Approved by L. S. Palmer

Approved C. H. Eckles

A STUDY OF DIFFICULT CHURNING

by

Robert Osborn Jr., B. S. in Agr.

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

in the

GRADUATE SCHOOL

of the

UNIVERSITY OF MISSOURI

1918

CONTENTS.

A STUDY OF DIFFICULT CHURNING.

INTRODUCTION.

There is at the present time a lack of definite knowledge as to why cream, at certain times, fails to give up its fat in the form of butter. This trouble occurs most commonly where only one or two cows are kept, the usual experience being that the cream gradually becomes more and more difficult to churn until it is apparently impossible to obtain butter by any amount of agitation.

Difficult churning of this kind also appears to be independent of the usual factors having a bearing on the churnability of the cream which are under the control of the buttermaker. For example, the control of such factors as the temperature of churning, the percentage of fat in the cream, and the proper use of commercial starters seem to be without effect on the cream.

Considerable financial loss is frequently involved where the butter fails to form, for the buttermaker frequently loses patience after several hours of apparently useless effort and throws away the cream as unfit for use.

The present investigation was undertaken with the view of studying the occurrence, causes, and possible remedies of difficult churning. It was hoped that the results would lead to a solution of this perplexing condition and at the same time extend our knowledge of the fundamental processes involved in the formation of butter.

HISTORICAL.

A thorough search of the literature fails to bring to light any extensive study of the factors causing that type of difficult churning which occurs near the end of a cow's lactation period. This type of difficult churning is characterized by the tendency to incorporate large quantities of air soon after agitation has been started. This foam is very persistent. The air is not liberated by continued churning as in normal cream.

A great deal of work has been done on the changes which occur in cow's milk as the stage of lactation advances. These studies have included the effect of stage of lactation upon the creaming of milk and also upon the proportion of the fat which may be recovered by churning, in the form of butter. Very little attempt has been made, however, to ascertain by experimental work which of these changes in the milk is to be associated with the problem of difficult churning.

Woll[1] found that with the advance in the stage of lactation the number of fat globules increased and that at the same time they were constantly growing smaller in size; also that the conditions which decreased the size of globules decreased the efficiency of churning.

Van Slyke[2] found that the "general tendency is toward a greater loss of fat in the skim milk as lactation advances------. The general tendency noticed was toward an increase in both temperature and length of time of churning as the period of lactation advances."

Jones[3] obtained results similar to Woll's in regard to the effect of the stage of lactation upon creaming. He studied the effect of size of globules as well as stage of lactation upon the creaming of milk.

Hills[4] divided seven cows into two groups containing three and four cows respectively, according to the relative size of the globules of the milk. He found that the milk containing relatively large globules gave one-third less loss in creaming than the milk containing relatively small globules.

Tests with fresh cows and cows late in lactation gave the results shown in Table 1.

Table 1.-Effect of Stage of Lactation on Creaming of Milk (Hills).

Method of Separation	Stage of Lactation	Fat in Skim milk
		Per cent
Deep Setting	Av. of fresh and late in lactation	0.34
Separator	do.	.24
Deep Setting	Fresh	.13
Separator	do.	.19
Deep Setting	Late in lactation	.55
Separator	do.	.29
Deep Setting*	do.	.29
Separator*	do.	.12
Deep Setting*	Strippers	1.65
Separator*	do.	.29

* From different experiment.

Robertson[5] conducted experiments to see what difference, if any, in the production of butter resulted from churning cream obtained by deep setting from the milk of three groups of cows at different stages of lactation. A summary of Robertson's work is shown in Table 2. It indicates the effect of the stage of lactation upon the completeness of separation and churning and upon the time required for churning.

Table 2.-Effect of Stage of Lactation Upnn Efficiency of Separation and Churning (Robertson).

	Group I*	Group II**	Group III***
Percentage of fat in whole milk	4.1	4.0	3.3
Pounds of cream	24.0	26.0	50.0
Percentage of fat in skim milk	1.38	1.1	0.58
Temperature at churning (^{o}F.)	66.0	64.0	62.0
Time churned in minutes	126.0	95.0	46.0
Speed of churn (R.P.M.)	63.0	65.0	66.0
Percentage of fat in buttermilk	0.33	0.27	0.23
Percentage of fat unrecovered	29.83	23.10	15.24

*7 cows 8 to 11 months in milk .
** 9 cows 5 to 7 months in milk.
*** 10 cows 1 to 3 months in milk.

This table shows that as the stage of lactation advanced the loss of fat in the skim milk and buttermilk increased. The time and temperature of churning increased with the advance in lactation.

Farenbach[6] studied the effect of the size of the fat globules on the rapidity and completeness of churning. He made eight series of experiments with sweet cream and six with ripened cream. The milk of two cows, distinguished by the size of fat globules, was mixed and from this mixture cream with large and with small globules was obtained. The two creams were alike in fat content and other respects except the size of globules. The average results with the sweet and ripened cream are shown in Table 3.

Table 3.-Effect of Size of Globules on Churning (Farenbach).

	No. of globules in 0.000,001 c.c. of fat	Time required for churning	Degree of Churnability
		Min.	Per cent
Sweet cream			
(a) large globules	30.03	46	95.12
(b) small globules	104.61	54	91.61
Ripened cream			
(a) large globules	28.80	42	97.13
(b) small globules	87.30	58	95.00

Rinkle,[7] in studying the factors that affect the ease of churning of cream, found that in every case in which the cream would not churn he had a combination of small fat globules and high protein. In order to show that both the fat and the serum were contributing to the difficulty Rinkle secured two lots of milk. The cream of one (209) was known to churn with great difficulty and the cream of the other (403) was known to churn easily. Each cream was divided into two parts. One part of each was ripened and churned as it was. The second part of cream 209 was mixed with the serum of 403. The second part of cream 403 was mixed with the serum of 209. These were reseparated, ripened and churned. The results obtained are given in the number of revolutions required to churn, in Table 4.

Table 4.-Relative Effect of Fat and Serum on Ease of Churning (Rinkle).

Source of Fat	Source of Serum	Revolutions to Churn	Temperature of Churning °F.
209	209	(9000 (4000 (3000	(64 (72 (78
403	403	1590	64
209	403	4750	64
403	209	5050	64

Practically the same results were secured by a second trial. The results show that when the fat of one is mixed with the serum of the other the number of revolutions necessary to churn was intermediate between the number of revolutions necessary to churn the original creams.

It may be pointed out in criticism of these experiments that the two mixed creams were run through the separator twice while the others were separated only once. If all of the creams had been separated twice easier churning might have been secured in the case of "209 fat in 209 serum." This point of view is supported by the observation that when cream is separated the second time by centrifugal force and the cream remixed with the skim milk small particles of partly churned fat can be found floating in the milk which results.

Robertson[8] found from eight tests that (1) when the milk from one fresh calved cow was added to that from eight cows which had been milking for periods exceeding six and one-half months each, 7.29 per cent more of the butter fat was not recovered than from cows milking less than six and one-half months; (2) the addition of the milk from one fresh calved cow to the milk of eight cows milking for periods exceeding six and one-half months resulted in the recovery of 18.55 per cent more butter fat than from the same cows when the milk of the fresh calved cow was not added; (3) from cows milking for longer than six and one-half months 26.21 per cent more fat was not recovered than from cows milking less than six and one-half months.

Eckles and Shaw[9] in their studies on the influence of stage of lactation upon the composition and properties of milk, found that "On the average the total protein represents 27 per cent of the total solids of milk and at the end of the lactation period constitutes about three per cent more than at the beginning."

"The casein increases slightly more than the total protein in per cent of total solids. On the average this constituent makes up 22.1 per cent of the total solids. The albumen averages 2.3 per cent of the total solids and shows a decline in relation to the total solids at the end of the lactation period."

"The fat represents 31.3 per cent of the total solids and shows a small variation during the lactation period in the way of an increase of about two per cent of the total solids near the end of the lactation period."

"The sugar represents 37 per cent of the total solids on the average. The ratio is quite uniform until about the ninth month when a decline begins which continues to the end of the lactation period."

The ash which shows practically no variation in amount during the lactation period represents on the average 5.3 per cent of the total solids.

In regard to the physical properties Eckles and Shaw state, "It may be observed that the fat globules are especially large shortly after the beginning of the lactation period--------. It will be seen that in every case the cows began their lactation periods with fat globules in relative size about twice the average for the milking period. The relative size declines sharply during the first six weeks, remains fairly constant for five or six months after which the decline is much more rapid to the end of the lactation period."

"On the whole the melting point seems to be little affected by the period of lactation when the feed is kept uniform, except at the extreme end, when abnormally high results may be expected." It is shown by the table of melting points in this bulletin that the melting point of the fat of three of the four cows which showed difficult churning was abnormally high.

The investigations which have been reviewed in the foregoing paragraphs reveal only three prominent changes which may be associated with difficult churning. These are, a decrease in the relative size of the fat globules, an absolute and also a relative increase in the protein portion of the total solids, and a rise in the melting point of the fat, which may be, at times, very great.

EXPERIMENTAL.

I. Control and Measurement of Factors Affecting Churning.

An attempt was made to control or measure all factors which at the time were thought to effect the ease of churning. Those factors which entered into the greater part of the experimental work were

1. Size, type and speed of the churns.
2. Temperature of churning.
3. Percentage of fat in the cream.
4. Methods of ripening cream, and its acidity.
5. Composition of the cream.
6. Size of the fat globules.
7. Viscosity of the cream.
8. Stage of lactation of the cow.

1. Size, type, and speed of churns.-The churns used in this experiment were standard two quart "Dazey" churns which were purchased at a local hardware store. Three of these were mounted upon a single shaft which

was belted to an electric motor. It was possible by this arrangement to conduct three churnings at the same time and at the same speed. The speed selected was 90 revolutions of the shaft per minute which gave 315 revolutions of the paddle per minute. A uniform speed was also obtained throughout all the experimental work. Each churn could be stopped by turning a set crew which fastened it to the shaft. It was necessary to select a point at which the churning would be considered complete. The point selected was that at which the butter formed a mass so firm that it stopped the paddle. This endpoint was found to give uniform results at any given temperature. The general arrangement of the churns with reference to the motor is shown in Plate I. Plate II shows the details of the three churns mounted upon a single shaft.

Two trials were conducted to determine whether or not duplicate results could be secured in the three churns. The results are found in Table 5.

