THE PRODUCTION OF MILK OF ABNORMAL COMPOSITION BY ANIMALS FREE FROM UDDER STREPTOCOCCI [1]

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INTRODUCTION

Chronic mastitis of the dairy cow has attracted much attention during the years of the present decade. Numerous papers have presented the findings of many research workers; varied suggestions, based on these findings, have been made to producers and to distributors. Regulatory agencies have been influenced. Indeed all agencies having to do with milk have become aware of chronic mastitis in the dairy cow.

The prevailing opinion is that:

1. Approximately 90 percent of the cases of chronic mastitis are due to *Streptococcus agalactiae;* the remainder to other bacteria, such as other kinds of streptococci, staphylococci, micrococci, and varieties of *Escherichia coli.*
2. *Streptococcus agalactiae* grows only in the udder.
3. It is carried from infected to noninfected udders.
4. The most frequent vehicle is the milker, human or mechanical.
5. The infected quarters do not produce a proportionate amount of milk.
6. The disease shortens the milk-production period.
7. The milk produced by infected quarters is changed in composition.
8. The organism is not pathogenic for man.

The acceptance of these statements leads the milk producer to the conclusion that the disease is of economic significance to him; the milk consumer to the conclusion that infected animals should be removed from the herd because the milk produced by them is abnormal. The public health official recognizes in acute mastitis an implied danger since it is known that *Streptococcus epidemicus* of the human type may cause a severe disturbance in the udder of the cow as well as septic sore throat in man. Milk free from all streptococci cannot cause septic sore throat; hence the consumer's desire to have his supply free from these organisms. Thus it would seem that it is to the interest of all persons concerned with milk to have cows with mastitis eliminated from the herds producing milk for the fluid-milk market insofar as this is reasonably possible. It is not sufficient to consider the qualitative aspects alone; the quantitative must also be considered, although the unity of interest may be disturbed by such a consideration of the problem.

Chronic mastitis is a bacteriological problem. Its diagnosis may be made in a direct manner by proving the presence of the causal organism, or indirectly by proving the milk to be abnormal in some respect. The direct manner of proof is expensive and involved; the

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indirect manner is cheap, easy, and adapted to use in the field. Practically all regulatory work is accomplished through the use of indirect methods rather than direct. The physical examination of the udder to detect changes in the tissue, due to the growth of organisms therein, is also widely used.

The purpose of this paper is to present certain findings and to consider their implication in relation to the problem of chronic mastitis. Normal animals and normal milk represent the ideal. If all cows are free from *Streptococcus agalactiae*, will the ideal of a normal milk be approached, or if all cows producing abnormal milk are removed, will only cows harboring this organism be removed? In other words, how safe are the foundations of our present ideas?

The standards which are commonly accepted in regard to normal milk are:

1. That it is free from visible particles.
2. That it contains less than 0.14 percent of chlorine in the form of chlorides (chlorine value).
3. That it yields less than 15 percent of its volume of 0_2 when mixed with hydrogen peroxide (catalase value).
4. That it has a true acidity represented by a pH of 6.5–6.8.

EXPERIMENTAL PROCEDURE

THE HERD

The animals were 1 year old at the time they were assembled. They were free from tuberculosis and Bang's disease and were placed on a farm on which there had been no cattle for 8 months. The stables had been cleaned and disinfected immediately after the removal of the previous herd. It was thought that both environment and animals would be free from *Streptococcus agalactiae*, and it was hoped that they would so remain during the first lactation period at least. The plan was to make no additions, other than a sire, to the herd, which was primarily for the study of pasture management. The animals had no contact with other bovines. It was therefore an isolated herd of Holstein-Friesian heifers that came to their first lactation period about 1 year after the animals were assembled.

THE MILK SAMPLES

The "routine" milk samples consisted of the first 30 cubic centimeters drawn from each quarter into a sterile bottle with no other precautions as to cleaning the udder and teats than were used in the usual procedure of milking. It was thought that additional precautions to prevent contamination of the milks from external sources were not necessary, a conclusion that has been justified by experience, since there is no reason to believe that such contamination has confused the picture. The foremilk samples are referred to in the discussion as "routine" samples. In the case of some of the animals they were supplemented by samples representing the remainder of the milk of each quarter for the particular milking. Such samples are referred to in the text as "special" samples and in table 5 as "whole milk."

