive off most of the alcohol. The soap solution was next poured into a liter and a half of water contained in a two liter flask and again heated. This second heating drove off most of the alcohol which remained and also heated the soap solution in preparation for the steam distillation, which was the next operation.

A solution of fifty per cent sulphuric acid was used to hydrolyse the soaps in order to get the free fatty acids but the preliminary experiment mentioned above showed that volatile acids were present, and in order to collect them it was necessary to distil them over in steam.

The Volatile Acids.

When the apparatus for steam distillation had been arranged, one hundred cubic centimeters of the sulphuric acid solution were added and the distillation started at once. The solution of volatile acids was collected in a flask and about six hundred cubic centimeters of distillate were collected. The distillate was rather cloudy and oily drops floated on the surface. The potassium salts of the acids were then made by neutralizing the distillate with potassium hydroxide solution. The volatile acids which might have been present were formic, acetic, propionic, butyric, caproic, caprylic, and capric, and tests were made for each one of these.

Capric Acid Group.

After adding an excess of potassium hydroxide solution to prevent hydrolysis which might result from the excess of water present, the solution was evaporated almost to dryness on the water bath. There is no satisfactory method for separating the acids by forming salts which will precipitate out, as in ordinary qualitative analysis, but the best method seems to be to isolate each individual acid where it

is possible and judge which one it is by its properties. When the potassium salts had been evaporated almost to dryness they were cooled and treated with a slight excess of dilute sulphuric acid in order to free caproic, caprylic, and capric acids,,which are insoluble in dilute sulphuric acid solution. An oily layer formed as soon as the sulphuric acid was added and the characteristic goaty odor of the group was apparent.

Perhaps the best method for determining which acids of the Capric Group are present in a mixture, when the amount of material is limited, is to cool the mixture and attempt to solidify it. Capric acid is a solid at ordinary temperatures, caprylic acid melts at 16.5 degrees, and caproic acid is a liquid even several degrees below zero. In making the test for the acids the solution was cooled in ice water for several minutes and as the oily layer remained a liquid and no crystals formed in it it was very evident that caproic acid was the only acid present. At least, if the others were present they were in such small amounts that they could not be determined. Since these three acids are usually found together in the milk of most animals it is very likely that they are present in human milk fat as well, and no doubt could be found in traces if a larger amount of butter fat could be taken for the determination.

It is interesting to note that Arnold (Zeitschr. f. Untersuchung d. Nahr., Vol. 23.) by his system of determinations from the molecular weights of the fractions he obtained claims that caprylic acid is present in the largest amount. The molecular weight of caprylic acid is 144, of caproic acid

is 116, and the molecular weight of the mixture of the capric acid group he found to be 128.9. From this he infers that it is caprylic acid which causes the high molecular weight.

Butyric Acid.

The solution left after the capric acid group had been removed was then tested for butyric acid. Butyric acid is insoluble in a sodium chloride solution and was easily salted out by adding an excess of common salt. An oily layer soon formed and it could easily be recognized as butyric acid by its characteristic odor, which was strong enough to fill the room. No exact quantitative determination could be made but the amount was greater than that of the capric acid group. It was estimated as being between two and three times as much.

Formic, Acetic, and Propionic Acids.

The butyric acid was removed by filtration and the filtrate treated with a small amount of ether in order to extract the lower acids if they were present. The ethereal solution was removed by means of a separatory funnel and diluted with water. Since ferric chloride is a test for any one or all of the acids that might be present it was the test that was used. Dilute sodium hydroxide was added to the solution of acids in order to form the sodium salts and after adding a few cubic centimeters of ferric chloride solution a small amount of hydrochloric acid was added. If any of the three acids had been present a red color would have formed

but the solution remained perfectly clear. So far as is known, no one has found any of these acids in human milk fat although formic and acetic acids have been found in the butter fat of cow's milk. It is very likely that if these acids are found in butter fat it is due to the fact that the milk was not kept clean and that bacterial action has begun.

Therefore, it would seem that the volatile acids present in human milk fat are butyric and caproic acids, with perhaps traces of capric and caprylic acids.

The Separation of the Saturated from the Unsaturated Acids.

The residue left in the flask after the steam distillation consisted of the saturated acids (less the volatile acids) and the unsaturated acids. The method used for separating these acids was to form the lead salts of the mixture and extract the lead salts of the unsaturated acids by means of ether in which they are soluble, while the lead salts of the saturated acids are insoluble in ether.

The solution of acids was acidified with an excess of acetic acid and then neutralized with an excess of potassium hydroxide solution in alcohol. The neutral point was very hard to determine owing to the fact that the solution had turned almost black during the saponification, as mentioned above. A ten per cent solution of lead acetate was made and heated to boiling and then added to the solution of acids until all the lead soaps were precipitated. The soaps clung to the sides of the flask and were a muddy yellow, waxy mass,

insoluble in water. After they had cooled they were washed well with water and then melted in fresh portions of water, the water being poured off each time. This was repeated until the washings were neutral.

