THE BACTERIOLOGY OF CREAM RIPENING AND CHURNING

THESIS FOR DEGREE OF M. S.

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THESIS

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PART I.

BACTERIA IN CREAM, THEIR NUMERICAL COUNT, TYPES AND THEIR ITINERARY IN THE MANUFACTURE OF BUTTER.

INTRODUCTION.

Milk produced under ordinary conditions always becomes contaminated with considerable, frequently with a very large number of bacteria. These organsims come from various sources and comprise a large number of species. Many of the species gaining access to the milk find conditions very favorable for growth and multiply more or less rapidly from the very start. By far the larger part of the bacteria found in ordinary milk gain access from external sources and it is due to the presence and growth of these that milk changes in taste and odor, undergoes the process of souring and various decomposition processes. In the separation the bacterial clumps are broken up and a large number of each of the different types present in the milk are carried over with the cream. Many of these cause

the same changes in cream as they do in milk while others have a very different action. The difficulties and problems involved in the handling of the cream for the manufacturer of butter are so numerous and complex that it is little wonder there is lack of uniformity in the finished product. Dairymen have realized that butter of the best quality cannot be made from cream that is produced under unsanitary conditions. The destruction of the natural, sweet, rich flavor of cream caused by the carelessness and neglect of the producer can be noticed in the butter. Scientific research has taken the matter into consideration and many interesting facts which have helped to make the manufacture of butter a science as well as a great commercial enterprise have been discovered.

The purpose of this paper is to make a study of the bacteria in cream, relative to the numerical count and prevailing types, and to trace these types through the different steps in the manufacture of butter; also to study their significance in cream and butter with some measures of control.

PREVIOUS INVESTIGATIONS.

"Dairying is an art the success of which depends almost entirely upon the extent to which we succeed in controlling the various fermentation processes" is a statement made by

Ernst Krämer (27). The fermentation that takes place in the dairy industry is largely due to bacteria. It is hoped that a knowledge of the nature and characteristics of these single cells belonging to the lowest type of plant life will help to throw light on some of those problems which are at present so difficult for the dairyman to solve. H. W. Conn (5) states that fresh cream contains commonly a very large variety of bacteria. This is especially so in cases of creameries where cream is collected from a wide territory, the number found under these circumstances being dependent in a measure upon the initial contamination and the age of the cream. The older the cream, the fewer are the types of bacteria found through bacteriological analysis. The total number increases as the cream ripens, this increase being due almost entirely to lactic acid bacteria which overshadow other groups. After churning many bacteria are removed from the butter with the buttermilk and many others with the wash water. The butter, however, contains great numbers of bacteria that do not find conditions as favorable as in the cream and that die off more or less rapidly. Their death is generally attributed to the small amount of moisture and oxygen present and also to the action of salt. W. W. Esten and C. J. Mason found the dirt that comes from the surface of the cow enormously stocked with bacteria. The contamination of milk by cow feces is the most objectionable as germs that cause putrefaction, gas production and various diseases are present.

The number of bacteria that can be washed from hay stems or leaves is sometimes as high as 76,000,000 per gram. Hay is the source of large numbers of bacteria in the barn and cow stable. The liquefiers from hay are the worst kind. The number of acid organisms on grass is much less in percentage and total numbers than in cured hay. Acid organisms are high in percentage on all grain feeds. W. A. Stocking, Jr. (24) in his study of the germicidal property of milk states that "Bact. lactis acidi and Bact. lactic aerogenes played an important part in milk or cream, the former occurring in much greater numbers than the latter". In the study of butter by Sayer, Rahn and Farrand (23), it was noted that the group of which Micrococcus lactis varians, a liquefying yellow coccus, is a representative, occurred most frequently in butter. Staph. pyogenes aureus belongs to this group. Members of the group are often found in the udder of the cow and on this account their frequency is easily explained. Micrococcus lactis aureus and M. l. albidus were also found present in fairly large numbers, both resembling M. l. varians in certain characteristics. The non-motile rod most frequently found was Bact. lactis lobatum, a slow, liquefying, orange bacterium. Bact. lactis album and Bact. lactis gorimi were present in varying numbers. The motile rods were present in small numbers. B. lactis cochleatus and B. l. gruchii were found several times. Both liquefying

and non-liquefying yeasts were frequently isolated. Three molds were found frequently, these being Oidium lactis, Pencillium glaucum and Aspergillus glaucus. None of the organisms were regularly present in butter except Bact. lactis acidi. Rosenau, Frost and Bryant (20) in their study of market butter found that the number of bacteria in butter diminished sharply with age. The average number of bacteria found per gram was five million, seven hundred thousand. They found no particular relation existing between the number of bacteria and any other constituent such as salt, moisture, etc. They found B. coli present in butter in six out of twenty-five samples. They found, however, that it soon dies out in the butter. Streptococci were found present in over half of their samples. B. welchii (B. enteritides sporogenes) was not found in any sample of butter. The tubercle bacilli was twice demonstrated.

The origin of the bacteria in butter is not always the same. Several types of organisms, lactic acid bacteria, M. l. varians, Oidium lactis, etc. are present in almost all milk. Other bacteria are derived from the washwater, B. fluorescens liquefaciens frequently coming from this source. W. W. Esten and C. J. Mason (7) grouped their udder organisms in order of occurrence as follows: M. l. acidi, M. l. albidus, M. l. varians, M. l. aureus and B. subtilis. Usually there is no harm coming from these, yet from a diseased udder, sore throat, tonsilitis and scarlet fever may be contracted

through the use of the milk. B. enteritides sporegenes, an anaerobic bacillus sometimes causing epidemic diarrhoea has its source in feces. According to W. L. Savage (22) and C. E. Marshall (15) - Staphylococci and Streptococci gain entrance through the orifice of the teats of healthy cows and multiply in the milk cisterns; they also come from manurial contamination, from stale milk left in dirty cans and from cows suffering from mastitis. The streptococci are of considerable importance as they are found in large numbers but as yet nothing very definite is known about them. B. coli and allied organisms are of importance as indicators of pollution. The presence of members of the colon group may be taken as signifying pollution from manurial source. The source of Bact. lactic acidi according to W. M. Esten (6) is the saliva of the cow.

W. L. Savage (22), H. A. Hopper (13), C. E. Marshall (15), M. J. Rosenau (19) and many others agree that in general the following include the sources of contamination:- Intramammary, introduction during the milking process, from the cow's coat, the udder, the teat, milking shed, milker, feces, utensils, strainers, coolers, bottlers, artificial cleansers, some in transit and much during the final distribution.

THE EXPERIMENTAL WORK.

Methods and Conditions.

This work was carried out as nearly as possible under commercial conditions so that our results should represent an average count and include the most common types of organisms present throughout the process of butter manufacture including the raw and finished product.

Source of Cream.- Cream was obtained from dairymen in the vicinity of the College Dairy.

Obtaining samples.- The samples were obtained from the College Dairy where butter is manufactured according to methods outlined by McKay and Larsen (16). Samples (about 50 cc. each of cream, pasteurized cream, starter, ripened cream and buttermilk), were obtained with sterile 10 cc. pipettes and placed into sterile 100 cc. Erlenmeyer flasks. Analysis of these samples were made immediately after collection. Four samples of butter, one before washing and salting and three when the butter was ready to tub were taken from the churn with sterile triers, the middle third of each trierful being then placed into sterile deep culture dishes. The sample taken before salting and one of the samples taken after were analyzed at once, the other two were stored at 40° to 45°F. and examined after seven days and one month respectively.

Bacterial Content and Isolation.- In all steps except those concerning butter, 1 cc. of the medium was added to 99 cc. of sterile physiological salt solution. This was then shaken and other dilutions made by introducing 1 cc. of this into another 99 cc. of sterile salt solution. Dilutions of 1:1000, 1:10,000, 1:100,000 and 1:1,000,000 were then plated out in litmus lactose agar and duplicates in casein agar. Butter was introduced into a small Erlenmeyer flask and placed into a water bath at 35°C. One gram (1.15 cc.) was measured by use of a sterile pipette into 99 cc. of sterile salt solution which has a temperature of 35° to 40°C. This was well shaken to a milky emulsion and other dilutions made as above. Samples were plated out in litmus lactose agar and casein agar, the same dilutions being used as for cream. After bacterial counts were recorde, many of the organisms were isolated and transferred from the agar plated to sterile nutrient bouillon.

Litmus lactose agar used for plating was made according to the rules adopted by the Committee on Standard Methods (Journ. Inf. Dis. Suppl. No. 1, 1905) to which 1 % lactose and o.o5 % azolitmin was added.

Casein agar was made up according to the formula given by S. H. Ayers (3).

