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The Bacterial Flora of Normal Cows Udder

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THE BACTERIAL FLORA OF NORMAL COW'S UDDER

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Brookings

Digest

1. Milk drawn aseptically from each quarter of the udder of forty cows gave an average bacterial count of 1,541 per cc.
2. Cell counts of the udders of the forty cows showed an average of 657,000 per cc.
3. Bacterial and cell counts compare quite closely. The coefficient of correlation between bacteria and cell counts was $+.6379 \pm .03364$.
4. Lactation exerted no appreciable effect on bacterial content of the udder.
5. The age of the cow exerted no effect except in the case of older cows past maturity in which both bacterial and cell counts were greatly increased.
6. Fore milk was considerably higher in bacteria than the middle milk or strippings. Strippings were found to run slightly less in bacterial count than milk from the middle of the milking.
7. Colostrum usually was very high both in bacterial and cell content although considerable variation was noted.
8. Bacteria of the udder are usually gram positive micrococci. They ferment sugar without the formation of gas and may or may not liquefy gelatin.
9. Udder cocci gave uniformly low total and volatile acidities.
10. In some cases, curdling of the milk was accomplished by an enzyme. Digestion of the curd was noted in several instances.
11. Milk from different cows seems to vary in germicidal power. Cultures of bacteria artificially introduced into the udder do not survive over a few days.

The Bacterial Flora of Normal Cows' Udders

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Facts pertaining to the bacterial and cellular composition of milk are vital in milk hygiene. Therefore, a study of the bacterial flora of normal udders is of importance to dairymen as well as bacteriologists.

Bacteriologists have long known that the udder of the dairy cow is not sterile and that aseptic conditions in milking will not reduce the germ content below what the udder contains before the milk is drawn. Harding and Wilson (1) report an average udder count of 428 bacteria per cc. from an examination of the udders of 78 cows. The same authors also classified the organisms into 71 groups, and these groups were characterized by a lack of motility of spore formation and of gas production. Most of the groups were micrococci and were gram positive. Udder bacterial counts have always been of importance to raw milk dealers, especially those producing certified milk. Hastings (2) reported an udder bacterial count exceeding 100,000 per cc. from an apparently normal cow. A few cows giving such high counts would cause serious trouble in a certified dairy.

The findings of Breed (3) from an examination of the milk of 122 cows show an average cellular content of 868,000 per cc. No attempt was made to demonstrate any correlation that might exist between cellular and bacterial counts. Although cell counts are not generally used in routine inspection of milk, their relation to bacterial counts may be of value especially in cases of infection. Cooledge (4) reported that milk from actively **Bact. abortus** infected udders is found to have an average cellular content over five times as high as the apparently normal average, and that **Bact. abortus** infection accounts for many of the samples of milk which might have high cell counts as drawn from apparently normal udders.

The effect produced in milk by the organisms inhabiting the udder is of equal interest. Gorini (5) has shown that normal udder cocci may play an important role in the ripening of certain kinds of cheese. Hastings (6) states that they produce no lactic acid, but only acetic, propionic, butyric and caproic acids. Off flavors in milk and cream have likewise been attributed to udder cocci.

Experimental Work

The data presented in this bulletin represent studies carried on over a period of two years. The investigation was divided into two main parts as follows:

Part I. Studies of the number of bacteria normally inhabiting the udder. Under this division were included total counts, distribution among the quarters, relation of bacterial counts to cell counts, relation of bacterial counts to the reduction of methylene blue, effect of age, stage of lactation, comparison of different portions of the milking and bacterial counts of colostrum.

PART II. Studies of the characteristics of udder bacteria together with the type of fermentation they produce in milk. Under this division were included a study of types of total acidity produced in milk by these bacteria, amounts and kinds of volatile acids produced, action of udder micrococci on the proteins of milk, effect of germicidal activity on udder organisms compared with other types, thermal death point of these bacteria and odors and flavors produced in the milk.

PART I

Studies of the Number of Bacteria in the Udder

Forty cows, representing the milking herd at the College farm, were used. The cows were of varying ages, extremes ranging from two to seventeen years. They also varied in stage of lactation. All of the cattle were tuberculin tested and free from other diseases which included udder infections.