Mixed cream from country patrons was used. It was ripened over night with commercial starter. This cream was used in Trial I. Equal amounts of the same cream were used in Trial II but it differed from

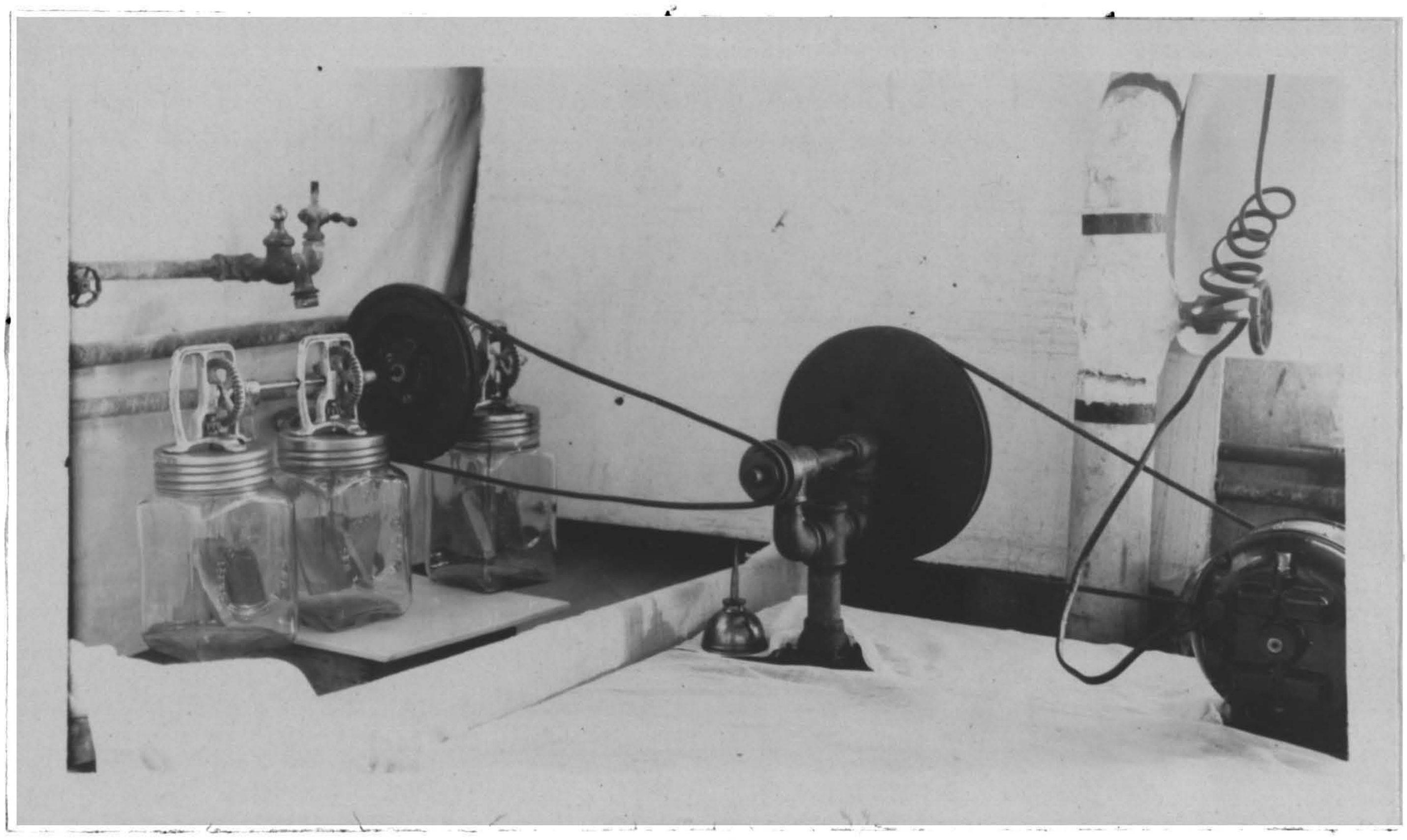

PLATE I

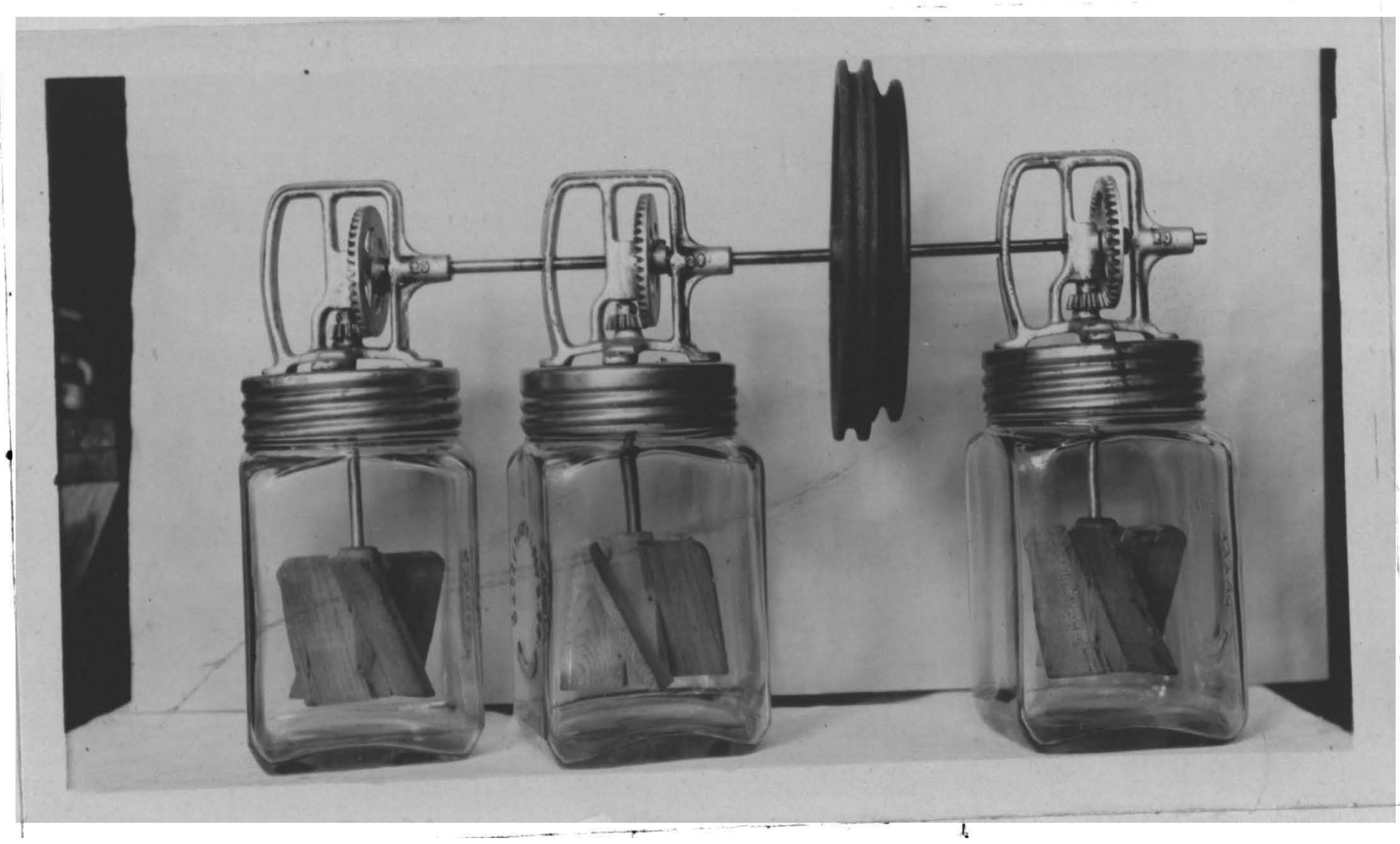

PLATE II

Trial I in that 2.39 grams lactic acid were added to each churning. This amount was found by calculation to double the acidity of the cream in Trial I.

Table 5.-Influence of the Churn Upon the Uniformity of the Time of Churning.

Trial: No.	Vol. of Cream	Acidity of Cream	Temp. of Cream	Time of Churning		
				Churn I	Churn II	Churn III
	c.c.	per cent	°F.	min.	min.	min.
I	800	0.3	63-66	90	80	88
II	800	.6	62-64	52	46	41

Since the same churn did not always give the most rapid churning it was concluded that the difference in time was due to insufficient mixing of the cream before it was divided and put in the churns.

2. Temperature of Churning.-In order to regulate the temperature of the cream during the churning process the churns were set in a frame in a water bath, the temperature of which could be controlled by steam and cold water. Butter churned at 64°F. was slightly too soft to give a sharp endpoint. In small churns 60°F. makes the cream churn quite slowly.

62^{o}F. gave the desired result so was used throughout the experimental work. In all of the experimental work proper the cream was brought gradually to 62^{o}F. and held for two hours in order to bring the temperature of the fat to that of the serum.

3. Percentage of fat in the cream.- Rinkle[10] found that cream of a high percentage of fat churns easier than cream of a low percentage of fat unless the cream is so high in fat that, due to its viscosity, it sticks to the sides of the churn. He found that 25 per cent of fat in cream gave better results than either 20 per cent or 30 per cent.

Preliminary investigations with 2 quart Dazey churns showed that 25 per cent cream gave good results therefore it was used where ever possible. The exact percentage of fat in the cream used is indicated in all the tables.

4. Methods and extent of ripening.- The cream for the several tests was ripened at from 70^{o} to 80^{o}F. until it became clabbered. This method was followed so as to make conditions conform as nearly as possible to conditions on the farm. This procedure gave an acidity often exceeding one per cent. It has been assumed in drawing conclusions that, other conditions.

being equal, the higher the acidity of the cream the more quickly it will churn.

Excepting the series of experiments tabulated in Tables 11 and 12 the acidities represent only titration values. Five grams of cream were weighed into 15 grams of neutralized water containing 3 drops of phenolphthalein. This was titrated with N/10 NaOH and the acidity calculated from the amount of alkali used. This is not the true acidity as will be explained later.

5. Composition of the cream.-The cream in all of these experiments except those in which some other source is mentioned was separated from the milk of a mixed herd of Holstein, Ayrshire, Shorthorn, and Jersey cows of the University of Missouri. Since the variation in composition of mixed milk from a large herd from day to day is small the percentage of the various solids not fat in the cream has been considered constant for a given per cent of fat.

6. Size of the fat globules.-In the review of literature considerable space has been devoted to the effect of the size of the fat globules upon the churnability of the cream. In order to control this factor it was necessary to obtain the large and small globules from the same milk. This was done by a

special method of separation that resulted in the smaller globules being obtained in one cream and the larger ones in another. It was found by trial that this could be accomplished in the following manner which is a modification of the method used by Shaw and Eckles.[11]

Mixed milk of the University herd was heated to 85^{o}F. and run through the Melotte separator so as to leave about .7 per cent of fat in the skim milk. To do this the crank was turned 27 revolutions per minute. Sixty revolutions per minute is normal speed for this machine. The cream slots were almost completely closed. The skim milk was then heated to 95^{o}F. and separated in the Iowa machine at normal speed with the milk screws set so as to deliver cream containing about 30 per cent fat. This was the small globule cream.

The cream from the Melotte was then mixed with the skim milk from the Iowa and reseparated at normal speed. This cream contained almost all of the large globules and many of the small ones. This was the large globule cream.

Samples were taken of these creams and the size of the fat globules in them determined. This was done by the Babcock method as used by Shaw and Eckles[11] and described by them as follows:

"The milk is diluted with distilled water to fifty times, or cream to one hundred times, its volume." The writer obtained more uniform results by diluting cream with water warmed to 35° or 40°C.

