U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—Bulletin No. 73.
D. E. SALMON, D. V. M., Chief of Bureau.

THE BACTERIA OF PASTEURIZED AND UNPASTEURIZED MILK UNDER LABORATORY CONDITIONS.

BY

LORE A. ROGERS,
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., May 11, 1905.

Sir: I have the honor to transmit herewith a manuscript entitled "The bacteria of pasteurized and unpasteurized milk under laboratory conditions," by Lore A. Rogers, expert in dairy bacteriology in this Bureau, and to recommend its publication as Bulletin No. 73 of the Bureau series.

Respectfully,

D. E. Salmon,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

Dy.—63.

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THE BACTERIA OF PASTEURIZED AND UNPASTEURIZED MILK UNDER LABORATORY CONDITIONS.

INTRODUCTION.

The rapidly increasing size of cities, with the resulting removal of a large proportion of the consumers from the source of supplies, has necessitated marked changes in the transportation and storage of various food products. This has been, with many articles of food, a simple question, but with others of a more perishable nature the problems involved have been more complex and difficult. This is especially true of milk. In this case we have a food in almost universal use that is subject to rapid changes, affecting not only its appearance and taste, but also its value and even its safety as a food. These changes are brought about by bacteria which are normally present in milk, even when it is collected under the best sanitary conditions, and which find there favorable conditions for rapid multiplication. Thus we frequently find that, as a result of this growth, in a comparatively few hours milk may become entirely unfit for use.

In the smaller cities and towns the producer usually delivers the milk directly to the consumer, and the problem of a good milk supply is simply one of sanitary barns and a proper cooling and handling of the milk. In the larger cities, however, the demand is too great for the immediately adjacent country to supply, and the producer is so far removed from the consumer that a middleman is necessary. A large proportion of the milk used in the larger cities of this country is shipped by rail, a few companies usually buying the milk from the farmers and distributing it from milk depots in the cities. Some of the milk used in Boston comes 140 miles ([1]).[a]

The spread of the city of New York into the surrounding country, together with the increasing value of land for truck farming, has forced the dairy farms farther and farther away, until now milk for use in New York City is collected along the banks of the St. Lawrence River, 350 miles away.([2]) In Chicago the conditions are somewhat different, and practically all of the milk used there is produced within 100 miles.

[a] Superior figures refer to the bibliography at the end of the bulletin.

Long shipments have necessarily lengthened very materially the time which elapses between the production of the milk and its consumption. A considerable part of the milk is held ten or twelve hours on the farm before it is delivered at the milk station, and another delay frequently occurs at the city depot before the milk is distributed. Much milk reaches Boston from 18 to 30 hours old.([1]) The greater part of the New York milk is from 12 to 36 hours old when it reaches the city. Great care is now used to hold the milk as nearly as possible in its original condition. On the long hauls the milk is cooled at the receiving station and ice is used to hold down the temperature in transit.

Notwithstanding all the precautions observed, the milk, as it is delivered to the consumer in the city, is usually in a state far from satisfactory. On the average farm the conditions are such that the milk becomes heavily contaminated with various kinds of bacteria. If the milk is not cooled at once, multiplication of these soon begins and continues with more or less rapidity, which depends largely on the temperature. Even in milk held at comparatively low temperatures the increase may be great.

A few examples of the bacterial content of city milk will serve to illustrate the extent of this contamination. Sedgwick and Batchelder ([3]) found in 57 samples of Boston market milk from 30,000 to 4,220,000 bacteria per cubic centimeter. Hill and Slack ([4]) tabulated the results of the examination of 2,394 samples, nearly all taken as the milk arrived in the city, as follows:

	Per cent.
Below 100,000 bacteria per cubic centimeter	42
Between 100,000 and 500,000 per cubic centimeter	29.75
Between 500,000 and 1,000,000 per cubic centimeter	9.75
Between 1,000,000 and 5,000,000 per cubic centimeter	12.75
Above 5,000,000 per cubic centimeter	5
Uncountable spreaders	0.75

Park ([5]) gives the average of 20 samples taken from cans immediately on arrival in New York in March as over 5,000,000 per cubic centimeter. The average of 13 samples delivered to tenement houses in midwinter was 1,977,692 per cubic centimeter, while early in September it was 15,163,600 per cubic centimeter. In the better districts at the same time the number was considerably less. Bergey ([6]) found the average of 10 samples collected at railroad stations in Philadelphia in July to be 4,802,355 per cubic centimeter. It is readily seen from these figures that the bacterial contamination of the city milk supply, especially of the large cities, is uniformly great.

It is well established that specific diseases are not infrequently spread through the milk supply. Aside from this important phase of the question, which can not be discussed here, it is well known that bacteria are able to bring about in milk many changes of a highly undesirable nature. These vary from decompositions scarcely affecting the taste to the formation of products of a very toxic nature; the most familiar change is the souring, or curdling, resulting from the acid fermentation of the sugar. While this spoils the milk for drinking purposes, the group of bacteria which brings about this change is not considered deleterious. Indeed, they are introduced in the form of artificially prepared cultures in the manufacture of butter and many kinds of cheese. On the other hand, there is normally present in milk a group of bacteria which acts on the nitrogenous constituents—the casein and albumen—and their decomposition products. These bacteria secrete enzymes, or digestive agents, which act entirely independently of the cell, and are capable of bringing about digestive action greatly in excess of the needs of the organism. The rennet usually secreted with the proteolytic, or digestive, enzymes curdles the milk, which is slowly digested until in time the curd may be entirely replaced by a clear serum. In some cases the digestion may go on without previous curdling. As milk is ordinarily used, this process is not sufficiently advanced to affect the appearance or taste appreciably, although it would probably be unusual to find milk twenty-four hours old in which some change of this nature had not taken place. The products of this decomposition may be entirely harmless even when taken in considerable quantities, or, on the other hand, they may have a very toxic action.

The cases of ptomaine poisoning from milk occasionally reported are in all probability due to bacteria of this class. Such cases are so infrequent and usually of such obscure origin that they need not be considered in a discussion of the general milk supply. However, it is generally recognized that children, and especially children under one year old, may be very seriously affected by milk containing many bacteria of this type. The relation between the condition of the milk and the amount of intestinal trouble in young children has been demonstrated by the investigations of Park and Holt ([illegible]) in New York City. They found that this relation held for very young children only, and did not hold for children over three years old, even when they were fed milk containing large numbers of bacteria. They were unable to find any relation between specific varieties of bacteria occurring in the milk and the health of children. Forty per cent of the cultures tested caused death when injected intraperitoneally, but only one of 139 caused illness or death when fed to young kittens.

It is probable that the intestinal troubles of children are not all caused by one or two specific varieties of bacteria, but that they may be caused by any one of a number of widely distributed species, which may under certain circumstances produce abnormal conditions in the digestive tract. This may be brought about either through the direct action of the bacteria in the intestines or indirectly by the formation of toxins in the milk itself.

The great bacterial contamination of milk is now well recognized, and the public interest in this question is shown in the municipal control which is now very generally exercised over the milk supply. The control usually takes the form of inspection for adulteration and the presence of antiseptics, licensing of dealers, and in some cases the inspection of the dairy farms, with certain requirements regarding their sanitary condition. In a very few cities it is required that the milk shall not be above a certain temperature when delivered, and that the bacteria shall not be above a certain limit. The city regulations of Boston require that milk shall not contain over 500,000 bacteria per cubic centimeter.

Another method which has been adopted with good results in some cities is the inspection of the dairies and milk by some organization, usually a medical society. Dairies meeting the requirements of the society are given a certificate and are allowed to advertise their milk as certified by the society. The so-called model, or sanitary, dairies furnishing milk under exceptionally clean conditions are becoming somewhat numerous, but, as they are obliged to sell their product at an advance over the usual price, they have not yet become an important factor in the general milk supply. All of these methods are designed to prevent the contamination of milk by bacteria or to inhibit their growth while the milk is being transported from the farm to the consumer. Although they have doubtless resulted in a distinct improvement, it is evident that they have by no means produced a perfect milk supply. An ideal milk would be collected under perfect sanitary conditions from healthy cows and held during transportation at such a temperature that the few bacteria which are found in it, even under the best conditions, would have no opportunity to develop. A milk supply of this nature will come only through a long slow process of education and regulation. In the meantime milk dealers have resorted to various methods to inhibit or destroy the bacteria already present in the milk. The objection to the use of antiseptics is so strong, in this country at least, that this method need not be considered.