Total bacterial and cell content.—Bacterial and cell counts were taken from each quarter of the udder of the forty cows. Both bacterial and cell determinations were made from the same sample, after which part of the sample was used to determine reduction time by the stand- and methylene blue method. The samples were collected under careful aseptic conditions. In all cases, approximately one-third of the milk was drawn from the quarter before taking the sample. Platings were made in duplicate in dilutions of .5 and 10 except in cases of cows showing high counts which were repeated using higher dilutions. Dextrose agar was used in plating with a four-day incubation period at 37 degrees C. Counting was done with a hand lens. Cell counts were made by the method suggested for this purpose by Breed (7), making duplicate smears and counting fifty fields per smear.

Table I presents the bacterial counts, cell counts and the reduction time of the milk.

TABLE I.—BACTERIAL AND CELL COUNTS AND REDUCTION TIME OF MILK FROM DIFFERENT COWS

Cow No.	Quarter	Bact.	Cells	Reduction Time, Hrs.
1	R.F.	1	387,840	20
	L.F.	0	327,240	20
	R.R.	416	2,139,000	13
	L.R.	1	278,000	17
2	R.F.	0	84,840	17.5
	L.F.	1	90,900	19
	R.R	1	124,000	18.5
	L.R.	1	60,600	18
3	R.F.	5,080	951,400	13
	L.F.	9	824,000	13
	R.R	450	618,000	13
	L.R.	2	296,900	19.5
4	R.F.	1,660	333,000	14
	L.F.	7,000	375,000	19.5
	R.R.	14,250	945,300	15
	L.R.	3,500	521,160	15
5	R.F.	7	333,300	13.5
	L.F.	180	199,900	17.5
	R.R.	9,450	406,020	15
	L.R.	28	230,280	12
6	R.F.	19	399,900	12.5
	L.F.	830	321,170	11
	R.R.	13	309,060	12
	L.R.	37	278,760	13
7	R.F.	2,165	454,500	14
	L.F.	47	278,760	13.5
	R.R.	780	284,800	13.5
	L.R.	540	224,200	14.5
8	R.F.	47	375,720	13.5
	L.F.	41	90,900	13.5
	R.R.	200	121,200	13
	L.R.	11	60,600	13
9	R.F.	285	478,740	17
	L.F.	1,320	606,006	16.5
	R.R.	14	72,720	14.5
	L.R.	4,850	824,160	13
10	R.F.	1	109,000	17
	L.F.	1	66,000	17
	R.R.	5	218,000	16.5
	L.R.	1	60,600	15
11	R.F.	0	169,000	22
	L.F.	blind	.....	....
	R.R.	0	139,000	22
	L.R.	4	187,000	22
12	R.F.	410	333,300	16
	L.F.	2,180	1,327,000	13
	R.R.	445	260,580	13.5
	L.R.	435	436,300	13
13	R.F.	1	48,480	14
	L.F.	0	54,540	14
	R.R.	0	36,360	14
	L.R.	1	12,120	14
14	R.F.	585	369,600	14
	L.F.	1	151,000	14.5
	R.R.	5	145,000	17
	L.R.	4	448,000	14
15	R.F.	2	72,720	14.5
	L.F.	3	121,200	17
	R.R.	6	154,440	16
	L.R.	4	127,260	17
16	R.F.	5	36,360	14.5
	L.F.	1,620	599,940	17
	R.R.	430	551,460	16
	L.R.	0	30,300	14.5
17	R.F.	1,390	284,460	17
	L.F.	4,225	309,060	14
	R.R.	131	333,300	17.5
	L.R.	1,910	448,440	17
18	R.F.	11	303,000	22
	L.F.	18	115,140	22
	R.R.	13	399,960	23
	L.R.	267	42,420	22
19	R.F.	2	393,900	20
	L.F.	2	327,240	21
	R.R.	1	599,940	20
	L.F.	1	399,960	18
20	L.R.	16,790	3,030,000	13
	R.F.	29,800	5,999,000	13
	R.R.	27,750	2,690,000	12
	L.R.	blind	.....	....
21	R.F.	580	42,400	13
	L.F.	35	412,080	13
	R.R.	1	109,080	14
	L.R.	1,205	290,900	12
22	R.F.	1,591	89,000	12
	L.F.	10	11,000	18
	R.R.	18	31,000	19
	L.R.	12	21,000	19
23	R.F.	12	158,000	22
	L.F.	50	4,389,000	8
	R.R.	11	1,125,000	12
	L.R.	112	1,239,000	12
24	R.F.	223	24,240	13
	L.F.	107	90,960	13
	R.R.	485	90,900	12
	L.R.	88	24,240	13
25	R.F.	35	24,000	12
	L.F.	44	66,660	14
	R.R.	1,100	230,280	10
	L.R.	200	72,720	12
26	R.F.	6	78,780	18
	L.F.	28	333,300	18
	R.R.	10	109,000	18
	L.R.	3,045	2,781,500	7.5
27	R.F.	660	6,660	12
	L.F.	5	12,100	18
	R.R.	5	30,300	18
	L.R.	1	18,180	24
28	R.F.	1	90,960	18
	L.F.	275	569,600	22
	R.R.	585	169,700	12
	L.R.	300	963,500	8
29	R.F.	0	72,720	15
	L.F.	15	87,870	16
	R.R.	4	127,200	15
	L.R.	21	757,500	14
30	R.F.	1,275	1,848,000	4
	L.F.	110	2,454,000	5
	R.R.	3,850	1,818,000	3
	L.R.	37	1,515,000	6
31	R.F.	995	4,090,000	4
	L.F.	480	1,212,000	9
	R.R.	305	15,599,000	4
	L.R.	3,500	3,423,000	8
32	R.F.	315	48,480	14
	L.F.	75	30,300	16
	R.R.	82	24,240	16
	L.R.	75	42,420	19
33	R.F.	14	96,960	12
	L.F.	18	42,400	16
	R.R.	72	18,200	16
	L.R.	8	18,200	18
34	R.F.	112,000	3,319,000	4
	L.F.	15,400	1,227,000	5
	R.R.	15,200	786,000	8
	L.R.	12,500	306,000	9
35	R.F.	540	2,598,000	8
	L.F.	12	17,760	14
	R.R.	200	155,000	12
	L.R.	100	61,200	12
36	R.F.	1,170	661,000	14
	L.F.	**768**	**366,000**	**16**
	R.R.	2,500	1,010,000	9.5
	L.R.	3,000	976,000	10
37	R.F.	43	257,000	18
	L.F.	30	263,000	18
	R.R.	16	164,000	17
	L.R.	5,200	586,000	11
38	R.F.	0	347,000	12
	L.F.	225	146,000	12
	R.R.	398	861,000	11
	L.R.	10	729,000	13
39	R.F.	2	72,700	14
	L.F.	1,620	599,000	17
	R.R.	131	333,000	17
	L.R.	267	42,400	22
40	R.F.	47	375,720	14
	L.F.	1,320	606,0000	16
	R.R.	5	218,000	16.5
	L.R.	4	187,000	22

