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TEMPERATURE EFFECTS UPON THE BACTERIA OF MILK

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Bacteria are microscopical one-celled plants, that reproduce by fission (cell division). Being of such minute size their great importance in the natural order of things about us is not realized until we study their habits and discover in a small measure their vast and unlimited extension over the whole earth.

Bacteria grow and develop into the mature state as one single cell. This cell then divides, forming two offspring which grow and multiply in the same way. Bacteria obtain their food by absorption through the protoplasmic medium of which the cell is made up. Some are able to live only in air while others are always found in the absence of air. The first are termed aerobic, and the latter are the anaerobic germs. Some are able to live in extremely high temperature; others at very low temperatures. In nature we find that, being the simplest form of life, bacteria are adapted only to the conditions in which they are found, that is, different species are found living in different conditions as regards light, heat, air, food etc., and only under those conditions are they capable of the freest existence. Place these germs in different conditions and they will not increase. Place them in an altogether different medium and if all the necessary conditions above mentioned are supplied, they will continue to increase in that medium. This would seem to indicate that bacteria, as found in nature, would not be very numerous with such varying conditions. This, however, is not the case, for with every varying condition we find species specially adapted to that condition. Bacteria reproduce with great rapidity in direct geometrical progression, providind all other conditions are equal. The average length of life from the time one cell divides until its offspring reproduce is but a few minutes to an hour. This may give some idea of the number of bacteria present about us.

Milk is one of the most favorable and nutritious medi~~um~~a for the rapid growth of bacteria, consequently, we find that milk handled without much extra care becomes contaminated with numerous kinds of bacteria, and we find it almost impossible with the greatest precautions to keep milk entirely free from germs. The germ most commonly found in milk and most difficult to keep out is the lactic acid germ, which changes milk sugar to lactic acid, and thus causes milk to sour. This germ grows best at about 70° F. It is, however, easily killed at a temperature of 176° F. This accounts for the fact that milk sours quickly when kept at this temperature (70°). Other germs which cause milk to become bitter, grow best at a little lower temperature, but in turn are easier to keep out of milk; but again are harder to kill, being more resistant than the lactic acid group. Those germs quite common with milk that has been handled carelessly with regard to sanitary conditions, and which cause foul odors and offensive tastes, have a wider temperature range for growth than the lactic acid germs. Many are as readily killed at a temperature of 176° F.

There are still many other kinds of bacteria often found in milk which are neither harmful nor beneficial, having no effect upon the milk whatever, and never becoming very numerous. Still other bacteria not so common are the ropy milk germ; the germ which causes milk to curdle sweet; pigment ~~but~~ bacteria which cause variously colored milk. The Ropy milk germ will perhaps withstand the lowest extreme of temperature of any germs effecting milk. It has been found in ice tanks where milk was being kept and it was even found that the germ, after being incased in ~~frozen~~ ice was still alive when the ice was melted and continued to live in the icewater.

Milk as a medium for the growth of bacteria, being subject to no change except as the bacteria themselves produce changes therein, the only varying condition is the temperature. As temperature is one of the most important conditions effecting the growth of bacteria, it is the one important means we have of promoting their growth, if desirable, and destroying them if undesirable. The subject of temperature effects upon the bacteria of milk thus becomes of great economic importance and should be more generally known, then we would be able to handle milk more intelligently, promoting its keeping qualities under sanitary conditions by regulating the temperature, destroying undesirable germs at a high temperature, or preventing their growth at low temperatures; to properly control and direct their growth as in the preparation of starters, and in the other case to effectively check their growth by proper refrigeration.

To know how to produce sanitary milk we must know how to control that which may cause the production of unsanitary milk--bacteria. To know how to control bacteria we must know now to use the controlling factor--temperature.

The effect of varying temperatures upon the growth of Bacteria in Milk.

Since different species of bacteria develop most rapidly at different temperatures, certain species gain the ascendency in numbers over others and thus check the growth of the others. If these species are such as produce bitter flavors or poisonous products, the milk will become unwholesome. If lactic acid species, though they may be in large numbers, the milk will not be unwholesome for most all other bacteria will then have been crowded out by the lactic bacteria.