"Fine capillary tubes are drawn out from the larger tubes, care being taken that the resulting tubes are round in cross section. They are then broken into pieces 2 to 3 centimeters in length and by means of forceps each tube is dipped into the diluted milk or cream. The tube fills instantly by capillary attraction. The two ends are sealed by vaseline. Three such tubes are used for each sample and these are laid parallel on a slide. A drop of glycerin is placed over the tubes and a cover glass applied. The slide thus prepared is allowed to remain on a leveled slab for half an hour. At the end of thirty minutes the slide is placed under a microscope supplied with an occular micrometer and a mechanical stage. The number of globules in 50 divisions of the micrometer scale is counted in three places in each tube, the internal diameter at each place of counting being first determined by throwing the ocular micrometer scale across the tube at right angles and carefully counting the divisions within the two walls. The total number of globules in 50 divisions is recorded, as well as the number under one division in diameter; also the number between one and two divisions in diameter and the number having a diameter greater than two divisions. A 1-inch ocular and a one-sixth inch objective are employed and with this combination the value of each division on the ocular micrometer with the division used is 0.00258 millimeter. In this way nine determinations are made for each sample. In order to compare results the number of globules which would be found in a standard tube 100 divisions in diameter and 50 divisions in height is calculated from the number observed in each tube and the average taken. In making this calculation the formula $\frac{10,000\ n}{d^2}$ is used. With the ocular used in this work the standard tube would have a volume of 0.006744 cubic millimeter and if the milk is diluted to 50 times its volume, 0.006744 divided by 500 or 0.00013488 cubic millimeter would be the volume of the original milk contained in the standard tube. To find the number of globules present in 0.0001 cubic millimeter it is only necessary to multiply the number in the standard tube by the factor 0.7414 obtained by dividing 0.0001 by 0.00013488.

The relative size is found by dividing the percentage of fat by the number of globules in 0.0001 cubic millimeter of the milk or cream. To avoid fractions the figure obtained is multiplied by 10,000."

In some instances counts were made upon both the cream and the milk from which it was taken.

7. Viscosity of the cream.-After a part of the experimental work had been done it was thought advisable to measure the viscosity of the cream. The viscosity was measured by means of a viscosity pipette, which was made in the following manner:

A 20 c.c. pipette with the lower stem removed was sealed to a one c.c. pipette where the tip of the small pipette had been removed. The tube through which the cream flowed and which was formed from the stem of the 1 c.c. pipette had a diameter of approximately 2 millimeters. The viscosimeter thus formed was suspended in a water bath as is shown in Plate III. The principle of this viscosimeter is the same as that employed in Oswald's apparatus.

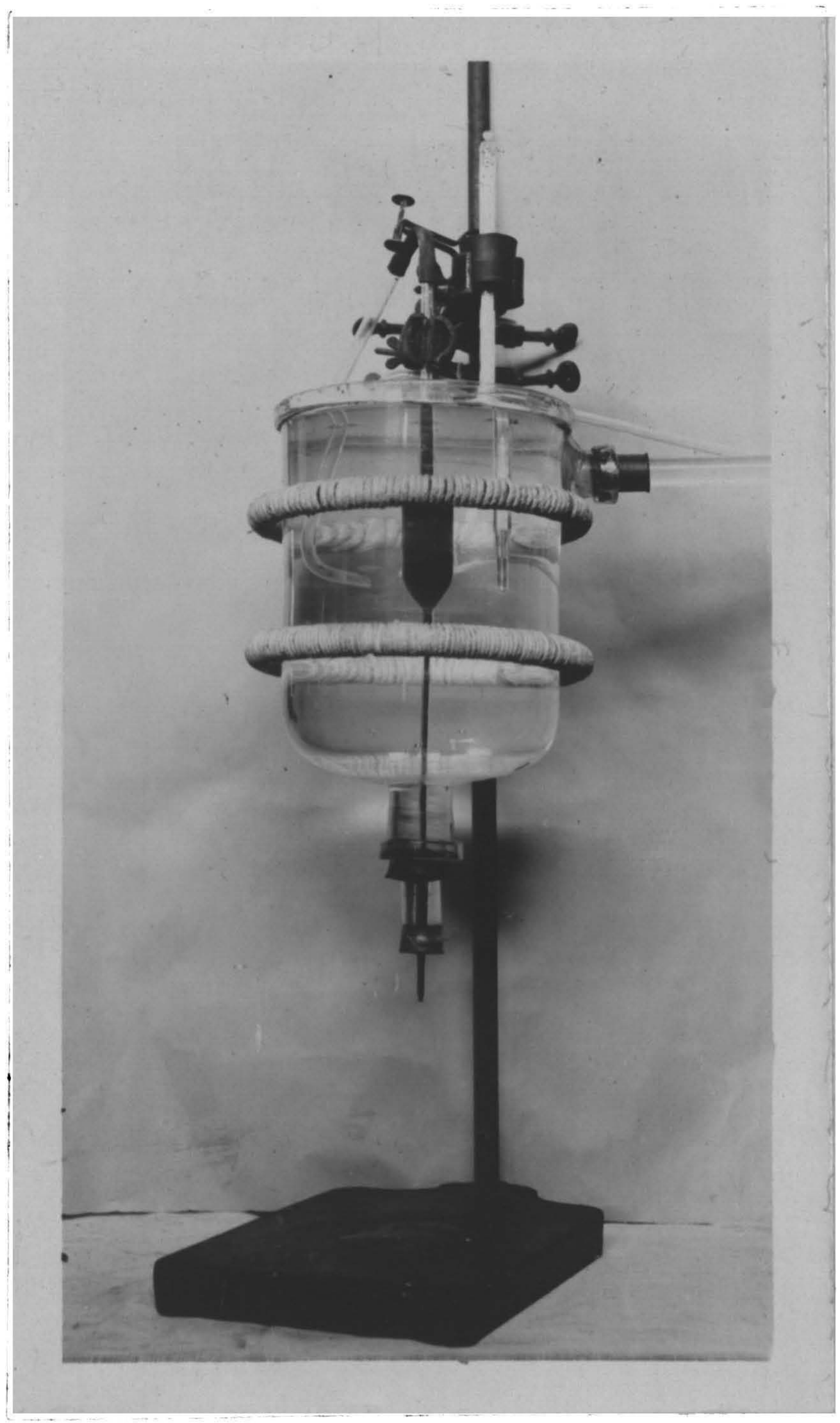

PLATE III.

The principle briefly stated is this; the more viscous the liquid, the more resistance it will meet in passing through a capillary tube and as a result the more slowly it will flow.

In order to measure the viscosity of any cream a sample was brought to approximately churning temperature which was 62°F. All readings were taken at this temperature. The bath was brought to exactly 62°F. The pipette was then drawn full of cream to the mark above the bulb and held there by means of a pinchcock on the rubber tubing. This was then let stand for a few minutes to let the cream come to the temperature of the bath. By the use of a stopwatch the time was taken that was required for the surface of the cream to pass from the mark above the bulb to a mark one inch below it.

Distilled water at 62°F. took 10 seconds to pass from the first mark to the second. The value taken for the viscosity of any given sample of cream was the ratio between the time required for the cream to flow through the tube and that required for distilled water. For example, if the time required for a sample of cream was 22.6 seconds the viscosity of the cream would be 22.6 divided by 10 or 2.26. This method of determining viscosity in cream indicates the true viscosity only to a point at which the acidity developed precipitates the casein. After this a sharp rise occurs in the time required due to the mechanical obstruction of the large coagulated casein particles.

8. Stage of Lactation of the Cow.-Except in the three experiments in which stage of lactation is specifically stated as being a factor, the effect was eliminated by the use of mixed milk from the University herd.

II. Attempts to Produce Difficult Churning.

As was previously stated, the literature cited points to the small size of the fat globules, the high protein content of the cream and the high melting point of the fat as the probable causes of the difficult churning of cream.

If difficult churning is caused by the small size of the fat globules, or the high protein content, or by a combination of both it should be possible to produce difficult churning in the laboratory from normal cream. Attempts to do this constituted the first part of the investigation.

1. By decreasing the relative size of the fat globules.- The first factor studied was the relative size of the fat globules. Milk was separated by the method which has been previously described so as to get creams containing relatively large and relatively small globules respectively. These creams were ripened and churned under as nearly identical conditions as possible. The results of several experiments of this kind are tabulated in Tables 6 and 7. Although the results are not entirely uniform, due probably, in part, to slightly varying conditions from time to time, the general effect of the difference in the size of fat globules is well shown in the averages of the respective tables.

Table 6.-Results of Churnings Having

Low Protein and Large Globules.

No. of Churning	Protein in Cream	Acid in Cream	Relative size of Globules	Time of Churning
	per cent	per cent		Min.
1	3.0	1.06	286	10
2	3.0	.66	255	60
3	3.0	.64	255	90
4	3.0	.60	255	15
5	3.0	.66	255	20
6	3.0	1.04	914	25
7	3.0	.69	914	68
8	3.0	.81	914	550
Average without 8	3.0	.76	448	47

Table 7.-Results of Churnings Having

Low Protein and Small Globules.

No. of Churning	Protein in Cream	Acid in Cream	Relative size of Globules	Time of Churning
	per cent	per cent		Min.
1	2.6*	0.60	105	76
2	3.0	.76	106	95
3	2.6*	.57	105	96
4	3.0	.67	small	203
5	2.6*	.52	105	35
6	2.6*	.60	105	50
7	3.0	--	111	545
Average without 4 and 7	2.68	.61	105	70

*By analysis.

The comparison of the averages of these tables shows that a longer time is required to churn small globule cream than large globule cream. The striking fact, however, is that none of the small globule creams shown in Table 7 except churnings 4 and 7 churned long enough to be considered very difficult churning. Certainly none of the samples in either table except Churning 8 in Table 6 and Churnings 4 and 7 in Table 7 had any of the characteristics of that kind of difficult churning which was the subject of this investigation. It is evident that some factor or factors other than size of fat globules also entered into the cases which, in a measure, resembled difficult churning. These factors will be discussed later.

2. By decreasing the relative size of the fat globules and increasing the concentration of the protein.- (a) With protein from Pasteur-Chamberland filters.- The next series of churnings was undertaken with the view of producing difficult churning artificially by increasing the concentration of the protein in the cream in addition to decreasing the size of the fat globules. Churnings carried out at the same time with cream containing high protein concentra-

tion and large globules were designed to show the effect of protein concentration alone.

Cream containing 25 per cent of fat normally contains about 3 per cent of protein. In order to increase this to a figure sufficiently high to affect churning, the data secured by Eckles and Shaw,[9] to which reference has already been made, were studied to ascertain the maximum protein concentrations of the milk of those cases of difficult churning which they report. This was found to be 5.5 per cent protein in the whole milk which would give a cream containing about 4.4 per cent protein.

It should be pointed out, in fact, that many of the cases of difficult churning reported by these investigators showed protein concentrations in the whole milk of less than 4 per cent. In this work, therefore, 5 per cent protein in the cream was considered sufficiently extreme if high protein concentration is an important factor in causing difficult churning.