PASTEURIZATION.

A method which was at one time looked upon as in some respects a solution of the question of the city milk supply is the application of heat to milk in such a manner that the greater part of the bacteria is destroyed without seriously affecting the character of the milk. This process is known as pasteurization, from the fact that it was first used on an extensive scale in the wine industry of France on the recommendation of Pasteur. In the application of this term to milk it is frequently confused with sterilization, which involves the complete destruction, not necessarily by heat, of all the bacteria present. As commonly practiced, however, pasteurization did not produce the results expected and its use gradually declined. Nevertheless, the statistics of 200 cities and towns collected by the Dairy Division [8] show that considerable milk is still pasteurized, and the practice is probably gaining somewhat in favor. Of the 163 cities answering an inquiry regarding the amount of milk pasteurized, 82, or about 50 per cent, stated that more or less milk was pasteurized. Usually a small amount only was treated, but in some cities it was given as from 10 to 50 per cent of the total supply. Various methods of pasteurization are employed, nearly all of them being efficient if properly used.

To be efficient, pasteurization should destroy practically all of the bacteria in the vegetative stage. This may be accomplished by the application of a comparatively high degree of heat for a short time or of a lower temperature for a longer period. In addition to being efficient, the method should not give the milk a decided cooked taste. In all probability this fault has been responsible for the decreased use of pasteurization. Milkmen have been careful to adopt a temperature low enough to avoid a cooked taste, and as a result a small part only of the bacteria has been destroyed, and the increased keeping quality of the milk has not been sufficient to repay the extra labor. Furthermore, a method to be practical must be economical of heat, labor, and time. A method applicable to a few gallons of milk can not be economically applied to several hundred or a thousand gallons. The simplest apparatus for pasteurizing milk is a vat surrounded by a water jacket, with some arrangement for heating the water. In the machines of this type the milk is heated to the desired temperature and held for some time, usually 15 to 30 minutes. The temperature required for efficient pasteurization is dependent on the length of exposure. The thermal death point of the tubercle bacillus, which is the most resistant of the known nonspore-forming pathogenic bacteria, is usually taken as the criterion for the proper pasteurizing temperature.

Recent work by Theobald Smith [9] and Russell and Hastings [10] has shown that this bacillus may be destroyed by an exposure for 15 to 20 minutes to a temperature of 60° C. (140° F.), provided that the milk is thoroughly stirred to prevent the formation of a film on the surface. In practice a slightly higher temperature (68°–71° C. (155°–160° F.)) is usually adopted. This has been found to be the highest temperature that can be used without causing a distinct cooked taste.

Another apparatus which has been generally adopted where milk is pasteurized on a large scale involves the continuous flow of the milk. In this case the milk flows over a heated surface, where it is raised to the required temperature, and passes on in a continuous stream over the cooler. In one machine of this type the milk passes into a cylinder, where it is thrown by rapidly revolving paddles in a thin sheet on the inner surface of a steam jacket. The milk is heated almost instantly to any temperature desired and passes into the cooler after a very short exposure. In an improved form of this machine the outgoing hot milk is partially cooled by flowing back over the incoming cold milk. One pasteurizer of this type is so arranged that the milk flows between revolving cylinders containing hot water. In another type the milk flows slowly through a vat holding revolving disks filled with water which is heated by a jet of steam.

On account of the short exposure the temperature necessary to insure efficient pasteurization is considerably higher than that used in the machines giving a longer exposure. Harding and Rogers, (11) working with a machine of the continuous rapid-heating type, found that it was very efficient at 80° or 85° C., but inefficient at 70° C. The higher temperatures (80°–85° C.) are recommended by Bang, (12) because the tubercle bacillus is certainly destroyed at these temperatures, even with the very short exposure secured with these machines, and the milk, in addition to having an improved keeping quality, may be guaranteed to be free from pathogenic bacteria. More recent work by Russell and Hastings (13) indicates that a temperature considerably below 80°–85° C. will insure the destruction of the tubercle bacillus, even with the short exposure obtained in continuous pasteurization.

Numerous objections, aside from the extra expense and labor, have prevented the general adoption of this practice. One of the most valid is that pasteurization may be used to correct the faults of insanitary conditions and thus retard the progress of hygienic methods. Milk collected under cleanly conditions and properly transported and delivered does not need pasteurization. Another serious objection in the eyes of the dealer is the changed condition of the fat globules. The cream separates out more slowly, giving the buyer the impression that the milk is deficient in fat. The taste of the milk is also changed more or less, especially if the continuous machines with the higher temperatures are used.

The question of the comparative digestibility of raw and cooked milk has been much discussed and investigated, but can not be considered as definitely decided. It is very generally believed that milk which is highly heated is somewhat less digestible than raw milk, and may in some cases cause pathological conditions due to improper nutrition. On the other hand, it has been shown in numerous instances that children thrive on pasteurized milk. The observations of Variot (14) made on 3,000 infants among the poorer classes of Paris fed on milk sterilized at 108° C. throw some light on this important question. Three or 4 per cent of these children could not use this milk, but the remainder, including those that had been retarded in their development by gastro-intestinal troubles, did well under this treatment. No scurvy or rachitis was observed in these children.

The statement has frequently been made, and is now generally accepted, that the bacteria developing in pasteurized milk are much more undesirable than those growing in raw milk, and that for this reason pasteurized milk may become actually dangerous as a food, while the taste and appearance remain unchanged. It is a matter of common observation that pasteurized milk frequently decomposes with a rank odor without souring, while unheated milk simply turns sour, curdles, and remains unchanged for some time. The reason for this is very evident. In the unheated milk the lactic bacteria develop enormously, forming acid so rapidly that the growth of the peptonizing bacteria is checked or completely prevented. In the pasteurized milk, on the other hand, the lactic bacteria are usually all destroyed, leaving a clear field for the development of the more resistant spore-forming peptonizing bacteria.

Conn and Esten ([15]) state that the development of lactic bacteria serves as a protection both to the milk and the person drinking it, since it prevents the growth of other bacteria. Flügge,([16]) on whose work most of the objection to pasteurized milk is based, heated milk for a short time at 90°–95° C. and isolated the surviving bacteria, which he studied in pure culture. These he found to be mostly of the peptonizing and the anaerobic butyric acid forms. Some of the former produced dangerous toxins.

Weber([17]) found that most of the bacteria developing in so-called sterilized milk were of the hay or potato bacillus type. Flügge's toxin-forming bacteria were found three times in the 150 flasks examined.

It is easy to see how the presence of considerable numbers of bacteria of this class in milk fed to infants might cause serious results either through the production of their toxins in the milk or after being carried themselves to the digestive tract. That this does not always hold true for pasteurized milk is well illustrated by some recent work by Park and Holt ([7]) in New York City. In this work the conditions were controlled as well as it was possible in investigations of this nature. About 50 babies were selected from the tenement houses and divided as accurately as possible into two equal lots. All were fed on milk modified at one of the Straus milk depots and were treated in the same way, except that one half were fed on milk pasteurized at 165° F. for thirty minutes and the other half received the same milk without heating. The raw milk averaged in the morning 1,200,000 bacteria per cubic centimeter and the pasteurized about 1,000 per cubic centimeter, while in the afternoon of the same day they contained respectively about 20,000,000 and 50,000 bacteria per cubic centimeter. In discussing the results of their investigations the authors say:

> Within one week 20 of the 27 infants put on the raw milk suffered from moderate or severe diarrhea, while during the same time only 5 cases of moderate and none of severe diarrhea occurred in those taking pasteurized milk. Within a month 8 of the 27 had to be changed from raw back to heated milk, because of their continued

illness; 7, or 25 per cent, did well all summer on raw milk. On the other hand, of those receiving the pasteurized milk, 75 per cent remained well, or nearly so, all summer, while 25 per cent had one or more attacks of severe diarrhea. There were no deaths in either group of cases.

Very similar results were obtained from a second trial made in the same manner during the following summer. Doctors Park and Holt were, however, unable to find that pasteurization of the milk affected the amount of intestinal trouble in older children.