The following tables show the results of keeping milk at different temperatures, the number of bacteria per cubic centimeter of milk after certain intervals of time, and the relative increase or decrease of the different species during the same time.

Samples of freshly drawn milk were taken and placed under the same conditions except that of temperature. The samples were kept at 32°, 40°, 55°, and 75°. One sample was allowed to change in temperature at different intervals from 32° to 60° to 45°, to note the effect of fluctuations in temperature. Agar plates were made and inoculated from the samples at intervals of one, six, ten, and twenty-six hours, and a few after a longer period. As many as possible of the different species were isolated from the colonies on the plates. The relative numbers showing decrease or increase of the different species was determined by differentiating the colonies upon the agar plates, made at the various intervals or stages in the period covered in the experiments. Twelve different species were isolated and seven were grown on media. Three of these were acid forming species; four species were neutral, of which one produced an enzyme which coagulated milk without producing acid. The first table shows the variations resulting at 32° F/ There was a decrease in nearly all species up to twenty-six hours, when the acid forming species began to increase and neutral bacteria continued to decrease until they disappeared. The colonies grew very slowly on the plates from this sample.

Table No. 2 shows the results at 40° F. The acid forming species apparently increased up to six hours and then decreased very rapidly until the tenth hour, when they increased to the end of the period. The neutral species increased slightly at first and then decreased to the end of the period. A large number and variety of species were present at the beginning but only two remained at the end of the period.

In table No. 3 the results from milk kept at 55° shows a predominance of the acid producing bacteria from the start. Only two species remain at the end of the period. No decrease is shown in the total number of bacteria at the first stage of the period, however, it is very probable that such a decrease took place between the first and sixth hours, for two species become extinct at this stage, and several decrease in number, while but one specie increases constantly throughout the period.

In table No. 4 are recorded the results of fluctuations in temperature during the same period, the temperature rising from 32° to 60°, and then to 50° and 45° F/ The fluctuations were not great enough to produce any visible effect. Three species disappear at the tenth hour and three remain, of which the acid species are predominant.

Table No. 5 shows a rapid increase of the acid producing group from the sixth hour, the growth during the first stage being quite slow. One neutral specie decreases from the start and disappears at the tenth hour. The colonies at this temperature developed rapidly on the agar plates and were vigorous in growth.

As seen in these tables, bacteria are able to grow at even 32° F/. though an increase in this case did not take place until after twenty-six hours. At the lower temperatures there are a greater number of species which persist with equal dominance. At 75° rapid development begins before the sixth hour, and though a greater number of species remain, at the end of the period there are always only a certain few which are predominant.

Table No I. at 32°

Name of Species.	after 1 hr.	after 6 hrs.	after 18 hr.	after 26 hrs.	after 192 hrs	Char. of Species	Description of the Colony.
Agrabilis.	quite numerous	decrease	Constant	Constant	very numerous	acid	Large round white; smooth surface.
B. Flourescens.	few	very few	Constant	Constant	extinct	Neutral	Lemon colored; small round
B. Antennaforiua	very numerous	decrease	Constant	Constant	very numerous	acid.	Small white; oval shaped.
—	quite numerous	increase	very numerous	decrease	extinct	—	Med. large round; pale translucent.
M. Rosettacens	very few	constant	Constant	constant	extinct	Neutral	Orange colored large round.
M. Lactis	few	very few	extinct	—	—	—	Triangular; very small, white.
—	very few	constant	constant	constant	extinct	Neutral.	Long narrow white; smooth surface.
Total number per c.c.	1,720	1,625	1,300	990	8,880		

Table No II. At. 40°.