The extremes in bacterial counts ranged from 0 per cc. to 347,000 per cc., with an average for 39 cows of 1,541 bacteria per cc. The cow whose milk yielded this maximum count was omitted from this average, although her udder was apparently normal. During her lactation period, numerous counts were taken; forty of these exceeded 50,000 per cc. and seventeen were over 100,000 per cc. The data compares favorably with that presented by Hardin and Wilson (1) in showing that the rear udders as a rule slightly exceeded the fore udders in counts. Seventy-eight per quarters gave an average count of 1,671 per cc. compared to 1,329 for the fore quarters. Cell counts showed a range in number from 11,000 to 15,500,000 with an average of 657,000 per cc. The rear quarters gave an average of 688,350 while the fore quarters contained 625,950 per cc.

In general, the bacterial and cell counts compared quite closely. In cases of cell counts over one million per cc., the bacterial counts were usually high, averaging 5,407 per cc. There were 20 such quarters with counts exceeding one million out of the 158 quarters examined. Tabulating all the counts, the coefficient correlation between bacterial and cell counts was found to be $+.6379 \pm .03364$. Comparing bacterial counts and reduction time at the aseptic samples, the correlation is not so close. This is attributed to the fact that many of the organisms reduce methylene blue slowly. However, in nearly all cases in which the methylene blue was reduced in much less than 12 hours, high bacterial counts were found. This phase of the problem was undertaken with a view of finding a means whereby the dairyman might trace his cows giving high counts without the labor involved in making bacterial determinations. While the method may hold possibilities, it appears at present only fairly reliable.

Effect of stage of lactation.—Four cows were selected and bacterial counts made from each quarter of the udder every five days for a period of 14 months to determine the effect of stage of lactation. This resulted in from five to six counts being run each month on each cow. The counts were then averaged for each month and totalled for the year. The monthly averages are shown in Table II.