Catalase.- The presence of catalase was determined through the use of the gum guaiac test. A tincture of gum guaiac was made by dissolving a little of the powdered resin

in alcohol. About 10 cc. of the cream was placed into a test tube, shaken with a few drops of hydrogen peroxide, then two drops of gum guaiac tincture were allowed to run down the sides of the tube coming in contact with the cream but not being mixed with it. A blue ring appearing within a few minutes is considered positive for catalase.

Reductase.- The presence of reductase was determined by adding 1 cc. of Schardinger's solution (190 cc. of distilled water, 5 cc. formalin and 5 cc. of alk. meth. blue) to 10 cc. of the medium to be tested, shaken to mix the color and milk uniformly and placed in a waterbath at 37°C. for half an hour. Decolorization is reductase positive.

Moisture.- The moisture was determined by heating 10 gms. of butter in an aluminum cup according to the Ames test (16). The sample was re-weighed and the percent of moisture obtained by multiplying the loss by 10.

Salt.- The amount of salt was determined by a slight modification of the Shaw test (25), the silver nitrate being of such a strength as to have 1 cc. represent .001 gm. of salt in butter or 0.1 % when 1 gm. samples are used, potassium chromate being used as an indicator.

Acidity.- The acidity was determined by titrating 10 cc. of the medium diluted with distilled water by the addition of N/10 NaOH and recorded as percent lactic acid.

Fat.- The percent of fat present was determined by the Babcock test (25).

Spores of anaerobic gas producers.- The anaerobic spore producers are tested for in every step of butter manufacture in the following way:- The medium to be examined was placed in tubes; 1 cc. in the first, 4 cc. in the second, 8 cc. in the third and 12 cc. in the fourth tube. Enough sterile milk was added to the first three tubes to make each approximately 12 cc. They were then heated in a water bath at 80°C. for ten minutes, cooled and the medium covered with one-half to three-quarters of an inch of sterile liquid paraffin to exclude the air. The tubes were incubated at 37°C. for two days. An abundant production of gas, the cream being torn and often thrown to the surface of the paraffin and coagulated masses of casein shows positive for spores of this group.

Coli-aerogenes group.- The presence of this group was determined by the inverted vial method (17), 1 cc., 0.5 cc., dilutions of 1:10, 1:100, and 1:1,000 of the sample being used. The production of gas in dextrose broth was considered positive.

Peptonizers.- To determine the number of peptonizing colonies, the casein agar plates, after counts were recorded, were flooded with N/10 lactic acid. The action of the lactic acid is to precipitate the casein in solution which produces an opaque white medium except about the peptonizing colonies where the casein has been dissolved by the peptonizing action of the bacteria. Colonies surrounded by a

clear zone and thus set off from the rest of the solid white medium are considered peptonizers.

Acid Organisms.- The lactics were determined by direct count of litmus lactose agar plates. They form either a distinct boat-shaped or round colony, this being small and of a distinct reddish-pink color.

Inert and Indifferent Organisms.- These were estimated by difference.

DISCUSSION OF DATA.

Types and Numbers of Organisms Found in the Raw Material and Finished Product.

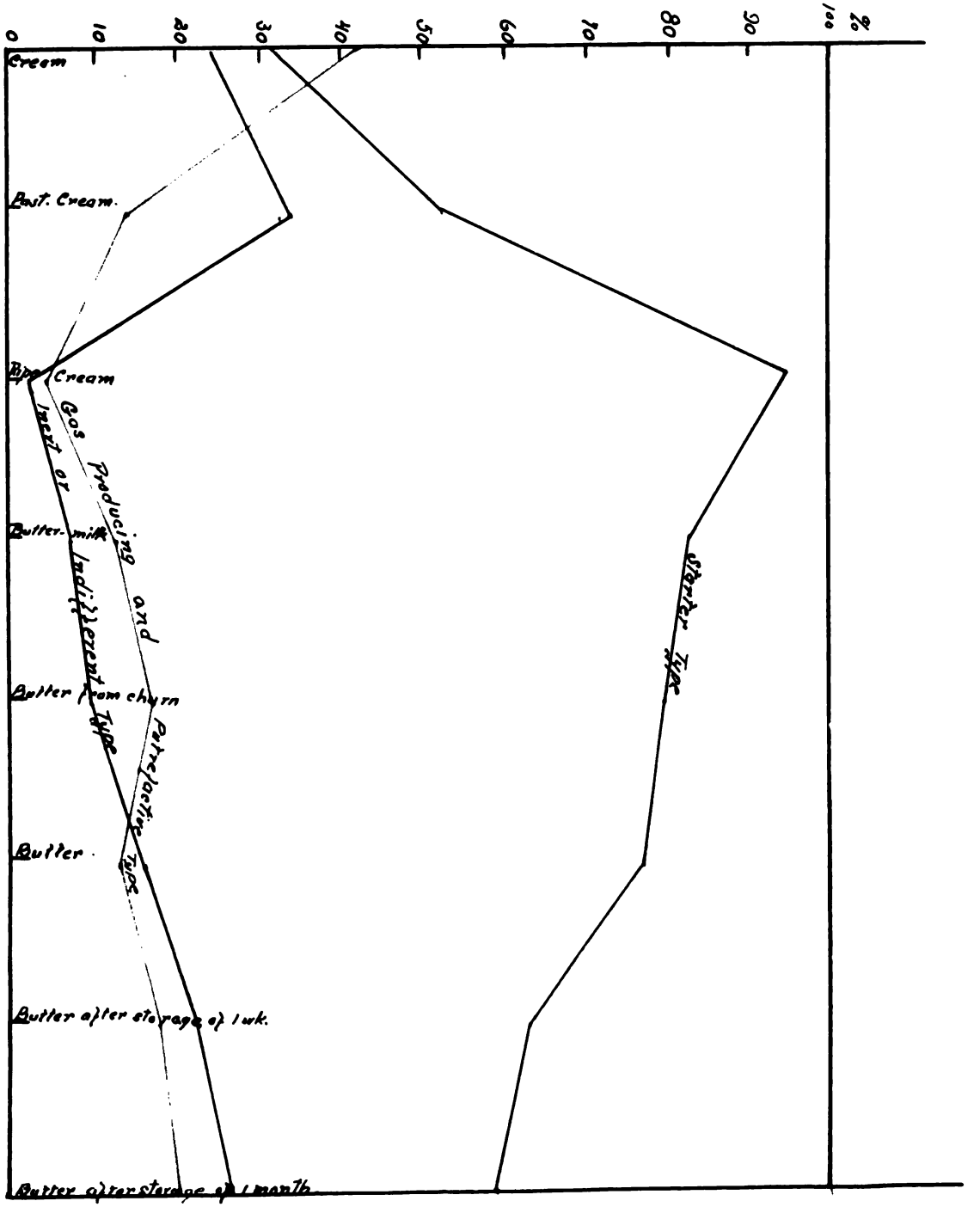
In our study of the bacteriology of cream ripening and churning, we have included in the following four groups of organisms:- First, those organisms which belong to the starter type; second, those that produce acid and gas; third, those that cause peptonization and putrefaction, and fourth, those that belong to the inert or indifferent group. By far the most important part played throughout the ripening and churning is taken by the starter and gas producing type of bacteria. The former is responsible for the ripening of the cream, giving it the thick, even, glistening appearance and

good flavor which are essential for good butter. Some members of the gas producing type cause much of the trouble that is often experienced in the manufacture of butter, as the formation of gas, bad odors, bad aroma and many other defects, causing the buttermaker much concern. Next comes the peptonizing and putrefactive group which is a constant source of trouble and which is present in greater or smaller numbers in all cream. They are mostly rods, motile or non-motile, capable of growing rather rapidly in cream and of performing marked changes. The casein in the cream is liquefied and digested with the formation of various products to which are due the undesirable and offensive odors and the bitter, stale and perhaps, the fishy flavors. Some products produced by members of this group are poisonous. The fourth group, inert and indifferent organisms, does not affect the cream or butter in any marked way, neither changing perceptibly the chemical nor the physical condition of the medium, but serves to swell the numerical count.

According to many investigators, the number of bacteria in "Top Milk" (cream layer) is much greater per cc. (2) than the number found in "Bottom Milk" (skim milk). In separation, the greater number of bacteria per unit volume leave the separator with the cream. The average number found in cream was 3,640,000. This great number was made up of groups of all kinds, but members of the gas producing and peptonizing groups predominated. In this first stage they

FIG. I. GRAPHIC SKETCH REPRESENTING THE DIFFERENT TYPES.