Name of Species	After 1 hr.	After 6 hrs.	After 10 hrs.	After 26 hrs.	After 122 hrs.	Char of Species	Description of colonies.
Aquatilis	few	numerous	increase	constant	extinct	acid	Large round; white, smooth.
B. Antennaformis	quite numerous	rapid increase	decrease	constant	increase	acid	Small oval; white
M. Rosettaceus	very few	extinct	—	—	—	Neutral	Med. large; round, yellow
M. Lactis	"	"	—	—	—	acid	Triangular white; very small.
M. Auses	few	constant	extinct	—	—	Neutral	very small oval; yellow.
—	very numerous	slow increase	decrease	constant	very numerous	—	Small round, white; smooth
Sar. Aurantiacia	very few	"	"	decrease	extinct	—	very small, round; yellow.
B. Flourescens Liquifaciens	"	very few	rapid increase	constant	extinct	—	Med. sized round smooth green.
Total number per c.c.	24,000	44,600	8,800	24,000	1,093,000		

Table No III. At 55°.

Name of Species.	after 1 hr.	after 6 hrs.	after 10 hrs.	after 26 hrs.	Char. of Species	Description of Colony.
Aquatilis	quite few	rapid increase	rapid decrease	extinct	acid.	Large round; smooth; white
B. Flourescens	few	extinct	—	—	Neutral	Large round; lemon color
B. Antennaformis	Numerous	very numerous	Constant	very numerous	acid	Small white; oval.
—	few	very few	increase	very numerous	—	Large round; pale transluscent.
M. Rosettaceus	"	"	extinct	—	Neutral	Large round, smooth; orange colored.
M. Keferstenii.	"	extinct	—	—	—	Med. large round pink color.
Total number per c.c.	2,015	3,600	3,750	54,000		

Table No. IV.

Name of Species.	after 1 hr. at 45°	after 6 hrs at 32°	after 10 hrs at 60°	after 26 hrs at 45°	Char of Species	Description of Colony.
M. Aquatilis	quite numerous	rapid decrease	increase	quite numerous	acid	Large round, smooth; white.
B. Flourescens	few	very few	constant	slight increase	Neutral	Large round smooth; lemon color.
B. Antennaformis	quite numerous	rapid decrease	very few	constant	acid	Small oval; white.
—	few	extinct	—	—	—	Large round; pale translucent.
M. Rosettacens	very few	constant	extinct	—	Neutral	Large round smooth; Orange color.
M. Keferstenii	"	extinct	—	—	—	Large round; pink color.
No. germs per c.c.	900	450	1850	1125		

Table No. V. At 75°

Name of Species	after 1 hr.	after 6 hrs.	after 10 hrs.	after 26 hrs.	Char. of Species.	Description of Colony.
Aquatilis	few	few	increase	great increase	acid	Large round smooth; white
B. Flourescens	"	"	extinct	—	Neutral	Large round; lemon color.
B. Antennaformis	very numerous	Constant	Constant	increase	acid	small oval; white.
—	few	quite numerous	very numerous	"	—	Large round; pale translucent.
M. Rosettaceus	"	few	increase	"	Neutral	Large round orange color.
Total number per c.c.	3,860	14,000	62,500	960,000		

6.

The so called Germicidal Quality in Milk.

Milk is unquestionably a most excellent medium for the growth of bacteria when in its natural state of condition. Because of this it is ordinarily supposed that bacteria multiply rapidly in milk immediately after it is drawn. Recent investigation, however, shows that the number of bacteria decrease for a certain length of time after milking. This fact was first announced by Fokker in 1890, then by Freudenrich in 1891, and later by Freeman in 1896. These investigators offered no theory for the cause of this unexpected condition but termed it a "germicidal action contained in freshly drawn milk." This "germicidal action" was made the subject of an extended series of experiments by Hunziker in 1901, and his conclusions were that this germicidal quality did exist and furthermore that it varied at different temperatures in length and intensity of its influence. He also stated that milk from different cows varied in its germicidal quality. Extreme heat, as 176°, he found destroyed this property. Quite recently experiments along this line have been made at the Connecticut Agricultural College/ The conclusion reached there was that a study of the different species of bacteria in the milk from the time it is drawn shows that many species do not find milk a favoragle medium in which to grow and reproduce, and hence the particular species so effected by the change of surroundings decrease in number and finally disappear altogether. To this diminution of the many species which find milk an unfavorable medium for growth, Storrs attributes the early decrease of bacteria in fresh milk, rather than to any germicidal property in the milk itself. This seems the more reasonable explanation for the phenomena, in that it presents the natural and inevitable result of a large variety of species finding their way into the medium of the fresh milk, and but a few species finding it a favorable medium for growth and therefore only these few species increasing, while the larger number are rapidly decreasing in number and many finally disappearing.