Examination of this table reveals that while there is always some variation from month to month, the number tends to remain fairly constant. This was true in nearly all cases and markedly so with cows number 259 and number 23. Number 259 was uniformly low in bacterial count for all quarters during the entire year, while number 23 was exceptionally high in all quarters for the lactation period. As far as known, this cow has never had an udder infection or inflammation during her life time. It was also found that differences in bacterial counts between quarters may be permanent. The right front quarter of number 306 almost always considerably exceeded the other quarters of the

udder in bacterial numbers. This was also true of the right front quarter of number 23. In no case did the stage of lactation seem to exert any influence on numbers except in the case of bacterial counts made within a week after parturition.

Effect of age.—To determine the effect of age on bacterial counts the forty cows were divided into three groups, under four years of age, four to seven years, and above seven years. The bacterial counts of all cows in each group were averaged and recorded in Table III.

Note that there is no appreciable difference due to age except in the case of cows over seven years of age. With the older cows, both the bacterial and cell counts were greatly increased. This increase is possibly explained by the fact that few cows go entirely through life without udder troubles or infections of some nature. The organisms causing the infection may persist in the udder even after recovery or the udder may be rendered susceptible to invasion by other types. In the case of the younger animals, the great majority were known to have always been free from udder infection or inflammation.

Bacterial content of different portions of the milking.—Many sanitary dairies engaged in producing and bottling raw milk discard the first few streams drawn from the udder. It is common knowledge that the first few streams drawn are relatively high in bacteria. However, to include this phase of the problem, trials were run using eight cows. Bacterial counts were made from the first two streams, from the middle of the milking and from the strippings of each quarter of the cows. This gave 32 comparisons. The results are tabulated in Table IV.

The left rear quarter of cow number 7 was omitted from the final average as this quarter had been injured, resulting in a slight infection. These results differ slightly from those reported by Harding and Wilson (1) in that the strippings averaged lower than the middle of the milking. The difference shown by Table IV is slight, however.

Bacterial and cellular content of colostrum.—Mention has been made that bacterial counts were frequently high when taken during the week following parturition. Whether there were more favorable conditions for growth due to inflammation of the udder that frequently follows partruition or whether the flora becomes more firmly established during the dry period is a question. As a rule, the high counts are only temporary. Bacterial and cell counts were determined on the separate quarters of six cows. In each case, the samples were collected within six hours after calving and in three cases before the calf had sucked. The counts are reported in Table V.

Table II—EFFECT F STAGE OF LACTATION.

Month	Cow No. 157					Cow No. 259					Cow No. 306					Cow No. 23				
	R.F.	L.F.	R.R.	L.R	Av.	R.F.	L.F.	R.R.	L.R.	Av.	R.F.	L.F.	R.R.	L.R.	Av.	R. F.	L.F.	R.R.	L.R.	Av.
April	19	35	19	54	46	130	29	41	70	71	911	12	10	26	245	9414	6619	4240	4404	6139
May	25	15	19	39	24	87	53	21	79	60	647	18	13	10	188	24328	5208	3214	4147	9404
June	13	18	40	26	23	61	10	17	24	28	911	39	25	35	243	17907	8681	9167	3726	9036
					*															
July	104	28	731	51	251	32	85	22	29	41	1108	12	31	45	311	70892	48850	31882	10420	40104
Aug.	13	37	32	23	26	7	20	14	7	12	726	23	43	21	203	107413	44819	50300	54550	64270
Sept.	32	4	24	15	22	10	14	15	25	16	328	129	14	38	127	87000	45000	25675	30020	49924
Oct	54	14	32	6	27	1	1	98	2	26	112	8	77	6	51	96400	31250	40300	58500	56612
Nov.	9	7	12	8	7	131	21	20	131	76	289	12	16	3	83	5505	5070	50883	2950	16115
Dec.	43	21	103	13	46	39	32	11	19	26	186	69	10	43	77	87387	44581	36694	25169	45415
Jan.	20	3	6	54	21	4	15	5	24	12	115	112	43	76	86	73000	19700	31500	9500	33425
Feb.	15	5	2	22	11	6	8	3	2	5	34	24	7	45	28	187400	7775	19033	10698	60705
Mar.	623	33	6788	115	1889	5	7	4	5	5	50	7	161	159	94	197915	13100	42300	17400	67687
										*										
Apr.	2	2	320	10	88	4	31	627	3	166	2018	8	176	1	550					dried up
May	1	0	316	105	105	0	0	3	1	1	5080	9	450	2	1385					
														dried up						
		Yearly	Av.		178					39					263					37,986

*Calved

Table III—EFFECT OF AGE OF THE COW ON BACTERIAL AND CELL COUNTS

Age	No. of Cows	Av. Bact. Count	Av. Cell Count
Under four years.....	12	331	420,300
Four to seven years....	17	541	388,500
Over seven years.....	11	8282	1,559,000

Table IV.—COMPARISONS OF BACTERIAL COUNTS OF DIFFERENT PORTIONS OF THE MILKING.