EXAMINATION OF THE MILK

During the first 8 months the samples were collected at the evening milking, were refrigerated overnight, and the next day were examined, before being mixed, for any physical abnormalities such as flocs,

wateriness, and sediment. Slides, to be stained according to the Newman method, were made from the cream layer in which bacteria and leucocytes would be concentrated. During the last 8 months the samples were collected in the morning and studied the same day. Microscopic examination was not continued during this period, since it supplied little or nothing of value beyond that furnished by the other tests.

During the early part of the study the milks were placed in two groups: One consisted of the milks containing less than 0.15 percent of chlorine; the other, of those showing a greater percent of chlorine. Later the chlorine content was quantitatively determined by titration with a solution of silver nitrate, dichlorofluorescein [2] being used as an indicator. Five cubic centimeters of milk was diluted with 20 cubic centimeters of distilled water, and the silver nitrate solution added until a faint pink color developed. Somewhat more consistent results can be obtained with dichlorofluorescein than with the chromate because the end point is more definite. The wet ashing method is believed to be the most accurate one for determining the chlorine content of milk. It yields lower results than are yielded by the titration method in which potassium chromate is the indicator. The results yielded by dichlorofluorescein fall between those supplied by the other two and, therefore, probably represent a closer approximation to the truth than those yielded by the chromate method.

The catalase value represents O_2 set free from a mixture of 2 parts of milk and 1 part of a 1.5-percent H_2O_2 solution in 24 hours at 20° C., expressed as percent of the milk. The catalase content is an indirect measure of the content of the milk in leucocytes and tissue cells, either in the form of recognizable cells or of cell debris. Fresh bovine blood serum, free from cells, contains no catalase. The bacteria in the milk as drawn were probably always too few to supply any appreciable amount of catalase.

The pH was determined by the colorimetric method suggested by Brown.[3] The total acidity was measured by titrating with N/10 NaOH solution, with phenolphthalein as the indicator.

The milks were cultured by spreading about one three-hundredth of a cubic centimeter of cream, or of the mixed sample, over the surface of a slope of glucose agar, free from water, and by placing an equal amount in a tube of litmus milk. The tubes of agar and of milk were incubated at 37° C. and examined after 24, 48, 72, and 96 hours for relative numbers of colonies, for colonies of streptococci, and for the characteristic changes produced in litmus milk by *Streptococcus agalactiae*, namely, an acid curd, and no reduction of the litmus, or reduction confined to the lower half of the column of milk. Suspicious colonies on the agar slopes and suspicious tubes of litmus milk were examined microscopically, and, if the circumstances indicated, pure cultures were studied in detail. The special samples were subjected to the routine tests and to more detailed cultural examination, such as plating on blood agar, on glucose agar made with veal infusion or with beef extract, and on the same agar containing enough sterile milk to give an evident opacity to the layer of medium in a Petri-dish culture. Some cultures were incubated under anaerobic condi-

[2] KOLTHOFF, I. M., LAUER, W. M., and SUNDE, C. J. THE USE OF DICHLOROFLUORESCEIN AS AN ADSORPTION INDICATOR FOR THE ARGENTOMETRIC TITRATION OF CHLORIDES. Jour. Amer. Chem. Soc. 51: 3273-3277. 1929.

[3] BROWN, J. H. THE COLORIMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF SMALL AMOUNTS OF FLUID. Jour. Laboratory and Clin. Med. 9: 239-244, illus. 1924.

tions. In fact numerous efforts were made to find an explanation for the conditions noted.

THE CRITERIA

The criteria used in classifying the milk as abnormal were a pH of 6.8 or above, a catalase value of 50 or above, and a chlorine content of 0.15 percent or above. These criteria may be questioned. Some may insist that a catalase value of 50 is too high. The data of table 1 present a general picture of the findings. At any period of observation the milk of any animal was classed as abnormal when the product from one or more quarters conformed to at least two of the three criteria of abnormality mentioned above. The data have been compiled with consideration of the influence of the colostral period and of late lactation on the composition of the milk. It is believed that the length of the period of observation, the number of observations, and the type of samples and of the tests were such as to give a basis for valid conclusions.