One feature, however, was not referred to in the Storrs' investigations, and that was the fact brought out by Hunziker that in pasturized milk no decrease resulted, but a constant increase in the number of bacteria occurred when such milk had been inoculated. This, however, may be accounted for in this way; by inoculating the milk which contains no germs, few germs are introduced and a less number of species. Although a decrease did take place with a majority of the species present in the inoculated milk, on account of the small number present it would not be a decrease sufficient to overbalance the very rapid increase of the few species to which the fresh milk was a medium favorable to rapid growth.

The accompanying table shows the results of experiments the writer carried out on this subject to determine the presence of this germicidal quality and to determine the effect of heat upon it. Samples were taken as soon as drawn. One sample was heated in every case to 176° for twenty minutes and cooled at once to the temperature at which it was to be kept. It was then inoculated from the other sample. Agar plates were made at regular intervals of one, three, five, ten, and twenty-five hours and the number of colonies counted after twenty-four to forty-eight hours. From this the number of bacteria in one cubic centimeter of the milk was calculated. The samples were kept at the different temperatures of 35°, 45°, 55°, and 70° F/, to determine the relations of initial decrease in the number of bacteria to different temperatures. The usual decrease occurred in every case excepting the sample kept at 70°, in which, however, the increase during the first few hours was very slow and it

Table No. VI. Effects of Pasteurization upon the Germicidal Quality of Milk.

	Temp.*	Hours at which agar plates were made.				
		1	3	5	10	25
Pasteurized	35°	140	110	120	130	290
Unpasteurized	35°	3,950	3,760	3,740	1,920	1,560
Pasteurized	45°	120	460	740	—	3,200
Unpasteurized	45°	8,120	3,780	6,360	—	24,000
Pasteurized.	55°	1,240	8,000	11,400	—	46,200
Unpasteurized	55°	12,760	11,340	2,180	—	42,600
Pasteurized	70°	1,740	4,000	17,200	76,000	innumerable
Unpasteurized.	70°	17,500	20,000	89,000	256,000	..

* Temperature at which each sample was kept during the period.

