# SOME FACTORS AFFECTING THE GERMICIDAL PROPERTY OF MILK

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**This is to certify that the**

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**Major professor**

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# SOME FACTORS AFFECTING THE GERMICIDAL PROPERTY OF MILK

By

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# INTRODUCTION

The freshly drawn milk of most cows possess to a greater or lesser extent the so called "germicidal property" of milk,which destroys a certain percentage of the bacteria in milk or inhibits their growth.

While milk is in the udder there is little or no increase in the number of bacteria.Those bacteria present in the udder of the healthy cow have gained access from outside.Thus,a variety of species of bacteria are to be found in the milk in the udder.However the micrococci predominate, whereas the other species are found in a very small minority. From this it appears that there must be some influence at work not only in the udder but also for some time after the milk has been drawn.This influence may be affecting different species to a greater or lesser extent.

Almost all the workers agree that such an influence exists,but each one has tried to explain it in his own way, and named it in the way he thought the best.Some called it the" germicidal property",whereas others name it as the "bactericidal property" and still others refer to it as the "inhibitory influence of milk" or "bacteriostatic property". Many views have been advanced in explanation of the phenomenon.

Most of the workers are of the opinion that the germicidal property is too feeble,variable and transitory

to be of practical value and so cannot be used successfully as an argument against the practice of heating milk for the purpose of destroying pathogenic bacteria.

At present, this delay in the increase in number of bacteria is utilized in the practical way in the dairy industry where regulations permit dairymen to bring uncooled milk to the plant if this is delivered within four hours after milk is drawn.

The purpose of this work is to find how the germicidal power which is at present of very little value can be economically utilized to the best advantage in practical dairying. With this point in view, studies have been made on the various factors that affect the germicidal property; keeping in mind, at all times, the controversial results obtained by different workers.

## REVIEW OF LITERATURE

General Discussion, Fokker (7) as early as 1890 probably was the first to call attention to the germicidal property in goats milk and since then the subject has been investigated by many observers but the conclusions derived were not in agreement.

Most workers favored the opinion that the milk substance was identical with blood alexin and was directly derived from the blood. On the other hand Rosenau and McCoy(31) suggested that the lower counts after incubation in raw milk were explicable on the ground of agglutination and phagocytosis by leucocytes contained in the milk may in part be responsible for the decrease. Stocking(33) suggested that the lack of adaptation for growth in milk of the organisms employed in the experiments may be responsible for the phenomenon. Others agree that milk had a definite bactericidal substance. This view was supported by Heineman(15), Chambers(3), Hanssen(14), and Jones and Little(22).

Breed Hunziker (19) as far back as 1901 reported that germicidal property was different for different breeds. His results are shown in the following table;

Growth of the sweet curdler in raw and heated Holstein and Jersey milk,and time of coagulation.

Bacteria per cubic centimeter

| sample | 0 hr. | 5 hr. | 9½ hr. | 23 hr. | coag. time |
|---|---|---|---|---|---|
| *Holstein raw | 65,000 | 11,550,000 | 43,000,000 | 86,800,000 | 6 |
| " heated | 64,900 | 12,600,000 | 47,600,000 | 131,600,000 | 5 3/4 |
| **Jersey raw | 64,000 | 5,250,000 | 40,810,000 | 82,000,000 | 9 ½ |
| " heated | 65,200 | 11,620,000 | 46,970,000 | 91,000,000 | 5 ½ |

* Plate count before innoculation 670
** Plate count before innoculation 1030

Curran (4) believed that raw Holstein milk had less germicidal property than raw Jersey milk.

Most of the workers including Chambers(3) and Roseneau and McCoy (31)seemed to agree with the results of the above workers.

Individuality Jones (21) thought that the bactericidal substance was present in the secretion of all cows although it was true that its concentration varied from animal to animal.

Works of Park (29),Hunziker (19),Jordon and Falk(26), Jones and Little(22),and Chambers (3) also showed that germicidal property varied in the milk of different animals.

Hunziker (19) found the following:

Number of bacteria in 1 c.c. of milk kept at 55°F.

| Name of Cow | Cow-warm | After 3 hr. | After 24 hr. | After 48 hr. |
|---|---|---|---|---|
| Ida | 35,560 | 25,440 | 23,420 | 20,040 |
| Rose | 500 | 600 | 460 | 1,740 |
| Clover | 37,190 | 28,340 | 24,980 | 28,110 |
| May | 440 | 320 | 340 | 240 |
| Dena | 4,980 | 3,720 | 3,910 | 11,860 |
| Peach | 2,330 | 2,510 | 2,170 | 21,800 |

Hunziker (19) in addition believed that the degree of germicidal action varied in the same animal taken at different milkings.

Age Jones and Little(22) stated that inhibitory action might be as strong in the milk of a young cow in the first lactation period as in that of an old cow,known to have resisted exposure to infection.

Various quarters Jones (21) observed that the inhibitory substance varied in the milk from different quarters of the same cow.He supported this by the data in the following table.

The effect of milk from various quarters on the growth of the mastitis streptococcus.

| Source | Time | | | | |
|---|---|---|---|---|---|
| | at once | after 2 hrs | after 4 hrs | after 6 hrs | after 8 hrs |
| Cow 83 Right fore quarter | 576 | 704 | 704 | 1,152 | 37,440 |
| Left fore quarter | 704 | 768 | 640 | 1,792 | 57,600 |
| Right hind quarter | 640 | 512 | 768 | 576 | 15,552 |
| Left hind quarter | 640 | 640 | 5,632 | inn. | inn. |
| Cow 07 Right fore quarter | 704 | 704 | 768 | 1,600 | 72,000 |
| Left fore quarter | 576 | 704 | 2,112 | 14,400 | inn. |
| Right hind quarter | 704 | 576 | 1,088 | 3,136 | 86,400 |
| Left hind quarter | 576 | 576 | 2,048 | 57,600 | inn. |
| all samples combined and boiled | 640 | 5,312 | 115,200 | inn. | inn. |

Season and Feed Hanssen(14) was of the opinion that bactericidal property of milk varied greatly in milk samples from the same cow at different seasons of the year.Oxidizing enzymes were affected by feed.So indirectly feed influences bactericidal property.

Jordon and Falk(25) stated "Carbohydrate deficiency in the ration results in decreasing content of milk sugar and increasing content of sodium chloride in order to maintain normal osmotic pressure.With salt rise the reaction changes and the germicidal property of the milk and udder disappears.A medium is formed that is most favorable for the growth of bacteria and mastitis develops because of decreased germicidal property".

