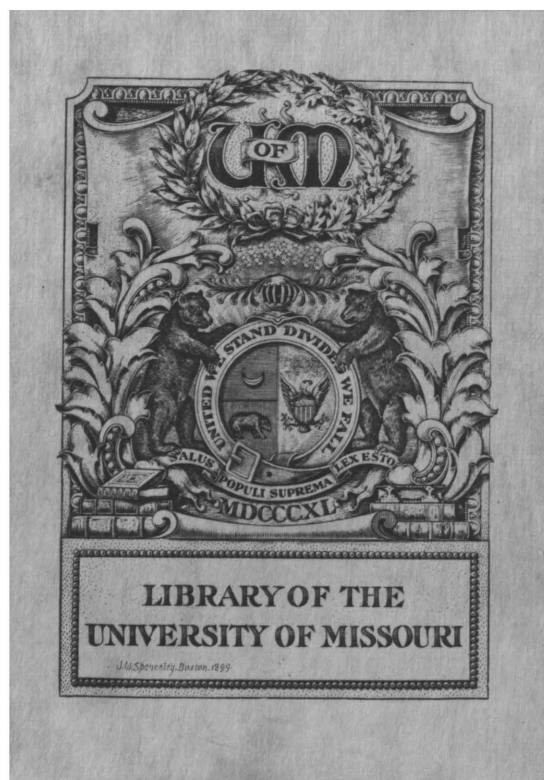


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THE PHYSICAL CHARACTERISTICS
" and
CHEMICAL CONSTITUTION OF HUMAN MILK FAT

by

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TABLE OF CONTENTS.

Introduction1
Review of Work Already Done.....4
Collecting and Caring for the Samples.....8
Physical Characteristics and Peculiarities of the
 Fat and the Physical And Chemical Constants.....12
Physical and Chemical Constants.....14
Summary.....24

INTRODUCTION.

Up to the present date there has been a large amount of work done on the special physical constants and the chemical constitution of certain better known and easily obtainable fats, including beef suet, milk fat especially from the different breeds of cows and some animal fats. There has been, however, a comparatively small amount of work done on the physical constants and chemical constitution of the fat from human milk.

It was the original intention in this paper to give all the physical constants of the fat from human milk which would in any way throw light on the chemical constitution of the fat, and to give a complete qualitative analysis with as near quantitative estimation of some of the constituents as accuracy of qualitative methods would permit.

The difficulty of obtaining samples, the limited time in which to complete the work and the unforeseen accidents and abnormalities in the action of the fat have prevented the completion as intended.

In this paper will be given the physical characteristics and physical and chemical constants of the fat from human milk and as many quantitative estimations as can be calculated from the constants thus obtained, with its summary of the probable constitution of the fat which will accord with the chemical constants obtained. The field of investigation is so extensive that only a small portion of it can be covered by such a paper as this.

It would perhaps be fitting to mention the field this investigation might easily cover.

- (1) Quantitative determination of the different fatty acids of the human milk fat.
- (2) Wherein it differs from ordinary butter fat and the reason for this difference.
- (3) Relation of milk fat to the body fat.
- (4) Relation of milk fat to food fat taken into the body.
- (5) Racial differences if any.
- (6) Individual differences. The data obtained for this paper were on composites obtained from several women.
- (7) Influence of the period of lactation on com-

position of the human milk fat.

Such an extensive investigation would require several years of work especially since the samples are so difficult to obtain in any workable quantities. It would also necessitate a large amount of preliminary work in perfecting methods which should be if possible more rapid and at the same time more accurate than the present best known methods. The present methods are far from being absolute, and some of the physical constants are more or less arbitrary. The writer hopes to be able to complete at least the quantitative separation and determination of the different constituents present in the average human milk fat and also if samples can be obtained in sufficient quantities to compare the chemical constitution of fats from the milk of the negro and the white race.

REVIEW OF WORK ALREADY DONE.

One of the first to do any work on milk fats was Chevreul¹. He was able to isolate from butter besides Oleic Acid, three volatile acids, (1) Butyric, (2) Caproic, (3) Caprinic. The non-volatile acids remaining he held to be a mixture of "Margarinic and Stearic Acids".

Lerch² shortly after this discovered a new volatile acid, Caprylic Acid

Later Heintz,³ in an elaborate series of researches based upon his own method of separation of the different fatty acids, was able to separate and identify most of the different fats of butter.