1	R. F.	1,110	71	21
	L. F.	1,600	51	18
	R.R.	2,710	101	13
	L. R	1,760	349	117
2	R.F.	2,570	64	72
	L. F.	2.100	184	110
	R.R.	2,100	45	22
	L. R.	1,145	321	32
3	R.F.	2,570	1,550	2,370
	L. F.	3,670	2,460	35
	R.R.	2,730	3,340	180
	L. R.	2,760	2,520	130
4	R.F.	25,200	175	295
	L. F.	31,000	1,400	420
	R.R.	5,000	600	500
	L.R.	1,800	265	640
5	R.F.	3,700	40	40
	L.F.	10,050	271	655
	R.R.	215	10	60
	L. R.	9,837	1,000	5,250
6	R.F.	265	10	29
	L. F.	50	9	7
	R.R.	35	62	13
	L.R.	535	22	13
7	R.F.	45	1	51
	L. F.	110	5	32
	R.R.	95	530	17
	L. R.	192,000*	2,750	790
8	R.F.	6,780	540	300
	L. F.	5,400	320	120
	R.R.	2,890	96	110
	L.R.	10,850	860	420
Average		5,989	557	415

Table V.—BACTERIAL AND CELLULAR ANALYSIS OF COLOSTRUM.

Cow No.	Quarter	Bacterial Count	Cell Count
1	R.F.	204,000	2,889.000
	L. F.	53,750	4,175,000
	R.R.	600	1,800,000
	L.R.	269,500	1,636,000
2	R.F.	100	943,000
	L. F.	200	692,000
	R.R.	9,700	4,140,000
	L.R.	14,200	86,670,000
3	R.F.	35	1,045,000
	L. F.	10	777,000
	R.R.	370	204,000
	L.R.	160	807,000
4	R.F.	20,350	13,350,000
	L. F.	4,300	4,320,000
	R.R.	18,200	10,755,000
	L. R.	23,300	13,350,000
5	R.F.	25,750	1,199,000
	L. F.	625,000	448,000
	R.R.	101,500	2,696,000
	L.R.	650,000	824,000
6	R.F.	230	946,000
	L. F.	4,250	18,940,000
	R.R.	890	7,460,000
	L.R.	10.200	14,235,000
	Average	84.858	8.095,000

A significant fact is that cow number 3 was not dry but was milked up to the evening before calving. Consequently, the first milk secreted after calving was more nearly normal. In the other cases, both the bacterial and cell counts were very high although all the cows showed extreme variation.

PART II

Studies of the Charasteristics of Udder Flora

In examining the plates made in taking the udder bacterial counts, the types of colonies growing on the plates were carefully noted. Typical colonies were used in a study of the type of fermentation produced in milk by these organisms. A detailed study of the characteristics and classification of the flora was not made because this phase of the problem was not of particular interest in this work.

General characteristics of udder flora.—However, the general characteristics of the predominating types were determined. Gram stains made direct from 100 typical 48-hour-old colonies on agar gave 98 positive and 2 negative stains. All of these slides when examined under a microscope were described as micrococci.

Before making these slides, the colonies were first picked into gelatin stabs and liquefaction noted. The tubes were incubated ten days at 37 degrees C, then cooled and examined for liquefaction, sixteen tubes showed decided liquefaction, nineteen slight liquefaction and the remaining sixty-five were completely solidified. The gelatin cultures were then transferred to both dextrose and lactose fermentation tubes. In every case, the sugar was fermented with the production of acid and the entire absence of gas. Brom cresol purple was added to the tubes to determine acid production.

The appearance of udder colonies on agar plates is quite characteristic. In the majority of cases, growth was quite luxuriant. Chromgenesis is another feature that is striking. In making the total counts recorded in the first part of this bulletin, the number of pigmented colonies was also recorded. Two thousand plates so examined showed the percentages of pigmented colonies recorded in Table VI.

Table VI.—NUMBER OF PIGMENTED COLONIES ON AGAR PLATES.