In order to standardize cream to 5 per cent protein it was necessary to have some form of solid or semi-solid milk proteins, readily soluble in milk and which had not been so treated as to change the character or properties of any of its constituents.

In the first series of tests the protein was secured by filtering skim milk under pressure through Pasteur-Chamberland filters using the Briggs[12] filtering apparatus. This process is exceedingly slow, it requiring from 10 to 12 days for one liter of milk to pass thru an 8 inch filter having a diameter of 1 inch using continuous pressure of three to four atmospheres. Studies made by Van Slyke and Bosworth[13] and by Palmer and Scott[14] show that the residue obtained on these filters consists of all of the casein as originally present in the milk, all the di-cal-phosphate of the milk, and 90 per cent of the albumin and globulin. This residue is, of course, contaminated with a little lactose and some non-nitrogenous matter. It is, however, readily soluble in milk or water which is the important point in connection with these studies.

Two Briggs filters each with a capacity of approximately one liter and fitted with Pasteur-Chamberland tubes, were set up and connected in parallel. A T-tube connected the filters to an oxygen tank which was used as a source of pressure.

A liter of skim milk, preserved with formaldehyde, 1 : 2000, was placed in each filter and the pressure maintained at three to four atmospheres until practically all the serum had passed through the filter. This required about two weeks.

The Pasteur-Chamberland tubes were then removed and the jelly-like material scraped off. This was dissolved in warm skim milk, practically all of the material going into solution with the aid of some maceration in a mortar. The amount of skim milk used in dissolving these materials was governed by the amount of skim milk required to standardize these samples of cream to 25 per cent of fat and to increase the protein concentration to the desired figure.

The samples of cream used in this test represented both small and large globules. The method used in securing cream with relatively large and relatively small fat globules by centrifugal separation was that which has already been described.

Four samples of cream were standardized in the following manner.

Sample 1.-Low Protein Small Globules

Cream, 47 per cent fat,small globules	532 gm.
Skim milk	418 "
Starter	50 "
	1000 gm.

Sample 2.-High Protein Small Globules

Cream, 47 per cent fat,small globules	532 gm.
Solids from 1 liter of skim milk and skim milk	418 "
Starter	50 "
	1000 gm.

Sample 3.-Low Protein Large Globules

Cream, 64 per cent fat, large globule	390.5 gm.
Skim milk	559.5 "
Starter	50 "
	1000 gm.

Sample 4.-High Protein Large Globules

Cream, 64 per cent fat, large globule	390.5 gm.
Solids from 1 liter of skim milk and skim milk	559.5 "
Starter	50
	1000 gm.

The results of this experiment are shown in Table 8.

Table 8.-Effect of High Protein From
Pasteur-Chamberland Filter and
Small Globules on Churning.

No. of Cream	Weight of Cream	Protein in Cream	Acid in Cream	Relative size of Globules	Time of Churning
	gms.	per cent	per cent		Min.
1	1000	3.0	0.67	small	203
2	1000	6.9*	1.12	small	155
3	1000	3.2*	.69	large	68
4	1000	5.4*	1.09	large	56

*By Analysis.

These results show that in each case the small globule cream was more difficult to churn than the large globule cream. They also show that the low protein creams required a longer time for churning than did the high protein creams containing the same size globules. The higher percentage of acid produced in the cream containing the higher percentage of solids may account in some measure for the greater speed with which the higher protein creams churned.

(b) With skim milk powder.-In the second and more extensive series of tests with high protein creams, the protein was added in the form of skim milk powder.

Skim milk powder was chosen for this work after a preliminary study of its aqueous solution indicated its identity with ordinary skim milk. This study showed that the casein could be readily precipitated from such a solution with dilute acetic acid. The clear filtrate which was secured gave a heavy coagulation on boiling, at the same time leaving a portion of soluble protein not coagulable but readily precipitated by Almen's tannic acid reagent. These results are similar in every respect with similar tests performed with normal skim milk. It may be stated further that solutions of the skim milk powder soured on standing and eventually formed the usual sour milk curd as in the case of normal skim milk.

The use of skim milk powder for the increase of the protein concentration of the creams thus presented so many obvious advantages over the Pasteur-Chamberland filtration that it was used throughout the remainder of the experimental work.

The method used for calculating the amount of skim milk powder to be used can be explained best by an example.

Given, cream containing 40 per cent fat and skim milk powder to make 800 grams of cream testing

25 per cent fat and 5 per cent protein.

First, the amount of fat required for 800 grams of 25 per cent cream is found. This is (800 x 0.25) 200 grams of fat. To get 200 grams of fat from 40 per cent cream, (200 ÷ 0.40) 500 grams of cream would be necessary.

Next, the grams of protein in the 500 grams of 40 per cent cream is calculated from data showing the percentage of protein in 100 parts of water in cream. Such data in tabular form are found in Richmond's "Dairy Chemistry.[14] This table shows that cream contains 4.05 grams of protein for each 100 grams of water. Forty per cent cream contains (100 - 40) 60 per cent water (serum). Five hundred grams of 40 per cent cream contains (500 x 0.60 x 4.05) 12.15 grams of protein.

Eight hundred grams of cream of 5 per cent protein would contain (800 x 0.05) 40 grams of protein. If the 500 grams of 40 per cent cream contains 12.15 grams of protein then (40 - 12.15) 27.85 grams of protein must be added to make 800 grams of cream containing 5 per cent protein.

The skim milk powder was assumed to contain

34 per cent* of protein. In order to add 27.85 grams of protein (27.85 ÷ 0.34) 81.9 grams of skim milk powder must be used. It was shown above that 300 grams of skim milk are needed to standardize 500 grams of 40 per cent cream to 25 per cent fat. The 81.9 grams of skim milk powder found by calculation is made up to 300 grams with distilled water** and this solution added to the 500 grams of 40 per cent cream. The result is 800 grams of cream testing 25 per cent fat and 5 per cent protein.

Table 9 gives the percentage of protein calculated by the above method and also the actual percentage of protein as determined by the Kjeldahl-Gunning method for several samples of cream. In each case the error introduced by calculation is seen to be less than 0.5 per cent which is within the limits of accuracy demanded by these churning experiments.

*Average of several analyses of skim milk powder made for the Dairy Department by the Department of Agricultural Chemistry.

**If skim milk was used as the solvent, allowance was made for the protein which it contained.

It is indicated in the tables throughout the text which of the protein percentages given are based upon actual determinations. The others are based on calculations.

Table 9.- Comparison of Percentage of Protein in Cream by Calculation and by Kjeldahl-Gunning Analysis.

No. of Sample	By Calculation	By Analysis
	per cent	per cent
1	3.0	2.88
2	3.0	3.15
3	3.0	2.61
4	3.0	2.84
5	3.0	2.97
6	4.1	4.13
7	4.1	4.14
8	4.1	3.72
9	5.0	4.58

Seventeen churnings were made in connection with the high protein studies; nine of them were made with large globule cream and eight with small globule cream.

The data secured from these churnings are given in Tables 10 and 11.

Table 10.- Results of Churning High Protein Large Globule Cream.

No. of Churning	Protein in Cream	Acid in Cream	Relative size of Globules	Time of Churning
	per cent	per cent		Min.
1	3.7*	0.75	194	25
2	5.0	1.01	286	70
3	3.7*	.77	194	7
4	3.7*	.72	194	30
5	5.0	1.09	large	56
6	5.0	.67	914	95
7	3.7*	.82	194	38
8	5.0	1.56	914	295
9	5.0	.85	194	30
Average without 8	4.7	0.84	334	44

*By analysis.

Table 10 shows considerable irregularity in the time of churning in the various samples. Churning 8 stands out particularly because of the much longer time which it required for the completion of churning. It seems evident that some factor other than high protein concentration entered into these results which was especially operative in the case of Churning 8.

Table 11.- Results of Churning High Protein Small Globule Cream.

No. of Churning	Protein in Cream	Acid in Cream	Relative size of Globules	Time of Churning
	per cent	per cent		Min.
1	4.1*	0.84	126	47
2	5.0	1.05	106	173
3	4.1*	.85	126	30
4	4.1*	.74	126	75
5	5.0	.41	small	265
6	5.0	1.12	small	155
7	4.1*	.87	126	68
8	4.1*	.90	126	37
Average	4.43	0.84	117	81

*By Analysis.

Table 11 shows even greater irregularity in the time of churning the various samples than Table 10.

These variable results cannot be explained either by the high protein concentration or the low relative size of the fat globules. The data again point to an additional factor or factors which not only caused the irregularity in the results but which are the fundamental factors causing the difficult churning typical of advanced lactation. It is true that a comparison of the averages of Tables 10 and 11 shows that of the creams containing a high concentration of protein those with large fat globules churned more easily than those with small fat globules. It should also be noted, however, that only four of the entire series of eighteen samples containing a high protein percentage required over two hours to churn, and that all the remaining samples churned comparatively easily. Furthermore, none of the four samples which required more than two hours to churn showed the usual characteristics of typical difficult churning and the one which required the longest time to churn (No. 8, Table 10) contained neither a high concentration of protein nor small globules.

A study of the data so far presented points conclusively to the fact that the two factors which were regarded of fundamental importance in causing the difficult churning characteristic of advanced lactation, namely small relative size of fat globules and increased protein concentration are of secondary importance only. It is obvious that a combination of high protein and small fat globules would contribute materially in bringing about an increase in the normal churning time of cream. It is also obvious, however, that the primary factors in causing typical difficult churning rest upon changes more fundamental than are revealed by a mere change in the protein concentration or in the relative size of the fat globules. Marked lack of uniformity characterized the attempts to combine these two factors in such a way as to artificially bring about difficult churning.

In the course of the experimental work several cases of difficult churning occured which imitated more closely than any of the others the kind of difficult churning which it was the purpose of this investigation to study. The data relative to these cases are brought together in Table 12. The surprising fact shown by these data is that the most difficult case of churning encountered, namely Sample 8, contained neither relatively small globules nor a high concentration of protein, while two of the easiest samples to churn of this lot, namely Samples 2 and 3, combined both of these factors.

Table 12.- Data on Samples Churning More Than Two Hours.

No. of Churning	Protein in Cream	Acid in Cream	Relative size of Globules	Time of Churning
	per cent	per cent		Min.
1	3.0		26	146
2	5.0	1.12	small	155
3	5.0	1.05	106	173
4	3.0	.67	small	203
5	5.0	.41	small	265
6	5.0	1.56	large	295
7	3.0		111	545
8	3.0	.81	914	550

In studying the data given in Table 12 with the view of finding an explanation of the cause of the difficult churning, particularly of Sample 8, which contained neither of the factors which had hitherto been considered as fundamental, it was found that a comparison of the time of churning of another sample of this same cream showed a close relation between the time of churning and the age of the cream when ripened. These data are shown in Table 13.

Table 13.-Effect of Age of Cream at Ripening on Churning.