Colostrum Jones(21),believed that the substance was present in colostrum of the first few days of lactation as well as later, but its effectiveness decreased as days after lactation advanced. He further stated that during the first few days after parturition the milk was rich in blood serum;this admixture of large quantities of serum protein may be capable in itself of causing considerable bacterial lag.When colostrum was first heated at 60°C.(140°F.)the inhibitory effect of blood alexin was eliminated and the true behavior of the milk fraction may be determined. The protocol of such an experiment is given in the following table;

The effect of colostrum and milk during early lactation on the mastitis streptococcus.

| | at once | after 2 hrs | after 4 hrs | after 6 hrs | after 8 hrs |
|---|---|---|---|---|---|
| Day of parturition | 1,152 | 1,404 | 11,456 | inn. | inn. |
| Day after " | 960 | 768 | 1,536 | 15,296 | 92,160 |
| 2 days after " | 960 | 896 | 7,168 | 69,220 | inn. |
| control 3 samples combined and boiled | 1,218 | 4,564 | inn. | inn. | inn. |
| 3 days after parturition | 1,024 | 832 | 2,880 | 10,816 | 40,896 |
| 4 " " " | 1,024 | 896 | 1,472 | 72,232 | inn. |
| 5 " " " | 960 | 896 | 7,168 | 69,220 | inn. |
| control 3 samples combined and boiled | 1,218 | 6,592 | inn. | inn. | inn. |
| 6 days after parturition | 1,088 | 1,024 | 2,368 | 9,408 | 34,560 |
| 7 days after parturition | 1,216 | 894 | 1,152 | 3,392 | 25,792 |
| 8 " " " | 1,152 | 1,216 | 960 | 2,048 | 28,800 |
| control 3 samples combined and boiled | 1,344 | 3,860 | 51,840 | inn. | inn. |

From this it may be concluded that though the inhibitory principle was present in the colostrum of the first day, it was more effective after a few days. According to him the maximum activity was marked after the sixth or seventh day.

Famulenir(8) concluded that antibodies(hemolytic antibodies) were transmitted from mother to offspring with the milk and not through the placenta. This was found to occur particularly through the colostrum.

Time and Temperature Most of the workers were of the opinion that the germicidal property of milk was marked in warm milk but lasted for a shorter time than the germicidal property in cold milk which was less marked but lasted for a longer time. Milk lost its germicidal property at higher temperature in a

very short time.

Hunziker(19),Fokker(7) and Freudenrich (12) believed that germicidal power was greatest at 70°F.and was lost at 149°F. for 40 minutes.

Heineman (15) found that the germicidal property was lost at 75°C,(167°F.)

Jones and Little(22)found that 62°C.(143.6°F.)for 20 minutes failed to affect appreciably the substance although 65°C.(149°F.) or 70°C.(158°F.) for the same period or boiling for five minutes completely inactivated it.

Frayer(9)recommended cooling the milk immediately to 40°F. rather than to 50°F. or 60°F. If cooling was delayed some adjustments took place within the cell,which later on led to a very luxuriant growth when the germicidal property had become inactive.

Hammer and Baker (13) believed that acid development was slowest in milk heated to 145°F.for 30 minutes,but increased with increase in temperature upto 160°F.or a little above.

According to Jordon and Falk (25) germicidal property was destroyed at 70°C.(167°F.)for fifteen minutes or at 80°- 90°C.(176°- 194°F.)for two minutes.At 15°C(59°F.)it was less marked but more prolonged.

Chamber (3) was of the opinion that agglutination and bactericidal inhibition were destroyed at 80°-90°C. (176°- 194°F.)

Hillard and Davis (18) were of the opinion that intermittent freezing of bacteria exerted a more effective germicidal action than continuous freezing;crystallization probably resulting in mechanical crushing was an important germicidal factor in causing the death of bacteria at 0°C (32°F.)and below.

According to the latest work of Morris(28) bactericidal substance was completely destroyed by heating to 53°C (127.4°F.)for one-half an hour,and that this destruction was critical to within 1°C.(1.8°F.)

Bacteria Hesse(17) as far back as 1894 noted that B.typhosus and the Cholera vibrio were prevented from multiplying in raw milk.Heineman (15) in 1903,found that milk contained for certain species of organisms a bactericidal substance,but the growth of other organisms was not inhibited.

Jones and Simms(24) had shown that when mastitis streptococci were introduced in small numbers into milk previously heated at 58°C(136.4°F.)for twenty minutes,there was a lag in growth of five or six hours succeeded by an abrupt increase which continued at a rapid rate.According to

them the inhibitory substance was not destroyed during this period since sufficient remained to inhibit the growth of a similiar culture for about four hours.A resistant form which was present from the start required some time to multiply to an appreciable number.Mastitis streptococci became adapted to the inhibitory agent.This was further supported by their experiment in which bacteria surviving the lag period were found to grow rapidly without lag,when placed in fresh milk. The scarlet fever streptococcus was unable to produce resistant forms and hence perished.Both organisms grew rapidly in milk that had been boiled for five minutes.

Chambers(3) found that the action was specific and depended on both the cow and the species of bacteria employed.

Sherman and Curran(33)who used Streptococcus lactis found a lag in the fresh milk,but the control tubes in which sterile milk was employed no lag occurred.

Hanssen(14) found that fresh raw milk inhibited the growth of B.typhosus and B. parathyphosus for one to four hours at 37°C (98.6°F.)

Jones and Little(22) inferred from their experiment that the action of the substance was not specific since by inoculating raw milk with one organism they were able to absorb the inhibitory agent for another.

Out of the 23 cultures that Frayer (9) studied,he found that there were some cultures that increased from the very beginning whereas others that were affected more or less by the germicidal property for several hours;and still others that maintained constant numbers.

Antibodies Many views have been advanced in explanation from as early as 1892 when Ehrlich (5) showed the presence of immune bodies in milk.In 1892 Brieger and Ehrlich (6) showed the presence of antitoxins.Widal and Sicard(34) were probably the first in 1897 to demonstrate the presence of agglutinins.Kane and Claypon(27) confirmed that the amboceptor and compliment took part in cytolysis of milk.According to Woodhead and Mitchell (35)opsonins may be present in milk. Rosenau and McCoy (31) thought that the presence of agglutinins was an important factor in bringing about the apparent reduction in fresh milk.They have shown that when milk was violently shaken the number of colonies was compared with the initial reading did not decrease and this led them to conclude that the milk after standing,caused bacteria to clump and that therefore fewer colonies appeared on the plates,although the actual number of cells may not have been reduced.This view seemed to be supported by the fact that in certain centrifuged

milk the colony count was greater than in the same milk before centrifugation.They further inferred that phagocytosis by leucocytes contained in milk may be in part responsible for the decrease.