Description of Colonies	Number Recorded	Percentage
White Colonies	1584	79.2 per cent
Lemon yellow colonies	220	11.0 per cent
Orange yellow colonies	160	8.0 per cent
Pink colonies	30	1.50 per cent
Brownish colonies	6	.30 per cent

Acid production.—In studying the action of udder bacteria in milk, typical colonies were picked from time to time in tubes of dextrose broth. After 24 hours' incubation 2cc. of these cultures were transferred to flasks of sterile skimmilk. In preparing the skimmilk, fresh mornings milk from the dairy barn was separated. The skimmilk was usually less than 3 hours old when flasked and sterilized. Approximately 600 cc. was placed in each flask. At first the flasks were sterilized intermittently, then incubated several days and the non-sterile samples discarded. Later, however, the milk was sterilized under pressure. This resulted in a browning of the milk to a slight extent.

The flasks of inoculated skimmilk were incubated 24 hours at 37 degrees C. Determinations were then made for total and volatile acidities. The action on casein was noted. Taste and aroma was also included on each sample.

Total acidities were determined using 18cc. of the milk sample, titrating with N-20 NaOH and dividing the results by 4. Acidities were calculated as lactic acid. The maximum total acidity was .67 per cent. Nine cultures were examined that gave acidities exceeding .50 per cent. The minimum acidity recorded was .17 per cent. Cultures picked from white colonies usually gave slightly higher total acidities than those from yellow colonies. Thirty-two cultures from white colonies gave an average acidity of .344 per cent, while 28 cultures from yellow colonies gave an average of .315 per cent.

The following technique was employed in making the volatile acid determinations: Duplicate distillations were made on each sample. A 250 cc. portion was distilled with steam. Before the distillation, 10 cc. of dilute phosphoric acid was added to each sample. A few grams of anhydrous Na2-SO4 were also added to prevent excessive foaming. Distilled water was used in the generator and was boiled for several minutes before connecting with the flask. The distillation was continued until 1,000 cc. of distillate was secured. The distillate was titrated with N-20 NaOH using phenolphthalein as an indicator. Results are expressed in terms of number of cc. of N-20 Na OH required to neutralize the first 1,000 cc. of distillate. In all except a few cases, a good agreement of duplicates was secured. Those titrations not checking closely were discarded in compiling the data.

The results of all the volatile acidity and a portion of the total acidity determinations are presented in Table VII.

In order to secure data to furnish a basis of comparison, total and volatile acid production was determined on milk cultures fermented with other organisms. The first used were good starters ripened at 21 degrees C. for 24 hours. Later, milk inoculated with L **acidophilus** and L. **viscosus** was used. The results are tabulated in Tables VIII. IX and X.

Table VII.—TOTAL AND VOLATILE ACID PRODUCTION OF UDDER MICROCOCCI

Culture No.	Tot. Acid. Per Cent	Vol. Acid.	Cul. No.	Tot. Acid. Per Cent	Vol. Acid Per Cent
1	.55	22.4 cc.	14	.25	14.4 cc
2	.32	24.0 cc.	15	.26	16.0 cc
3	.32	15.2 cc	16	.32	16.0 cc
4	.34	28.0 cc	17	.38	19.2 cc
5	.65	23.2 cc.	18	.36	17.6 cc
6	.20	14.4 cc.	19	.34	22.4 cc
7	.25	13.6 cc.	20	.21	12.0 cc
8	.25	12.8 cc.	21	.305	11.9 cc
9	.35	25.1 cc.	22	.51	21.6 cc
10	.60	43.2 cc.	23	.415	24.5 cc
11	.50	26.4 cc.	24	.58	27.9 cc
12	.31	8.8 cc.	25	.39	22.2 cc
13	.40	21.6 cc.	26	.355	24.0 cc
			Average	.358	20.36 cc

Table VIII:—TOTAL AND VOLATILE ACID OF STARTERS RIPENED IN 24 HOURS AT 21 DEGREES C.

Starter Number	Total Acidity	Volatile Acidity
1	.77%	70.6 cc
2	.71%	68.9 cc
3	.84%	67.3 cc
4	.62%	61.4 cc
5	.78%	76.9 cc

Table IX.—TOTAL AND VOLATILE ACID PRODUCTION OF CULTURES OF LACTOBACILLUS VISCOSUS INCUBATED 24 HOURS AT 10 DEGREES C.