No. of Churning	Protein in Cream	Acid in Cream	Age at Ripening	Time of Churning
1	3.0	1.04	2	25
2	3.0	.81	14	550

A series of experiments was next conducted to obtain additional data on the relation between the age of the cream and the time of churning.

3.-By decreasing the relative size of globules, increasing the protein concentration and increasing the age at ripening.- Large, medium, and small globules creams were secured for the following experiments by separating milk from cows of different breeds. Small globule cream was secured from Holstein milk, large globule cream from Jersey milk, and medium or mixed globule cream from approximately equal parts of Jersey, Holstein, and Shorthorn milk. The creams were all cooled in ice water as soon as separated and were standardized to 25 per cent fat.

Twelve 800 cc. Erlenmeyer flasks were cleaned and dried. Four were filled with Holstein cream, four with mixed and four with Jersey cream. The initial acidities and viscosities were taken.

As was previously stated, typical cases of difficult churning are characterized by excessive whipping. A number of investigators who have studied the factors causing cream to whip have found that by increasing the viscosity of cream the tendency to whip is greatly increased. It therefore seemed advisable to measure the viscosity of the cream in the remainder of the samples churned in order to have a basis for comparison in case the property of whipping should appear again in the course of the experimental work.

The acidities taken in this work were the true and not the apparent acidities. Bosworth and Van Slyke[15] have found that the true acidity of fresh milk toward phenolphthalein can be measured only after the neutral calcium phosphates have been removed. For this purpose they added a small amount of a saturated solution of neutral potassium oxalate before the titrations of the initial acidities were made. This method was followed in the remainder of the experimental work. The titrations made after the calcium salts were precipitated represented the true initial acidity plus the lactic acid actually developed in the cream. It was accordingly possible to calculate readily the actual amount of acid developed.

The following technique was used in taking the acidities of the creams of this series. Twenty-five grams of cream was weighed into a 200 cc. flask. To this 75 cc. of recently boiled distilled water were added. One cubic centimeter of a saturated solution of neutral potassium oxalate and 5 drops of phenolphthalein were used for each 25 cc. of cream. Titrations were then made with tenth normal sodium hydroxide solution.

The viscosities were taken just before ripening each set of churnings. No viscosities were were taken after the cream became so sour that it would not flow freely from the viscosimeter. Acidities were taken each time before and after ripening, in addition to the initial acidities.

After the creams had ripened enough to clabber they were brought to 62^{o}F., held for two hours and churned. By the fifteenth day the creams still in the cooler had developed sufficient acidity for churning and were not ripened further.

Another set of the same creams was made up in the same way except that before the cream was put into the flasks it was standardized to approximately 4.1 per cent protein with skim milk powder. Five flasks of each cream were put in the cooler. The same data were taken on these as on the low protein creams.

The composition of the two sets of cream is given in Table 14. Five per cent of starter was added to each at the time they were set to ripen.

Table 14.-Composition of Creams in Experiments on Effect of Age at Ripening Churning.

Source of Cream	Protein* in Cream	Relative size of Globules	Fat in Cream
	per cent		per cent
Low Protein			
Holstein	2.6	105	23.9
Mixed	2.8	168	23.9
Jersey	3.0	255	23.9
High Protein			
Holstein	4.1	126	23.9
Mixed	4.1	145	23.9
Jersey	3.7	194	23.9

*By Analysis.

The results of these experiments are shown in Table 15 and Table 16.

Table 15.-Effect of Age of Cream at Ripening on Time of Churning (Low Protein).

Kind of Cream	Acid before ripening	Acid after ripening	Vis-cosity	Age at ripening	Time of Churning
	per cent	per cent		days	Min.
Holstein	0.065	0.599	2.23	1	76
Mixed	.050	.558	2.12	1	40
Jersey	.061	.664	2.12	1	60
Holstein	.071	.572	2.15	3	96
Mixed	.066	.616	2.10	3	45
Jersey	.077	.644	2.00	3	90
Holstein	.457	.517		6	35
Mixed	.552	.572		6	17
Jersey	.563	.599		6	15
Holstein	.601			15	50
Mixed	.616			15	10
Jersey	.662			15	20

Table 16.-Effect of Age of Cream at Ripening on Time of Churning (High Protein).

Kind of Cream	Acid before ripening	Acid after ripening	Vis-cosity	Age at ripening	Time of Churning
	per cent	per cent		days	Min.
Holstein	0.091	0.835	2.80	1	47
Mixed	.083	.810	2.86	1	35
Jersey	.070	.749	2.40	1	25
Holstein	.096	.853	2.95	3	30
Mixed	.092	.860	3.08	3	10
Jersey	.081	.778	2.46	3	7
Holstein	.709	.742	3.25	6	75
Mixed	.715	.749	3.39	6	55
Jersey	.679	.720		6	30
Holstein	.864	.866		10	68
Mixed	.891	.878		10	43
Jersey	.818	.824		10	38
Holstein	.900			19	37
Mixed	.929			19	27
Jersey	.853			19	30

The samples, both high and low protein, developed gassy fermentations after the fifth or sixth day. The low protein ones developed more than the others. This unexpected fermentation may have affected the results to a considerable extent.

It will be seen from an examination of Table 15 that there was a slight drop in the viscosities from the first to the third day. There was a direct relation between the drop in viscosity and the increase in the time of churning. On the other hand, in the high protein series, Table 16, the reverse is true, namely, there was a direct relation between the increase in the viscosity and the decrease in the time of churning from the first to the third day. These results were exactly contrary to what was expected.

The increase in the age of the cream at ripening did not increase the time required for churning. The rapid development of acid is offered as an explanation for the failure for the creams to become more difficult to churn. This is true for both the high and the low protein creams. The theoretical aspects of the effect of the slow and rapid development of acid will be discussed later.

III. Study of Typical Cases of Difficult Churning.

1. Comparison of Effects of adding normal cream and of reseparating the fat from normal skim milk on churning of difficult cream.- In connection with the attempts to produce difficult churning some naturally occuring cases were studied. One of these cases was reported by E. F. Sippel of Clifton Hill, Mo., who was milking only one cow, a Jersey six years old. The writer went to Clifton Hill to obtain samples of the cream and to make a study of the circumstances under which this trouble developed.

The cow had calved in June, six months previously and was due to freshen in four months. She was being fed cane and clover hay and corn for grain. The trouble in churning, accompanied by a bitter taste and rancid odor to the milk and cream began about the time grass failed, near October 1. The cream gradually became harder to churn until finally butter refused to come even after being churned for several hours on two successive days.

A study was made of the method of handling the milk and cream. As soon as the milk was drawn and strained it was set in stone jars in the milk house. At the time of the year in which the investigation was made the temperature of the milk house remained at from 40 to 55 ^{o}F. The milk seemed normal when drawn but after standing 12 hours a bitter taste and a rancid odor was found to have developed in both the milk and the cream. The cream was placed in a stone jar and set on a shelf near the stove. The cream was well clabbered by the time enough had been collected to churn, which usually required about three or four days.

The churn used was a cylindrical can having inside flanges. The dasher was of the type of those used in the experimental churns used in this investigation.

As samples the writer obtained one-half gallon of sour cream, three-fourths of a quart of sweet cream and one-fourth quart of fresh whole milk. These samples were taken to the laboratory and prepared for the churning tests in the following manner.

Two-thirds of the sour cream was put in Churn 1 to be used as a check sample. The other one-third of the sour cream was mixed with an equal amount of normal cream of the same per cent of fat from the milk of the University herd. This mixed cream formed Churning 2. The sweet cream was standardized to 4 per cent milk with skim milk from the University herd and reseparated in a centrifugal separator. It was then standardized to the same per cent of fat as the others. This sample was called Churning 3.

The creams were ripened the same length of time and churned. The data from these churnings are shown in Table 17.

Table 17.-Effect of Adding Normal Cream and of Reseparating from Normal Skim milk on Difficult Churning

No. of Churning	Fat in Cream	Acid in Cream	Relative size of Globules	Time of Churning
	per cent	per cent		Min.
1	22	1.01	90	346
2	22	.53		86
3	22	.66	90	146

Although no cause other than stage of lactation was found for the difficult churning, the methods of treating the cream showed how the difficulty in churning may be greatly lessened.

Churning 1, the check sample, was in every way a typical case of difficult churning. At 62^{o}F. it churned for five hours and four minutes without giving any evidence of butter being produced. It was thought that the union of the fat globules might be facilitated by the addition of some butter to the cream if the butter was in a more or less liquid condition.* For this purpose 100 c.c. of melted butter was added while the churn was running. It produced no immediate effect but soon the foam started to break down and butter came in 42 minutes after the melted butter was added. Other attempts to aid difficult churning by the addition of melted butter will be described later.

Churning 2 shows that the addition of normal cream to difficult churning materially reduces the churning time of the latter. The reason for this result has not been worked out but it seems,

*There is an old idea that butter which is hard to churn may be made to come by throwing a hot horseshoe into the churn.

however, to be due to a lessening of the intensity of the factors causing difficult churning rather than to their elimination.

Churning 3 shows that reseparation of the fat from normal skim milk also aids greatly in reducing the time required to churn. This method, however, does not cause as great a reduction in the time of churning as does the method used in Churning 2. It appeared that the addition of melted butter in Churning 1 of Table 17 caused the butter to come. In order to test this further melted butter was added to another sample that showed difficult churning. The sample used was the seventh churning already shown in Table 7. This churning was made soon after the one just described.

After Churning 7 of Table 7 had whipped persistently for several hours and showed no signs of churning, the following small amounts of butter were added, at 35 minute intervals, to the churning cream: 14 cc., 14 cc., 20 cc., 20 cc., and 25 cc. Then after 10 minutes 25 cc. more was added at about 130°F. Each addition of butter raised the temperature about 2°F. but the bath immediately cooled the cream down to 62°F. The butter came in 155 minutes after the last addition of melted butter. The total amount of butter added was 118 cc. The total time of churning was 545 minutes as is shown in Table 6. It was concluded that the addition of melted butter in small amounts would not materially reduce the churning time.

Since the cream in the foregoing test whipped to twice its original volume before any fat was added, it was thought that this might have influenced the results. Another churning of the same cream was therefore prepared, to which 25 cc. of melted butter was added before the whipping commenced. Forty minutes later 40 cc. of butter at 130°F. was added. Even this produced no sign of the completion of churning. The churn was permitted to run for 245 minutes from the time of starting. At this time 1.9 grams of pure lactic acid were added in hopes of breaking the air emulsion. No effect was obtained. The churning was then stopped and the cream heated to 140°F. At this temperature the air came rapidly to the surface and escaped. Large globules of the melted butter could be seen through the sides of the glass churn. It was then cooled and placed in the cooler for 48 hours. It was again churned for 120 minutes with no results except instantaneous whipping. Sixteen grams of butter salt was added. This did not help the butter to come. The cream was again set in the cooler during which time it wheyed off. The air, however, did not leave the curdy mass that was above the whey.