Heineman and Glenn (16) stated,"The agglutination of certain bacteria in milk serum seems to bear some relation to the apparent decrease in number of bacteria observed in fresh milk but this is probably not the only factor causing such reduction.Bacterial substances are destroyed by heating at 56°C for thirty minutes."

Chambers(3) wrote,"No common relation between agglutination and bacterial inhibition is noted except that both are destroyed by heating the milk."

Rogers(30) agreed with many of the workers when he wrote,"There is in blood and in other body fluids a bactericidal power.Several kinds of antibodies are known to take part in the destruction of bacteria in the blood-agglutinins,precipitins, bacteriolysins,and opsonins.Since the fluid part of the milk with many of its constituents is derived from the blood,it is not surprising that the milk should be found to contain the same kinds of antibodies as the blood".

Jones and Little(22) and Jones (21) found that agglutination of streptococcus was not found on microscopic examination and so they rule out the agglutination theory put

forth by Rosenau and others.

Chambers (3) emphasized the specificity of the germicidal property and suggested an immunity of organisms constantly present in the cows' own udder.

Jones (21) seemed to rule out Stockings' theory of lack of adaptation,as well as theory of agglutination and phagocytes.Jones and Little(22) inferred from their experiment that the inhibitory substance originated in the udder and not from the blood.

Jones(21) wrote that,"The inhibitory substance is not absorbed by casein,fat,kieselguhr,keolin,or bolus alba though considerable is taken out by animal charcoal.The principle passes through a coarsest Berkefeld filter although a considerable portion is obtained by N.Candles".

Lactinin Jones and Simms(23)called the bacterial growth inhibitory substance found in milk as'Lactenin'.According to them lactenin was stable for one and a half hours at pH value ranging from,4 to 10.If the time of exposure be lengthened,then the material must be kept closer to the neutral point.They had shown that it was not associated with salts and carbohydrates and could be seperated from them by dialysis. Lactenin was associated with the proteins of whey but was not

removed with casein during rennet coagulation.Like proteins it did not pass through permeable collodion membranes.It and some of the proteins resisted the digestive activities of trypsin but the split products of the proteins hydrolyzed by trypsin are removed by dialysis without impairing lactinic activity.They were not sure if lactenins were also a protein. Even the removal of fat did not decrease the lactenin activity. On dry weight basis the dried material with salt solution was 200 times as active as the original milk.

## EXPERIMENTAL PROCEDURE

Raw milk for the different experiments was obtained from the Michigan State College Dairy barn. Machine drawn milk of different cows was collected in sterile Erlenmeyer flasks. These flasks during transit from barn to the laboratory were kept in thermos jugs to prevent temperature variations. About fifteen to twenty minutes elapsed from the time the milk was drawn, to the time milk was plated for zero-hour reading. All the zero-hour readings were taken fifteen to twenty minutes after the cow was milked.

For measuring the reduction in bacterial count in milk, caused by the germicidal property, standard plate counts were made by the method described in the Standard Method for the Examination of Dairy Products.(1)

For each sample, three standard plates, each of different dilutions were made. After introducing the desired portions of milk or its dilutions 10 to 12 ml. of the liquid T.G.E.M. (Tryptone-glucose-extract-milk agar) was introduced at 41°C to 44°C (105.8-111.2°F.) and mixed. After solidifying the agar, the plates were inverted and incubated for 48 hours at 37°C (98.6°F.). At the end of 48 hours the cultures were counted with the aid of the Quebec Colony Counter. Plates with a colony count between 30 and 300 were used for the data.

Escherichia coli and Staphylococus aureus were the two organisms used for innoculation.A 24-hour culture of the two above organisms in F.D.A.(Food and Drug administration broth)kept at 37°C. for 24 hours was used for innoculation. Innoculation was necessary to facilitate a more marked change caused by the germicidal property.To every 100 ml.of milk 0.1 ml.of broth culture was added.

Composition of tryptone-glucose-extract-milk agar was as follows:

Agar..... 1.5 percent

Beef extract..... 0.3 "

Tryptone..... 0.5 "

Glucose..... 0.1 "

One percent sterile skim milk was added just after final melting in all cases where dilutions greater than 1: 10 were made.

Composition of F.D.A.broth was as follows:

Peptone .....10 gm.

Liebigs beef extract..... 5 gm.

salt ..... 5 gm.

Water..... 1000 c.c.

Boil..... 20 minutes.

Table 1. Effect of time and temperature on the growth of E.coli when inoculated into colostrum

| Time (Hours) | Standard plate count when the milk was held at(°F.) | | |
|---|---|---|---|
| | 40 | 60 | 80 |
| | COW NO.A21 | | |
| 0 | 1,270,000 | 1,270,000 | 1,270,000 |
| 1 | 1,170,000 | 980,000 | 1,080,000 |
| 2 | 1,040,000 | 1,200,000 | 880,000 |
| 4 | 910,000 | 1,080,000 | 960,000 |
| 8 | 980,000 | 1,010,000 | 2,040,000 |
| 18 | 870,000 | 7,600,000 | 74 ,040,000 |
| 24 | 840,000 | 11,600,000 | 322,000,000 |
| 48 | 960,000 | 48,000,000 | 372,000,000 |
| 72 | 740,000 | 772,000,000 | 521,000,000 |
| 96 | 1,140,000 | 800,000,000 | coagulated |
| 120 | 790,000 | 880,000,000 | |
| | COW No.A 27 | | |
| 0 | 890,000 | 890,000 | 890,000 |
| 1 | 780,000 | 890,000 | 768,000 |
| 2 | 417,000 | 940,000 | 880,000 |
| 4 | 1,060,000 | 1,280,000 | 5,310,000 |
| 8 | 8,300,000 | 1,280,000 | 112,000,000 |
| 18 | 760,000 | 4,400,000 | 620,000,000 |
| 24 | 1,030,000 | 27,400,000 | 800,000,000 |
| 48 | 650,000 | 101,000,000 | 1,420,000,000 |
| 72 | 800,000 | 421,000,000 | 940,000,000 |
| 96 | 1,040,000 | 2590000,000 | 41,100,000,000 |
| 120 | 670,000 | 2620000,000 | 340,000,000 |

## RESULTS

The germicidal property of colostrum.