(% of total flora)

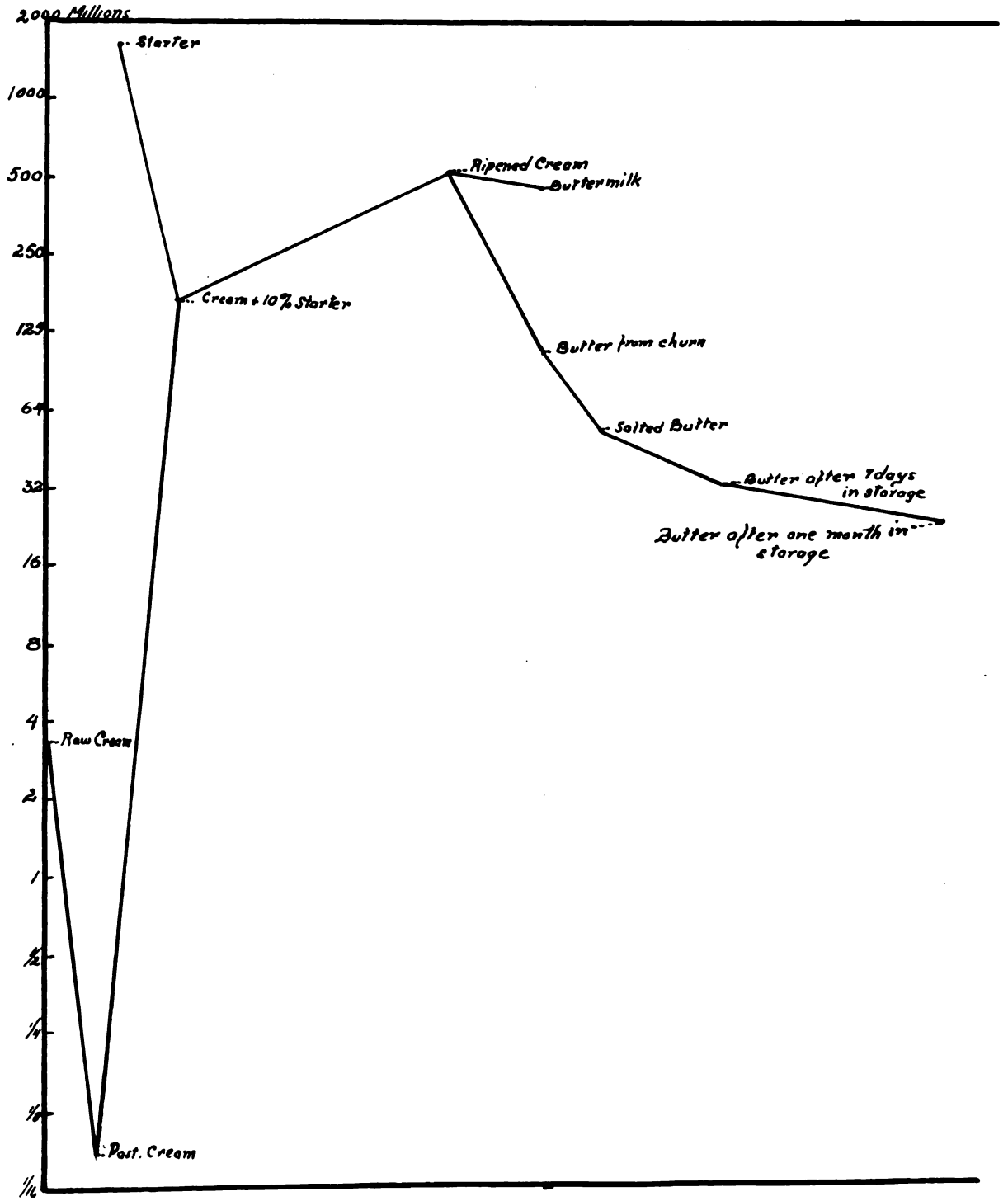


are more numerous than either the atarter type or the inert and indifferent group. Pasteurization greatly reduced their numbers. It is in the pasteurized cream that the starter group of organisms is first given the lead and in consequence of the addition of the starter is able to keep its numbers far above that of the other types present. According to the bacterial counts given in our tables, it is seen that pasteurization kills 98 % of the bacterial flora. The gas producing and peptonizing types are especially affected by pasteurization, the relative percentage to the total flora being reduced two-thirds. In the addition of pure culture starter we have added many desirable starter organisms for every surviving organism in the pasteurized cream and as a direct result we have a great percentage of the total flora of ripened cream made up of the starter type. It is also apparent from our table that a large number of the bacteria present in the ripened cream are removed after churning with the buttermilk. Many more are washed out of the butter during the washing and working process. Butter as a finished product contains a little more than one-tenth as many organisms as did the ripened cream. It seems therefore entirely plausible to assume that the other nine-tenths are removed with the buttermilk and the wash water. Three-quarters of the bacterial flora of fresh butter are the desirable starter type of bacteria. Upon these, salt and low temperatures have a deleterious effect and as a result they die off rapidly at

FIG. II. GRAPHIC SKETCH REPRESENTING THE NUMBER OF MICROORGANISMS IN THE MANUFACTURE OF BUTTER.

(Average of 12 experiments)

(Based on the log of 2)



first, then more gradually until after some time in storage their numbers as compared with the numbers of the other types is very much reduced. The relative percentage of the Inert and Indifferent type increases with the time in storage. The average number of bacteria found alive in fresh butter was 56,000,000. Storage and salt, however, seem to have so great a deleterious effect on the butter flora that after storage of one month, less than half of this number remain alive.

Adaptation of Casein Agar and Litmus Lactose Agar for Numerical and Differential Count.

The two types of media which are of great importance in determining the types of bacteria present are casein agar and litmus lactose agar. Throughout the whole process, the bacterial counts on the two media were very close together. The casein agar counts, however, were generally lower than the litmus lactose counts. One of the disadvantages of casein agar is that it must be allowed six to eight days for incubation, whereas litmus lactose agar needs only three to five days when the common temperature of 30° to 35° C. is used. After total counts and isolations were made, the casein agar plated are valuable for the detection of peptonizers. The work with casein agar bears out in general the work of Ayers (3) in that the casein media favored

the inert, alkali forming and peptonizing groups of bacteria, while acid forming organisms were favored and more readily distinguished on the litmus lactose agar.

Catalase and Reductase in Cream and Butter.

The action of the reducing and oxidizing enzymes was studied in order to determine if possible the reason of the disappearance or inactivation of catalase and reductase found in fresh cream. In Table II it is to be noted that neither catalase nor reductase was detected in ripe starter, ripened cream and buttermilk from the churn. Positive results were obtained for both oxidases and reductases throughout the rest of the process. It is known that catalase is concentrated with the cream when milk is centrifuged (14). It is also believed that ordinary lactic acid bacteria are not able to produce catalase while many other typical milk organisms are able to produce it in large quantities. The amount of catalase is measured in most cases by the amount of oxygen liberated in two hours, at 30° to 37°C. from a definite measure of standard hydrogen peroxide mixed with a definite volume of milk, but in our work the previously described color method for detection could be used with great satisfaction. We found that the catalytic activity is influenced by the amount of hydrogen peroxide added. When either too much or too little is added the test is either

retarded or negative. According to Koenig and Leon (13), catalase exists in milk partly free and partly combined. The combined catalase does not act until after twenty-four hours have elapsed but upon addition of a weak alkali the combined catalase becomes at once active. We know that the starter does not ordinarily produce catalase but even if the organism could produce catalase, the high acidity developed by it would either temperarily or permanently inactivate it. Ripened cream and buttermilk are also very high in acid and there is little doubt that this factor is the explanation of the negative results obtained in these steps of the ripening and churning process. The catalase, however, is not permanently inactivated as a direct result of the acidity as positive tests for it are obtained in the butter which may be explained by the low acidity of the butter. To further prove what the direct action of acid would be on catalase, to tubes of fresh cream, which gave positive tests for catalase, was added N/1 lactic acid until the cream was almost visibly curdled. On repeating the tests for catalase, negative results were obtained. The cream was then brought down to a normal acidity (15° Fuller's scale) by the addition of N/1 sodium hydroxide and then positive results were obtained, thus confirming our assumption that high and low acidity are factors in determining negative or positive results in catalase determinations.