Culture Number	Total Acidity	Volatile Acidity
1	.185%	8.8 cc
2	.18 %	8.2 cc
3	.185%	15.2 cc
4	.17 %	11.2 cc
5	.195%	8.0 cc

Table X.—TOTAL AND VOLATILE ACID PRODUCTION OF CULTURES OF LACTOBACILLUS ACIDOPHILUS INCUBATED 24 HOURS AT 37 DEGREES C.

Culture Number	Total Acidity	Volatile Acidity
1	1.11%	37.6 cc
2	1.18%	28.4 cc
3	1.21%	34.4 cc
4	1.03%	44.0 cc
5	.94%	37.2 cc

From the results given in Tables VIII, IX and X, it is evident that the total volatile acidities produced by cultures of udder bacteria were quite low, especially when compared with starter acidophilus cultures. The results obtained with the starters compare favorably with that reported by Hammer (7) except that in his report the volatile acidities are expressed in terms of N-10 Na OH. L. **acidophilus** cultures gave higher total but lower volatile acidities than did the starter, while L. **viscosus** gave very low total and volatile acidities. It would appear that as far as amount of acid production is concerned, bacteria of udder origin, even should they predominate, would not play a very important part in milk fermentations.

It has already been reported by others (6) that the volatile acids produced consisted of acetic, propionic, butyric and caproic acids. Identification of the volatile acids was attempted by a microscopical examination of crystals. The distillates, after neutralization, were evaporated to approximately 20 to 30 cc., acidified with a few drops of sulfuric acid, warmed to liberate any CO^2 present, neutralized again with NH^4 OH and then mixed with a solution of calcium chloride. This was heated to boiling and a drop put on a slide under a miscroscope to compare the form of the crystals with those of known calcium salts of low volatile fatty acids. Using this method, the formation of crystals had to be carefully watched because many forms were observed in progressive stages. Calcium acetate was found to predominate while calcium butyrate also appeared. Part of the neutralized distillate was also used in the preparation of volatile ethers (8). A few drops of sulfuric acid were added and the distillate slowly warmed, noting the odors of the vapors of the liberated organic acids. Both acetic and propionic acids were indicated by the volatile esters driven off.

Effect on Milk Proteins.—The remaining part of the milk cultures, usually about 75cc., was used in studying the action of the udder organisms on the casein. In 12 cases, of the 60 cultures made, the casein was undoubtedly curdled by the acid produced. There were 17 other cultures that showed decided curdling in which the total acidity was too low to have been wholly responsible. In these cases, the casein precipitation was attributed to an extra cellular enzyme. There were several cases in which peptonization and digestion of the curd was evident. This was not surprising when it had previously been found that nearly 50 per cent of the udder bacteria liquefied gelatin. Ten of the cultures were examined for products of casein digestion. After filtering the partially fermented milk through paper, a part of the filtrate was tested for protein, using both the Biuret and Millon's tests. The results are given in **Table XI.**

Table XI.—TESTS FOR PROTEIN ON THE FILTRATES OF CURDLED SAMPLES.

Sample	Biuret Test	Millon's Test
1	strong +	strong +
2	—	—
3	—	very weak +
4	weak +	weak +
5	—	—
6	strong +	strong +
7	weak +	weak +
8	—	weak +
9	weak +	weak +
10	weak +	weak +

All of the remainder of the filtrates were then saturated with powdered zinc-sulfate, allowed to stand an hour and filtered again. According to Sherman (9), all proteins except peptones are precipitated. The clear filtrates were again tested for protein reactions. Results are shown in Table XII.

Table XII.—TESTS FOR PROTEIN ON FILTRATES AFTER TREATING WITH ZINC SULFATE.

Sample	Biuret Test	Millon's Test
1	strong +	strong +
2	—	—
3	—	weak +
4	weak +	weak +
5	—	—
6	strong +	strong +
7	weak +	weak +
8	—	—
9	weak +	weak +
10	—	—

Two of the samples that gave weak protein reactions at first, gave negative reactions after precipitating with zinc sulfate and refiltering. The other samples gave the same reactions after the second filtration as before. This would indicate, in some cases at least, a partial breaking down of milk proteins with the production of peptones. Many of the inoculated samples became bitter in taste. The decomposition products of the milk proteins may be responsible for this.