The soaps were then removed from the flask and all the adhering drops of water were removed by means of filter paper, after which they were treated with fifty cubic centimeters of ether and warmed on the water bath. The lead salts of the unsaturated acids dissolved easily, while the lead salts of the saturated acids were left as a fine powder in the bottom of the flask. The ether solution was filtered off and the saturated acids were washed well with ether, the washings being poured into the solution of unsaturated acids.

The filtrate was run into a separatory funnel and treated with dilute hydrochloric acid which broke up the lead salts, giving lead chloride and the free fatty acids. The solution of lead chloride and hydrochloric acid was run off and the fatty acids washed two or three times with dilute hydrochloric acid, after which they were washed well with distilled water and run into a flask. They were then dissolved in ether and the solution dried over night with anhydrous sodium sulphate.

The method of determining which acids are present in a mixture of unsaturated acids is by means of bromination and resolving the brom-derivatives into their constituents by means of their solubilities in petroleum ether. The dibromostearic acid, which is formed from oleic acid, is very soluble in petroleum ether as well as in all the other sol-

vents for fats. The tetrabromostearic acid, which is formed from linoleic acid, is soluble in warm petroleum ether but precipitates out in the cold. It is very soluble in diethyl ether, however. The hexabromostearic, which is a derivative from linolenic acid, is practically insoluble in petroleum ether and acetic acid but dissolves in hot benzene.

To make the determinations, most of the ether was very carefully evaporated on a warm water bath and the mixture dissolved in about twenty-five cubic centimeters of glacial acetic acid. The solution was then treated with bromine, a drop at a time, until the yellow coloration persisted. The principle of the bromination is that the unsaturated carbon atoms of the acids become saturated with bromine and thus form the brom-derivatives, the amount of bromine taken on depending on the degree of unsaturation of the carbon. The solution was kept cooled in ice water for three hours and then examined for a precipitate, which would be the hexabromostearic acid. In this determination a slight precipitate was formed. It was filtered off and washed well with ethyl ether in which it did not dissolve, but when it was treated with hot benzene it was easily dissolved. There was not enough of the material to make further tests by taking the melting point or estimating the amount of bromine in the compound but its nonsolubility in ethyl ether and acetic acid and its solubility in hot benzene are fairly confirmatory. Therefore, linolenic acid was evidently present.

The filtrate was treated with water three or four times in order to dissolve out the acetic acid and after the

water had been run off through a separatory funnel the mixture of brom-derivatives was dissolved in ether and dried over anyhydrous sodium sulphate. The ether solution was filtered and the sodium sulphate washed with ether and the washings added to the filtrate. After the ether had been removed by warming gently over the water bath the mixture was dissolved in petroleum ether and then cooled down to about three degrees in an ice water bath. A slight turbidity formed which disappeared on warming the mixture and reappeared when it was again cooled in the ice water. Tetrabromostearic acid is insoluble in cold petroleum ether and soluble in warm petroleum ether so it is more than probable that it caused the turbidity. It was also present in too small an amount to make any further confirmatory tests. Tetrabromostearic acid is the derivative from linoleic acid.

The solution left was dibromostearic acid which is the derivative formed from oleic acid.

Therefore it would seem that the principal unsaturated acid present was oleic since linoleic and linolenic acids were present in very small amounts. There was more linoleic acid present than linolenic.

The Saturated Acids.

The residue left in the flask after the lead soaps of the unsaturated acids had been dissolved out with ether was the lead soaps of the saturated acids. When they were melted they were a very light yellow but when solid they were almost a pure white.

The saturated acids were freed from lead by the same method employed in getting the free unsaturated acids, viz., by treatment with dilute hydrochloric acid. They were poured out of the flask and the part clinging to the sides was washed out with water, into a beaker. Dilute hydrochloric acid was added and the mixture of acids was melted over the water bath. The treatment with dilute hydrochloric acid was repeated three times and the free fatty acids finally washed well with water. They were then melted in water two or three times in order to wash out all the lead chloride which might be held in the acids and the water was run off. The saturated acids themselves differ very little from their lead salts as far as color is concerned, except that they may perhaps be a little lighter yellow when they are melted.

There are three or four methods that have been used for freeing the individual acids from the mixture and the one first tried was to precipitate the barium salts in the hope that those with the highest molecular weight would precipitate out first. The method was to dissolve the acids in hot alcohol and then add a very dilute solution of barium acetate, much less than the amount necessary to precipitate the total acids. The solution was allowed to cool slowly and the first crystals were collected and examined. Melting points were taken and were found to vary so greatly that it was very evident that pure substances had not been obtained. The method was tried several times and found to be unsatisfactory for this work. It could very probably be used where not more that two acids were present and only approximate quantitative

results were desired but where there was a mixture of acids it was not practicable.

Following out the same idea that was used in trying to crystallize the barium salts of the acids an attempt was made to crystallize the free acids themselves. This method gave the same difficulties that the previous one did, viz., the products were not pure and did not have constant melting points.