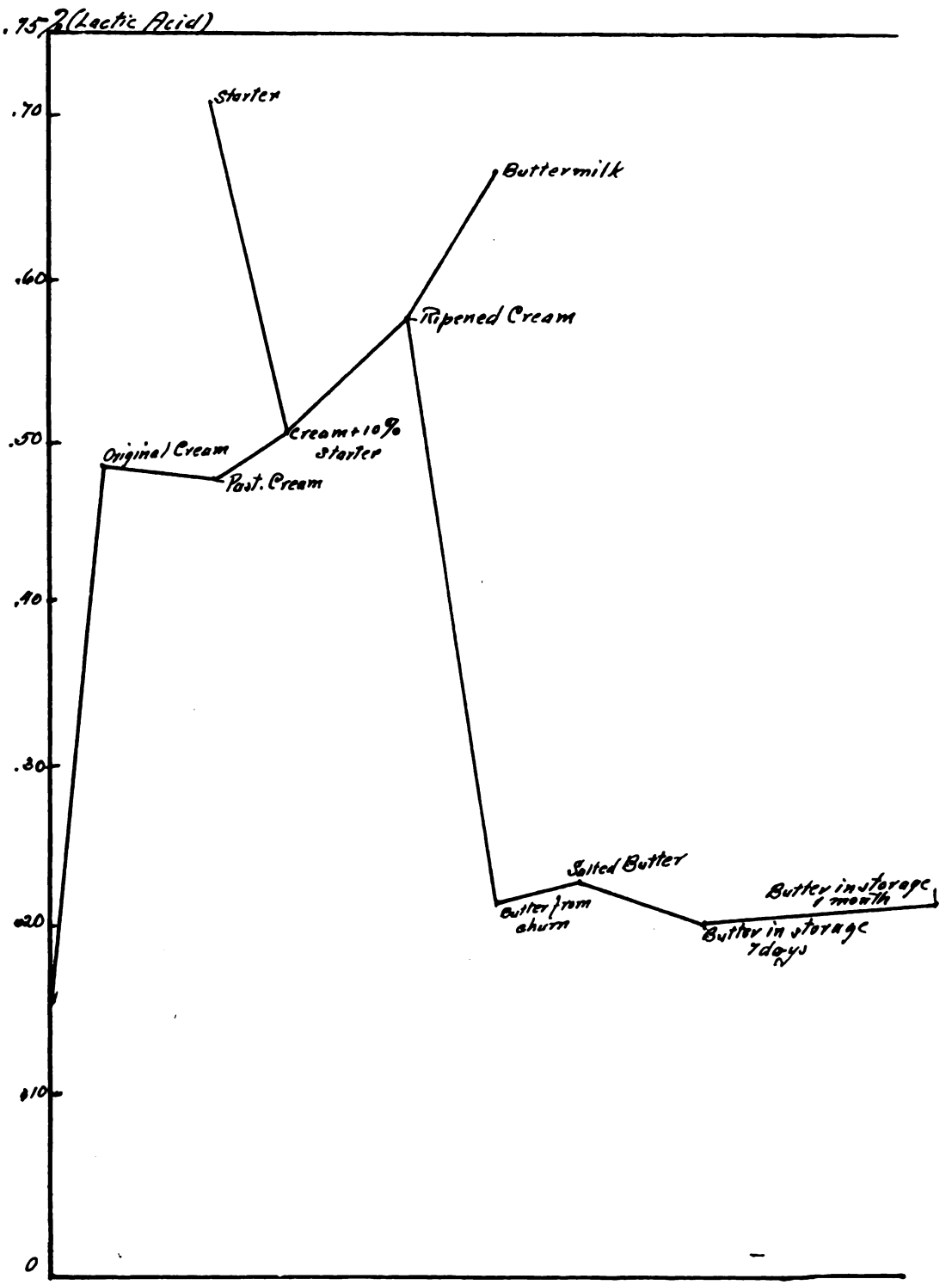
Reductase is similarly affected by acidity, it being

inactivated by the addition of lactic acid and its activity restored by the addition of a weak alkali. This causes us to state that reductase as well as catalase is only temporarily inactivated by a high acidity of the medium in which it is determined and may again be activated by the addition of weak alkalies.

Acidity of Cream and Butter.

As we note the different steps passed through in the ripening of the cream and the churning process, it is apparent that great differences in the acidity exist. The original acidity does not differ greatly from that of pasteurized cream. The starter, having a high acidity, raises the acidity of the cream to a considerable height. Buttermilk is also possessed of a high acidity. Butter, as a finished product, possesses a comparatively low acidity. In storage the acidity of the butter seems to change but very little. Acidity not only plays a great part in the quality of the butter but also shows its influence upon the keeping quality. Although investigators believe sweet cream to possess as good keeping quality as butter made from ripened cream, it is the opinion of many that butter made from sweet cream does not possess as good keeping qualities as butter made from ripened cream. However, acidity is by no means the only factor to be considered in the determina-

FIG. III. GRAPHIC SKETCH REPRESENTING CHANGES IN ACIDITY.



tion of the keeping quality of butter. Acidity also affects many other changes throughout the process as enzymatic action, physical structure, chemical equilibrium, etc.

Microörganisms.

Description, Morphology, etc.

Table II gives counts of organisms obtained throughout the whole process of cream ripening and churning, showing also the percentage of the different groups of organisms present. In every step of the ripening and churning, organisms were isolated from plates and planted in bouillon. From the bouillon culture agar plates were made and pure cultures assured. These were then transferred to litmus milk bouillon, agar slants and gelatin stabs. The characteristic growth of each was then recorded and some of the more prevalent organisms were identified. First, the bacteria were grouped according to the morphology into coccus, bacterium and bacillus, yeast and mold. Next they were subdivided into liquefying and non-liquefying and into acid and non-acid groups. These were then divided into spore-forming or non-spore-forming organisms. In identification, "The Classification of Dairy Bacteria" by Conn, Esten and Stocking was used and it was comparatively easy to trace the more preva-

lent organisms to their proper group and name.

In the following tables the morphology of the organisms is shown. The number of times that each of the organisms was found present is also shown in the tables.

The organism present in greatest numbers and the only one present throughout the different steps of ripening and churning was Bact. lactis acidi. It occurs in the smallest numbers in storage butter. Other organisms present in greater or smaller numbers were identified as Micrococcus lactis aureus (80), Micrococcus lactis varians (21), Micrococcus lactis albidus (19). These three belong to the coccus group and have very slight differences by which one may be distinguished from the other. M. l. varians liquefies gelatin while M. l. aureus does not liquefy it. Their color varies from orange yellow to white. M. l. albidus may be designated as a white variety of M. l. varians.

Very few rod-shaped bacteria were found. B. coli (126) was perhaps the most prevalent throughout but was not found in butter. B. fluorescens liquefaciens (123) was present in all steps except in pasteruized cream and buttermilk. Only twice out of the twelve experiments did it occur in fresh cream. B. subtilis (103) was found present in all stages except in the finished product.

Yeast and torula, both of a liquefying and non-liquefying type, were found present and as we have at hand no good

guide for their classifications, they were grouped only as to their ability to liquefy gelatin and according to their color. Yeasts and torulae were especially prevalent in the original cream. The part they play in the manufacture of butter is minimized by pasteurization since their numbers are greatly reduced.

A few molds were found but these with one exception, Oidium lactis, were discarded as they were thought to have little influence in butter.

Possible Source.

Under the subject of "Previous Investigations" the possible source of organisms in general has been fairly well covered. It is, however, necessary to give a few of the possible sources of the specific organisms occurring throughout the process. Bact. lactis acidi is generally believed to be present in the saliva or on the tongue of the cow. It has also been found present on plants but only where some bovine has had access. The organism is spread about the dairy in insufficiently cleaned or unsterilized utensils. Hair from sides of the cow and other material which at some time has been moistened with saliva is responsible for its dessemination. The gas formers, B. coli communis and Bact. lactis aerogenes have as their source usually fecal matter, dust or dirt from the animal's

coat or the upper layer of the soil. The micrococci are commonly found in the udder. B. fluorescens liquefaciens is found in the soil and water, gaining entrance to butter by washing the utensils and the butter with an impure water. The bacterial content of the washwater is a subject to be given consideration since many harmful organisms are from this source. Filtering and heating of water has been suggested to avoid this source of contamination. B. subtilis is added to the milk from hay, dust of barn and soil. In general the outside contamination would be due to the milker, air and dust of the stable, the milk pail, water supply, cooler, cans, transportation, ripener, churn, buttermaker, package and distribution.

Spores of Anaerobic Gas Producers.

Throughout our work, tests for B. enteritidi sporogenes spores (B. welchii) were made by the method previously given. This group of anaerobic spore producers is frequently found in milk and cream. This would lead one to suppose it would be found in butter also. In all the steps of ripening and churning, this organism was found present with the exception of butter. It is indeed surprising that it is not found in the finished product yet the fact remains that many investigators who have worked on the same subject have

had negative results, for which no reason has been given.

Coli - aerogenes Group.

This group includes a considerable variety of organisms. According to Hastings (10) they are classed among the facultative anaerobes and differ greatly in morphology, cultural characteristics and amounts of their by-products. He also states that they are to be found in every sample of market milk in varying proportions. Tests for this group were made during each of the different steps of butter manufacture. The same results were obtained in these tests that were obtained in the B. enteritidi sporogenes tests, the organism being present in every stage except in butter. For this no reason could be given other than that the butter contains less than ten percent of the milk serum present in the cream, unless the agitation during churning or plasmolysis of the cell due to the added salt kills the organisms. Reinman (18), Jensen (13), Rosenau, Frost and Bryant (20) and a number of other investigators have found this group in butter though always in rare instances whereas some authors have made the group conspicuous by stating that they have never found it present in butter. The only reason, therefore, that may be ascribed to the rare appearance of the group must be the one given above.