Heintz's method was briefly the obtaining of fractions by fractional crystallization and precipitation with Ba. Mg. and Pb setting free the acids from the salts and determining the melting points of the fractions. Comparing these melting points with the melting points of known mixtures such as he assumed his fractions to be composed of, he showed that the Margarinic Acid of Chevreul was a

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1. Ann. Chem. Pys. 22: p 356 f.
 2. Liebig's Annalen. Bd. 49: S.212 f.
 3. Erdmann's Jour. Vol. 66, 1855, ppl-51.



mixture of Stearic and Palmétic acids. He also found a new acid which he called Butinic. Ernst Wein later showed that Heintz's Butinic was really Arachidic. Wein also claimed to have found Formic and Acetic acids.

The fatty acids up to this time supposed to be in butter fat were then Formic, Acetic, Butyric, Capronic, Caprylic, Caprinic, Myristic, Palmitic, Oleic, Stearic and Arachidic.

In 1899 C. A. Browne, Jr.⁴ went through a quantitative separation and determination of the different constituents of butter fat. The fats ~~he~~ he found and determined quantitatively were:-

Oleic	Capric
Palmitic	Caproic
Stearic	Caprylic
Myristic	Butyric
Lauric	Di-Oxy-Stearic

Browne also gave all the physical constants of an average butter fat including some constants not usually determined in regular butter analysis.

The work done so far on the fats of human milk consists of a considerable amount of work on the Iodine Number and the varying percentage of Oleic acid with time of taking sample and the period of

4. Jour. Am. Chem. Soc. Vol.21. pp 612 and 807.

lactation. a little work on the volatile acids and one qualitative separation of some of the fatty acids from human milk fat.

On some of the common physical constants of human milk fat Thiemich,⁵ Gogetidse,⁶ Sauviatre,⁷ and Engel⁸ have worked. Engel spent all his time on the Iodine number of human milk fat. His conclusions were (1) The percentage of Oleic acid in different human milk fats is subject to variation within moderate limits, (2) The Iodine number of any individual's milk fat is subject to daily variation which may be expressed as a general rule for the time of day taken.

Laves⁹ with a limited amount of fat, (16 gr.) was able to determine the relative amounts of the three classes of fats, (3 fractions). (1). Volatile water soluble, (2) Volatile water insoluble and (3) Non-volatile fats. In the fraction of non-volatile fats he determined quantitatively the Oleic acid. His results were Volatile Soluble Acids 1.4%, Volatile insoluble acids 1;9%. Total Volatile 3.3% , Non-Volatile 96.7%. The total Oleic Acid calculated from Iodine number was 53.5% of the total acids or 49.4% of the non-volatile acids.

5. Thiemich

6. Gogetidse: Zeitschrift Bio#. N. F. Bd.28.

7. Sauvaitre.

8. Engel. Zeitschrift. Physiologische Chemie. Bd.44, 1905.

9. Laves. Zeitschrift fur Phys. Chem. Bd. 19. p.369.,1894.

Ruppel ¹⁰ made a separation and approximate determination from 200 grams of fat from human milk. He mentions the presence of Formic acid which no other investigation has found. In summary, not considering the doubtful presence of Formic acid he gives to human milk fat the following fatty acids: Butyric, Caproic, Caprinnic, Myristic, Palmitic, Stearic and Oleic. But he does not give percentage of each present.

The preceding contains all the work of note that has been done on the milk fats of any nature.

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10 Ruppel. Zeitschrift fur Biologie. Bd 31. S 1-12.

COLLECTING AND CARING FOR THE SAMPLES.

The first difficulty in attempting a work of this kind is the obtaining of any material at all, let alone obtaining it in such quantity as is necessary.

The samples collected were all from negro women. They were taken regularly every twelve hours and consisted of the entire output of milk from the left breast in every instance. The women were all in good health and of good physical development. By far the greater percentage of the milk was obtained from women of from twenty-five to thirty-three years of age; one only being without this limit (eighteen years of age). The milk was collected at different periods of lactation. One peculiarity or abnormality should be noted. From the woman whom I shall designate as "A", milk was collected during the seventh month of lactation, then again during the twenty-second and twenty-third months of the same lactation period.