TABLE 1.—*Occurrence of foremilk of abnormal composition in animals free from udder streptococci*

Animal no.	Length of the period of observation	Observations	Times milk was—		Rating of animal	Animal no.	Length of the period of observation	Observations	Times milk was—		Rating of animal
			Normal	Abnormal					Normal	Abnormal	
	Days	*Number*	*Number*	*Number*			*Days*	*Number*	*Number*	*Number*	
1	245	17	17	0	Normal.	27	156	14	12	2	Normal.
2	183	12	12	0	Do.	28	344	25	7	18	Abnormal.
3	240	15	2	13	Abnormal.	29	250	22	4	18	Do.
5	321	34	3	31	Do.	31	318	36	17	19	Do.
6	307	33	33	0	Normal.	32	309	30	25	5	Normal.
7	285	30	29	1	Do.	33	198	20	20	0	Do.
8	322	32	32	0	Do.	34	315	31	17	14	Abnormal.
10	268	18	9	9	Abnormal.	35	259	14	1	13	Do.
11	275	23	0	23	Do.	36	233	19	17	2	Normal.
13	371	29	22	7	Normal.	37	371	32	32	0	Do.
14	317	30	26	4	Do.	38	363	33	32	1	Do.
15	349	34	5	29	Abnormal.	39	320	29	21	8	Abnormal.
19	329	27	3	24	Do.	40	274	31	23	8	Do.
21	268	30	5	25	Do.	42	244	11	11	0	Normal.
24	198	20	20	0	Normal.	44	294	29	24	5	Do.
26	163	15	14	1	Do.						

THE FINDINGS

The results of the examination of the 31 cows, mentioned in table 1, and of the 3,100 samples therefrom for *Streptococcus agalactiae* were wholly negative. The abnormalities noted in the milks were due to other causes than this organism. This point will be discussed in greater detail later.

It is evident from table 1 that only 8 animals (i. e. those that yielded no abnormal samples) out of the 31 can be considered as having supplied no evidence of mastitis throughout the period of observation. Any one of the remaining animals might have been judged as producing milk unfit for direct consumption if a strict interpretation of the tests had been made. The assumption might also have been that the udder was infected with *Streptococcus agalactiae* and the removal or segregation of the animal advisable to lessen the spread of chronic mastitis in the herd.

TABLE 2.—*Condition of the herd as indicated by chlorine, catalase, and pH values of milk of each animal on different dates*

Date	Data for cow No.—																														Total	
	1	2	3	5	6	7	8	10	11	13	14	15	19	21	24	26	27	28	29	31	32	33	34	35	36	37	38	39	40	42	44	Normal	Abnormal
																																Number	*Number*
Apr. 1, 1935	----	----	----	+	−	−	−	----	+	+	−	−	----	+	----	----	----	----	----	−	−	----	----	----	----	−	−	−	−	----	−	12	4
May 1, 1935	----	----	----	+	−	−	−	----	+	+	−	+	+	+	−	----	----	−	----	+	−	−	−	----	----	−	−	−	−	----	−	14	7
June 1, 1935	−	----	----	−	−	−	−	−	+	+	−	+	+	+	−	−	−	−	−	−	−	−	−	----	----	−	−	−	−	----	−	22	5
July 1, 1935	−	−	+	+	−	−	−	−	+	−	−	+	+	+	−	−	+	+	+	+	+	−	+	----	−	−	−	−	+	----	−	16	13
Aug. 1, 1935	−	−	+	+	−	−	−	−	+	−	−	+	+	+	−	−	−	−	+	+	+	−	+	+	−	−	−	+	−	−	−	19	12
Sept. 1, 1935	−	−	+	+	−	−	−	+	+	−	−	+	+	+	−	−	−	−	+	+	+	−	+	+	−	−	−	+	+	−	−	17	14
Oct. 1, 1935	−	−	+	+	−	−	−	+	+	−	−	+	+	+	−	−	−	+	+	+	+	−	+	+	+	−	−	+	+	−	+	15	16
Nov. 1, 1935	−	−	−	+	−	−	−	−	+	−	−	+	−	+	----	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	18	12
Dec. 1, 1935	−	−	+	+	−	−	−	+	+	−	−	+	+	+	----	----	----	+	−	+	+	----	+	+	−	−	−	+	----	−	−	13	13
Jan. 1, 1936	−	−	−	+	----	----	−	+	+	−	+	+	+	----	----	----	----	+	+	----	−	----	+	+	+	−	−	+	----	−	+	9	13
Feb. 1, 1936	−	----	+	----	----	----	----	+	+	−	−	----	+	----	----	----	----	+	----	----	----	----	----	+	----	−	+	----	----	−	----	5	7
Mar. 1, 1936	----	----	+	----	----	----	----	----	+	−	−	----	+	----	----	----	----	+	----	----	----	----	----	+	----	----	----	----	----	−	----	4	6
Apr. 1, 1936	----	----	+	----	----	----	----	----	----	−	−	----	+	----	----	----	----	+	----	----	----	----	----	+	----	----	----	----	----	−	----	3	4