T A B L E V.

THE NUMBER OF PLATINGS IN WHICH AN ORGANISM WAS FOUND IN THE DIFFERENT CHURNING EXPERIMENTS.

No. of Organism.	Churning I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII.
1	1	0	1	0	0	0	0	2	0	0	0	0
2	5	3	0	0	0	3	2	0	2	2	0	2
5	1	2	0	0	0	2	1	2	0	2	0	2
6	0	0	2	0	1	2	2	0	0	2	2	2
9	2	0	2	2	2	2	1	0	0	2	2	0
19	0	3	2	2	3	0	2	0	2	0	0	3
20	1	1	0	0	0	0	3	0	0	0	0	0
21	3	2	3	3	0	4	4	0	3	0	3	2
26	0	2	2	0	2	1	1	2	0	1	1	0
29	2	2	0	1	0	0	4	0	0	0	2	1
31	0	0	4	2	3	2	2	0	0	0	2	0
54	0	0	2	2	3	2	0	0	1	0	2	2
63	0	0	3	3	2	0	0	0	2	0	2	1
68	0	0	2	3	3	2	0	0	2	0	0	0
69	0	0	3	0	0	1	0	0	3	0	1	0
71	0	0	1	3	0	2	3	0	0	3	0	0
74	8	8	8	8	8	8	8	8	8	8	8	8
80	0	3	0	2	3	0	0	3	0	0	2	2
84	0	0	0	3	0	0	1	1	1	1	0	2
90	0	3	2	2	2	0	2	2	2	2	1	2
102	2	0	0	3	0	0	0	0	2	2	2	2
109	0	0	0	0	2	1	0	2	0	2	3	2
111	0	0	0	0	0	3	0	2	0	2	0	0
119	7	3	2	0	0	0	3	2	0	3	0	0
122	1	0	0	0	2	0	0	2	0	0	0	0
123	0	2	0	2	0	1	0	2	2	0	2	2
126	4	4	3	2	4	4	4	4	4	3	4	4

Note:- Just before the final typewriting that part of Table IV which contained the results of Churnings Nos. IV, V and VI was irretrievably lost.

The Occurrence of Microörganisms.

In the preceding tables the occurrence of microörganisms with the exception of yeasts and molds have been recorded. Yeasts were present in every churning in varying numbers. They are most prevalent in the cream before it has undergone pasteurization. Molds were found in but few cases with the exception of Oidium lactis. Most of those found were in butter which had been kept in storgae for one month and undoubtedly had some relation with the condition of the butter at that particular time. Members of both the Penecillium and Aspergillus types were represented but neither a morphological nor physiological study was made of them. Table V also shows the number of platings in which an organism was found in the different churning experiments.

General Significance.

In the first part of the discussion we have taken up the influence of certain types of bacteria found throughout the process of ripening and churning. It remains, however, to discuss the general influence of all types found present throughout the work, laying special emphasis on their effect on the finished product. Sweet cream butter as a rule soon undergoes deleterious changes especially when made from un-

pasteurized cream. On the other hand, butter made from cream which has been properly ripened differs greatly in flavor from that prepared from sweet cream and is believed by most investigators to possess better keeping quality than sweet cream butter. Butter made from cream which is fresh and sweet when it is delivered to any creamery is of higher grade and has better keeping quality than butter made by the same buttermaker from cream which is old and sour. The acid fermentation of the ripening process gives intensity and character to the flavoring compounds. It should be our aim to eliminate to a great extent undesirable organisms as those of the coli-aerogenes group, etc. and to allow the desirable Bact. lactis acidi type to predominate at all times. This of course means that we should use the pasteurization process to help eliminate many of the undesirables and employ a good starter which is responsible for the desirable flavor and uniformity in quality of the finished butter. According to Herter (11), the coli-aerogenes group sometimes induces excessive fermentations of lactose and other sugars with the production of irritating acids (especially lactic and acetic) and at the same time liberates an excessive amount of gas. They cause a sharp taste in the finished product very noticeable especially when butter is made from unpasteurized cream. Members of the group are present frequently in large numbers due to fecal contamination. The peptonizing bacteria or casein digesting

group digest vasein either with or without coagulation. They often develop strong putrefactive odors and are in many cases the cause of abnormal flavors in butter, but are in general kept down to such small numbers that their influence is generally not heavily felt. The yeasts were present in all churnings and although we know very little as yet of their influence on the keeping quality of butter, yet we do know that "oily" butter and certain abnormal flavors are due to them. Moldy butter due to the growth of certain molds on the lining of the butter tubs or the paper in which the butter is wrapped, is also a factor to be taken into consideration. Oidium lactis is always present in butter and seems to decrease in number with the age of the butter. Hastings (10) states that itself is not a good substratum for mold growth. The remedy recommended or rather a preventative is the paraffining of butter tubs and the heating in hot water of the paper linings and wrappers.

SOME METHODS OF CONTROL.

Pasteurization.

In any discussion pertaining to butter manufacture it is almost a necessity as well as a matter of great interest to fully discuss pasteurization and and its real influence

on the finished product. About a quarter of a century ago, Storch, the noted Danish scientist isolated certain bacteria that were necessary in the ripening of cream. The best results in ripening were not always obtained when these isolated cultures were added to cream already crowded with a great number of bacteria and on this account he started to work to obtain a cleaner field for his cultures by destroying by means of heat the organisms that already existed in cream. This process in later years, greatly modified, received the name of pasteurization. We now define pasteurization as the process in which milk or cream is heated to a sufficiently high temperature to destroy a portion of the bacteria and then is rapidly cooled to prevent growth of the surviving organisms. The two main purposes of the process are to destroy all pathogens and to improve the quality of the product. In the process of buttermaking it enables the buttermaker to eliminate undesirable taints due to many bacteria in cream and to produce a more uniform flavor and quality by allowing the starter organisms a good, clean field. It also enhances the keeping quality of butter. As a control, efficient pasteurization first of all removes all dangers of pathogenic organisms. Flügge (9) lays particular stress upon peptonizing bacteria, most of which form spores. The spores survive pasteurization and would then have a field free for growth and activity. Ayers and Johnson (4) state that many inert and alkali forming organ-

isms resist the heat of pasteurization. They have also found strains of Bact. lactis acidi, B. coli, B. lactis aerogenes and types of streptococci which survive pasteurization. It seems, therefore, that pasteurization when used alone as a control would not accomplish the desired end.

Acidity.

Acidity is taken up as a factor which may control the type of organism. The tolerance of several organisms for acid, on agar and in milk was tried and it was found that all of the organisms used in the experiments could grow on a medium containing .4 % lactic acid. The organisms used were M. l. varians, M. l. albus, M. l. albidus, B. subtilis, B. fluorescens liquefaciens, Oidium lactis, a liquefying and a non-liquefying yeast, (59, 37). The results were similar in both milk and agar. It is thus apparent that an acidity of .4 % is not sufficient to prevent growth of the common types of organisms existing in butter.

Judicious Use of the Starter.

It has been previously mentioned that when a considerable number of species of bacteria are present in milk or cream, not all of them are able to multiply at the same rate. Most species of bacteria thrive best on a neutral or slightly alkaline medium. In nearly all milk, however, as it comes from the milk pail, there are a number of lactic acid organisms which soon produce sufficient acidity in the milk to check the growth of a number of species of bacteria which do not thrive in the presence of acid. On the basis of antagonism of mixed cultures, it is hoped we can establish a control of the organisms that occur in butter. First, in considering the starter organism, let it be remembered that a culture of Bact. lactis acidi noted for its characteristics should be used. This should have good vitality and fermentative power, self preservation, flavor producing properties and be able to form a curd of uniform consistency with no presence of gas. We know that of the great number of organisms present in the original cream, only two percent or less are alive after pasteurization, i. e. ninety-eight percent are killed by the heat of efficient pasteurization. In the addition of ten percent starter we add 5,000 or more of the beneficial starter type of bacteria for every organism in the pasteurized cream. This plurality gives the starter organism so great an advantage

that under proper conditions they will develop in the ripening cream the desired flavor and aroma. At the same time the starter organism also exerts an action strongly antagonistic to the members of the group of peptonizers and putrefiers growing in the same cream. When the ripening is well under way, this group is almost completely held in check and their harmful changes are arrested. The inert or indifferent group are really of little importance except in so far as they increase the total count of bacteria. It is easily seen that a judicious use of the starter can do much along lines of control. There is, however, a danger of allowing the starter to use its influence for too long a duration, giving as a result over-ripened cream which imparts to the butter a sour cream flavor and impairs the keeping qualities. In the majority of cases the starter cannot do its full work of control if the cream which is to be ripened is not previously pasteurized for it usually contains so large a number of injurious bacteria that the favorable influence of the pure culture is greatly diminished. It is thus seen that a good starter and pasteurization represent the most efficient control of microorganisms in cream and in butter.