The next attempt was to make fractional distillations of the ethyl esters of the acids in Bruhl's vacuum apparatus. This was tried for the reason that the esters have much lower boiling points than the acids and for that reason it was thought they would be less liable to decompose when they were distilled. The boiling points of the esters as given in Leath, The Fats are: ethylstearate, 200; ethylpalmitate, 185; ethylmyristate, 102; ethyllaurate, 232. The first three boiling points were taken at 10 mm., the last at 760mm.

To form the esters the acids were boiled under a reflux condenser for four hours with anhydrous ethyl alcohol and a few cubic centimeters of sulphuric acid. The esters were then well washed to free them from the sulphuric acid and most of the alcohol and were distilled in the vacuum apparatus. The alcohol, of course, came over first and as soon as most of it was boiled off the mixture began to decompose and to become dark brown in color. About five cubic centimeters of a clear liquid came over up to about one hundred degrees, and was proven to be acetic acid. During the distillation, while the temperature was rising after the alco-

hol had distilled off, the apparatus filled with clouds of smoke, the boiling was very irregular, and there was a great deal of trouble from bumping. Nothing more came over until the temperature went up to about one hundred and ninety degrees although it was noticed that drops of water formed in the tubes of the apparatus. From this temperature up to about two hundred and thirty degrees almost five cubic centimeters of a clear, oily liquid came over and soon solidified. A melting point determination was made but the substance did not melt very near the melting point of any of the pure esters. It was very likely a mixture of the decomposition products as at that temperature all of the esters should have distilled over. Nothing more came over even though the temperature was raised to two hundred and forty degrees, which was as high as it would go. It is not known why the esters decomposed but it is possible that if they had been distilled in the presence of some inert gas they would have distilled more readily without decomposition.

The next and final attempt was to distil the free acids in vacuo and the pressure used was 10 to 15 mm. The acids supposed to be present in butter fat as saturated acids are: lauric, myristic, palmitic, and stearic, with boiling points of 176, 200.5, 215, and 232, respectively. In distilling the mixture of acids it was intended to save the fractions coming over five degrees before and five degrees after the boiling point of each acid.

Ten grams of fat were taken for each distillation and it was hoped that quantitative results could be obtained,

but this had to be given up for all but lauric acid. It was very hard to get fractions coming over at constant boiling points and only approximate results could be obtained. Nothing came over until the temperature registered about one hundred and thirty degrees, when an oil began to distil over. The first portion was discarded and the fraction which came over between one hundred and seventy to one hundred and eighty-two or eighty-three was saved. The oil solidified and the melting point was found to be 42, the true melting point of pure lauric acid is 43.5. The amount which came over was a little more than one gram.

When the next fraction was attempted it was found that the acids could not be distilled over quantitatively and this had to be given up. A small amount of a substance was collected between one hundred and ninety and two hundred and five and had a melting point of between 52 and 53. The melting point of pure myristic acid is 54.

A very small amount of palmitic acid was collected in the same way but for this determination only the portions coming over within two or three degrees of the true boiling point of the palmitic acid were saved. By doing this a fraction was obtained which had a melting point of between 61 and 62. The true melting point is given as 62.6.

It was not possible to get pure stearic acid by this method as the substance which came over after the boiling point of palmitic acid was passed did not have a constant melting point. The distillation was discontinued after the temperature had gone up to about two hundred and thirty-five

degrees as decomposition began then. The residue left in the flask was dissolved in alcohol and washed out into a beaker, after which the alcohol was allowed to evaporate over a warm water bath and the acids cooled. Since the acids had been heated high enough to drive off all the lauric and myristic acids it was very probable that the substance remaining was a mixture of stearic and palmitic acids.

In order to estimate the percentage of stearic acid in the mixture a melting point was taken. By comparing the melting point of the unknown mixture with the melting point curve of known percentages of palmitic and stearic acids it could be estimated how much stearic acid was present. It was found that 3.8 per cent stearic acid in palmitic acid had a melting point of 48 while the melting point of the unknown mixture was 47. While no definite test for stearic acid could be made, it is very probable that it was stearic acid which lowered the melting point of the palmitic acid mixture.

Therefore, the acids present as saturated acids were lauric, myristic, palmitic, and stearic.

Any one who has worked with the fatty acids can realize the difficulties which come up in a work of this kind. Our work, like that of others who have worked on the fatty acids of human milk, was, as can well be imagined, greatly hampered by the difficulty of securing sufficient material at the right time from the proper sources. It is apparent that a more delicate apparatus is needed for distilling small quantities at a constant pressure in vacuo. A special piece of apparatus was ordered for this work but,

through an unavoidable delay in importation, it did not reach us in time to be used, and none could be obtained in this country.

I desire to express my sincere thanks to Professor Sidney Calvert, under whose direction this work was done, for his help and suggestions in choosing this subject, and for his assistance in the various difficulties I encountered.

Acknowledgement must also be made to Professor C. A. Eckles, who very kindly placed at our disposal the remaining portions of the butter fat from human milk which had been collected for Mr. Bristow's research.

THE END.

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Approved,
S. Calvert.

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