The milk samples were kept separate for each woman and at the end of each week the fat was separated in a small cream separator. The resulting cream was then diluted to several times its volume with dis-

tilled water at 36° C and again separated . This washing was generally repeated in order to remove as completely as possible the peculiar slimy caseinogen which made churning of the samples so difficult. Indeed, it was found impossible to churn the cream obtained from nurse "A" in her twenty-second and twenty-third month of lactation though the sample was churned for several hours and the temperature was manipulated in a way that made all other samples easy to churn. After churning a few minutes the bottle of cream was placed in an electric oven at 60° C and allowed to stand until a thin layer of melted fat was to be found on top. The sample was then cooled to 15° C without disturbing the upper layer of fat and diluted with cold water. Then Upon churning the fat would immediately separate out. This difficulty in churning was undoubtedly due to the peculiar character of the caseinogen and would offer an interesting problem in the line of milk proteins and the influence of period of lactation on their composition.

The separated milk and washings also the butter milk from the churning were combined and with difficulty extracted with ether. The amount of fat

thus obtained was so extremely small that it was disregarded in subsequent separations.

In every case where possible the separated cream was churned and the resulting butter was melted in an electric oven at 50°-60° C. and the fat thus obtained filtered. The fat samples were then kept as a composite for nurse "B", "C", etc.

In the case of nurse "A" where it was found impossible to churn the sample the separated cream was placed in the electric oven at 60° and the fat allowed to rise to the top. The lower layer of water, casein, etc was then siphoned off and the fat samples were kept in ground glass stoppered bottles away from light and in a cooler, at a temperature of from 33°-40° F. The different composites were finally made into one large composite when it was seen that the time and money available would not permit the collection of large enough individual samples to be of use for the work already planned. The large composite was composed from the following fractions.

Nurse "A", 7th month.....	63.60	grms.
" "B",	61.10	"
" "B",	65.00	"
" "A", 22nd and 23rd month.....	137.00	"
" "D",	11.25	"
Small Fractions from Nurses, "E", "F", "G", "H", "I", "K".....	13.75	"
Total	<u>361.75</u>	"

--11--

This large sample was kept in a tightly stoppered bottles, away from light and in the cooler, kept at from 33^o-40^o F.

PHYSICAL CHARACTERISTICS AND PECULIARITIES
of the
FAT AND THE PHYSICAL AND CHEMICAL CONSTANTS.

The fat when solid has scarcely any color, being a very light yellow, tallow-like solid. When liquid, the fat has a distinct yellow color, though rather dark as if slightly tinged with brown. In comparison with butter fat, it is not nearly so brilliant in color, nor is it so clearly transparent.

In connection with the free fatty acids, there has been a peculiar phenomena noticed. Upon saponification with glycasol soda in the Reichert-Meissl determination, the fats turned a very dark brown, resulting in a brown soap solution. The free fatty acids from this soap solution gave such a dark brown layer as to be almost black. In saponification with alcoholic KOH solutions, the fats turned dark brown immediately on addition of the alkali. In the Reichert determination, it was noticed that on discontinuing the steam distillation, the free fatty acids separated into two distinct layers, the upper and larger layer being a light

golden yellow and the lower layer a very dark brown. The same thing was noticed in the Acetyl number.

The fat has a peculiar musty odor similar to that of Castor oil. The volatile acids have the same odor while the non-volatile acids are free from any trace of it.

In the titrations for Glycerol Number, Acetyl Number, Free Acidity and Saponification numbers the end points were not very sharp and were hard to decide upon owing to the above mentioned peculiar color of the solutions.

PHYSICAL AND CHEMICAL CONSTANTS.

(1) Melting point	31.65
(2) Refractive Index	1.4644
(3) Sp. Gr. 40°/40°	0.98392
(4) Iodine Number	52.54
(5) Saponification Number	207.50
(6) Free Acidity (1 gr.)	4.89 cc N/10
(7) Acid Number	19.56
(8) Ether Number	187.94
(9) Reichert Number (0.932) reduced to One gram fat	0.374
(10) Reichert-Meissl Number (1.325) "	0.265
(11) Acetyl Number	25.25
(12) Glycerol calculated	10.004%
(13) Glycerol by Analysis	9.645%
(14) Volatile insol. Acids from 1gm. fat	7.0 cc/N/10 Alk.
(15) Neutralization Number (Calculated)	216.7
(16) Mean Molecular weight of fatty acids	259.15

(1) The melting point of the fat was determined by Wiley's disk method ¹¹ and the average of five determinations taken.

(2) The refractive index was taken at 40° C, with an Abbe Refractometer ¹¹ and reduced to 25°.

This is readily done since every degree change in temperature produced a change of fraction 0.000365 in the refractive index. In this case to reduce to 25°, the fraction 15×0.000365 must be added.

(3) The specific gravity was taken at 40°C with a Geissler pycnometer.