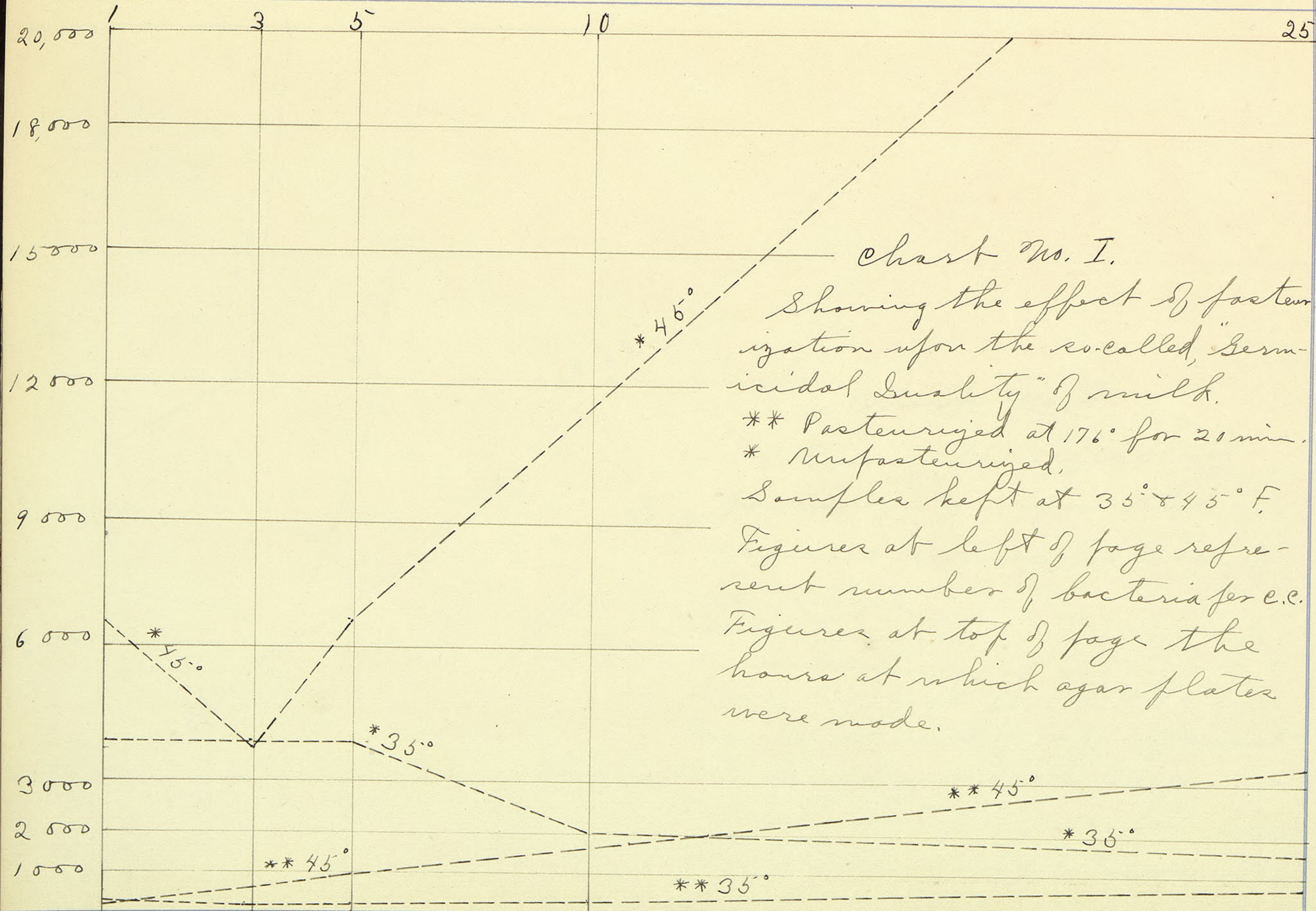


Chart No. I.

Showing the effect of pasteurization upon the so-called "Germicidal Quality" of milk.

** Pasteurized at 176° for 20 min.

* Unpasteurized.

Samples kept at 35° & 45° F.

Figures at left of page represent number of bacteria per c.c.

Figures at top of page the hours at which agar plates were made.