A study was made to ascertain if the germicidal property attributed to milk was present also in colostrum. To this end data were obtained on colostrum for two cows. The colostrum was obtained in both cases about three days after parturition. According to Jones(21), the maximum germicidal property might be expected about the sixth or seventh day after parturition. The data obtained are presented in Table1. Here it will be noted that the germicidal effect in colostrum was quite marked when the colostrum was cooled immediately to and stored at 40°F. The germicidal property was maintained as long as 120 hours at this temperature. The data show further that the germicidal property of colostrum persisted for approximately 8 hours when the milk was held at 60°F. However, some inconsistencies appear in these data for the germicidal property of colostrum from cow A27 at 60°F. did not last as long as that of cow* . At 80°F., however, the colostrum period did not extend much longer than 2 hours. Again the persistency of the germicidal property was not consistent in the colostrum from both cows. In one case, that of cow * the germicidal period lasted 4 hours, whereas in colostrum from cow A27 the germicidal property period extended only 2 hours.

* A21

Growth of E.coli in autoclaved milk,intermittently sterilized milk and F.D.A.broth;as compared with raw milk.

This study was necessary to determine some factors besides the germicidal property which were responsible for the decrease in bacterial count during the first eight hours after the milk was drawn.With this end in view intermittently sterilized and autoclaved milk were used in which the germicidal property was supposedly destroyed.Again these two milks were compared with F.D.A.broth,to see if the culture in the F.D.A. broth reacted the same way as in the other two milks.

Milk was autoclaved by exposing it for fifteen minutes to a temperature of 120°C(248°F.)in an autoclave.After this the milk was cooled to a respective temperature and inoculated with E.coli at the rate of 0.1 ml. for 100 ml.of milk.The milk was divided into three Erlenmeyer flasks which were kept at 40°F.,60°F.,and 80°F.respectively.Plate counts were taken at the end of 0,1,2,4,8,18,24,48,96 and 120 hours or until milk had curdled.Four trials were run on the milk of two different cows and the results were averaged and tabulated as in Table 2.

Intermittent sterilization of milk consisted in heating the milk for five minutes in steam and then leaving the milk in the incubator at 37°C(98.5°F.)for 24 hours.This was done for three consecutive days,after which it was treated in the same way as was the autoclaved milk.

In case of raw milk,the inoculation was made to raw milk,after which it was treated in the same way as the above two.Five trials were run on two different cows,the results of which were averaged and tabulated in Table 2.

Instead of milk,F.D.A. broth was used in the last case,and it was treated in the same way as the raw milk.

All the samples in the experiment kept at 40°F. showed a decrease for the first four hours or so.In case of the raw milk sample,the decrease was much more marked,as compared with the other three samples kept at the same temperature. This definite decrease in raw milk was attributed to the germicidal property of milk.In the other three cases the decrease was due to the change of media which some of the organisms could not withstand and so they died.The remaining organisms soon became accustomed to the new surrounding and soon started growing at a fast rate.This did not last very long for soon the organisms started decreasing in numbers.This decrease may be due to the unavailability of the food material or to the production of toxic substances by the cells at that low temperature or to other factors.The same thing happened in case of pasteurized milk kept at this low temperature.

In case of samples stored at 60°F.there was a decrease in bacteria count for the first few hours for the same reason as in the case of milk stored at 40°F.,but the

decrease in this case was of a shorter duration.Again in raw milk the decrease was more prominent due to the germicidal property.After about four hours in all the cases,the count increased due to the organism getting accustomed to the new surroundings.

In case of raw milk stored at $60^{\circ}$F.there was a marked residual germicidal effect observed at 24 hours.

In case of samples stored at $80^{\circ}$F.there was a slight decrease for the first two hours or so and then a rapid increase.In case of the raw milk,there was a distinct decrease for the first four hours,undoubtedly due to the germicidal property.

Table 2 Growth of E.coli in different media at 40°F, 60°F. and 80°F.

| Time in hours | Bacteria count (in thousands) | | | |
|---|---|---|---|---|
| | Autoclaved milk | Intermittently sterilized milk | raw milk | F.D.A. |
| | STORAGE TEMPERATURE 40°F. | | | |
| 0 | 1,560 | 2,430 | 1,104 | 1,720 |
| 1 | 1,320 | 1,940 | | |
| 2 | 1,000 | 1,800 | 698 | 1,420 |
| 4 | 1,100 | 1,650 | 680 | 1,150 |
| 8 | 1,085 | 2,050 | 853 | 1,630 |
| 18 | 1,100 | 1,560 | 2,468 | 1,530 |
| 24 | 1,245 | 1,800 | 955 | 560 |
| 48 | 733 | 1,500 | 2,155 | 1,870 |
| 72 | 465 | 1 ,600 | 3,920 | 1,620 |
| 96 | 210 | 1,050 | 8,740 | 1,630 |
| 120 | 161 | | 5,934 | 1,460 |
| | STORAGE TEMPERATURE 60°F. | | | |
| 0 | 1,560 | 2,430 | 1,104 | 1,890 |
| 1 | 1,418 | 1,700 | 943 | 1,980 |
| 2 | 1,295 | 1,900 | 1,122 | 1,760 |
| 4 | 1,490 | 1,810 | 855 | 2,050 |
| 8 | 1,353 | 2,650 | 5,486 | 3,070 |
| 18 | 3,550 | 16,400 | 8,947 | 22,300 |
| 24 | 5,474 | 45,000 | 1,296 | 14,300 |
| 48 | 83,800 | 920,000 | 35,050 | 476,000 |
| 72 | 537,000 | 1,140,000 | 599,500 | 1,140,000 |
| 96 | 1,610,000 | | innumerable | 20,900,000 |
| 120 | 8,460,000 | | | 1,720,000 |
| | STORAGE TEMPERATURE 80°F. | | | |
| 0 | 1,560 | 2,430 | 1,104 | 2,130 |
| 1 | 1,415 | 1,940 | 1,073 | 2,070 |
| 2 | 1,443 | 2,750 | 253 | 2,090 |
| 4 | 13,600 | 6,780 | 867 | 2,500 |
| 8 | 166,750 | 226,000 | 6,400 | 219,000 |
| 18 | 704,250 | 131,000 | innumerable | 900,000 |
| 24 | 1,133,750 | 1,350,000 | | 1,800,000 |
| 48 | 7,800,000 | 1,480,000 | | 1,800,000 |

Growth of E.coli broth culture added in three different amounts(0.1ml;0.01ml and 0.001 ml,to 100 ml of freshly drawn raw milk.)