[1] −=milk was normal; += was abnormal.

An examination made on any date would have indicated only a portion of the animals as abnormal. Thus, an inspection at monthly intervals throughout the year would have yielded the results presented in table 2. It is to be noted that, on the basis of the total number of examinations made, animals 1, 2, 6, 8, 24, 33, 37, and 42 in table 1 would be classed as normal since none of these supplied a sample of abnormal milk. On the basis of an examination made on the 1st of every month for 13 months, the same animals would have been classed as normal, and also no. 7 which, as is shown in table 1, yielded but one sample of abnormal milk.

If the herd had been inspected for mastitis on June 1, 1935, 5 of the then 27 milking animals would have been considered affected. An inspection as of December 1, 1935, would have revealed 26 milking animals, 13 of which would have been classed as abnormal.

It is to be noted from table 1 that some of the animals were classed as normal in spite of the occurrence of abnormal milk. Animal 13 is an example. The distribution of the abnormal samples was such as to relate the abnormality to the colostral period or to some transitory condition in a quarter. Inasmuch as the purpose of this paper is to present the record of certain of the animals about the abnormality of which there can be no question, any discussion about either the normal or the questionably normal has been omitted. There were 17 such in the total of 31 and 14 definitely abnormal animals. Detailed data are presented for seven of these. The data for the others are omitted since they would add nothing to the picture.

THE ABNORMAL ANIMALS

A table has been prepared in which the figures represent the approximate number of days from the beginning of the lactation period to the date of sampling. A plus sign means that the sample satisfied two of the three criteria previously mentioned and that the foremilk was, therefore, classed as abnormal; a minus sign that two of the three criteria were not satisfied and that the milk was considered normal. Thus, it is seen from table 3 that quarter 1 of cow 3 supplied no abnormal samples; quarter 2, 2; quarter 3, 4; while the milk from quarter 4 was normal but twice.

It is to be noted from table 3 that one quarter of cow 11 produced abnormal milk throughout the entire period of observation, 275 days, while the other quarters always produced normal milk. The detailed data of the routine samples from this animal are given in table 4. It is seen that every sample from quarter 2 was abnormal in respect to each of the criteria used in the routine examinations except the pH in the first and last samples, while every sample other than the first two taken from quarter 1 was normal or did not satisfy any two of the three criteria of abnormality. Samples of foremilk and of the remainder produced by each quarter were taken at five periods (table 5). It is to be noted that quarter 2 produced a much smaller quantity of milk than the other hindquarter, no. 3, and that the abnormality was not confined to the foremilk but was present in the entire quantity, termed "whole milk" in the table, from quarter 2, at any milking. The pH of both foremilk and the entire quantity was generally high and the titratable acid low. Quarter 2 thus differed significantly from the others as regards the composition and yield of milk.

TABLE 3.—*Normality or abnormality of the foremilk of the four quarters of the udder of the abnormal cows at various times after their lactation periods began, as indicated by the ability or inability of the sample to satisfy the three criteria mentioned in the text* [1]