These results may be taken as conclusive evidence that, under certain circumstances at least, poor city milk is rendered a safer infant food by pasteurization. It must be remembered, however, that this work was done under carefully controlled conditions and that the milk was given out in small bottles only, so that it had to be used before there was time for any great development of deleterious bacteria.

Park and Holt, in the paper cited above, express the belief that intestinal troubles in children fed on poor milk which had been pasteurized were due to changes in the milk which were not neutralized by heat. Lübbert, ([18]) on the other hand, states that the toxic properties of a milk culture of one of Flügge's peptonizing bacteria were destroyed by heating. The results touching on this question are not conclusive, but it seems probable, when the comparatively stable nature of the known toxins is considered, that the toxicity of badly infected milk would not be decreased by the heat received in ordinary pasteurization.

We have seen by this brief review of the literature on milk supply that, notwithstanding the increasing stringency of municipal regulations and efficiency in their enforcement, the milk is usually badly contaminated, frequently even to the point of becoming positively dangerous when used as a food for infants; that some authorities consider pasteurization an advisable remedy for this condition, and that this method is used in many American cities. On the other hand, it appears that some people consider that the bacteria which develop in milk after pasteurization make it more dangerous than poor raw milk.

EXPERIMENTS WITH RAW AND PASTEURIZED MILK.

It is undoubtedly true that bacteria of a very undesirable nature may develop in the clear field left by the destruction of the lactic bacteria, but how soon they develop after pasteurization, what numbers they must attain before the milk becomes dangerous, and how their development compares with the growth of similar bacteria in unheated milk, are questions which have not yet been answered. The writer has been unable to find the results of any investigations giving the quantitative bacteriological examination of pasteurized milk beyond the number present immediately after pasteurization. In undertaking the work, the results of which are given in this paper, it was not intended to attempt to demonstrate that it is advisable to pasteurize or that it is inadvisable, but to determine the rapidity of bacterial development in heated milk and to compare it, quantitatively and qualitatively, with the flora of raw milk. It was planned at the beginning to make this investigation more comprehensive, but certain circumstances have interfered to prevent its completion at this time. It is believed, however, that the results already obtained are of sufficient value to warrant their publication in this incomplete condition.

The milk used in this work was all obtained from a large dairy with an excellent reputation. When delivered, the milk, which was the mixed milk of several herds, was 24 to 36 hours old. The first nine samples were held overnight in a refrigerator to allow bacterial development corresponding to that of the oldest city milk. The remaining samples were pasteurized when received—that is, when 24 to 36 hours old. Although no comprehensive bacteriological examinations of the milk supply of Washington, D. C., have been published, the results of the following determinations, made (with the exception of Nos. 1 and 2) in March and April, indicate that these may be taken as fairly representative of the milk delivered by the large Washington dairies during the warm months:

TABLE 1.—*Bacteria in milk from various sources, at Washington, D. C.*

No.	Obtained from—	Lactic acid.	Total bacteria.	Peptonizing bacteria.
		Per cent.	*Per c. c.*	*Per c. c.*
1	Sanitary dairy		168,000	
2	.....do		118,500	102,000
3	Private city dairy		13,660	0
4	Grocery store	0.144	3,775,000	137,500
5	City dairy	.126	16,880,000	
6	.....do	.126	89,000	

In carrying out the work outlined in the introduction it is essential first of all that the pasteurization be efficient. The method by which this result is obtained is not important. For the sake of convenience a small model made for this work was used, with a temperature of 85° C. (185° F.). This apparatus consisted of a small trough, with an inlet and an outlet tube at opposite ends; a series of revolving hollow disks, so arranged that steam could be blown through them, served the double purpose of heating and stirring the milk. The milk flowed in at one end, was heated to the required temperature, and passed out and over the surface of a cooler which brought the temperature down to about 20°-25° C. The temperature adopted (185° F.) is higher than is ordinarily used, and milk treated in this way has a distinct cooked taste;

but, since this work was arranged only to study the bacteria growing in efficiently pasteurized milk, this fact was of no importance in this instance. Three samples were collected in sterile flasks—one from the milk before pasteurization and two of the pasteurized milk. One of the latter was placed in a refrigerator kept uniformly at 10° C. (50° F.), and the other was kept (with the exception of a few samples which were held in the laboratory) in an incubator holding uniformly at 20° C. (68° F.). In like manner half the samples of raw milk were held at 20° C. and the other half at 10° C. Gelatin plates were made from each sample at the time of pasteurization, and at intervals of 6, 12, and 24 hours, up to 96 hours, or until the milk curdled. Two and one-half per cent lactose gelatin was used. A series of dilutions in sterile water was made so as to get as nearly as possible 300 or 400 colonies on a plate. When the lactic bacteria became numerous the liquefiers were counted on plates containing several thousand colonies. Although this method is probably inaccurate it enables one to count many of the less numerous species, which would be missed in a dilution suited to the lactic forms. All small spherical solid colonies were counted as lactic-acid bacteria. While this grouping would probably include many inert forms the error would not affect the results seriously. The plates were incubated at 20° C. until the spread of liquefying colonies made it necessary to count. In many cases this became necessary before some of the slower-growing colonies had appeared. No attempt was made to study the various species beyond what was necessary to determine the relationship of the colonies occurring on the different plates. In the tables the bacteria are arranged in four different groups—the total bacteria, the acid-forming bacteria, the peptonizing, or liquefying, bacteria, which are subdivided into the slow and rapid liquefiers, and the inert bacteria, or those having no appreciable effect on milk. The action on milk was determined by transferring a number of type colonies from each sample to litmus milk and incubating for several days at 30° C.

The acidity of each sample of milk was titrated against $\frac{N}{10}$ NaOH with phenolphthalein as an indicator when the gelatin plates were made, and the appearance and taste of the milk usually noted when it curdled, or at the end of 96 hours.

TABLE II.—*Physical condition and taste of the milk.*

Sample No.	Treatment.	Temperature.	Age.	Condition.
		° C.	*Hours.*	
11	Raw	Room.	72	Acid curd.
21	Pasteurized	Room.	72	Curdled with slight digestion and gas.
31	.....do	10	120	Appearance unchanged; sweet taste.
12	Raw	10	96	Acid taste.
22	Pasteurized	Room.	96	Curdled; peculiar nutty odor.
32	.....do	10	96	Unchanged; slight off taste.
13	Raw	Room.	48	Partly curdled.
23	Pasteurized	Room.	24	Lost.
33	.....do	10	96	Marked odor of raw oysters.
14	Raw	10	96	Not curdled; pleasant acid taste.
24	Pasteurized	Room.	96	Curdled; bitter taste.
34	.....do	10	96	Slight nutty flavor.
15	Raw	Room.	72	Curdled; pleasant acid taste.
25	Pasteurized	Room.	96	Slight off flavor.
35	.....do	10	96	Sweet; unchanged.
16	Raw	10	96	Not curdled; acid taste.
26	Pasteurized	20	48	Curdled; pleasant acid taste.
36	.....do	10	96	Sweet.
17	Raw	20	48	Curdled; acid with slight cheesy taste.
27	Pasteurized	20	48	Curdled; pleasant acid taste.
37	.....do	10	72	Sweet.
18	Raw	10	96	Acid taste.
28	Pasteurized	20	48	Rennet curd.
38	.....do	10	96	Sweet.
19	Raw	20	48	Curdled; gas.
29	Pasteurized	20	48	Rennet curd.
39	.....do	10	96	Sweet.
110	Raw	10	96	Acid curd.
210	Pasteurized	20	48	Rennet curd, disagreeable taste.
310	.....do	10	96	Sweet.
111	Raw	20	48	Acid curd.
211	Pasteurized	20	48	Curdled.
311	.....do	10	96	Sweet.
112	Raw	10	96	Not curdled; cheesy odor.
212	Pasteurized	20	48	Curdled, bitter astringent taste.
312	.....do	10	96	Sweet.
113	Raw	20	24	Curdled, clean acid taste.
213	Pasteurized	20	48	Rennet curd
313	.....do	10	96	Sweet.
114	Raw	10	96	Sour taste, not curdled.
214	Pasteurized	20	48	Curdled, slight digestion.
314	.....do	10	96	Sweet.