CONCLUSIONS.

1. The organisms in butter are for the greater part those which are present in cream.

2. Neither the buttermilk nor the freshly churned butter taken from the churn before the buttermilk was drawn contains per unit volume as many living bacteria as the ripened cream. The average from our data shows that about 30 % of the organisms were killed in the churn, doubtless due to mechanical agitation during churning.

3. The process of washing and salting removes fifty percent of the microorganisms.

4. Plates made with casein agar give a total count a trifle lower than litmus lactose agar and such plates aid in the detection of the peptonizing organisms. Litmus lactose agar is well adapted for the detection of acid producers.

5. Positive tests for catalase and reductase were obtained in the cream and butter but not in the starter, ripened cream or buttermilk. Negative tests are due to a temporary inactivation by high acidity.

6. Spores of anaerobic gas producers and members of the *coli-aerogenes* group were not detected in butter although they were present in greater or less numbers in cream.

7. Bact. lactis acidi is the predominant type throughout the manufacture of butter. Other organisms appearing frequently in our samples are M. l. varians (21), M. l. aureus (90), M. l. albidus (119), S. l. fulvus (19), Bact. lactis album (2), B. coli (126), liquefying and non-liquefying yeasts, Oidium lactis.

8. The growth in milk of organisms frequently found in dairy products was not entirely prohibited by the presence of 0.4 % lactic acid.

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PART II.

FACTORS INFLUENCING THE RESISTANCE OF LACTIC ACID BACTERIA TO PASTEURIZATION.

The ready acceptance of the fact that organisms survive the pasteurization process because of the great bulk of cream and milk used or because of their high thermal deathpoint, has deterred dairy investigators from discovering the real cause or protective factors which allow their survival. In 1912 J. J. Kinyoun (8) found in commercial pasteurized milk of Washington, D. C., high counts of colon bacilli and streptococci, and contributed their presence to dirty milk, inefficiently pasteurized. C. E. Marshall (10), in 1897, found that a large percentage of the samples of milk pasteurized at 68°C. for 20 minutes, loppered with the production of acid, although no true lactics were found on plating. Among those isolated were four non-spore-forming bacteria, three of which were able to survive 80°C. for 20 minutes in bouillon and the fourth withstood 70°C. for the same length of time. H. D. Pease (11) found that members of the coli group are more difficult to kill by pasteurization than Bact. tuberculosis. A number of streptococci and members of the colon group, whose thermal deathpoint is high, were isolated by

Harrison (6) in 1905. N. L. Russell (12) says - that to a large extent the lactic acid bacteria are destroyed by the pasteurization process. Gage and Stoughton (5) in 1906 found a strain of B. coli whose thermal deathpoint was 80°C. and attempted to increase its resistance to heat by subculturing from the surviving few, but no increase in the resistance was obtained. Many investigators have found streptococci amd members of the Bact. lactis acidi and colon groups that can survive at least 70°C. for 20 minutes. In 1910 Ayers and Johnson in their work on "The Bacteriology of Commercially Pasteurized and Raw Market Milk" (3) say that "temperature (62.8°C. kept for 30 minutes) would be sufficiently high to afford protection against pathogenic bacteria and yet would leave in the milk the maximum proportion of lactic acid bacteria and the group proportions would be very similar to those of all grades of market milk". In their conclusions they also assume the fact that the souring of pasteurized milk is due to the development of lactic acid bacteria which on account of their high thermal deathpoint survive pasteurization or which come from subsequent infection during cooling or bottling. They also claim that the thermal deathpoint of one lactic acid organism which was isolated from milk was 74.4°C. in broth and 75.6°C. in milk when heated in Sternberg bulbs for thirty minutes.

Theobald Smith (15) in 1899, working with Bact. tuberculosis states that the organism suspended in water, normal

salt solution, bouillon and milk are destroyed at 60°C. while the pellicle which forms on milk during the exposure at 60°C. may contain living organisms even after 60 minutes. The work of H. L. Russell and F. G. Hastings (13) in 1902 concludes that the destruction of bacteria in milk by means of heat, depends upon the conditions under which the exposure was made; the formation of a pellicle protects any organism within the pellicle. The resistance of bacteria in the surface membrane is thought to be due not entirely to the lowering of the temperature, but is affected by the nature of the enclosing membrane itself. In closed vessels as Sternberg bulbs they state that milk offered no protection greater than whey or bouillon. The opinion was expressed by A. Wollf (17) in 1908 that the lactic acid bacteria (B. Guntheri) pasteurized at 70°C. for thirty minutes are protected by the heat forming around the cell an acid coagulum of albumin. Heat resisting strains of B. coli were found in milk by T. Zelensky (18) who states that their thermal deathpoint is higher in milk than in broth and suggests that the protein and fat in milk act as a protecting factor.

Nearly every investigator working with pasteurized milk agrees that members of the B. coli and Bact. lactis acidi groups survive pasteurization and too that there exist members of these groups whose thermal deathpoint is sufficiently high to carry them through commercial pasteurization. Many are of the opinion that the thermal deathpoint is the same whether

determined in bouillon or in milk, especially if the milk is sealed from the air to prevent the formation of a surface pellicle or membrane; while others have found that the thermal deathpoint of a number of organisms when determined in milk is a little higher than in bouillon.

The object of this work is to study:

1. The variation in temperature during commercial pasteurization (holding process).

2. The thermal deathpoint of some organisms surviving pasteurization.

3. Subsequent infection during cooling and bottling.

4. The protective agencies in milk and how they affect different types and strains of bacteria.

The Variation in Temperature During Commercial Pasteurization by the Holding Process.

This work was carried on in coöperation with the College Dairy Department, a "Perfection" Pasteurizer (300 gal. capacity) being used. The Holding process of pasteurization (145°F. for 30 minutes) has been used by the department with good results. The determinations of temperature in this experiment was made in cream pasteurized by this process. The temperature at the four corners and in the center of the pasteurizer was taken at one minute intervals. The results in

Table I show that the temperature did not vary over two degrees during the entire process. The slight variation in temperature may be to the credit of the special type of pasteurizer used as the spirals stirred the cream thoroughly. It is evident from the data obtained that the question of bulk does not answer for the protection of the organisms through uneven distribution of the pasteurization temperatures in a pasteurizer of this type.

TABLE I.

Variation of Temperature During Pasteurization.

Min.	Corner I.	Corner II.	Corner III.	Corner IV.	Center.
0	145°	145°	144°	144°	145°
1	144°	144°	145°	145°	145°
2	145°	145°	144°	145°	144°
3	145°	145°	145°	144°	145°
4	144°	145°	144°	146°	145°
5	146°	145°	145°	144°	145°
6	145°	144°	146°	145°	146°
7	145°	145°	145°	145°	145°
8	145°	145°	145°	145°	144°
9	144°	145°	145°	145°	145°
10	145°	145°	144°	144°	144°
11	144°	146°	145°	146°	145°
12	145°	145°	144°	144°	144°
13	146°	144°	144°	144°	145°
14	146°	145°	145°	144°	146°
15	145°	145°	146°	145°	144°
16	145°	145°	145°	145°	145°
17	145°	145°	145°	144°	146°
18	145°	145°	145°	145°	145°
19	145°	145°	145°	145°	144°
20	145°	145°	146°	145°	146°

The Thermal Deathpoint of Some of the Organisms Surviving Pasteurization.

Method.

By thermal deathpoint we mean the lowest temperature which caused death to vegetative forms on exposure for ten minutes (4). The bacteria were grown in nutrient broth for twenty-four hours and then one cc. of the culture was placed into tubes of 10 cc. sterile nutrient broth. Duplicate tubes were heated in a waterbath for 10 minutes at definite temperatures ranging from 50°C. to 78°C, each set being exposed to a temperature 2°C. higher than the one preceding. The temperature was not allowed to vary one-half degree C. The tubes were then quickly cooled below 20°C., one cc. inoculated into 10 cc. of sterile litmus milk and placed at optimum temperatures (30°C. for Bact. lactis acidi and 37°C. for B. coli.). The organism was not considered killed until it had been incubated for 5 days and neither tube showed growth. Other tubes were treated by the same method as above only the time of heating to which they were subjected was lengthened to 30 min. so that it would correspond to the length of time used in commercial pasteurization. Another type of technic on the effect of a lack of oxygen during determinations of a thermal deathpoint was tried through the addition of a layer of paraffin one-half inch in depth on top of the media in which the thermal deathpoint was to be determined. Cream

and skimmed milk were used as the media, inoculated with the different cultures and covered with paraffin; then the thermal deathpoint was determined. Later parallel tests were run by the use of the Sternberg glass bulb method (7). The tube was filled by warming the bulb slightly to expand the air and then the stem was at once inserted into the bacterial suspension which is drawn by suction into the bulb as it cools. The neck was then sealed as it cooled. The heating was done with the bulb held suspended by a wire and completely immersed in a waterbath. After being quickly cooled below 20°C. the contents were emptied into a tube of sterile litmus milk and this set aside at optimum temperatures.