Effect of Germicidal Action.—The effect of germicidal action on freshly drawn milk is well known. With this action taking place in the udder, the question arises as to whether the common udder micrococci are more resistant than are other species. Another question was whether the germicidal activity of milk from different cows varied to any extent. If this is true, it might explain why the udder flora is so largely micrococci and why different cows vary so much in total udder counts. The four cows mentioned in a study of the effect of lactation were used as they showed considerable variation in total counts. Samples of 500 cc. were taken from the middle of the milking from each quarter of the

udder. Plate counts were made immediately. Each flask was then inoculated with 3cc. of a broth culture of an organism previously isolated from the quarter from which the flask of milk was drawn. This organism was the type that predominated in that quarter. The flasks were shaken vigorously and counts made immediately. The flasks were then inocubated two hours and again plate counts were made. These were compared and the increase or decrease noted. The procedure was repeated three times with each cow. Table XIII shows the results obtained after averaging the different trials.

Table XIII.—EFFECT OF GERMICIDAL ACTIVITY ON NORMAL BACTERIA OF THE UDDER

Cow No.	Quarter	Count Before Inoculation	% Inc. or Decrease	Av. % Inc or Decrease
306	R.F.	307	+ 34	+ 36
	L.F.	13	— 16	
	R.R.	8	+ 43	
	L.R.	9	+ 86	
257	R.F.	8	— 17	— 14
	L.F.	5	— 17	
	R.R.	5	+ 2	
	L.R.	20	— 24	
259	R.F.	164	+ 1	+ 4.9
	L.F.	33	+ 8.7	
	R.R.	28	— 1.0	
	L.R.	128	+ 10.5	
23	R.F.	37,710	+ 80	+ 94
	L.F.	12,130	+ 109	
	R.R.	5,798	+ 136	
	L.R.	9,203	+ 48	

The results shown in Table XIII would indicate that the germicidal action was more noticeable in milk from cows having relatively low bacterial counts; or possibly the bacterial count was low in the udder of such cows because of more pronounced germicidal action of the milk.

Russel and Hastings (10) injected three types of organisms into cows' udders to learn if they would persist there. The types used were suspensions of **S. lactis, B. prodigiosus,** and a liquefying cocci. None of the organisms were capable of surviving more than a brief period of time. No conclusions were drawn, although they suggested that possibly it was due to the effect of germicidal agents.

A comparison was made of the effect of germicidal action on organisms not found in the udder. **Aerobacter aerogenes** was used. This is one of the most common organisms encountered in the barn yet it was never isolated from the udder during this work. In taking the samples, 500cc. of milk was collected aseptically from the middle of the milking. Initial counts were made immediately. Two cc. of a broth culture of the **A. aerogenes** was then added to each flask and counts again made. The flasks were set in a water bath at 37 degrees C. for two hours after which plate counts were made. Table XIV includes the results.

Table XIV.—EFFECT OF GERMICIDAL ACTION OF FRESHLY DRAWN MILK ON CULTURES OF AEROBACTER AEROGENES

Cow	Initial Bacterial Count	Bacterial Count just after Inoc.	Bacterial Count after 2 hoursr incubation at 37 deg. C.	% Increase or Decrease
1	111	719,000	441,000	—38 %
2	21	1,023,000	5,775,000	+464 %
3	9	927,000	174,000	— 81 %
4	1820	576,000	789,000	+ 28 %
5	268	1,042,000	18,223,000	+1648 %
6	473	823,000	1,130,000	+ 37 %
7	4545	1,003,000	9,304,000	+829 %
8	335	524,000	281,000	— 46 %
9	117	1,263,000	12,366,000	+871 %
10	7350	1,006,000	2,970,000	+195 %
11	106	641,000	261,000	— 59 %
12	6	1,010,000	4,165,000	+312 %
			Average	+346 %

The results of this table were unexpected. Four of the cows showed a decrease in count during the two-hour incubation period but the remaining eight gave quite large increases. A suspension of the organism was then injected thrcugh the teat into the right fore quarter of cow number 12. Within 24 hours, the quarter became swollen and enlarged, although no inflammation or feverish condition was observed. The milk became thick and lumpy. Bacterial counts were made each day afterwards and are recorded in Table XV.

Table XV.—BACTERIAL COUNT OF MILK FROM INOCULATED UDDER

Days	Bacterial Count
1st	4,060,000
2nd	14,300
3rd	8,000
4th	700
5th	900
6th	130
7th	290

Although these organisms showed an increase of 312 per cent when inoculated into milk for two hours, they could not survive in the udder more than a few days.

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