This experiment was carried out to determine if the amount of inoculum of E.coli influenced the rate of percentage of destruction of these organisms by the germicidal property of milk.With this end in view the same milk sample was divided into three equal parts and each one of them was inoculated with different amounts of the E.coli culture. (0.1 ml,0.01ml,0.001 ml)In each case the colony counts were taken at 1,2,4,8,18,24 and 48 hours.The data are shown graphically in Figure 1.

Results indicated that milk that was inoculated with a larger amount of E.coli culture,showed a greater destruction of organisms although the percentage destruction was not as great.

This was also noticed in milk that contained naturally a large number of bacteria as compared with the milk containing fewer bacteria.

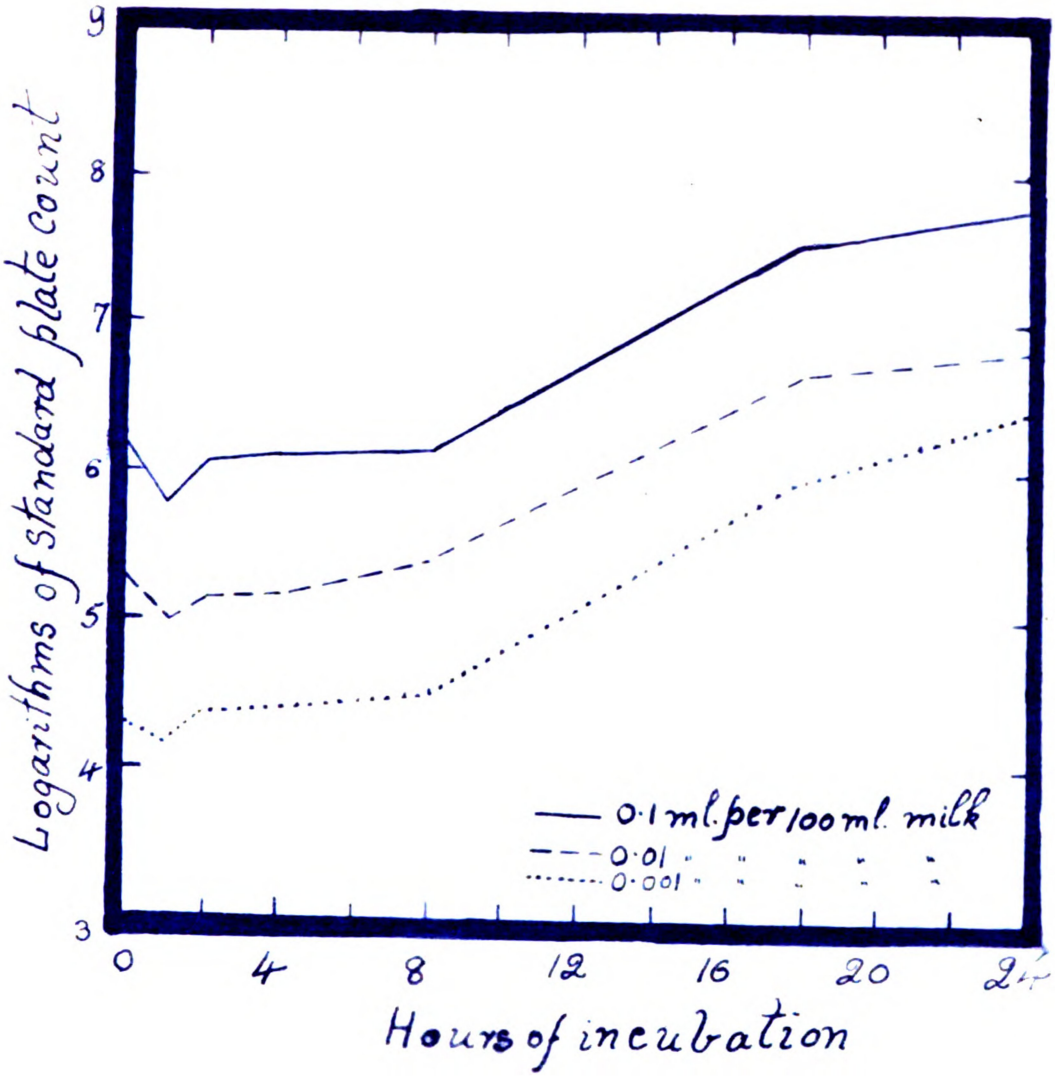


Figure 1.- Growth of broth culture of E. coli added in freshly drawn raw milk.
(Kept at 60°F.)

Growth of E.coli skimmilk culture added in three different amounts(0.1ml,0.01ml,and0.001 ml,to 100 ml of freshly drawn raw milk.)

Everything was done the same way as in the previous experiment except that skimmilk E.coli culture was used instead of the broth E.coli culture. The data are presented graphically in Figure 2.

In this case the milk with the highest amount of E.coli inoculation did not show as much decrease as the ones with lesser inoculation.This was due probably to the fact that the organisms grown in skimmilk were adapted to grow in milk and so did not show any marked lag phase.In the other two cases in which less inoculum was used,the decrease was due to the marked reduction in the bacteria present in the milk, and not due to the reduction in the E.coli added.In the previous case the reduction was not very marked as the inoculation was heavy and hence the reduction was hardly visible.

The difference in the growth curve of the ones inoculated with 0.01 and 0.001 ml was due to the germicidal action which had a more marked destructive effect on the milk of high bacterial counts.