Comparison of Methods.

The three methods described above, namely, the open tube, closed tube and paraffin tube method, were studied for the relative merits of each and to see whether one would have any greater or less influence on the thermal deathpoint of microorganisms. To show the influence exerted by the different technic, cream and skimmed milk, inoculated with the lactics Nos. II A, IV A and Knop, B. coli V. and Bact. bulgaricum were compared. In the following table it is seen that no difference which would influence results was found in any of the trials.

TABLF II.

Comparison of Open and Closed Tubes.

Names of Organisms	Open Tubes		Closed Tubes		Paraffin Tubes	
	Cream	Milk	Cream	Milk	Cream	Milk
Bact.l.acidi No.II A	69°C	60°C	69°C	60°C	69°C	6C°C
Bact.l.acidi No.IV A	71	63	71	63	71	63
Bact.l.acidi Knop	71	63	69	63	69	63
B. coli No. V.	73	65	73	65	73	63

Determination of Thermal Deathpoint.

In this phase of the work non-spore forming bacteria were used. The thermal deathpoint of twelve cultures of Bact. lactis acidi isolated from pasteurized cream and butter, four cultures of B. coli isolated from pasteurized cream and one of Bact. lactis citronis isolated from butter were studied. The variation in the thermal deathpoint of the different organisms proved to be great, varying from 56°C. to 78°C.

TABLE III.

Name Organism	Thermal Deathpoint. 10 min.	20 min.
Bact. lactis acidi I	58°C	56°C.
" " " II	72°C	68°C.
" " " III	76°C	74°C.
" " " IV	62°C	58°C.
" " " V	56°C	52°C.
" " " VI	76°C	72°C.
" " " VII	78°C	74°C.
" " " VIII	68°C	66°C.
" " " IX	66°C	62°C.
" " " X	72°C	68°C.
" " " XI	74°C	72°C.
" " " XII	64°C	62°C.
Bacillus coli I	74°C	70°C.
" " II	60°C	58°C
" " III	70°C	68°C
" " IV	56°C	52°C.
Bact. lactis citronis	64°C	62°C.

We observe that some bacteria whose thermal death point is below the pasteurization temperature, survive pasteurization; due to some agency or factor which milk brings into play and which the nutrient broth is unable to manifest.

TABLE IV.

Constancy of Thermal Death Points.

Name of Organism	1 min.		2 min.		3 min.		4 min.		5 min.	
	10	20	10	20	10	20	10	20	10	20
Bact. lactis acidi I	56	56	58	56	58	56	56	54	58	54
" " " II	72	68	72	66	72	66	70	68	72	68
" " " III	76	72	74	72	76	74	74	74	76	74
" " " IV	60	56	62	56	60	56	60	56	60	56
" " " V	56	52	56	50	56	52	56	52	56	52
" " " VI	76	70	78	70	74	72	76	72	76	72
" " " VII	78	76	76	74	76	74	76	74	78	76
" " " VIII	68	66	70	66	70	66	70	66	68	66
" " " IX	64	62	64	60	64	60	66	60	66	60
" " " X	70	68	70	68	70	68	70	66	70	66
" " " XI	74	70	74	72	74	70	74	72	74	70
" " " XII	62	62	64	62	64	62	62	62	62	60
Bacillus Coli I	74	70	74	70	74	70	74	70	74	70
" " II	60	56	60	56	58	56	58	56	60	56
" " III	70	68	70	68	70	68	70	68	70	68
" " IV	56	50	56	50	56	50	56	52	56	50
Bact. lactis citronis	64	62	62	60	62	60	64	60	64	60

Constancy of Thermal Death Points.

The constancy of the thermal death points was determined by obtaining five times at three day intervals the thermal death points of each organism formerly studied by the test tube method heating for both ten and twenty minutes. The results which are tabulated in Table IV show a variation for the same organism heated for the same length of time of not

more than two degrees which is only one step in our determination.

Subsequent Infection During Cooling and Bottling of Milk.

Three sterile liter flasks were used, the first being used for a sample of the milk as soon as pasteurization by the "Holding" process was completed; the second for a sample which had been cooled in the pasteurizer and the third for the milk that had been bottled. As soon as possible after collecting, each sample was plated. From the results record-

TABLE V.

Subsequent Infection Table. (Average of 20 trials).

Milk after	Total	Lact. Acid Bacteria	Alkali and inert per cc.	Peptonizers per cc.
Pasteurization	46,000	34,000	3,000	8,000
Cooling	69,000	39,000	14,000	11,000
Bottling	86,000	63,000	17,000	6,000

ed in Table V we assume that Ayers and Johnson in their assumption that the souring of pasteurized milk is due to subsequent infection with acid-forming bacteria during cooling and bottling are only partly right as the increase in number found immediately after pasteurization may be accounted for partly by multiplication. The process of cooling and bottling takes about half an hour. The remaining increase in numbers is through subsequent infection from utensils during the process of cooling and bottling.

The Protective Agency or Factors in Milk.

Bact. lactis acidi and B. coli were grown in nutrient broth for twenty-four hours at their respective optimum temperatures. One cc. was then introduced into each of the following: 10 cc. of sterile distilled water; 10 cc. of sterile broth and 10 cc. of sterile litmus milk (skim milk plus 0.05 % azolitmin). Then the thermal death point was determined in each of the three by the methods previously described. It becomes evident from this data, the average of

TABLE VI.

Thermal Death Point Average of 12 Determinations.

Organism		Water	Broth	Milk
Bact. lactis acidi	XII	63½°C.	64° C.	68° C.
Bacillus coli	III	68½°C.	69½°C.	72° C.

which is recorded in Table VI, that milk exerts some influence which protects the bacteria from heat, an influence which is not so marked or is absent in water and broth. The thermal death point of the cultures showed a difference of 3½° to 4°C. between broth and milk and about 4½° between water and milk.

Higher Thermal Death Point Due to the Physical Nature of Milk.

The different constituents of milk were next respectively dissolved or suspended in distilled water in the proportion found in fresh milk, placed in test tubes, sterilized in steam and then inoculated with one cc. of a twenty-four hour broth

culture. The thermal death point of the organism introduced into the solution or suspension of each constituent (water, fat, lactose, ash, casein and albumin)* was determined. It

The constituents were present in the following proportions (14).

Constituents	Percent	Range	Percent
Water	87.00	82.4 to	89.6
Fat	3.50	2.5 "	6.0
Casein	3.25	2.5 "	4.0
Albumin	0.50	0.5 "	0.8
Milk sugar	5.00	4.3 "	6.0
Ash	0.75	0.6 "	0.8
Solids	13.00	10.4 to	17.6

was found that in the presence of casein, albumin and fat, the thermal death point was higher than with the other constituents.

TABLE VII.

Thermal Death Points. (Average).

Organism	Water	Fat	Casein	Albumin	Sugar	Ash
Bact. lactis acidi No. XII.	63½°C.	65°C.	68°C.	68°C.	64°C.	64½°C.
Bacillus coli No. III.	68½°C.	71°C.	72°C.	72°C.	70°C.	70 °C.

*Much difficulty was encountered in trying to dissolve and suspend the different constituents.
Fat was emulsified by constant shaking in a shaker machine, casein was dissolved in a weak solution of sodium hydroxide and then neutralized. Albumin was made slightly alkaline and boiled.

The results recorded in Table VII indicate that it is both the nitrogenous matter and fat which act as the protector of the bacteria when these are subjected to pasteurization. Fat does not seem to exert as much protection as the albumin and casein. To determine how each of the nitrogenous compounds would act in combination with fat, mixtures of casein and fat, of albumin and fat, and of casein, albumin and fat were made in the proportions in which they are present in milk and sterilized. In these the thermal death point of B. coli and Bact. lactis acidi were determined. From the results in Table

TABLE VIII.

Organism	Casein and Fat	Albumin and Fat	Albumin, Casein and Fat.
Bact. lactis acidi No. XII.	68°C.	68°C.	68°C.
B. coli III.	72°C.	72°C.	72°C.

VIII we would infer that a combination of nitrogenous matter and the fat of milk does not tend to materially increase the ability of organisms to resist heat nor raise the thermal death point over that of either of the notrogenous constituents used alone.