It will be noticed that the unheated milk held at 20° C. usually curdled in 48 to 72 hours with an acid taste, although at times there were indications of peptonization. In the raw milk at 10° C. the curdling was much retarded and the taste was affected by presence of digesting bacteria in one or two cases. The pasteurized milk held at 20° C., on the other hand, usually had a rennet curd at the end of 48 hours, with a disagreeable taste, although in a few instances the lactic bacteria were evidently not entirely destroyed and formed an acid curd in 48 hours. The pasteurized milk held at 10° C. almost without exception remained perfectly sweet to the taste and unchanged in appearance for 96 hours or more.

TABLE III.—*Acidity of milk expressed as per cent of lactic acid.*

No.	Treatment.	Temperature.	Percentage of lactic acid after lapse of—						
			0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
		°C.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
11	Raw	Room.	0.126	0.133	0.140	0.234	0.594	0.720	
21	Pasteurized	Room.	.126	.126	.110	.110	.126	.560	
31	....do	10	.126	.126	.126	.126	.126	.126	0.126
12	Raw	10	.144	.216	.270	.324	.432	.468	.522
22	Pasteurized	Room.	.144	.144	.162	.144	.144	.180	.645
32	....do	10	.144	.144	.162	.144	.144	.162	.162
13	Raw	Room.	.126	.162	.234	.342	.648		
23	Pasteurized	Room.	.126	.126	.126				
33	....do	10	.126	.126	.126	.144	.144	.216	.306
14	Raw	10	.144	.144	.126	.162	.230	.486	.630
24	Pasteurized	Room.	.144	.144	.126	.126	.126	.180	.216
34	....do	10	.144	.108	.108	.126	.126	.126	.126
15	Raw	Room.	.162	.162	.180	.270	.504	.702	
25	Pasteurized	Room.	.162	.126	.108	.144	.144	.162	.216
35	....do	10	.162	.126	.126	.144	.144	.126	.126
16	Raw	10	.126	.144	.162	.144	.162	.216	.230
26	Pasteurized	20	.126	.126	.108	.126	.666		
36	....do	10	.126	.126	.126	.108	.126	.126	.126
17	Raw	20	.144	.216	.414	.694	.864		
27	Pasteurized	20	.144	.144	.144	.144	.702		
37	....do	10	.144	.144	.144	.144	.144	.144	
18	Raw	10	.144	.144	.162	.180	.234	.342	.612
28	Pasteurized	20	.144	.144	.144	.144	.162		
38	....do	10	.144	.144	.144	.126	.126	.126	.126
19	Raw	20	.126	.180	.252	.486	.810		
29	Pasteurized	20	.126	.144	.126	.126	.162		
39	....do	10	.126	.126	.144	.126	.126	.126	.144
110	Raw	10	.144	.162	.162	.162	.270	.648	.666
210	Pasteurized	20	.144	.144	.126	.126	.144		
310	....do	10	.144	.126	.126	.126	.144	.126	.126
111	Raw	20	.108	.162	.252	.448	.756		
211	Pasteurized	20	.108	.126	.126	.126	.468		
311	....do	10	.108	.126	.126	.126	.126	.126	.144
112	Raw	10	.126	.126	.144	.144	.162	.306	
212	Pasteurized	20	.126	.126	.126	.126	.126		
312	....do	10	.126	.126	.126	.126	.126	.126	.126
113	Raw	20	.126	.162	.306	.666			
213	Pasteurized	20	.126	.126	.126	.126	.144		
313	....do	10	.126	.126	.126	.126	.126	.126	.126
114	Raw	10	.126	.126	.144	.126	.144	.252	.324
214	Pasteurized	20	.126	.126	.126	.126	.144		
314	....do	10	.126	.126	.126	.126	.126	.126	.126

For convenience of comparison these results are averaged and arranged in Table IV.

TABLE IV.—*Average increase in acidity of milk expressed as per cent of lactic acid.*

Treatment.	Percentage of lactic acid after lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Raw milk kept at 20° C.	0.131	0.168	0.251	0.449	0.696	0.711	....
Pasteurized milk kept at 20° C.	.134	.131	.127	.130	.251	.271	0.359
Raw milk kept at 10° C.	.136	.152	.167	.177	.233	.388	.497
Pasteurized milk kept at 10° C.	.131	.129	.131	.129	.129	.130	.132

As would naturally be expected, the increase in acidity of the unheated milk was much more rapid at 20° C. than at 10° C. The average of the increase in acidity of the pasteurized milk at 20° C. does not express the true state of affairs, as the increase was usually very slight, except in a few instances when the rapid multiplication of lactic bacteria caused a sudden and marked increase in the acidity. In the pasteurized milk at 10° C. there was usually no increase whatever, and in one lot only was there sufficient acid to affect the taste.

The complete results of the bacteriological counts are given in Table V. These are summarized in Table VI, which contains the mean of the total bacteria, and in Table VIII, which contains the mean of the peptonizing bacteria.

TABLE V.—*Bacteria per cubic centimeter of milk, arranged in groups.*

SAMPLE NO. 11.—RAW MILK KEPT AT ROOM TEMPERATURE.

Group.	Number of bacteria found after lapse of—						
	0 hours.[a]	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	835,000	12,825,000	75,032,500	671,500,000	560,625,000	150,937,000	....
Total lactic	....	....	....	....	....	....	....
Total liquefiers	6,500	25,000	225,000	1,932,500	500,000	0	....
Rapid liquefiers	....	....	....	....	....	....	....
Slow liquefiers	....	....	....	....	....	....	....
Inert	....	....	....	....	....	....	....

SAMPLE NO. 21.—PASTEURIZED MILK KEPT AT ROOM TEMPERATURE.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	48	198	220	99,700	(b)	132,000,000	....
Total lactic	....	....	....	....	....	132,000,000	....
Total liquefiers	2	4	80	1,000	1,700,000	0	....
Rapid liquefiers	....	....	....	....	....	....	....
Slow liquefiers	....	....	....	....	....	....	....
Inert	46	....	140	82,000	....	....	....

SAMPLE NO. 31.—PASTEURIZED MILK KEPT AT 10° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	48	318	536	4,570	2,115	197,000	38,830,000
Total lactic	0	0	0	0	0	0	0
Total liquefiers	2	7	0	30	355	2,200	920,000
Rapid liquefiers	....	....	....	....	....	....	....
Slow liquefiers	....	....	....	....	....	....	....
Inert	46	311	536	1,540	1,780	194,800	37,410,000

[a] The age of the milk given in the tables is reckoned from the time of pasteurization.
[b] Dilution too low.

TABLE V.—*Bacteria per cubic centimeter of milk, arranged in groups*—Continued.

SAMPLE NO. 12.—RAW MILK KEPT AT 10° C.

Group.	Number of bacteria found after lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	103,925,000	139,500,000	153,000,000	266,000,000	618,000,000	152,500,000	354,000,000
Total lactic	....	....	....	....	....	....	....
Total liquefiers	....	75,000	100,000	....	500,000	....	....
Rapid liquefiers	....	....	....	....	....	....	....
Slow liquefiers	....	....	....	....	....	....	....
Inert	....	....	....	....	....	....	....

SAMPLE NO. 22.—PASTEURIZED MILK KEPT AT ROOM TEMPERATURE.

Total bacteria	70	23	50	1,200	1,475,000	40,740,000	308,000,000
Total lactic	....	....	....	....	1,175,000	40,440,000	298,750,000
Total liquefiers	4	0	8	800	7,500	150,000	....
Rapid liquefiers	....	....	....	800	....	....	....
Slow liquefiers	....	....	....	0	....	....	....
Inert	....	....	....	....	225,000	150,000	9,250,000

SAMPLE NO. 32.—PASTEURIZED MILK KEPT AT 10° C.

Total bacteria	70	70	30	60	70	6	60,600
Total lactic	....	....	....	....	....	....	59,800
Total liquefiers	4	4	2	10	0	....	300
Rapid liquefiers	....	....	....	....	....	....	....
Slow liquefiers	....	....	....	....	....	....	....
Inert	....	....	....	....	....	....	500

SAMPLE NO. 13.—RAW MILK KEPT AT ROOM TEMPERATURE.