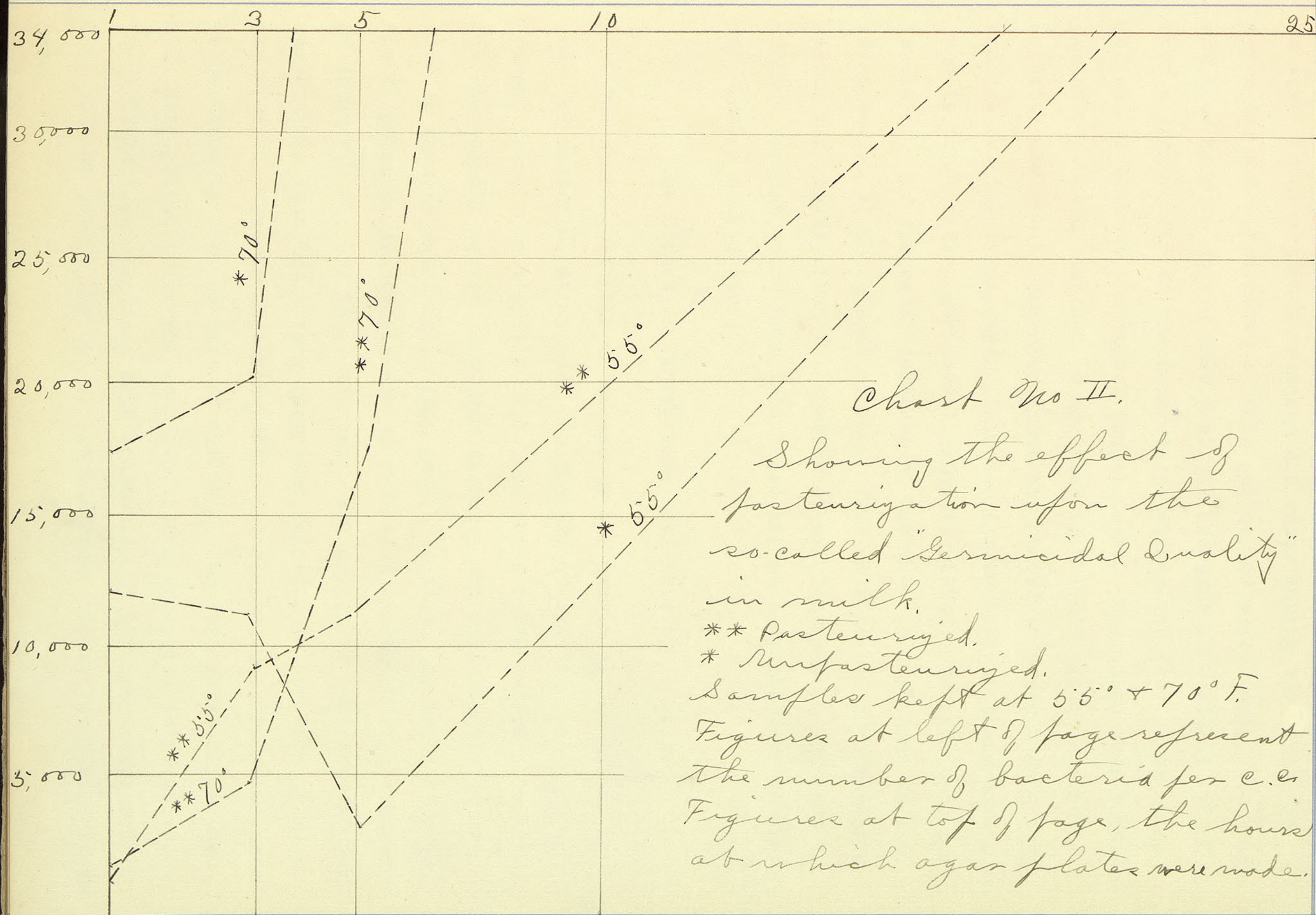


Chart No II.

Showing the effect of pasteurization upon the so-called "Germicidal Quality" in milk.

** Pasteurized.

* Unpasteurized.

Samples kept at 55° & 70° F.

Figures at left of page represent the number of bacteria per c.c.

Figures at top of page, the hours at which agar plates were made.

is very probable that a decrease did occur during the period between one and three hours of this particular experiment. The table and accompanying charts show that the decrease in bacteria extended over a longer time at the lower temperatures and over quite short periods of time at the higher temperatures. They also show that apparently no such decrease as occurred in the milk which was not heated, resulted in the milk which was heated to pasteurizing temperature for twenty minutes. This would show that if there exists in freshly drawn milk a germicidal property, this is destroyed by pasteurizing. If the usual decrease of bacteria is explained as the disappearing of those species which find milk to be an unfavorable medium for growth, then the absence of any decrease in the pasteurized samples which were inoculated may be accounted for in the way already stated above.

The Effect of Temperature Upon Acidity in Milk.