Upon starting the churn again 50 grams of salt were added. This was enough to make the salt content of the cream equal to that of butter. After 2 hours more the temperature was raised to 70°F. Two and one-half hours later the butter came in a very soft condition. It is evident that had the melting point of the fat been at fault in this sample of cream the butter would have come in a firmer condition or would have churned more quickly. The total time of churning was 630 minutes. The fat percentage was 25 at the beginning but was increased by the addition of 65 cc. of melted butter. The acid at the time the butter came was very high, probably nearly 3 per cent.

2. A comparison of effect of gravity and centrifugal separation on the time of separation.-

Difficult churning most frequently occurs where only one cow is milked in which cases the milk is separated by gravity and not by a centrifugal separator. One experiment was run to obtain data on the effect of centrifugal separation on the ease of churning when compared with gravity separation. The milk of Cow 248, a Holstein in the University herd, was used for this test. She had been in milk 14 months and was giving about three pounds of milk daily. Her milk was saved for two and one-half days to get a sample of gravity cream and for the next two days to get the sample of centrifugally separated cream. These were ripened and churned. The acidity of the gravity cream was 1.9 per cent and the fat content 17 per cent. The acidity of the separator cream was 2.1 per cent and the fat content 23 per cent.

These creams were first churned for 135 minutes then set in the cooler for 84 hours during which time the whey separated from the curd. One-half its own weight of cream from a Holstein cow which had been fresh only two weeks was then added to the gravity cream. Fifty minutes later 100 cc. of skim milk from the same fresh cow was added to the centrifugally separated cream. After 70 minutes more the temperature of the creams was raised to 68°F. Butter came in the gravity cream 50 minutes after the temperature was raised. The total time of churning was 305 minutes. Churning was not continued for the separator cream.

The only definite conclusion to be drawn from this trial is that centrifugal separation has very little if any effect upon the ease of churning of difficult churning cream. If the separation should be repeated several times some effect might be shown.

The general conclusions drawn from these studies of typical cases of difficult churning are:-

(1) The addition of melted fat does not appear to aid materially in overcoming difficult churning.

(2) Mixing difficult churning cream with cream from a comparatively fresh cow aids greatly in getting the butter to come. Changing the serum of difficult churning cream by mixing with skim milk from cows not advanced in lactation, and reseparating

aids to a limited extent the churning of difficult churning cream.

(3) No amount of acid up to and perhaps beyond 2 per cent will alone insure easy churning.

THEORETICAL.

In order to get a clear understanding of the factors causing difficult churning it is necessary to understand what takes place during the churning process. No satisfactory explanation of churning has been advanced which is generally accepted. The changes occuring in the cream during the process of churning must, however, be closely related to the physical state of the milk constituents in the cream and also to the physical and physical-chemical properties of these constituents. These properties, as well as the physical-chemical status of the milk constituents are understood much more clearly when viewed in the light of the process by which they secreted in the cow's udder.

1. Theory of Milk Secretion.- Many theories have been put forward to explain the secretion of milk in the mammary gland but probably the most recent and the one which offers a particularly interesting working hypothesis for studying the factors operative in the phenomena of difficult churning has been put

forth by Fischer.[16] According to this authority milk secretion is merely a change in the form of an emulsion. "As histological study shows," Fischer states, "the alveoli of an active mammary gland consist originally of a single layer of nearly cubical gland cells. In the process toward milk production, these cells increase in length, pushing toward the lumen of the alveolus. The portions farthest from the basement membrane to which these cells are attached (farthest, we would prefer to say from the capillaries which supply oxygen to the gland cells) begin to show the appearance of fatty droplets in them. When morphological pathologists describe the process they say that the peripheral portions of the secreting cell under go 'fatty degeneration.' The facts are that the distal portion is swollen often granular ('cloudy swelling') and studded with fatty granules. These peripheral portions of the cell finally swell so much that they'go into solution' and thus form 'milk'. The portion of the cell which remains and is attached to the basement membrane is then again cubical and again begins the series of changes just described.

"Expressed in terms of emulsion chemistry, this set of cellular changes represents the original emulsion of fat in the concentrated hydrophilic colloid (corresponding to the original cubical cell) becoming richer in water. One of the colloids in the cell swells while a second is precipitated. Together, this yields the 'cloudy swelling' which, however, in terms of emulsion chemistry means not only a decrease in the concentration of the hydrophilic colloids of an emulsion but a decrease capacity for holding water on the part of some of them. The tiny fat droplets of the original emulsion therefore tend to run together, to form larger ones which begin to appear in the distal portions of the cell (fatty degeneration). When this coarsened emulsion is still further diluted, when, in other words, the cells swell to the 'solution' point, there results an emulsion of fat droplets in a dilute mixture of proteins (albumins and globulins) to which we are in the habit of attaching the name 'milk'."

Fischer has extended his ideas of the colloidal character of milk constituents to explain the changes which occur when cream is churned into butter. These views will be presented later in connection with others bearing upon the process of butter formation.

2. The state of fat globules in milk.- Since it is the fat in globular form in the cream which is united in churning to form butter it is especially necessary to understand the physical status of these fat globules in the milk and cream, as well as their physical-chemical properties. These questions have been the subject of much discussion during the last seventy-five years. Certain writers believe that there is a definite membrane around the fat globules, in other words that the fat globule is a cell filled with fat. This view, however, is accepted by very few at the present time.

The generally accepted view is that the globule is surrounded by a more or less dense coating of proteins which keeps the globules from uniting. Decidedly different opinions are held by various investigators as to the character and cause of the presence of this coating around the fat globule.

Storch's[17] ideas on the covering of the fat globules are unique in that he is of the opinion that the covering which he terms a "mucoid substance" is an entirely different protein from the protein of the milk serum. He states that he was able to wash fat globules free from all cream serum until the wash water was perfectly clear. From the washed globules Dr. Storch claims to have secured a mucilaginous precipitate which had chemical and physical properties very different from the casein and albumins usually separated from milk.

This substance was insoluble in dilute sodium hydroxide or ammonia water but on standing for five or six weeks in a very dilute solution of ammonia water to which ten per cent of alcohol had been added it became so swollen that it formed an opaque viscous fluid when heated to boiling.

Dr. Storch separated this material and estimated it to be 60 per cent of the total protein of butter. Dr. Storch thinks he was working with the substance which Danilewsky and Radenhausen designated as "the protein of milk globules."

This theory of a special substance other than the commonly known milk proteins seems to be well supported by Storch's work. Recently Osborne and Wakeman[18] have isolated a new protein from milk which has a number of properties closely resembling the substance with which Storch worked.

Ramsden,[19] Babcock,[20] Cooper, Nuttal and Freak,[21] Richmond,[22] and Fischer[23] agree that the covering of the globules in milk consists of casein and albumins although no reports were found indicating any investigation such as Storch made of the composition of the covering. The authors just mentioned give different explanations of the cause and composition of this covering of the fat globule. All of them agree, however, that the covering exists as a concentrated layer of milk solids on the surface of the globule.

Ramsden[19] states that, "The persistence of many emulsions is determined largely among other facts by the presence of solid or highly viscous matter at the interfaces of the two liquids. Direct measurements of the various surface tensions concerned are not available but the close resemblance of the phenomenon to those occuring at a free surface points to the view that the accumulation of solid matter at the interfaces of the above emulsion pairs occurs because the 'surface energy' is thereby diminished." From this he concluded that the proteins of milk collect around the fat globules because the fat has a lower surface tension than the protein solutions.

Babcock[20] shows the resemblance of milk to artificial emulsions. He shows the resemblance in the following ways;

1. The microscopical appearance of the fat globules is identical.

2. Separation of cream on standing is the same in both.

3. Churning produces the same results in both.

4. The action of ether on the fat of milk and artificial emulsions is the same.

Babcock points out that when small amounts of ether are added to an emulsion there is evidence that it goes into combination with the fat. "The slight differences in the effect of ether upon milk and artificial emulsions are differences of degree only." Bechamp found the same to be true but Storch found that the milk globules were not changed by the presence of a small amount of ether. Babcock states that not only do milk and artificial emulsions behave alike in respect to the points mentioned, but the resemblance is equally marked in all others to which his experiments were directed "so that the conclusion is inevitable that milk itself is an emulsion."

Cooper, Nuttall, and Freak[21] state, "In our opinion the divergent results obtained and the views held by the different workers will all be reconciled on the theory that the constituents of the milk serum are absorbed to the surface of the globule." They also state that "a number of artificial emulsions, cyllin in water for example, behave exactly like milk in this respect," that is that ether will not remove the fat until the emulsifying agent is broken down.

Richmond[22] gives this statement in favor of Storch's conclusions: "Butter in which the globules are certainly more naked than in milk, can be prepared containing about 85 to 86 per cent of fat; this is solid because the solid fat globules are in such close proximity; cream on the other hand cannot be prepared with more than about 72 per cent of fat and as this has the same consistency as butter at the same temperature it may be assumed that the globules are in equal close proximity. This would agree with the view that each globule is surrounded by a layer which increased the effective size."

Richmond explains that the difference between the results of Storch and Bechamp in regard to the effect of ether on the fat globules is due to the fact that Bechamp worked with freshly drawn milk in which the fat is liquid while Storch probably worked with fat at a low temperature. Liquid fat will absorb ether but solid fat will not.

Richmond states that the following facts definitely disprove Storch's view of the membrane around the fat globule.

(1) "The ratio of milk sugar and protein in the cream are the same as in separated milk.

(2) "When milk or cream is stained no layer can be detected around the globules until the aqueous portion is washed away. There are, however, many stained particles (probably mucoid protein) quite independent of the fat globules.

(3) "The mucoid protein can only be separated with the fat globules if the density of the serum is increased by the addition of cane sugar till it is greater than the density of the mucoid protein (1.0228); this proves that it is independent of the fat globules."

According to Richmond, "The general conclusion of opinion among chemists who may be considered as authorities on this point is that the fat in milk is not surrounded by a membrane and, therefore, that it is a true emulsion. There is only very little doubt that a layer exists around each fat globule; this is probably formed by an attraction due to the surface energy, a force akin to that which causes the phenomenon known as capillary attraction. Much of the evidence which has been taken as evidence of the presence of a membrane round the globules is only evidence of the presence of a layer of some sort but not necessarily membranous."

Fischer[23] takes the same view of the covering of the fat globules as Richmond in that it consists of particles of the emulsifying agent held to the surface of the globule by surface tension, thus placing it in the category of true emulsions. He was able to make artificial milk by using amphoteric casein, water, and fat and later adding the lactalbumin, lactoglobulin, milk sugar, and salts. He was able to make solutions or emulsions by the use of any hydratable colloid such as soap, acacia, egg white, blood or muscle protein, or many other emulsifying agents. He was able to use as his source of fat any animal or vegetable fat, and these were sometimes replaced with mineral oils.

It seems to be well proved, then, that cream and milk are true emulsions. It sould seem, therefore, that the explanation of changes in this emulsion such as those which are involved in the formation of butter must be sought in a study of emulsions chemistry and its related phenomena.