Total bacteria	33,707,500	83,062,500	168,812,500	231,750,000	947,250,000	....	....
Total lactic	32,945,500	80,830,500	165,625,000	226,937,500	946,750,000	....	....
Total liquefiers	762,000	2,232,000	3,187,500	4,812,500	500,000	....	....
Rapid liquefiers	....	....	....	1,812,500	....	....	....
Slow liquefiers	....	....	....	3,000,000	....	....	....
Inert	....	....	....	....	....	....	....

SAMPLE NO. 23.—PASTEURIZED MILK KEPT AT ROOM TEMPERATURE.

Total bacteria	4,040	29,320	(*a*)	(*b*)	....	....	....
Total lactic	....	....	....	....	....	....	....
Total liquefiers	11	50	....	....	....	....	....

SAMPLE NO. 33.—PASTEURIZED MILK KEPT AT 10° C.

Total bacteria	4,040	6,390	46,150	843,000	Liquefied.	34,375,000	247,750,000
Total lactic	....	....	....	....	....	1,875,000	29,250,000
Total liquefiers	12	190	720	4,500	....	5,000,000	3,000,000
Rapid liquefiers	....	....	....	....	....	2,625,000	....
Slow liquefiers	....	....	....	....	....	2,375,000	3,000,000
Inert	....	....	....	....	....	27,500,000	215,500,000

[a] Dilution too low.

[b] Flask broken.

TABLE V.—*Bacteria per cubic centimeter of milk, arranged in groups*—Continued.

SAMPLE NO. 14.—RAW MILK KEPT AT 10° C.

Group.	Number of bacteria found after lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	11,125,000	16,125,000	49,250,000	388,500,000	266,500,000	432,750,000	1,102,000,000
Total lactic							
Total liquefiers	140,000	157,000	3,312,000	5,250,000	9,337,500	18,000,000	11,625,000
Rapid liquefiers	55,000		750,000				
Slow liquefiers	85,000		2,562,000				

SAMPLE NO. 24.—PASTEURIZED MILK KEPT AT ROOM TEMPERATURE.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	50	50	30	8,500	49,600,000	471,500,000	1,613,000,000
Total lactic							
Total liquefiers	10	0	10	2,750	8,360,000	90,000,000	61,000,000
Rapid liquefiers	10		10	2,750	1,540,000	37,500,000	
Slow liquefiers					6,820,000	52,500,000	
Inert				5,750	41,240,000	381,500,000	

SAMPLE NO. 31.—PASTEURIZED MILK KEPT AT 10° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	50	50		160	148,000	21,600,000	277,250,000
Total lactic							
Total liquefiers	10	0		50	30,900	11,200,000	46,750,000
Rapid liquefiers	10			50	11,500		
Slow liquefiers					19,400		

SAMPLE NO. 15.—RAW MILK KEPT AT ROOM TEMPERATURE.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	3,123,750	13,725,000	66,625,000	158,750,000	795,000,000	686,500,000	
Total lactic	2,991,250	12,400,000	63,475,000	138,500,000	773,500,000	672,625,000	
Total liquefiers	132,500	900,000	1,800,000	10,250,000	3,000,000	1,125,000	
Rapid liquefiers	70,000	450,000	750,000	5,000,000	500,000	2,750,000	
Slow liquefiers	62,500	450,000	1,050,000	5,250,000	5,500,000	1,375,000	
Inert		125,000	1,350,000	10,000,000	18,500,000	9,750,000	

SAMPLE NO. 25.—PASTEURIZED MILK KEPT AT ROOM TEMPERATURE.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	110	50	50	200	1,000	3,525,000	
Total lactic							
Total liquefiers	0	0	0	200	1,000	3,525,000	
Rapid liquefiers				200	1,000	3,525,000	
Slow liquefiers							
Inert							

SAMPLE NO. 35. PASTEURIZED MILK KEPT AT 10° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	110	50	10	2,790	5,860	6,430	7,720
Total lactic							
Total liquefiers	0	30	0	0	0	30	20
Rapid liquefiers						20	20
Slow liquefiers						10	
Inert				2,790	5,860	6,400	7,700

TABLE V.—*Bacteria per cubic centimeter of milk, arranged in groups*—Continued.

Group.	Number of bacteria found after lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
SAMPLE NO. 16.—RAW MILK KEPT AT 10° C.							
Total bacteria	5,325,000	21,500,000	30,875,000	75,000,000	314,000,000		480,500,000
Total lactic	4,550,000	18,000,000	29,200,000	70,550,000	283,250,000		378,000,000
Total liquefiers	125,000	1,500,000	1,400,000	2,500,000	24,250,000	Plates	99,000,000
Rapid liquefiers	75,000	500,000	1,100,000	2,500,000	21,250,000	liquefied.	65,500,000
Slow liquefiers	50,000	1,000,000	300,000	....	3,000,000		33,500,000
Inert	650,000	2,000,000	275,000	1,750,000	6,500,000		3,500,000
SAMPLE NO. 26.—PASTEURIZED MILK KEPT AT 20° C.							
Total bacteria	20	40	220	1,040,000	1,581,000,000	....	....
Total lactic	....	....	....	960,000	1,581,000,000	....	....
Total liquefiers	0	30	90	80,000	....	....	....
Rapid liquefiers	....	10	90	10,000	....	....	....
Slow liquefiers	....	20	....	70,000	....	....	....
Inert	....	....	....	....	....	....	....
SAMPLE NO. 36.—PASTEURIZED MILK KEPT AT 10° C.							
Total bacteria	20	40	20	30	110	2,430	67,900
Total lactic	....	....	....	....	....	2,430	67,880
Total liquefiers	0	30	10	20	10	0	20
SAMPLE NO. 17.—RAW MILK KEPT AT 20° C.							
Total bacteria	37,950,000	136,000,000	317,000,000	78,000,000	452,500,000	....	....
Total lactic	34,725,000	110,750,000	314,750,000	....	452,350,000	....	....
Total liquefiers	3,075,000	24,500,000	1,750,000	1,000,000	25,000	....	....
Rapid liquefiers	25,000	1,250,000	500,000	....	....	....	....
Slow liquefiers	3,050,000	23,250,000	1,250,000	1,000,000	25,000	....	....
Inert	150,000	750,000	500,000	....	125,000	....	....
SAMPLE NO. 27.—PASTEURIZED MILK KEPT AT 20° C.							
Total bacteria	2,220	2,800	75,500	15,650,000	1,227,500,000	....	....
Total lactic	....	....	72,920	15,552,500	1,218,250,000	....	....
Total liquefiers	10	0	280	30,000	750,000	....	....
Rapid liquefiers	10	....	....	....	500,000	....	....
Slow liquefiers	....	....	280	30,000	250,000	....	....
Inert	....	....	300	67,500	7,500,000	....	....
SAMPLE NO. 37.—PASTEURIZED MILK KEPT AT 10° C.							
Total bacteria	2,220	2,860	2,350	3,130	36,500	6,715,000	....
Total lactic	....	....	....	....	....	....	....
Total liquefiers	10	0	10	10	120	70,000	....
Rapid liquefiers	10	....	....	....	90	....	....
Slow liquefiers	....	....	....	....	30	....	....
Inert	....	....	....	....	36,380	6,645,000	....

TABLE V.—*Bacteria per cubic centimeter of milk, arranged in groups*—Continued.