(4) The Iodine number or the percentage of Iodine absorbed by the fat was determined by the method of Hubl¹¹ and the average of four almost perfect checks taken. The percentage of Olein is calculated from the Iodine number by the formula $x=100/86.2 I$. Since 86.2 is the theoretical Iodine number for pure Olein and where x is the percent sought and I the Iodine number found.

(5) The saponification number or milligrams of KOH or NaOH required to saponify 1 gram of fat was obtained by the Regular official methods¹¹ with alcoholic KOH.

(6) The free acidity or the number of c.c. of N/10 KOH necessary to neutralize the free acids in one gram of fat was obtained by dissolving about 1.5 grams of fat in hot neutral alcohol and cooling to about

11. Bulletin 107, U. S. Department of Agr. "Official Methods".

40°C and titrating with N/10 NaOH, ^{using} phenolphthaline as an indicator. As has been stated the end point was extremely difficult to decide upon in this determination, though good duplicates were obtained. The Acid Number of a fat is the milligrams of KOH necessary to combine with the free acids in one gram of fat.

(7) The Ether Number is obtained by subtracting the Acid Number from the Saponification Number.

(8) The Reichert Number, or the number of c.c. of N/10 Alkali necessary to combine with the volatile soluble acids obtained from 2.5 grams of fat, was obtained by steam distilling the free fatty acids from 2.5 grams of fat to six liters of distillate; the last fraction of the distillate requiring 0.1 c.c. N/10 alkali to neutralize the volatile soluble acids. The volatile insoluble acids from this Reichert distillation were then dissolved in neutral alcohol and titrated with N/10 Ba(OH)₂ using phenolphthaline; the result was calculated to one gram of fat. This

was done simply because it was noticed that the volatile insoluble acids were high in comparison to the volatile soluble acids and it was desired to get a number which would express this relation.

Volatile Soluble Acid (1 gm. fat)	0.374 N/10 Alk.
Volatile Insoluble Acid (1 gm. fat)	4.7 cc.N/10 "

(9) The Reichert-Meissl number or the number of c.c. N/10 Alk. required to neutralize the volatile soluble acids obtained from 5 grams of fat by the Reichert-Meissl distillation process ¹¹ was obtained as outlined in official methods.¹¹

(10) The Acetyl number furnishes a measure of the Hydroxy acids in a fat. It is the milligrams of KOH necessary to neutralize the Acetic Acid that has been combined with 1 gram of Acetylated fat. The Acetyl number was run by the method of Benedikt And Lewkowitsch. ¹² Five grams were refluxed for two hours with twice its weight of Acetic Anhydride. The Acetylated fat was then washed until the washings were no longer acid to litmus. Recover the layer of Acetylated fat and dry to constant ^{weight}. Saponify a weighted portion of the dried fat, set free the fatty acids

12. Lewkowitsch Oils, Fats, & Waxes. Vol. 1.

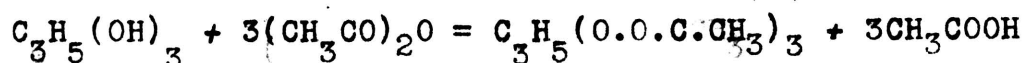
by making acid with H_2SO_4 and determining the number of c.c. of N/10 KOH required to neutralize the volatile soluble acids in the distillate obtained by Reichert distillation. On subtracting the Reichert number from this, the correct amount of Acetic acid from the Acetylation of the OH groups is determined. The percent of oxy-acids is then calculated as percent di-oxy-stearic acid by the formula:

$$x = \frac{100 \text{ c m}}{a(56100 - 42a)}$$

where x is the percent of oxy acid sought and c , the acetyl number, a , the number of Hydroxyl groups acetylated and m , the molecular weight of the acid.

(11) The Glycerol was obtained by the Benedict-Cantor Acetin process.¹² Fifteen grams of fat were saponified with alcoholic KOH and the alcohol driven off on a water bath. The resulting soap decomposed with dilute H_2SO_4 and the liberated fatty acids filtered off. The filtrate was then made neutral with $BaCO_3$ and evaporated on the water bath almost to dryness. The residue is then extracted with a mixture of equal parts of alcohol and ether, the ether-alcohol evaporated off on the water bath and the crude glycerine dried in a vacuum dessicator.

This process is based on the quantitative conversion of Glycerol into tri-Acetin by heating with Acetic Anhydride according to the equation:-



If the product of this reaction is then dissolved in water, and the free acetic acid carefully neutralized with KOH, the dissolved tri-acetin can be easily estimated by saponifying with a known volume of standard alkali and titrating back the excess alkali with standard acid-N/2 HCl preferred.