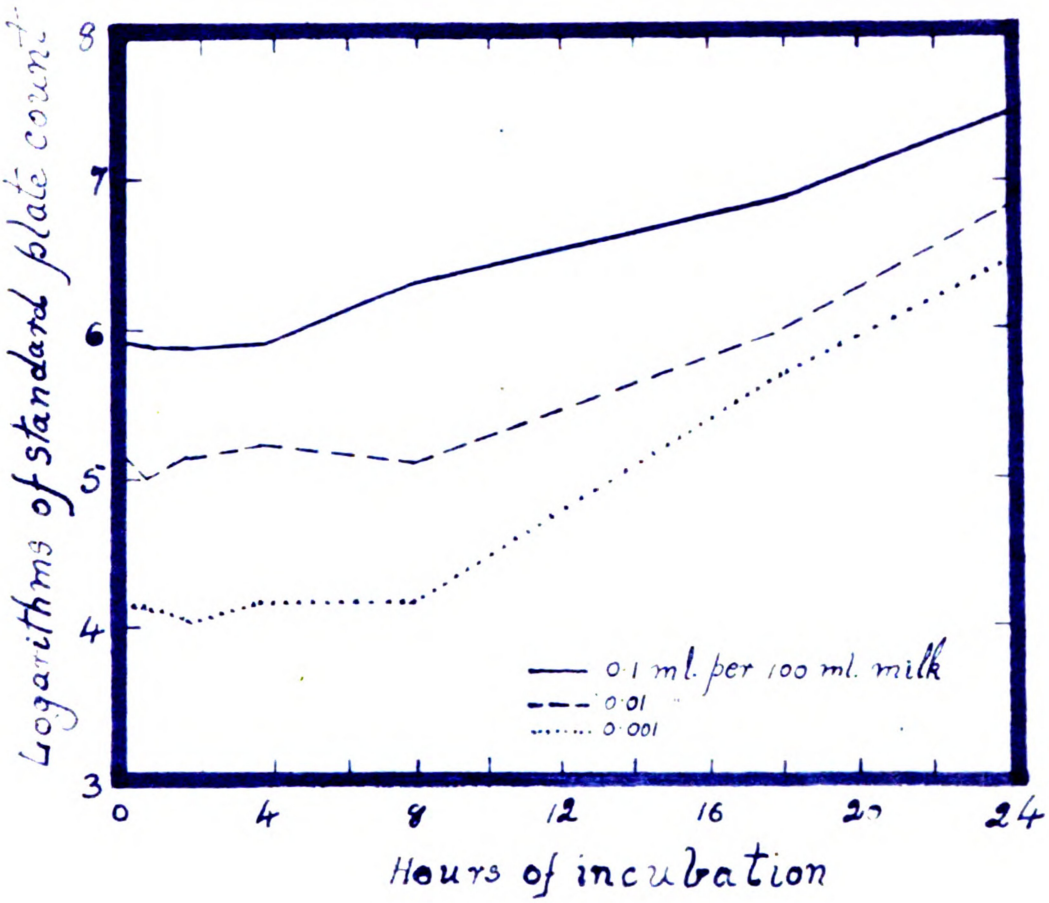


Figure 2. - Growth of a skimmilk culture of E. coli added in freshly drawn raw milk. (Kept at 60°F)

Comparison of the growth curves of broth and skimmilk E.coli culture in fresh raw milk.

On studying the data of Figures 1 and 2 it was noticed that the decrease in bacteria count as shown in Figure 2 was not marked as that of Figure 1.This was probably due to the fact that the organisms once growing in milk do not show any marked lag phase when inoculated into milk.Organisms grown in broth require a longer period for adjustment when transferred to milk than do organisms grown in milk and transferred to milk.Therefore,the decrease after inoculation is more apparent in the former case than in the latter.During the prolonged adjustment period the organism may be more susceptible to the germicidal action of milk,others may not be able to survive in the new medium.

This was further supported by the following data: Fresh milk was inoculated with 1/10th its amount with 30-hour old milk inoculated with E.coli.The results follows:

| Time | Bacteria Count |
|---|---|
| At 0 hours ..... | 17,900,000 |
| After 3 hours ..... | 19,900,000 |
| " 4 hours ..... | 30,150,000 |
| " 6 hours ..... | 49,580,000 |

In this case no lag phase was noticed as a resistant form was developed which was not affected by the germicidal property.

The effect of the germicidal property on different organisms kept under the same conditions.

Ever since the first studies had been made on the germicidal property of milk this has been an important subject with most of the workers.

Two organisms, E.coli and Staph. aureus were therefore studied under the same conditions. In each case milk from the same cow was studied on four days under the same conditions as far as possible. The data of all the four trials were averaged and tabulated in Table 3.

On studying the data it was noticed that each organism gave a different growth curve as compared with the uninoculated milk. In case of Staph.aureus the decrease was marked for 96 hours whereas in the case of E.coli there was a distinct decrease for 18 hours after which the number increased at a very fast rate. At the end of 120 hours the number of E.coli was almost double that of Staph.aureus although the number of E.coli at 18 hours was a little less than half of the Staph.aureus number.

On comparing the inoculated milks with the uninoculated milk it was observed that the decrease in case of the uninoculated milk was not so very great. This was due to the inhibitory substance in milk being more effective against foreign organisms, than against the one already present in the milk as it comes from the udder of the cow.

Table 3- The effect of germicidal property on different organisms kept under the same conditions ( 40°F.)

| Time in hours | Uninoculated milk | Inoculated with E.coli | Inoculated with Staph.aureus |
|---|---|---|---|
| 0 | 154,950 | 1,377,000 | 1,064,000 |
| 2 | 45,700 | 577,750 | 227,475 |
| 4 | 59,312 | 513,125 | 684,125 |
| 8 | 126,080 | 526,750 | 306,875 |
| 18 | 117,450 | 155,666 | 246,875 |
| 24 | 167,240 | 1,214,000 | 655,000 |
| 48 | 129,620 | 3,023,222 | 560,666 |
| 72 | 188,750 | 3,921,250 | 502,500 |
| 96 | 303,000 | 16,680,000 | 875,000 |
| 120 | 337,500 | 25,079,333 | 12202,500 |

Effect upon the germicidal property when evenimg milk was mixed with morning milk from the same cow.

The mixing of evening milk with morning milk or vice versa is a common practice.This mixing is done either at the body temperature or is done after cooling the milk at favorable low temperatures,before the milk is sent to the plant for processing.

With the hope of finding an economically favorable mixing temperature several trials were made.

Samples of morning milk of four different cows were taken and each of them was divided into two parts,one was kept at 40°F.whereas the other was kept at 60°F.Bacterial counts were taken at 0,1,and 8 hours.At the end of eight hours, milk of the same cows was mixed with the above samples at 40°F. and at body temperature.Counts of the mixed as well as of the unmixed milk were taken at 0,2,4,8,24,and 48 hours respectively.