3. Theories of Churning.- Dr. Storch[17] presents a theory of churning which is in accord with his ideas of the mucilaginous membrane around the fat globules. Storch states that the fat globules are not destroyed in churning but that they may still be seen in the butter if it is spread out in a thin layer. He also has made stained preparations in which he claims to have been able to distinguish the individual globules. This indicates that even in butter there is a something which keeps the globules from uniting just as is the case in cream. He states that the form and the size of the globules have been changed and that a very large number of fluid drops

are to be found among them.

According to Storch the milk globules cannot unite into butter until the slimy layer which surrounds them is partly removed. This layer surrounds the globule completely and it is impossible to remove it entirely by mechanical means. Storch believes that the agitation of churning gradually wears off the outer part of the slime, and when the slime layer is sufficiently thin a large number of globules unite and the butter "comes." The outer part of the slimy layer which is torn loose during churning clumps together between the globules at the moment of formation of the butter and, according to Storch, form the very large number of fluid drops which are very readily seen in butter under the microscope. In addition Storch states that small clumps which form first gradually form larger clumps and also enclose between them cream serum or buttermilk. These latter droplets, according to Storch, form the liquid which is removed by working but the smaller ones cannot be removed. He also believes that these smaller droplets contain the aromatic and flavoring elements of the butter.

Storch advances the hypothesis that just as fat exists in cream and milk in the form of an emulsion the small particles of water exist in the butter in the same state.

Fleishman's[24] view is that the globules in milk are in a superfused condition and that churning is a process of solidification. Richmond[25] holds that, "This view is untenable since we know that the fat in milk at churning temperatures is solid." He also disagrees with Soxhlet's theory that churning consists in breaking the enveloping membrane.

Richmond states his theory in these words; "As it is improbable that this layer is elastic, the effect of the impact of one globule upon another will be to squeeze out the layers between them and bring the globules within the reach of each others attraction. In this way nuclei will be formed which will on continued churning increase in size; as the nuclei get larger and larger the resistance, owing to the fluid friction on their surfaces will gradually bear a smaller and smaller proportion to the force tending to bring them to the surface and at a given moment the butter will come. This theory is in accord with all known facts. By microscopical examination of cream during churing the formation of nuclei of irregularly shaped masses of fat globules is noticed. As an irregular shaped mass will occupy a greater apparent volume than a sphere the transformation of spherical globules into irregular nuclei should be attended with thickening of the cream which is in accord with facts; as the nuclei increase in size, the layer condensed by the surface energy round them will rapidly become less, so that the cream will gradually decrease in thickness; this decrease in thickness of the cream should take place later than the increase mentioned above which is also the case."

Fischer's [23] theory is stated in this way. "Whole unchanged milk shows little tendency to form butter. The tendency to do so is increased, first, by allowing the fat globules in the unit volume of hydrated colloid to concentrate (through letting the cream rise or by mechanical separation of the cream), and second, by aiding dehydration of the colloids to which the water is so largely bound as by allowing the cream to sour. Since the hydrated colloids tend to collect in surface layers between the fat particles and the aqueous phase of the cream, efforts are made to break these layers and so to hasten coalescence of the fat droplets by churning. The combined efforts therefore bring about a progressive increase in the concentration of the oil with a decrease in the concentration of the hydrated colloid until the instability of the oil-in-hydrated colloid emulsion becomes so great as to 'break' and yield the hydrated colloid-in-fat emulsion which we call butter."

4. Probable causes of difficult churning.- The experimental work done in this investigation has shown that whipping is the predominating characteristic of the special type of difficult churning which was studied. In every case of difficult churning encountered the cream whipped much more readily than normal cream, and , as mentioned before, the foam was very persistent.

It seemed evident that the fundamental factors involved in the formation of air emulsions, particularly those of the more or less permanent type, of which whipped cream is an example, were present in these cases of difficult churning. A study of the literature on whipped cream therefore seemed important in order that a more comprehensive view of this subject might be available in the study of the persistent whipping which, as has been stated, characterized difficult churning.

Melick,[26] who carried on extensive investigations on whipped cream found that the acidity of cream was a very important factor. He states that, "The fat content proved to be the more important factor up to 20 per cent than the acid, above 20 per cent the acid content is more important." This investigator carried out a series of experiments in which various amounts of lactic acid were added to cream and found that, "In every case, the cream, to which the lactic acid had been added, whipped in less time and produced a denser foam than that to which no acid had been added." Judging from this result, there seems to be a relation between the acidity of the cream and the density of the foam. If it is true as Melick states that, "Lactic acid, either commercial or natural, at low temperatures effects a gelatinous consistency of the casein and albumin which renders it capable of holding a considerable quantity of air, thus facilitating whipping," then we may have the explanation of why cream whips so readily after standing for several days at low temperatures. This may also explain in part, at least, the difficult churning encountered in Churnings 7 and 8 in Table 12. These two samples had stood for a considerable length of time at low temperatures and, although they were churned at 62^{o}F. they whipped excessively in the churns and churned with great difficulty.

The work of Miss Dumaresq[27] on the viscosity of cream throws some additional light upon this subject. Her results show that the increase in acidity of cream is accompanied by a gradual rise in viscosity until the "critical acidity" is reached. At this point the viscosity increases very rapidly but this rapid rise of viscosity is probably the effect produced by the coagulated particles of caseinogen, since this "critical acidity" is about 0.6 per cent acid. It is of interest to note also that "For separated milk," according to Miss Dumaresq, "there is no critical acidity, proving that this is a property of the fat globule or rather of its envelop."

Kohler[28] has shown that milk increases in viscosity as the stage of gestation advances.

Ramsden[19] states that, "The high pressure required to force solutions of sopanin and albumin through capillary tubes when bubles of air are present is due largely, among other factors, to the presence of solid surface membranes around the air bubbles and the increased resistance to de-formation brought about." Ramsden also refers to a like "solid surface membrane at the interfaces of caseinogen solutions and pure neutral olive oil or butter fat." The effect of this solid surface at the interfaces of liquids could be interpreted to explain the critical acidity in cream as opposed to the lack of it in separated milk which would be practically free from interfacial solids.

If the action of acid at low temperatures on the colloidal particles collected at the surface of the globules is the same as that described by Melick that is to "effect a gelatinous consistency" it is reasonable to suspect that this gelatinous mass would greatly retard the union of the fat globules to form butter as well as to facilitate whipping which in its self would also tend to retard churning.

The effect of acid upon the proteins of milk as related by Melick[26] shows a great resemblance to the action of acid upon other proteins studied by Fenn[29], Loeb,[30] Fischer,[31] Paule,[29] Ostwald,[29] Proctor and

Ehrenberg[29] and many others.

Acids retard the precipitation of gelatin by alcohol according to Fenn who showed a relation between his alcohol number* curves and Schroeders viscosity curves for gelatin. The retarding of precipitation is related to the swelling of the proteins, which the acid causes.

Fenn also shows that weak acids, to which class lactic belongs, have a more gradual effect on proteins than strong acids; some of the weak acids which he studied gave alcohol number curves which passed through a maximum and then made a gradual decline, while others which he followed showed a continual rise without reaching a maximum within the limits of his experiments.

Applying the results of Fenn's work to difficult churning it is obvious that lactic acid which develops in cream would be expected, first, to cause a gradual swelling of the proteins. There would then follow a retarding of the precipitation of these swollen proteins by acid, which would itself make the churning more difficult since the literature points

*Alcohol number is the number of cubic centimeters of 95 per cent alcohol required to precipitate 5 grams of gelatin from a gelatin and electrolyte solution.

to the fact that the proteins must be removed from the fat globules before they can unite and the butter be made to "come."

It also follows that, swollen proteins or as Melick describes them, the proteins of gelatinous consistency would be much more difficult to remove from the fat globules than precipitated proteins.

It would seem, however, that there should be some critical acidity for the precipitation of even these swollen proteins. Just what critical acidity would be necessary to bring this about is not clear although some of the experimental work which was carried out showed that acidities as high as two per cent were not effective in preventing difficult churning. This may be explained, in part, by Fenn's work which showed that certain salts increase the amount of alcohol necessary to precipitate gelatin from its solutions. Salts containing a di-basic cation were found by him to be particularly effective in this regard. It is therefore not improbable that the calcium salts in milk tend to augment the difficulty of precipitating the proteins and also to increase their gelatinous consistency.

Babcock and Russell[32] worked on the effect of viscogen (a saturated solution of $Ca(OH)_2$ in a 35 per cent solution of cane sugar) on restoring the viscosity of pasteurized cream, but in their explanation of factors which cause the changes in the viscosity of the cream, no reason is offered for the part which the viscogen plays in this change.

Melick[26] also showed that viscogen immediately restored the viscosity of pasteurized cream but he offered no explanation further than that the sugar furnished food for the lactic acid bacteria and the statement that the calcium might have some effect. Interpreting this in the light of Fenn's work, the soluble calcium salts merely accentuate the effect of the slight amount of acid present in the cream.

It has been found out by practical creamery men that the pasteurization of the cream temporarily destroys its viscosity. This has been explained on the basis that heat changes the soluble calcium salts to insoluble ones. Melick has shown that if pasteurized cream is allowed to stand until a slight acidity develops, the viscosity is restored. This is thought to be due to the action of the acid in changing the calcium salts back to a soluble form. These explanations are in accord with the results of Fenn's work and they also give an explanation of the action of viscogen on pasteurized cream since in the addition of viscogen soluble calcium salts are introduced.

Babcock and Russell interpret their microscopic examinations of viscous cream to mean that the clumping of the fat globules, which they observed, is the cause of the viscosity, although they admit that some samples of viscous cream do not show this clumping. A more probable explanation of the clumping of the fat globules observed by Babcock and Russell, and one in accord with the results of Fenn's and Melick's work is that the swelling of the proteins forms a gelatinous mass around the fat globules and this gelatinous mass holds the globules in clumps. Sufficiently large clumps should make the cream appear thicker, according to the view stated in Richmond's[25] theory of churning.

The increase in the protein content of milk which occurs at the close of the lactation period, and which was regarded in the experimental work as of fundamental importance in causing difficult churning, would be interpreted in the light of Fischer's[23] work as an increase in the number of colloidal particles in the milk. According to the work of Fenn and Melick, the action of acid upon this increased protein content would be to form a greater viscous layer around the fat globules and thus tend to make churning more difficult.

This was borne out in the experimental work in which large amounts of milk solids were added to cream. While churning was retarded to a certain extent in these experiments it was also shown that this factor alone was not of fundamental importance.

The preceding review of the effect of various reagents on proteins point to the conclusion that the action of acid and calcium salts upon milk proteins when let stand at low temperatures not only increases the tendency to whip but also makes the covering around the fat globule of such consistency as to resist being removed by friction or precipitated by other means.

While some of the experiments carried out in this study point to the above factors as being the fundamental ones involved in bringing about difficult churning it must be admitted that other portions of the data do not admit of such an interpretation.

It is, therefore, clear either that difficult churning may be due, under different conditions, to entirely different changes in the physical-chemical properties of the cream, or that the fundamental factor causing difficult churning is yet to be discovered.