These titrations are difficult under favorable conditions but in this case as has been stated before the end points were hard to decide upon and only fairly good checks were obtainable. The percent of Glycerol is calculated in the following manner:-

Blank Alkali required.....	153.12 cc Acid
Excess " "	<u>67.53 " "</u>
Alkali necessary for saponification	85.59 " "

Acid used was 0.48227 N

1 c.c. N Acid = $\frac{0.092}{3}$ or 0.03067 gm Glycerol.

1 c.c. 0.48227 Acid = 0.014791 gm. Glycerol.

85.59 c.c. 0.48227 Acid = 1.26598 gm. Glycerol.

Percent then is calculated as pure Glycerol in the

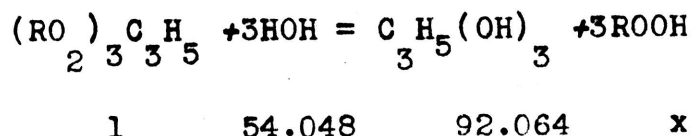
entire sample of fat. The Glycerol may also be readily calculated from the ether number by the formula,

$$G = 0.0005465 E.$$

The percentage of Fatty Acids from 1 part of fat may then be calculated from the Glycerol number by the formula:

$$T = 1 - \frac{54.048G}{92.064} - G$$

where T = % fatty acids and G = Glycerol number,
Since in saponification:-



The neutralization number N and the mean molecular weight m, of the fatty acids can be calculated from the Ether number, E, and saponification number, s, of the fat by the formula, $N = \frac{s}{1 - 0.0002257E}$

$$m = \frac{56158}{N}$$

Neutralization Number = 216.7

Mean molecular weight of fatty acids = 259.15.

After obtaining all these constants about 150 grams (exactly 148.94 grams) of the filtered dried

fat were saponified with alcoholic KOH, the alcohol driven off from the resulting soap which was then dissolved in water and the acids set free by adding HCl to 2 drops in excess of neutral. An apparatus was then set up for steam distillation such that a blank 500 c.c. distilled over in the same time as was required for a 500 c.c. distillate from the fatty acids. The volatile water insoluble acids were then separated from the water soluble volatile acids by filtration. The water soluble acids being titrated immediately with N/10 Ba(OH)₂ using phenolphthaline as an indicator. The insoluble volatile acids were dissolved in neutral alcohol and titrated with N/10 Ba(OH)₂ using phenolphthaline as an indicator. It was found necessary to make the filtration of volatile soluble from volatile insoluble at a fixed temperature as relative percentages of these two varied with the temperature of the distillate when filtered. 20° C was chosen as a convenient temperature easy to obtain and at which the volatile insoluble acids were solid.

For the first 3 liters of distillate there was a regular and very marked decrease in the number of cubic centimeters of N/10 Ba(OH)₂ required to neutralize

the volatile acids. After the 5th liter of distillate had been collected the number of cubic centimeters of N/10 alkali required to neutralize the volatile soluble acids became fairly constant and remained so even after the distillate had reached the 60th liter, at which time for some unaccountable reason the entire amount blew up scattering glass and non-volatile fatty acids all over the laboratory. The intention was in this method to obtain 3 main fractions as a preliminary separation. These fractions to contain 1, Volatile water soluble acids, 2, Volatile water insoluble acids, 3, Non-volatile acids.

The different constituents of volatile fractions could then be separated and ^{determined by} distilled ~~in~~ in vacuo; and the non-volatile fraction separated by fractional crystallization and fractional precipitation.

The volatile water soluble acids from 148.94 gms. in 60 l. of distillate required exactly 186.05 c.c. of 0.1017 N Ba(OH)₂ to neutralize. The amount of 0.1017 N. Alk required to neutralize the volatile soluble fatty acids which were obtained from the first 10 l. of distillate however, was only 65.15 c.c. which was not unnaturally above the theoretical amount calculated from the Reichert number. When we bear in mind the fact

that from about the 5th liter of distillate on, the amount of volatile acids were almost constant and that the decomposition or whatever it was that caused this phenomena had probably already set in. The average amount of $\frac{n}{\lambda}$ alkali required to neutralize 1 liter of distillate from the fatty acids, after the first 10 l, was 2.51 c.c. There was only slight deviation from the amount throughout, while the blank each time did not vary over 0.15 c.c. seldom over .05 c.c. The volatile water insoluble acids were also practically constant for each liter of distillate and required a total of 370.3 c.c. 0.08522 N. $\text{Ba}(\text{OH})_2$ to form the Ba. salts of the fatty acids.