The data are presented in Tables 4 to 7 inclusive.

From the data in these three tables it will be seen that on the whole there was a change in the bacteria count of the unmixed milk as compared with the mixed milk held at the same temperature.The decrease was very marked in case of the milk held at 40°F.and to which evening milk cooled to 40°F. was added.In case of the milk held at 40°F.and to which evening milk at body temperature was added,the decrease takes place

but it was not as marked as in the previous case.

Milk from three of the four cows held at 60°F. and to which evening milk at body temperature was added showed a decrease.However,in the milk from Cow No.37 the count increased.The milk held at 60°F. and to which evening milk cooled to 40°F.was added decreased in count more than the previous one.

The decrease in all thecases was due to the residual effect of the germicidal property.In case of the milk held at 40°F.the germicidal property was not very marked,but it lasted for a longer time and was marked for 24 hours or even more.The reduction of count was due not only to the residual germicidal property of the morning milk but it was also reduced a great deal by the addition of the fresh evening milk which had its own germicidal property.

In case of the milk held at 60°F. the germicidal property was almost lost by the time the fresh milk was added to it.The germicidal property of the fresh milk when added to the milk held at 60°F.was not marked as the growth continued at a very fast rate and the germicidal property of the fresh milk was not high enough to make it appreciable.

In concluding,it might be stated that cooling plays a very important part in keeping the bacterial count at a low number.When both milks were at 40°F.at the time of mixing the

best results were obtained.Cooling of either the previous days milk or the fresh milk to be added showed quite a marked change.Practically speaking,cooling of the morning milk was the best,so that the addition of warm milk does not alter the bacterial count to a great degree.As the milk after this treatment was taken to the plant within three or four hours.

Table 4 Influence of mixing morning milk with evening milk from the same cow upon the germicidal property(Cow No.60)

| Time in hours | Bacteria count in morning milk | Time in hours | Standard plate count in Evening milk | Standard plate count in mixed at body temp | Standard plate count in Mixed at 40°F. |
|---|---|---|---|---|---|
| HELD AT 40°F. | | | | | |
| 0 | 15,600 | | | | |
| 1 | 11,600 | | | | |
| 2 | 12,500 | | | | |
| 8 | 22,000 | 0 | 90,000 | 62,000 | 61,800 |
| 10 | | 2 | 75,000 | 52,000 | 49,400 |
| 12 | | 4 | 113,000 | 75,000 | 45,000 |
| 16 | | 8 | 76,000 | 39,100 | 45,100 |
| 32 | | 24 | 76,000 | 73,000 | 49,000 |
| 56 | | 48 | 118,000 | 62,000 | 52,000 |
| HELD AT 60°F. | | | | | |
| 0 | 15,600 | | | | |
| 1 | 16,300 | | | | |
| 2 | 14,100 | | | | |
| 8 | 24,800 | 0 | 101,000 | 45,000 | 47,000 |
| 10 | 19,700 | 2 | 70,000 | 77,000 | 56,000 |
| 12 | 54,000 | 4 | 39,600 | 86,000 | 115,000 |
| 16 | 258,000 | 8 | 161,000 | 116,000 | 240,000 |
| 32 | 11,000,000 | 24 | 17400000 | 40,000,000 | 36,400,000 |
| 56 | greater than 3 B. | 48 | 270000000 | 850,000,000 | 430,000,000 |

Table 5 Influence of mixing evening milk with morning milk from the same cow upon the germicidal property.

(Cow No.224)

| Time in hours | Bacterial count in morning milk | Time in hours | Standard plate count: evening milk | Mixed at body temp. | Mixed at 40°F. |
|---|---|---|---|---|---|
| HELD AT 40°F. | | | | | |
| 0 | 105,000 | | | | |
| 1 | 19,100 | | | | |
| 2 | 18,400 | | | | |
| 8 | 133,000 | 0 | 2,380 | 65,000 | 70,000 |
| 10 | | 2 | 75,000 | 52,000 | 49,000 |
| 12 | | 4 | 113,000 | 75,000 | 45,000 |
| 16 | | 8 | 4,000 | 30,000 | 80,000 |
| 32 | | 24 | 2,480 | 89,000 | 153,000 |
| 56 | | 48 | 3,120 | 286,000 | 294,000 |
| HELD AT 60°F. | | | | | |
| 0 | 105,000 | | | | |
| 1 | 17,300 | | | | |
| 2 | 16,200 | | | | |
| 8 | 1,500,000 | 0 | 2,490 | 730,000 | 400,000 |
| 10 | 2,900,000 | 2 | 3,600 | 1,310,000 | 1,396,000 |
| 12 | 9,500,000 | 4 | 1,900 | 9,200,000 | 6,100,000 |
| 16 | gr.than 300M | 8 | 5,000 | 20,000,000 | 7,900,000 |
| 32 | 2,910,000,000 | 24 | 900,000 | 352,000,000 | 95,000,000 |
| 56 | | 48 | 17,900,000 | | |

Table 6 Influence of mixing evening milk with morning milk from the same cow upon the germicidal property.

Cow No. 221

| Time in hours | Bacteria count in morning milk | Time in hours | Standard plate count: Evening milk | milk at body temp. | mixed at 40°F. |
|---|---|---|---|---|---|
| HELD AT 40°F. | | | | | |
| 0 | 7,340 | | | | |
| 1 | 9,800 | | | | |
| 2 | 10,500 | | | | |
| 8 | 12,600 | 0 | 10,000 | 10,700 | 10,000 |
| 10 | 5,000 | 1 | | | 8,900 |
| 12 | | 2 | 10,400 | 15,800 | 10,500 |
| 16 | 10,400 | 4 | | 10,400 | 10,100 |
| 32 | | 8 | 14,500 | 9,800 | 8,900 |
| 56 | 26,000 | 48 | 4,800 | 51,000 | 17,300 |
| HELD AT 60°F. | | | | | |
| 0 | 7,340 | | | | |
| 1 | 10,600 | | | | |
| 2 | 16,600 | | | | |
| 8 | 15,000 | 0 | spreads | 15,500 | 17,300 |
| 10 | 35,000 | 1 | | 14,900 | 13,700 |
| 12 | | 2 | 10,600 | 27,600 | 21,700 |
| 16 | 1,100,000 | 4 | | 99,000 | 72,000 |
| 32 | | 8 | 15,900 | 420,000 | 250,000 |
| 56 | 9,280,000 | 48 | 84,600,000 | 92,800,000 | 68,000,000 |