The souring of milk is caused by the growth of the lactic acid group of bacteria, which have the faculty of changing the milk sugar present in milk into lactic acid. As these bacteria are allowed to develop rapidly or are kept from growing, the milk becomes quickly sour or remains sweet for a long period of time. As before stated, the temperature most favorable to the rapid growth of these bacteria is about 70° F., and they will cease all growth at low temperatures. Temperature then has a very important relation to the keeping quality of milk. The accompanying table and charts show the relation of temperature to the per cent of acidity. Samples of freshly drawn milk were placed under the same conditions excepting temperature. The different samples were kept constantly at the temperatures 35°, 45°, 50°, 60°, 75°, and 90° F/ At different intervals of time tests ~~were~~

were made of the acidity of each sample as indicated in the table and charts. These show a more rapid increase in the per cent of acidity as the temperature becomes higher, increasing very rapidly at 90°F/ At 35° the acidity shows no increase to any extent throughout the period of 110 hours. At 45° and 50° the acidity remains constant for 72 hours and then gradually increases to the end of the period. At 60° there is a very gradual increase up to 34 hours, when the increase becomes very rapid up to 1.04% at 58 hours. At 75° a very rapid increase begins at 10 hours and continues up to 1.22% at 34 hours. At 90° a more rapid increase begins at 10 hours and continues only up to .95% at 24 hours, after which the rise is very slow. At the lower temperatures there is a constant rise and fall in the per cent of acidity. This is caused by the increase and subsequent decrease of certain alkali producing species of bacteria, which neutralize the acid produced by the lactic bacteria. When the latter finally gain the ascendency there is a constant increase in the acid content of the milk. At the higher temperatures the increase continues until the great amount of acid causes the milk to become an unfavorable medium for further frowth, when further increase ceases. The highest per cent of acidity was gained in the sample kept at 75°.

These results show that milk if properly handled, may be kept sweet for an indefinite length of time at 35°, although the quality of the milk may deteriorate through the growth at this temperature of certain species which produce bitter flavors in the milk. For economic uses, however, milk, as shown by these results, may be kept sweet a sufficient length of time at 50°F/. if properly handled, which is slightly below the temperature of ordinary deep well water in summer-time. Thus is shown the close relation between the keeping quality of milk and the temperature at which it is kept.

The rapid souring of milk during days of rain and thunder storm is often and quite commonly attributed to an effect upon the milk, caused by the atmosphere being surcharged with electricity. It has been proven, however, by experiments, that electricity has no stimulating effect upon the growth of bacteria. Furthermore it has been shown that a warm temperature combined with a moist, humid atmosphere does greatly increase the rate of growth of the lactic bacteria of milk. The rapid souring of the milk is then not due to the thunder-storms, but to the warm, humid condition of the atmosphere, common at such times, which cause a more rapid growth of bacteria and hence more rapid souring of the milk.