The Barium Salts of the volatile acids were dried and preserved in 3 fractions. (1) The Ba salts of the volatile water soluble acids from the first 10 liters of distillate which were supposed to contain the bulk of the true volatile soluble acid content. (2) The Ba salts of the volatile soluble fatty acids after the 10th liter of distillate after the decomposition had evidently set in. (3) The Ba salts of the Volatile water insoluble acids.

SUMMARY.

Concerning the composition of human milk fat the things most to be noted are:-

(1) The very low percentage of volatile fatty acids. The Volatile water soluble acids represented by the Reichert number (0.932) shows this extremely small percent. The volatile water insoluble acids were high in comparison with the volatile soluble acids, though this might have been due for the most part to steady decomposition of higher fatty acids, from the prolonged steam distillation.

(2) The extremely high percentage of Oleic Acid or Glycerol Tri-Oleate present. Calculating from the Iodine number already given by the formula $\text{O} = 100/86.2 \text{ I. O}$ or percent Olein = 60.95%.

(3) The above high percent of Olein is all the more remarkable when the high percent of Oxy-Acids present is considered.

The Hydroxy-acids present is undoubtedly due to oxidation of the double bond in Oleic Acid and is present as di-oxy-stearic acid. Calculating from the Acetyl Number by the formula, already given (p-18-)

$$x = \frac{100 \times 25.25 \times 314.27}{2 (56100 - 42 \times 25.25)}$$

x= 7.209 % Di-oxy-stearic Acid.

The Acetyl number is one that has never before been run on human milk fat and but a few times on ordinary butter fat, so there is practically no basis on which to make comparisons. Browne found the di-oxy-stearic acids in cow's butter to be 1% but mentions two other instances in which the Acetyl number of fat has been found to be considerably higher.

It has been generally accepted that Oleic Acid is the only unsaturated acid present in milk fat and di-oxy-stearic acid is consequently the only hydroxy acid present.

The extremely high Iodine and Acetyl numbers for human milk fat would seem to open again, the question whether or not Oleic Acid is the only unsaturated acid in milk fat, especially in human milk fat, or whether there is not probably at least one unsaturated acid of the di-olefine series which would absorb much more Iodine than Oleic and would give rise to a tetrahydroxy acid. This seems even more probable when one considers the calculated mean molecular weight of the fatty acids (259.15) together with the percent Olein (60.95) %, Molecular weight of Oleic Acid 282.27, and

percentage di-oxy-stearic acid (7.209% molecular weight of di-oxy-stearic acid 314.27).

(4) The extremely high free acidity as shown by the acid number. This was due at least in part to the length of time the fat was kept before work could be begun on it, since the acid number of the fat increased from 19.56 to 23.80 in about 4 months time, though every precaution had been taken in keeping the sample. The weight of free acid expressed as Oleic acid in 1 gram of fat may be calculated from the acid number by the proportion:-

$$282.27: 40::x :.01956$$

$$x = .1380 \text{ gram Oleic acid.}$$

Free acidity of a fat is most probably not due entirely to Oleic acid but it is usually calculated as entirely Oleic in expressing percent free acidity.

Most of the numbers obtained are in close accord; for instance the Refractive Index, Specific Gravity, and the calculated Mean Molecular Weight; also the Reichert number and Saponification number are in close agreement. The Melting point, refractive index and Iodine number furnish good checks the one upon the



other and the Specific Gravity and Reichert-Meissl numbers compare well when the effect of the high percent Olein and oxy-acids upon the specific gravity is considered.

The free acidity presents the most striking abnormality in composition of the fat and needs repeating on a large number of samples. The high percent of hydroxy-acids should also be especially investigated on a large number of samples to ascertain if this is a special characteristic of human milk fat or is a variation from the normal to be found only in the composite from which these data were obtained.

This work was done in the Laboratory for Dairy Research maintained by the Dairy Division of the U.S. Dept. of Agriculture in cooperation with the University of Missouri Experiment Station.

Note for Graduate Faculty
and for editor university publications.

This work was done in the Laboratory
for Dairy Research working in cooperation
with the University of Mo. Exp. Station
as stated in the footnote - both thesis.
It was mutually agreed by Prof. Eckles &
Calvert that publication should be pursuant
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