Table 7 Influence of mixing evening milk with morning milk of the same cow upon the germicidal property. Cow No.37

| Time in hours | Bacteria count in morning milk | Time in hours | Standard plate count | | |
|---|---|---|---|---|---|
| | | | Evening milk | Mixed at body temp. | Mixed at 40°F. |
| HELD AT 40°F. | | | | | |
| 0 | 12,800 | | | | |
| 1 | 11,400 | | | | |
| 2 | 14,100 | | | | |
| 8 | 12,900 | 0 | 5,400 | 10,000 | 9,100 |
| 10 | 9,900 | 1 | | 7,800 | 9,500 |
| | | 2 | 5,300 | 10,600 | 8,400 |
| 12 | | 4 | | 8,000 | 9,400 |
| 16 | 5,800 | 8 | 10,200 | 13,500 | 9,100 |
| 48 | | | | | |
| 56 | 34,800 | 48 | 15,100 | 92,000 | 10,500 |
| HELD AT 60°F. | | | | | |
| 0 | 12,800 | | | | |
| 1 | 12,900 | | | | |
| 2 | 12,600 | | | | |
| 8 | 13,800 | 0 | 5,400 | 10,900 | 8,800 |
| 10 | 18,700 | 1 | | 10,100 | 9,100 |
| 12 | | 2 | 4,400 | 9,900 | 10,600 |
| 16 | 143,000 | 4 | | 13,300 | |
| 32 | | 8 | 8,400 | 62,000 | 86,000 |
| 56 | 11,840,000 | 48 | 50,000,000 | 168,000,000 | 114,800,000 |

The germicidal action in milk of different cows.

On observing data in Tables 4 to 7 inclusive it was seen that in all the cases milk held at 40°F. showed no appreciable increase for at least eight hours. In case of cow No.221 there was no decrease for eight hours. In case of cow No.60 and 221 there was a very slight decrease and in the case of cow No.224 there was a remarkable decrease in the bacteria count. This property was greatly affected by a great difference in the bacterial contamination and its population.

In case of the milk held at 60°F. the germicidal property was marked for a shorter time. Even in this case the milk of each animal presented a different effect. In case of cow Nos.60 and 221 there was a very slight decrease whereas in the case of cow No.37 there was no marked decrease. In case of cow No.224 there was definitely a marked decrease. From this it was clear that the difference in the germicidal property of individual animals was not very clearly observed in case of milk kept at 40°F. However the difference in the germicidal property of individual animals was clearly observed in the milk kept at 60°F.

Further study of the same tables show us that different milking of the same cow on the same day have a decided variation in germicidal property.

## DISCUSSION

These experiments were in agreement with those of other workers who found that milk possessed a property which caused the bacterial content of milk to be decreased instead of increased during the first few hours after milking.

Results of these experiments seem to concur with those of Jones and Simms(24),Chambers (3),Curran (4), Heineman and Glenn (24),Heineman (15) and Frayer(9) with regards to its more or less specificity of influence.

These experiments further revealed in agreement with Hunziker (19),Park (29),Jordon and Falk(25),Jones and Little(22) and Chambers (3),that this germicidal substance varied with the individual cow.In addition to this it was also found that the germicidal substance varied to some extent in the milk of the same animal for two consecutive milkings or milk of two consecutive days.The colostrum contained the inhibitory substance but its effect was not very much different from that found in normal raw milk;except when studied at 40°F. Jones (21)believed that the maximum concentration of the inhibitor was not reached until the 6th or 7th day after calving.These experiments were performed on colostrum obtained for three days after parturition and so these results cannot be compared with those of Jones.

Stockings' (32)theory of adaptation was not found to be entirely groundless as pointed out by some of the workers.It was very clearly observed when the skimmilk and broth cultures were compared,that the sample of milk to which milk culture was added showed less lag phase than the sample from the same milk in which broth culture had been added.

No studies were made of the agglutination theory, however the observations of Heineman and Glenn(16) that agglutination may function together with factors which destroy bacteria seemed quite plausible.

Another factor which was responsible in decreasing the bacteria count was the fact that certain bacteria apparently found milk an unfavorable medium and rapidly died,whereas others required time to get accustomed to the new environment. This was clearly seen in the experiments in which raw milk was compared with autoclaved milk,intermittently sterilized milk and F.D.A. broth.

Experiments conducted by mixing evening milk with morning milk at various temperatures showed no favorable results.But still it was clearly noticed that mixing of 40°F. cooled evening milk with 40°F.cooled morning milk gave the best results with respect to bacterial counts.Mixing of body temperature evening milk with 40°F.cooled morning milk was found to yield quite satisfactory results in the laboratory

but the former was undoubtedly better. Mixing of larger quantities of milk may yield results that are quite different.

The rate of increase of organisms was determined chiefly by temperature, type of bacteria present, and the number present from the beginning and contamination of the milk later on.

According to most of the workers the germicidal property was destroyed at high temperature. These experiments show that the germicidal property was not entirely lost in case of autoclaved and intermittently heated milk. The lag phase, which might be of a shorter duration, was always present. This slight lag was also noticed in case of the experiment in which F.D.A. broth was used instead of milk.

On observing the modern practices as in practical dairying, it was found that, these are more or less in harmony with the results obtained on the germicidal property and very little seems necessary to be altered.

When one observes the above results, one believes Hunziler(19) was quite correct when he wrote, "such germicidal action of milk however, cannot be depended upon". Park(29) seems to be of the same opinion for he wrote, "Over the germicidal quality of the milk we have no power."

## SUMMARY

Germicidal property was found in the milk of all the animals studied,but its intensity varied from animal to animal,and even in the same animals for different milkings.Germicidal property was also found in the colostrum.

The rate at which the organisms increased in milk was determined primarily by the temperature at which the milk was kept,and the amount and kind of contamination.

The germicidal property was more or less specific, depending on the animal and the species of bacteria.

The germicidal property has greater destructive effect on milk with higher contamination.

Lack of adaptability seems partly responsible for the lag phase.

Mixing of evening milk at 40°F.to morning milk kept at forty seemed to produce most beneficial results.Mixing of evening milk at body temperature to morning milk kept at 40°F. were quite satisfactory.Milks stored and mixed at 60°F. seem to be fairly satisfactory,but every hour the milk was stored at this temperature reflected adversely on its future quality.

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