One possibility in this connection is offered in a study of the "mucoid" substance which Storch separated from cream and regarded as a membrane layer around the fat globules. That portion of Storch's work, in particular, which indicated a relation between this "mucoid" substance and the whipping properties of cream, seems to have a special bearing upon the problem of difficult churning.

Storch found that cream which had been washed free from all proteins save the mucoid protein, by means of a sugar solution and then water, whipped extraordinarily readily and retained its form for several days, while the unwashed cream, when whipped, went down in a day. This phase of Storch's work has never been repeated and therefore lacks the substantiation which would make it directly applicable to the excessive whipping encountered in connection with difficult churning. The question obviously deserves further study, however, inasmuch as considerable evidence points to the fact that milk proteins, and the physical-chemical changes to which they are subject, bear an important relationtto the problem of difficult churning and its accompanying phenomena. In the past the proteins which have been regarded as important in this connection have been the casein, albumin, and globulin of milk, whose existance in the milk has been definitely proved. The substantiation of Storch's work, however, admittedly presents the possibility of relating the whole problem to a specific, hitherto unknown protein in milk, which may, under certain conditions, normally cause difficult churning, or which may increase with advancing lactation and thus account for the difficult churning so frequently associated with the last stage of the lactation period.

Most authorities have refused to accept Storch's work associating a special protein with the fat globules of milk. The work of Aberhalden and

and Völtz[33] has, however, been interpreted by some authors to support the correctness of Storch's view. They obtained glycine, which is not present in casein or lactalbumin, on acid hydrolysis of the proteins which they found to be associated with the fat globules of milk that had been washed once by allowing them to rise once through a 50 cm. column of water. There is a probability, however, that the glycine in their work may have come from the lactoglobulin of milk serum, inasmuch as the animal globulins are known to contain glycine.

Still greater support is given the possibility of the presence in milk of a protein like Storch's mucoid substance in the recent work of Osborne and Wakeman[18] to which reference has already been made. This protein, which is characterized especially by its solubility in alcohol, as is also Storch's "mucoid" protein, was isolated not from fat globules, but from casein. This fact, together with the numerous points of similarity between Osborne and Wakeman's alcohol soluble protein and Storch's "mucoid" protein, is very suggestive of a probable relation between the rise in the casein content with advancing lactation and difficult churning. This phase of the problem deserves more extensive study. The time limits placed upon the experimental work of a dissertation of this kind, did not permit this to be undertaken.

There remains still another phase of the problem of difficult churning which deserves consideration from a theoretical point of view, namely the factors involved in Fischer's[26] hypothesis that butter formation is merely the change from an emulsion of fat in hydrated-colloid to one of hydrated-colloid in fat. There is much evidence to support this view of Fischer's, but the circumstances which determine the relative stability of the two kinds of emulsion and the factors which cause the fat in hydrated-colloid emulsion to "break" and pass over into the other kind of emulsion are not yet sufficiently understood to warrant a consideration such as they deserve.

Bancroft[34] holds that oil emulsions are always accompanied by a third phase between the two materials emulsified in each other. According to Clowes[35] this interfacial film is bent in one direction or the other by changes in surface tension induced by various substances. In one case there would accordingly, be an oil-in-water type of emulsion, and in the other one of water-in-oil. These results were secured by a study of more or less simple systems. Whether they also hold true for a more complex system like milk remains to be determined, although it is evident that this deserves a more extended study than it was possible to give the question in this work. It is not improbable that the solution of difficult churning may be found by extending the experimental work along these lines.

SUMMARY.

Studies were made of the chemical and physical-chemical changes occuring in cow's milk which were thought to be fundamental in causing that type of difficult churning which often occurs near the end of a cow's lactation period.

Relatively small fat globules and relatively high percentages of protein in cream are shown to increase the time required for churning. Evidence is presented, however, pointing conclusively to the fact that these are not the fundamental factors causing the type of difficult churning mentioned.

The tendency to whip into a very persistent foam is shown to be intimately associated with difficult churning and to be one of its predominant characteristics.

No amount of acid in cream up to two per cent alone insured easy churning.

The addition of cream from a comparatively fresh cow to difficult churning cream was found to aid materially in reducing the time of churning.

The mixing of difficult churning cream with

skim milk from a comparatively fresh cow and re-separating it, was also found to reduce the time of churning, but, to a limited extent.

A recent theory of milk secretion and several theories of the state of the fat globules in milk, as well as the theories of the formation of butter during the churning of normal cream were reviewed in order to correlate some of the phenomena connected thereto with difficult churning.

The probable causes of difficult churning were discussed with a view of associating some recently discovered properties of proteins with the problem of difficult churning.

A correlation of older views regarding a special protein in milk, particularly associated with the fat globules, with recent work on milk proteins is also discussed with reference to this problem.

BIBLIOGRAPHY.

1. Woll, F. W.,

   1889 Digestion Experiments with Corn Ensilage and Fodder Corn. Sixth Ann. Report, Agr. Exp. Sta., U. of Wisc., pp. 70, 95, 122.

2. Van Slyke, L. L.,

   1891 Influence of Advancing Lactation on the Production of Butter. Twelfth Ann. Report. N. Y. Agr. Exp. Sta., pp. 369-386.

3. Jones, L. R.,

   1890 Study of Milk Globules and Influence of Lactation. Fourth Ann. Report, Vt. Agr. Exp. Sta., p. 67.

4. Hills, J. L.,

   1893 Miscellaneous Notes on Handling of Milk. Seventh Ann. Report, Agr. Exp. Sta., Vt., p. 111.

5. Robertson, J. W.,

   1891 Experiments in Churning Cream. Canada Exp. Farms Report, pp. 88-104 and Tables 13,14,15.

6. Fahrenbach,

   1897 Some New Experiments in Churning. Ag. Inst. at Leipsic. Abst. Molk. Ztg. No. 52, pp. 847, 848.

7. Rinkle, L. G.,

1910 Conditions Which Affect the Ease of Churning of Cream. Thesis, U. of Mo.

8. Robertson, J. W.,

1892 Experimental Dairy Work. Canada Exp. Farms Report, p. 71-76.

9. Eckles, C. H., and Shaw, R. H.,

1909 The Influence of the Stage of Lactation upon the Composition and Properties of Milk. U. S. D. A., B. A. I., Bul. 155, pp. 54-57, 73.

10. Rinkle, L. G.,

1910 Conditions Which Affect the Ease of Churning of Cream. Thesis, U. of Mo., p. 7.

11. Shaw, R. H., and Eckles, C. H.,

1909 A Chemical and Physical Study of the Large and Small Fat Globules in Cow's Milk. U. S. D. A., B. A. I., Bul. 111, pp. 8, 11.

12. Briggs, L. J., (Filters)

1906 Colorimetric, Turbidity, and Titration Methods. U. S. D. A., Bur. Soils, Bul. 19, p. 31 and

1902 Capillary Studies and Filtration of Clay. Bul. 31, pp. 12-16.

13. Bosworth, A. W., and Van Slyke, L. L.,

1914 Condition of Casein and Salts in milk. N. Y. Exp. Sta. Technical Bul. No. 39, pp. 1-17

14. Palmer, L. S., and Scott, Robert G.,

1917 Unpublished Data Dairy Chemistry Laboratory, U. of Mo.

15. Van Slyke, L. L. and Bosworth, A. W.,

1914 Conditions of Casein and Salts in Milk, Tech. Bul. 37, N. Y. Agr. Exp. Sta., p. 6.

16. Fischer, Martin H.,

1917 Fats and Fatty Degeneration, John Wiley and Sons, New York, p. 105-106.

17. Storch, V.,

1897 Abstract of the work of Prof. V. Storch with regard to investigation of several important faults in the consistency in butter and their origin and also the formation of the milk globules from the 36 Bericht des Kgl. Velerinär -- und Landbauhochschuli -- Laboratoriums, Abstract in Milch Zeitung X, pp. 282 - 229, 243-45, 257-259.

18. Osborne, T. B., and Wakeman, Alfred J.,

1918 Some New Constituents of Milk. Third Paper: A New Protein Soluble in Alcohol. Jour. Biol. Chem. xxxiii, No. 2, p. 243.

19. Ramsden,

1903 Observations on Surface Tension Membranes, Bubbles, Emulsions, and Mechanical Coagulations. Chem. News, lxxxviii, pp. 49-51.

20. Babcock, S. M.,

1885 A Study of the Fat Globules in Milk. Fourth Ann. Report, N. Y. Agr. Exp. Sta., p. 294.

21. Cooper, W. F., Nuttall, W. H., and Freak, G. A.,

1913 Observations on the Fat Globules in Milk. Jour. Agr. Science, v. p. 340-341.

22. Richmond, Henry D.,

1914 In Dairy Chemistry Ed. 2, Lippincott and Co., Philadelphia, pp. 1-7.

23. Fischer, Martin H.,

1917 Fats and Fatty Degeneration, John Wiley and Sons, New York, pp. 93, 94.

24. Fleishman, W.,

1903 The Book of the Dairy. Trans. by Aikman and Wright. D. Van Nostrand Co., New York, p. 159.

25. Richmond, Henry D.,

1914 Dairy Chemistry, Ed. 2, Lippincott and Co., Philadelphia, p. 305.

26. Melick, C. W.,

1909 Whipped Cream. Md. Agr. Exp. Sta. Bul. 136.

27. Dumaresq, F. K. M.,

1912 The Viscosity of Cream. Proc. Roy. Soc. Victoria, Ser. 25 (N13) No. 2, pp. 307-322.

28. Kohler, B.,

1908 Investigations in the Viscosity and Surface Tension of Milk. Arch. Physiol. (Pflüger) 125 No. 1-2 pp. 1-72, fig. 5.

29. Fenn, W. O.,

1918 Effects of Electrolytes on Gelatin. Jour. Biol. Chem. xxxiii No. 2, pp. 279-294, Review of work of Pauli, Lacquer, Sackur, and Zoja, Ostwald, Proctor, and Ehrenberg.

30. Loeb, Jaques,

1918 Swelling of Gelatin, Jour. Biol. Chem. xxxiii No. 2.

31. Fischer, Martin,H., and Hooker, Marion O.,

1918 On the Swelling of Gelatin in Polybasic Acids and Their Salts. Jour. Amer. Chem. Soc. xl, No. 1, p. 272.

32. Babcock, S. M., and Russell,

1896 Restoring the Viscosity of Pasteurized Cream. Agr. Exp. Sta. Bul. 54, U. of Wisc.,

33. Abderhalden, Emil, and Völtz, W.,

Beitrag zur Kenntnis Zusammensetzung und der Vatier der Hüllen der Milchkügelchen. Zeit. f. Physiol. Chemie 59.

ACKNOWLEDGMENTS.

The author wishes to express real appreciation to L. S. Palmer, assistant professor of dairy chemistry, for the careful supervision of the experimental work and the helpful suggestions in the preparation of this thesis; also to C. H. Eckles, professor of dairy husbandry, for his excellent counsel and advice.