Thus far the constituents of milk which had been used were commercial products. It was thought that there might be a difference in the effects of the constituents of milk as they exist in natural milk and those which are commercially prepared. To determine any difference in the action of the

natural and commercial constituents the following method was used: Whole milk was tubed (10 cc. per tube). Then another portion was skimmed and the skimmed milk was tubed. To a part of the skimmed milk rennet was added and the casein coagulated and separated by filtration and the milk serum tubed. All of these tubes were sterilized in steam, then the thermal death points of both B. coli and Bact. lactis acidi were determined in each of the solutions. The difference in the thermal death point in the whole milk and in the skimmed milk shows the protection exerted by fat, while the difference between the skimmed milk and milk serum shows the amount of protection given organisms by casein and albumin. Then, since the organisms are the same strains as those used in the previous experiments with broth, water and milk, still further comparison as to protection of the other substances besides fat and casein that are present in milk and not present in broth and water can be made. From the results obtained

TABLE IX.

Organism	Whole Milk	Skim Milk	Milk Serum	Broth	Water
Bact. lactis acidi No. XII.	70°C	67°C	64½°C	64°C	63½°C
B. coli	74°C	71½°C	69½°C	69°C	68½°C

in Table IX it is evident that the casein and fat are the main protective factors which exist in milk. There is also some protective action in milk serum as the thermal death point is higher in it than it is in either broth or water.

TABLE X.

Thermal Death Point in Acid Media.
(Average of 20 trials).

Acidity of Milk	No. II A.	No. IV A.	Knop
0.25 % (Lactic Acid)	57°C.	61°C.	61°C.
0.40 % (Lactic Acid)	57°C.*	61°C.	61°C.
Acidity of Broth			
0.25 % (Lactic Acid)	53°C.	55°C.	55°C.
0.40 % (Lactic Acid)	53°C.	55°C.	55°C.

*Except in four cases when the acidity of the medium was higher than 40° the milk curdled and the thermal death point was higher than any temperature used during the trials.

TABLE XI.

Thermal Death Points.

Medium	51	53	55	57	59	61	63	65	67	69	71	73	75	77	79°C.
Bact. lactis acidi No. II A.															
Cream	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Whole Milk	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Skim Milk	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Milk Serum	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Bouillon	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Bact. lactis acidi No. IV A.															
Cream	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Whole Milk	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Skim Milk	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Milk Serum	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Bouillon	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Bact. lactis acidi "Knop".															
Cream	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Whole Milk	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Skim Milk	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Milk Serum	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Bouillon	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

(Table 11), Concluded.

Medium	Thermal Death Points. 51	53	55	57	59	61	63	65	67	69	71	73	75	77	79°C
B. coli No. V.															
Cream	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Whole Milk	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Skim Milk	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Milk Serum	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Bouillon	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Non-acid B. No. 16.															
Cream	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Whole Milk	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Skim Milk	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Milk Serum	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Bouillon	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Non-acid B. No. 14 A.															
Cream	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Whole Milk	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Skim Milk	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Milk Serum	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Bouillon	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Bact. bulgaricum. 69	71	73	75	77	79	81	83	85	87	89	90	91	93	95°C
Cream	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Whole Milk	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Skim Milk	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Milk Serum	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Bouillon	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

It is also evident that the commercial constituents and the constituents as they exist in milk act differently in protecting an organism from the pasteurization temperature, i.e., the natural constituents exert a greater protective influence.

Influence of Cream.

The fact that the thermal death point of two organisms is found to be higher in whole milk than in separated milk led to the determination of the thermal death points of Bact. lactis acidi Nos. II.A, IV.A.and Knop, B. coli No. V. and Bact. bulgaricum and of two inert bacilli Nos. 16 and 14 A. in cream, whole milk, skim milk, milk serum and bouillon. The results in Table X show that the presence of fat enables the various organisms to withstand higher temperatures. In the case of B. 14 A. the thermal death point shown in bouillon was over ten degrees lower than the temperature of pasteurization by the "Holding" process, yet this organism was one isolated from pasteurized cream, its thermal death point, through the influence of the fat in the cream being raised above that of the pasteurization temperature.

Erw. F. Smith (16) in his book on "Bacteria in the Relation to Plant Diseases" Vol. I, states in a footnote that an acid medium protects organisms from heat which would be destroyed in a medium containing less acid, neutral or alkaline. To verify this statement, tubes containing litmus milk which had an acidity of 25° and 40° Fuller's scale (made acid by the addition of N/1 lactic acid) and tubes of bouillon of like acidity were inoculated with lactics II A., IV A. and Knop and their thermal death points determined. Results as shown in Table X lead us to the belief that acid, when used in small quantities has no effect on the thermal death point.

When it is used in large enough quantities to curdle milk an increase of the thermal death point results which is probably due to the coagulation of the casein in milk.

General Discussion of Possible Influencing Factors.

Organisms that are non-spore formers are found to survive pasteurization. Among these are the lactic acid bacteria. Many theories and assumptions have been presented and each new one is asserted by the author to be the true and most feasible explanation. For the average layman, the most easily believable theory is that bacteria survive pasteurization on account of the huge amount of milk or cream pasteurized at a time, because the temperature is not evenly distributed throughout. This assumption might be true if we were to use the Flash system for pasteurization or pasteurized in a plain tank containing an inefficient stirring device, thus not causing distribution of heat evenly throughout the medium which is being pasteurized. But our modern commercial pasteurizer contains spiral coils which stir the liquid constantly and there is little reason why the temperature should vary greatly at any part of the tank. This theory, so easily believable must therefore only be taken into consideration in those establishments which pasteurize their milk or cream by the use of the Flash system or have inefficient pasteurizers. The assumption

that some organisms found in pasteurized milk do not survive pasteurization but gain access during the process of cooling, bottling and capping was found to be true, contamination from imperfectly sterilized apparatus, the room air etc. being the cause. This source when properly controlled adds so few bacteria, most of these being of the harmless type, that it is almost negligible, yet it is a factor which allows bacteria to enter milk after it is properly pasteurized. This means of entrance of bacteria could be avoided by thorough cleansing and sterilization of utensils and general cleanliness in the dairy. Organisms which have a high thermal death point have been found by many authors and have been given as one cause of bacteria surviving pasteurization. Ayers and Johnson as well as Zelonki found members of the colon group which varied greatly in thermal death points. The same authors found streptococci which showed great variation in their ability to resist heat. This then is one of the reasons whereby bacteria of the lactic acid type are found in milk after proper pasteruization. Russell and Hastings found that a pellicle formed during heating of the milk would exert a protective influence. The pellicle not being observed to form during any of the trials made, their proof is accepted as a factor which influences the death of microorganisms in milk by heat. The shutting off of air during thermal death point determinations as tried in the closed and paraffin covered tube in comparison with the open tube has little if any influence. This

theory may almost be entirely neglected in attempting to group the true reason of bacteria surviving pasteurization. The nitrogenous constituents and the fat in milk or cream do protect organisms, the fat having a greater influence than the casein. The action of casein and albumin is probably one which was assumed by A. Wolff , namely, the heat forming around the cell an acid coagulum of albumin. Fat must act in a way very similar to that of the nitrogenous constituents of milk or cream in protecting the organisms. A high acidity (0.4 % lactic acid) has no effect on the thermal death point. An acidity high enough to cause the curdling of the milk during thermal death point determinations raises the thermal death point several degrees. The breed of cattle from which the milk is drawn, the general health of the cow and the condition of the udder, the products resulting from bacterial growth previous to pasteurization, heating under reduced or increased pressure and the relative specific heat of milk or cream may also be factors that protect organisms during heating.

SUMMARY AND CONCLUSION.

1. The thermal death points of seventeen different strains of bacteria isolated from pasteurized milk varied greatly when heated for 10 minutes or for 20 minutes.

2. Fifty-three percent of the cultures which were used in this experiment had a thermal death point below the temperature commonly used for pasteurization (62½°C.)

3. The variation of temperature during commercial pasteurization (Holder process) is very slight. This shows conclusively that the variation in the bulk of cream pasteurized does not account for the survival of the bacteria.

4. The thermal death point of some of the organisms used was very high, this accounting for their survival of pasteurization.

5. Contamination during cooling and bottling is very slight, since the count obtained directly after the pasteurization is more than half as much as that obtained after bottling. This increase is accounted for, partly by multiplication and partly by contamination from utensils.

6. The protective agency in milk is the nitrogenous matter (casein and albumin) and fat. This is true even when commercial constituents of milk are used.

7. When constituents of milk in its natural state are used, the fat and casein offer greater protection from heat.

8. Commercial constituents of the milk in its natural state differ in their protective influence. With commercial

constituents the fat, the casein and the albumin exert almost an equal protection. But with the fat and casein in the natural state the presence of the fat adds additional protection to the suspended organisms.

9. A sealed tube or a tube closed with liquid paraffin does not protect organisms to greater extent that an ordinary open tube.

10. A low acid medium has no effect on the thermal death point while a high acid medium may raise it several degrees.

11. Factors which account for the presence of organisms in pasteurized milk are the contamination during cooling, and bottling, pellicle formation during heating, a high acidity of the medium, the high thermal death point of some organisms and the protection given by the fat and nitrogenous contents of the milk or cream.

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