Group.	Number of bacteria found after lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
SAMPLE NO. 18.—RAW MILK KEPT AT 10° C.							
Total bacteria	2,600,000	9,575,000	24,250,000	58,500,000	189,500,000	191,500,000	215,500,000
Total lactic	2,425,000	8,635,000	22,700,000	52,500,000	183,500,000		189,700,000
Total liquefiers	125,000	800,000	1,225,000	5,750,000	4,500,000		1,800,000
Rapid liquefiers	25,000	575,000	775,000	3,500,000	1,500,000		1,475,000
Slow liquefiers	100,000	225,000	450,000	2,250,000	3,000,000		325,000
Inert	50,000	150,000	325,000	1,250,000	1,500,000		
SAMPLE NO. 28.—PASTEURIZED MILK KEPT AT 20° C.							
Total bacteria	80	30	420	115,000	9,100,000		
Total lactic							
Total liquefiers	0	10	290	75,000	875,000		
Rapid liquefiers	0	10	170	55,000	725,000		
Slow liquefiers			120	20,000	150,000		
Inert							
SAMPLE NO. 38.—PASTEURIZED MILK KEPT AT 10° C.							
Total bacteria	80	60	10	10	70	8,500	532,500
Total lactic							
Total liquefiers	0	0	0	0	10	750	0
Rapid liquefiers					10	750	
Slow liquefiers							
Inert						7,750	532,500
SAMPLE NO. 19.—RAW MILK KEPT AT 20° C.							
Total bacteria	10,287,500	[a]9,500,000	([a])	40,875,000	93,600,000		
Total lactic	10,062,500			40,550,000	93,500,000		
Total liquefiers	175,000			225,000	50,000		
Rapid liquefiers	75,000			100,000	50,000		
Slow liquefiers	100,000			125,000			
Inert	50,000			100,000	50,000		
SAMPLE NO. 29.—PASTEURIZED MILK KEPT AT 20° C.							
Total bacteria	15	220	140	110	([a])		
Total lactic							
Total liquefiers	10	5	10	110			
Rapid liquefiers	10	5	10	110			
Slow liquefiers							
Inert							
SAMPLE NO. 39.—PASTEURIZED MILK KEPT AT 10° C.							
Total bacteria	15	80	10	90	70	1,900	9,000
Total lactic							
Total liquefiers	10	10	10	10	10	200	
Rapid liquefiers	10	10	10	10	10		
Slow liquefiers						200	
Inert						1,700	9,000

[a] Dilution too high.

TABLE V.—*Bacteria per cubic centimeter of milk, arranged in groups*—Continued.

SAMPLE No. 110.—RAW MILK KEPT AT 10° C.

Group.	Number of bacteria found after lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	252,500	1,082,500	8,200,000	57,000,000	211,250,000	537,000,000	661,250,000
Total lactic	185,000	862,500	5,600,000	40,040,000	181,375,000	516,550,000	637,000,000
Total liquefiers	67,500	210,000	2,575,000	15,960,000	26,875,000	16,750,000	19,250,000
Rapid liquefiers	40,000	132,500	1,225,000	5,210,000	19,875,000	10,000,000	13,175,000
Slow liquefiers	27,500	77,500	1,350,000	10,750,000	7,000,000	6,750,000	6,125,000
Inert		10,000	25,000	1,000,000	3,000,000	3,700,000	5,000,000

SAMPLE No. 210.—PASTEURIZED MILK KEPT AT 20° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	40	60	40	150	[a] 7,500		
Total lactic							
Total liquefiers	0	20	10	150	2,500		
Rapid liquefiers		20	10	150	2,500		
Slow liquefiers							
Inert							

SAMPLE No. 310.—PASTEURIZED MILK KEPT AT 10° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	40	30	35	40	30	200	2,800
Total lactic							
Total liquefiers	0	10	5	30		150	2,800
Rapid liquefiers			5	20		150	
Slow liquefiers		10		10			2,800
Inert							

SAMPLE No. 111.—RAW MILK KEPT AT 20° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	7,050,000	106,450,000	229,437,500	543,000,000	799,500,000		
Total lactic	6,847,500	105,300,000	225,187,500	540,750,000	799,300,000		
Total liquefiers	180,000	825,000	3,500,000	2,000,000	125,000		
Rapid liquefiers	20,000	175,000	500,000	625,000			
Slow liquefiers	160,000	650,000	3,000,000	1,375,000	125,000		
Inert	22,500	325,000	750,000	250,000	75,000		

SAMPLE No. 211.—PASTEURIZED MILK KEPT AT 20° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	70	835	185	38,700	([b])		
Total lactic				36,900			
Total liquefiers	20	35	90	1,400	397,500		
Rapid liquefiers	20	10	30		22,500		
Slow liquefiers		25	60	1,400	375,000		
Inert		800		400			

SAMPLE No. 311.—PASTEURIZED MILK KEPT AT 10° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	70	95	1,000	920	1,630	273,900	25,920,000
Total lactic						273,000	25,917,900
Total liquefiers	20	20	25	10		900	2,100
Rapid liquefiers	20			10		300	
Slow liquefiers		20	25			600	2,100
Inert							

[a] Dilution too high. [b] Dilution too low.

TABLE V.—*Bacteria per cubic centimeter of milk, arranged in groups*—Continued.

SAMPLE NO. 112.—RAW MILK KEPT AT 10° C.

Group.	Number of bacteria found after lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	217,500	965,000	3,012,500	25,162,500	155,812,500	221,500,000	
Total lactic	170,000	900,000	2,587,500	23,162,500	144,247,500		
Total liquefiers	32,500	52,500	325,000	1,950,000	3,690,000	11,000,000	
Rapid liquefiers	12,500	12,500	75,000	500,000	375,000		
Slow liquefiers	20,000	40,000	250,000	1,450,000	3,350,000		
Inert	15,000	12,500	100,000	350,000	7,875,000		

SAMPLE NO. 212.—PASTEURIZED MILK KEPT AT 20° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	192	315	495	1,184,800			
Total lactic							
Total liquefiers	5		495	1,184,800			
Rapid liquefiers			495	1,184,800			
Slow liquefiers	5						
Inert							

SAMPLE NO. 312.—PASTEURIZED MILK KEPT AT 10° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	192	132	12	110	110	55,600	
Total lactic							
Total liquefiers	5	15	0	5		50	
Rapid liquefiers		5		5		50	
Slow liquefiers	5	10					
Inert						55,550	

SAMPLE NO. 113.—RAW MILK KEPT AT 20° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	1,802,500	157,137,500	629,000,000	1,184,500,000			
Total lactic	1,776,759	155,137,500	628,000,000	1,180,925,000			
Total liquefiers	20,000	950,000	425,000	275,000			
Rapid liquefiers	750	150,000	75,000				
Slow liquefiers	19,250	800,000	350,000	275,000			
Inert	5,750	1,050,000	575,000	300,000			

SAMPLE NO. 213.—PASTEURIZED MILK KEPT AT 20° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	120	815	500	1,054,000	13,075,000		
Total lactic							
Total liquefiers	10	25	500	1,054,000	13,075,000		
Rapid liquefiers	10		500	1,054,000	13,075,000		
Slow liquefiers		25					
Inert							

SAMPLE NO. 313.—PASTEURIZED MILK KEPT AT 10° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	120	130	397	110	1,610	386,950	11,037,500
Total lactic							
Total liquefiers	10	10	20	15	20	250	3,250
Rapid liquefiers	10	10	10	10	10	250	3,250
Slow liquefiers			10	5	10		
Inert					1,590	386,700	11,034,250

TABLE V.—*Bacteria per cubic centimeter of milk, arranged in groups*—Continued.

SAMPLE NO. 114.—RAW MILK KEPT AT 10° C.

Group.	Number of bacteria found after lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	38,000		260,000	3,025,000	27,687,500	313,000,000	
Total lactic	33,250		202,500	2,725,000	25,862,500	305,125,000	
Total liquefiers	3,250	12,500	50,000	225,000	1,600,000	5,375,000	
Rapid liquefiers	2,500	12,500	45,000	225,000	1,600,000	3,750,000	
Slow liquefiers	750		5,000			1,625,000	
Inert	1,500		7,500	75,000	225,000	2,500,000	

SAMPLE NO. 214.—PASTEURIZED MILK KEPT AT 20° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	122	50	520	25,000	1,275,000		
Total lactic							
Total liquefiers	10	10	460	25,000	1,275,000		
Rapid liquefiers	10	10	460	25,000	1,275,000		
Slow liquefiers							
Inert							

SAMPLE NO. 314.—PASTEURIZED MILK KEPT AT 10° C.

Group.	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Total bacteria	122	60	40		380	2,000	54,255,000
Total lactic							
Total liquefiers	10	10	10	15	10	100	4,000
Rapid liquefiers	10	10	10	15	10	100	4,000
Slow liquefiers							
Inert					370	1,900	54,251,000

TABLE VI.—*Average number of bacteria per cubic centimeter in all samples under each treatment.*

Description and treatment of sample.	Number of bacteria after the lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Raw milk kept at 20° C	13,522,331	74,142,857	247,651,250	457,910,714	608,079,166	568,718,500	
Pasteurized milk kept at 20° C	245	426	6,028	1,501,335	320,337,388	236,911,250	975,500,000
Raw milk kept at 10° C	17,640,428	31,457,833	38,406,785	124,783,928	254,678,542	308,041,666	562,650,000
Pasteurized milk kept at 10° C	245	308	378	1,026	15,119	2,462,492	37,088,456

Probably the most striking thing in these tables, especially to the person not familar with the bacteriology of milk, is the enormous number of bacteria usually found in the raw milk after standing two or three days. This number always reached several hundred million per cubic centimeter, and occasionally went as high as 1,000,000,000. Even higher numbers have been obtained ([15]).