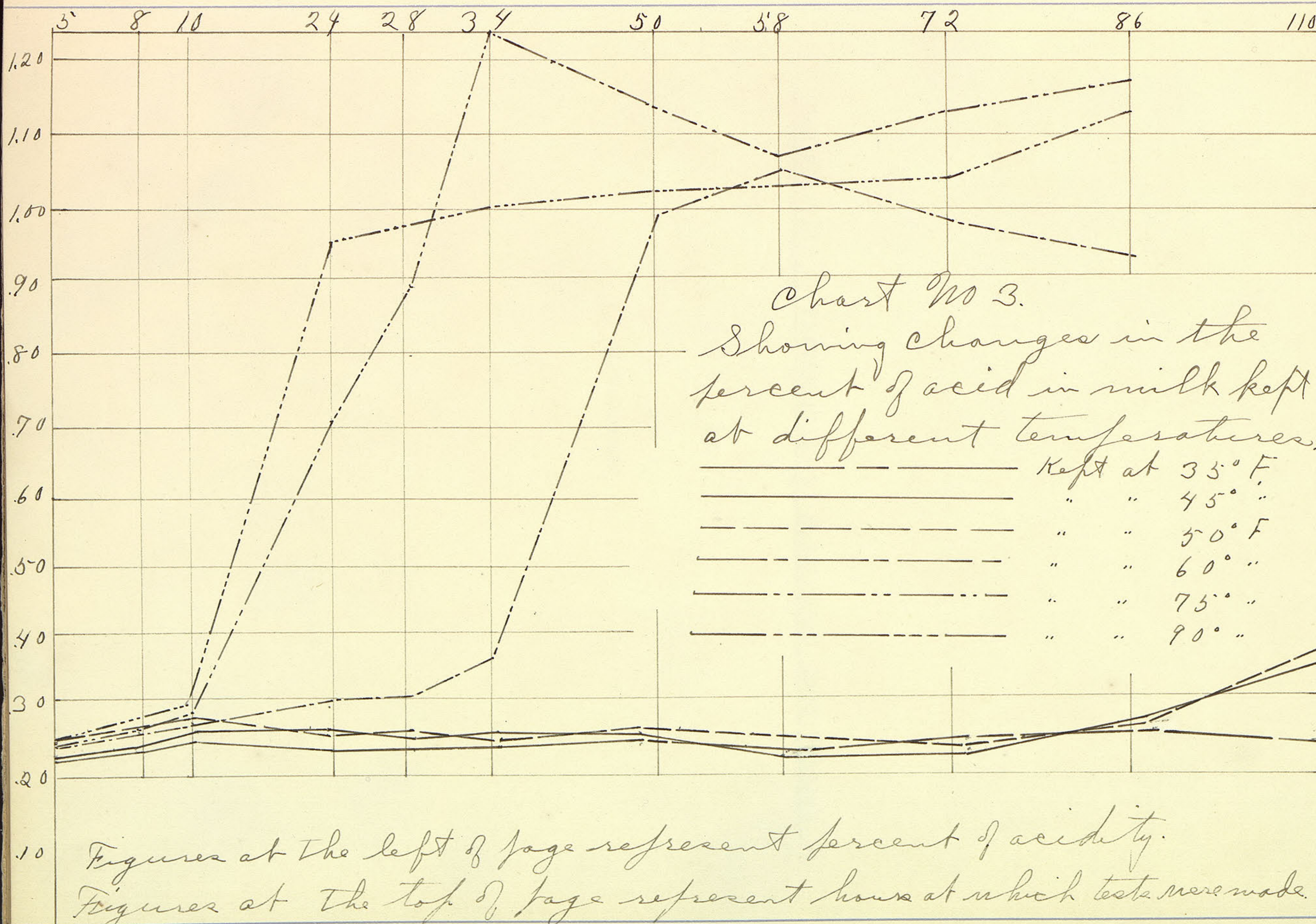
Thus we see that the influence of temperature upon the bacterial flora extends over a wide range and is very effective in producing changes in the relative activity of different species of bacteria in milk. That a knowledge of the effects of different temperatures upon various species of bacteria in milk becomes of great economic importance in the realms of dairying is readily recognized. Without such knowledge, milk will not be handled judiciously and with the greatest profit at the dairy farm and creamery.

Table No. VII. Comparative increase in acidity of milk kept at different temperatures.

Temp.	Hours at which acid tests were made.										
	5	8	10	24	28	34	50	58	72	86	110
35°	23	234	233	237	237	24	244	238	237	248	244
45°	237	237	248	248	24	252	25	23	234	26	36*
50°	252	266	268	252	255	248	256	24	23	252	374*
60°	24	255	259	288	29	36*	98**	1,04	98	91	
75°	24	259	263	70	89**	1,22	1.13	1.06	1.12	1.17	
90°	258	26	28	95**	98	1.00	1.02	1.02	1.03	1.13	

* Distinctly sour to taste
** Curdled.

Figures in column opposite temperatures represent percent of acidity.


3
8
10
24
28
34
50
58
72
86
110
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
Chart No 3.
Showing changes in the percent of acid in milk kept at different temperatures.
Kept at 35° F.
" " 45° "
" " 50° F
" " 60° "
" " 75° "
" " 90° "
Figures at the left of page represent percent of acidity.
Figures at the top of page represent hours at which tests were made.