Accepting Cohn's ([19]) calculation of the weight of a single bacillus as 0.000,000,001,571 milligram, the weight of 1,000,000,000 bacteria, a number not infrequently found in a cubic centimeter of milk, would be over 1½ milligrams. In other words, over 1½ tenths of 1 per cent of the total weight of the milk has been converted into plant tissue. But even this does not tell the whole truth. In the course of their growth the bacteria have converted a considerable proportion of the milk solids into by-products of growth. If, as is usually the case, when such large numbers are found, the bacteria are of the lactic-acid class, the decomposition is of a comparatively harmless nature, although in each cubic centimeter of milk 5.7 milligrams of lactose may have been converted into 6 milligrams of lactic acid; on the other hand, if many bacteria of the liquefying group are present, each tiny cell, by secreting its digestive enzymes, minute in the individual but potentially great in the aggregate, is able to produce results all out of proportion to its infinitesimal weight.

The influence of low temperatures in retarding the growth of bacteria is well known, but it may be of interest to compare the increase under the conditions of this experiment. In considering the multiplication of a single cell in the raw milk, it should be remembered that at the time the count began the bacteria had already passed the period of rapid increase. For this reason the increase of the bacteria in the pasteurized milk, as tabulated in Table VII, can not be compared with the increase in the raw milk under similar conditions.

TABLE VII.—*Increase of a single bacterium in one cubic centimeter of milk.*

Description and treatment of sample.	Number of bacteria found after the lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Raw milk at 20° C..........	1	5.5	18.3	33.9	41.9	42.5	....
Raw milk at 10° C..........	1	1.7	2.2	7.1	11.1	17.4	31.1
Pasteurized milk at 20° C ..	1	1.7	21.2	6,128	307,199	967,107	3,981,632
Pasteurized milk at 10° C ..	1	1.2	1.5	4.1	6.2	10,051	143,218

It will be seen that the progeny of a single cell in the raw milk held at 10° C. had not reached in 96 hours the number attained in the raw milk at 20° C. in 24 hours. In the pasteurized milk the conditions are different in that the count beginning when the milk contained a very few bacteria only extends over the period of rapid increase. The bacterial multiplication was very rapid in the pasteurized milk held at 20° C., a single organism reproducing itself three hundred thousand times in 48 hours. The pasteurized milk held at 10° C. presents a striking contrast; the multiplication was very slow for the first 48 hours, and at the end of 96 hours the increase was far below that in the pasteurized milk at the higher temperature.

Returning to the actual numbers of bacteria in the milk we find that at the time of pasteurization the mean of the samples held at 20° C. was 13,000,000 per cubic centimeter, while the milk held at 10° C. shows a slightly higher number. This probably represents poor rather than average city milk, but may be taken as a fair sample of milk needing pasteurization. The reduction of this number to 245 per cubic centimeter may be considered as efficient pasteurization, probably a higher efficiency than would be obtained under ordinary commercial conditions. The variation from the mean was slight; the minimum number found was 20 per cubic centimeter and the maximum 4,040. The latter was so exceptional that the results from this sample (No. 33) were excluded in making up the means.

Milk under ordinary conditions is held in a refrigerator at 10° to 15° C. and is consumed usually within 12 hours or at most 24 hours after delivery. The milk held at 10° C. will therefore represent normal conditions more nearly than that held at 20° C., but it will be of interest to study the bacterial development in the milk held at the higher temperature.

In the raw milk over 200,000,000 bacteria per cubic centimeter were found at 12 hours, while the same quantity of pasteurized milk contained about 6,000 only; 12 hours later the latter number had increased to over 1,000,000, and at 48 hours the difference in the total bacteria present in the two cases was small. There is more or less variation from these means in individual cases, but the frequency of their occurrence makes it safe to predict that pasteurized milk held at 20° C. for 24 hours or more will have a high bacterial content.

The growth of bacteria in the pasteurized milk at the low temperature is so slow that at 48 hours after pasteurization the number of bacteria was less than that usually found in sanitary milk, and even at 96 hours was not much greater than the total number in the milk before it was heated. The results obtained at this temperature were uniform, showing much variation at 72 hours and 96 hours only. At these two periods two or three exceptionally high results have raised the general mean considerably above that of the remaining counts.

Milk showing an acidity of about 0.25 per cent has a distinct sour taste, and would not be considered fit for use after it had reached this condition. The average raw milk had reached this stage at 48 hours, while the pasteurized milk was, so far as the total bacteria show, in good condition 24 and even 48 hours later. At 20° the difference was somewhat less although, as will be pointed out later, the nature of the bacteria developing in the pasteurized milk at the higher temperature has some influence on the length of time during which it may be considered fit for use.

It is, however, very unsafe to judge the quality of the milk by the total number of bacteria present without regard to their nature. A few hundred thousand bacteria of certain species may be more harmful than a hundred million of another class. For this reason it will be more profitable to consider the bacteria grouped under the head of peptonizing bacteria, as this group includes the bacteria generally acknowledged to be the cause of the most undesirable changes in the milk. It is difficult to secure accurate counts of liquefying bacteria, especially when they are growing with lactic-acid bacteria, but the results given in Table VIII may be taken as a fair representation of the growth of this group under these conditions.

TABLE VIII.—*Average number of peptonizing bacteria per cubic centimeter of milk.*

Description and treatment of sample.	Number of bacteria found after the lapse of—						
	0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
Raw milk at 20° C.	621,571	4,905,333	1,814,583	2,927,857	700,000	1,375,000	....
Pasteurized milk at 20° C.	7	11	188	259,831	2,411,163	31,225,000	....
Raw milk at 10° C.	82,208	505,333	1,518,666	5,272,500	11,708,750	12,781,250	32,918,750
Pasteurized milk at 10° C.	7	11	9	15	3,143	*856,219	4,251,219

In making up the mean for the raw milk at 20° C. those determinations in which some unusual circumstance made the count evidently inaccurate are not included. In this milk the mean shows a rapid increase of liquefying bacteria for the first 6 hours, followed by a gradual decline. In the samples held at room temperature, which at the time this work was done was usually below 20° C., the maximum was reached in all cases at 24 hours, but a few high numbers in the later samples held at 20° C. bring the mean maximum down to 6 hours. The variation in the actual number of peptonizing bacteria present in the different samples was large, ranging from a few thousand to 24,000,000 per cubic centimeter.

In the pasteurized milk held at 20° C. the peptonizing bacteria, freed from the inhibiting influence of the lactic group, increased uniformly. The average number found at 24 hours was a little over 200,000 per cubic centimeter, increasing to over 2,000,000 at 48 hours and to 30,000,000 at 72 hours. The number present during the first 12 hours was insignificant. There is considerable variation at this temperature and more or less inaccuracy, owing to the difficulty in securing a correct count of a large number of rapid liquefiers.

In the raw milk held at 10° C. the multiplication of liquefiers was less rapid, but continued for a longer period, and reached in one case (sample No. 16) 99,000,000 in 96 hours, although the lactic bacteria were present in large numbers from the beginning. An examination of Table III shows that the acidity of this milk at 96 hours was 0.23 per cent only, which is a little below the mean for 48 hours. Excluding this exceptionally high figure the mean number of liquefiers present at 96 hours would be 10,891,666, indicating a slight falling off after 72 hours.

The counts of the liquefying bacteria in the pasteurized milk at 10° C. indicate that there was scarcely any growth for 48 hours, and even after 96 hours the number found was no greater than that of the unheated milk 72 hours earlier. In other words, the pasteurized milk was in better condition, so far as bacteriological examination shows, when 48 hours old than the unheated milk at the time of pasteurization. In many cases there were very few liquefiers even at 96 hours.

The variation in species occurring in these samples of milk was not great. Three or four species of liquefiers were almost uniformly present and all multiplied more or less, although, after the influence of the lactic-acid bacteria began to be felt, this number was usually reduced to one or two varieties. The most common and persistent of these was the hay bacillus.

Two or three species of liquefiers usually survived pasteurization. In the heated milk held at 20° C., the great increase was confined, almost without exception, to the hay bacillus. The rapid growth of the colonies of this organism usually prevented the determination of the slower growing, less abundant species.

The hay bacillus developed much more slowly in the pasteurized milk at 10° C. For this reason it was possible to ascertain the presence of a few other species, none of which could be called predominant.

The bacteria included under the heading "inert" were mostly of a group forming white colonies spreading slightly on the surface of the gelatin. In the pasteurized milk, however, and especially the part held at 10° C., there frequently occurred a considerable increase of a variety forming small round colonies resembling the colonies of the lactic group, but without appreciable effect on milk. The lactic-acid bacteria which survived pasteurization were found, in nearly every case, to be identical with the predominating lactic species in the corresponding unheated milk.

The qualitative difference in the flora of the pasteurized and unpasteurized milk consisted in the absence, with a few exceptions, of the lactic group from the latter, the small reduction in the number of varieties of peptonizing bacteria, the rapid multiplication of the hay bacillus in the heated milk at 20° C., and the presence in some cases of large numbers of inert bacteria in the pasteurized milk held at 20° C.

INFLUENCE OF LACTIC BACTERIA ON THE DEVELOPMENT OF PEPTONIZING BACTERIA.

Taking up the question of the protective action of the lactic group, we find that when both the lactic and the peptonizing bacteria are growing together in milk, as in the unheated milk in these experiments, there was a rapid increase in the latter for a short time, followed by a period of slow decrease, or at least a complete prevention of multiplication. This was not confined to a single species, but included all liquefiers found in these milks. Reference to Table IX shows that this inhibition was coincident with the development of from 0.25 per cent to 0.3 per cent acidity:

TABLE IX.—*Relation of acidity of milk to development of peptonizing bacteria.*

		Percentage of acidity and number of bacteria after the lapse of—						
		0 hours.	6 hours.	12 hours.	24 hours.	48 hours.	72 hours.	96 hours.
20°	Acidity	.131	.168	.254	.449	.696	.711	....
	Peptonizing bacteria	621,577	1,905,333	1,844,583	2,927,000	700,000	1,375,000	....
10°	Acidity	.138	.153	.168	.183	.245	.417	.551
	Peptonizing bacteria	73,650	306,400	1,512,400	5,827,000	9,200,500	12,781,250	10,891,666

In the raw milk at 20° C., the growth of the liquefiers was checked between 6 hours and 12 hours; in this period the acidity increased from 0.166 per cent to 0.254 per cent. In the raw milk at 10° C., the continued development of the liquefiers was proportionate to the slow increase in the acidity. If we exclude No. 16, which, as has already been explained, showed an exceptionally slow increase in acidity, there was little increase after 48 hours, when the acidity had reached 0.245 per cent. The samples (Nos. 21, 22, 26, and 27) in which there was a sudden multiplication of lactic-acid bacteria, with a correspondingly increased acidity in the pasteurized milk, were accompanied by a sharp decrease in the liquefying bacteria.

Bouska, [20] who has done some extensive work along this line, states that, while the major part of the inhibitory action exercised by the lactic bacteria on *B. subtilis* is due to lactic acid, it is also evident in media containing no sugar.

Thus it seems evident that the protective influence of the lactic group of bacteria becomes effective only when the acidity has reached a point that renders the milk undesirable for food. The tables given in the paper by Conn and Esten, [15] previously quoted, show a more or less rapid increase of liquefiers for a time, depending on the temperature at which the milk was held. Although the acidity is not given, the number of lactic bacteria present makes it probable that the liquefiers increased until the milk was at least slightly sour.

This applies, however, to the number of liquefiers present, and does not take into account the effect of the acid reaction on the proteolytic enzymes secreted by this group of bacteria. The pasteurized milk held at 20° C. frequently curdled 48 hours after pasteurization, with a very disagreeable taste, although the mean number of peptonizing bacteria found at this time was only 2,500,000 per cubic centimeter, while the raw milk containing several times as many bacteria of this group, in addition to the lactic bacteria, usually remained unchanged except for a slight sour taste. It must be remembered, however, that the acidity was sufficient to obscure any change except a decided one.

In one instance (sample No. 16), in which the raw milk contained 99,000,000 peptonizing bacteria per cubic centimeter, these bacteria had not appreciably affected the taste. In view of the tryptic nature of the bacterial proteolytic enzymes and the well-known sensitiveness of this enzyme to acid reactions, it is probable that the acid produced by the lactic bacteria has a very distinct inhibiting effect on the bacterial enzymes in milk and that their protective value is due to this fact rather than to their retarding influence on the growth of the organism itself. That this action is not entirely effective is shown by some of the work cited in the introduction to this paper.

CONCLUSIONS.

With the conditions under which this investigation was carried on, milk held at 10° C. was found to be in good condition from a bacteriological standpoint for 48 hours after pasteurization. The same milk if held at 20° C. could not be considered a safe food longer than 24 hours after pasteurization. The presence of a large number of peptonizing bacteria in the pasteurized milk usually caused such a marked change in the taste that it would not be used after these organisms had become very numerous. The bacteria of this class were frequently found in the raw milk and were found especially in the raw milk held at the lower temperature in greater numbers than in the pasteurized milk under similar conditions, but some influence, evidently the acid, so retarded their activity that they usually did not appreciably affect the taste of the milk.

Inasmuch, however, as the present experiments were carried on in a laboratory under carefully controlled conditions, it would be unsafe to apply the same deductions to pasteurization on a commercial scale. If milk could be pasteurized commercially in such a way that the bacteria would be reduced to a few hundred per cubic centimeter and held at a low temperature until used, it would be perfectly safe for 48 hours or even 72 hours. Under these circumstances it would probably be in better condition after this long period than ordinary city milk at the time it is delivered. How closely these conditions could be approximated commercially is another question.

SUMMARY.

Briefly summarized, the facts brought out in the first portion of this paper are as follows:

Examination of milk by many bacteriologists shows that the milk used in American cities is usually badly contaminated by bacteria.

Increased public interest in the milk supply has resulted in more rigid municipal regulations and inspection, but progress is necessarily slow and pasteurization is frequently resorted to in order to increase the length of time that the milk will remain sweet and to reduce the danger from spread of infectious diseases.

The objection is frequently made that pasteurization, by destroying the lactic-acid bacteria, allows the development of other less desirable bacteria, which, without affecting the taste of the milk, make it actually dangerous, especially as a food for young children.

It is well established, however, that under certain circumstances the intestinal troubles of children may be reduced by pasteurization of the milk.

The experimental work may be summarized as follows:

Milk was pasteurized under laboratory conditions in a continuous machine at 85° C. (185° F.), the bacteria being reduced from over 10,000,000 per cubic centimeter to less than 500 per cubic centimeter.

Milk held at 20° C. (68° F.).—In the unheated milk the lactic bacteria increased rapidly and the milk became acid in about 12 hours. The peptonizing bacteria increased in 6 hours to about 5,000,000 per cubic centimeter and then decreased slowly.

In the heated milk the peptonizing bacteria increased rapidly after 12 hours, and the milk was usually curdled in 48 hours, with a disagreeable taste and odor. Occasionally lactic bacteria survived pasteurization and multiplied rapidly after 24 hours, completely inhibiting the peptonizing bacteria.

Milk held at 10° C. (50° F.).—In unheated milk the growth of the bacteria and the consequent curdling of the milk was much retarded. The average milk did not contain sufficient acid to affect the taste until it was over 48 hours old. The proportion of peptonizing to lactic bacteria was greater than at the higher temperature, and the taste of the milk occasionally showed the influence of the former.

In the pasteurized milk the bacteria increased very slowly, and in nearly every case the milk was unchanged in taste and appearance 96 hours after pasteurization. In only 2 of 14 cases was there a marked increase of peptonizing bacteria. The predominating bacteria were species having little or no effect on milk.

The lactic bacteria inhibited the development of the peptonizing bacteria only when they had developed sufficient acid to render the milk unfit for use.

It seems probable that the acid had a distinct inhibitory action on the proteolytic enzymes of the peptonizing bacteria.

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