Days after lactation period began	Date for indicated quarter [2] of cow no.—																															
	3				11				15				29				19				21				5				31			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
7	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−				
14	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−				
21	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−				
28	−	−	+	+	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−				
35	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
44					−	+	−	−	−	+	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
51									+	+	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
56	−	−	−	+	−	+	−	−	−	+	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	+	−	−				
63					−	+	−	−	+	+	+	−	−	−	−	+	−	−	−	+	−	+	+	−	−	+	−	−				
70	−	−	−	+	−	+	−	−	−	−	+	−	−	+	−	−	−	−	−	+	−	+	−	−	−	+	−	−				
75									+	+	+	−					−	−	−	+	−	+	−	−					−	−	−	−
79					−	+	−	−					−	−	−	+					−	+	−	−	−	−	−	−				
85					−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	+	−				
92	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	+	+	−	−	−	+	−	−	−	−	−
98									+	+	+	−					+	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−
104					−	+	−	−													−	+	−	−	−	−	−	−	−	−	−	−
112					−	+	−	−													−	+	−	−	−	−	−	−	−	−	−	−
115									+	+	+	−	−	−	−	+									+	−	−	−	−	−	−	−
121	−	−	−	−					+	+	+	−	−	−	−	+	+	−	−	+	−	+	−	−	+	−	+	−	−	−	−	−
126					−	+	−	−	+	+	−	−	−	−	−	+					−	+	−	−	+	−	+	−	−	−	−	−
135	−	−	−	+					+	+	+	−					+	−	−	+	−	+	−	+	−	+	+	−	−	−	−	−
142									+	+	+	+					−	−	−	+	−	+	−	+	+	−	+	−	−	−	−	−
147	−	−	−	+					+	−	−	−									−	+	−	+	−	−	+	−	−	−	−	−
155					−	+	−	−	+	+	+	−	−	−	−	+	−	−	−	+	−	+	−	+	−	−	+	−	−	−	−	−
161									+	+	+	−					−	−	−	+	−	+	−	+					−	−	+	−
167																					−	+	−	−	+	−	+	−				
173									+	+	+	−													−	−	+	−	−	−	−	−
176	−	−	−	−																									−	−	−	−
181					−	+	−	−					−	−	−	+	−	−	−	−	+	+	−	+	−	−	+	−	−	−	−	−
188					−	+	−	−	+	+	+	−	−	−	−	+	+	−	−	+									−	−	−	−
198									+	+	+	−																	−	−	−	−
203									+	−	−	−									−	+	−	−	−	−	+	−	−	+	−	+
210	−	−	−	+	−	+	−	−									+	−	−	−												
215													−	−	−	−									−	−	+	−	−	−	+	−
224					−	+	−	−	+	+	+	−									−	+	−	−					−	−	+	−

[1] Plus signs indicate that the sample satisfied 2 of the 3 criteria for abnormality mentioned in the text, minus signs that it did not.
[2] 1 is the right forequarter; 2, the right hindquarter; 3, the left hindquarter; and 4, the left forequarter.

TABLE 3.—*Normality or abnormality of the foremilk of the four quarters of the udder of the abnormal cows at various times after their lactation periods began, as indicated by the ability or inability of the sample to satisfy the three criteria mentioned in the text*—Continued

Days after lactation period began	Date for indicated quarter of cow no.—																													
	3				11				15				29				19				21				5				31			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
237	−	−	−	+					+	−	+	−					−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−
245					−	+	−	−	+	+	+	+																	−	+	+	−
250									+	+	+	−	−	−	−	+									−	−	+	+	−	+	+	−
260																	−	−	−	−	−	+	−	−					−	+	+	−
266																					−	+	−	−	−	−	+	−	−	+	+	−
275					−	+	−	−	+	+	+	+					−	−	−	+												
282									+	+	+	+																	−	+	+	−
292																									−	−	+	−				
302									+	+	+	−					−	−	−	−					+	−	+	−	−	+	+	−
321																									−	−	+	−	−	+	+	−
328																	−	−	−	+									−	+	+	−
349									+	+	−	+																	−	+	+	−
378																													−	+	+	−

TABLE 4.—*The pH, chlorine content, and catalase value of the foremilk from the different quarters*[1] *of the udders of the definitely abnormal cows at various times after their lactation periods began*

COW NO. 11

Days after lactation period	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	pH	Cl[2]	Catalase[3]	pH	Cl[2]	Catalase[3]	pH	Cl[2]	Catalase[3]	pH	Cl[2]	Catalase[3]
		Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
4	6.6	+	113	6.4	+	175	6.4	+	34	6.5	+	22
12	6.6	0.183	75	7.0	0.332	150	6.4	0.155	7	6.6	0.172	21
19	6.4	.152	9	7.0	.230	20	6.4	.178	10	6.5	.148	7
25	6.5	.141	2	7.0	.273	172	6.5	.141	2	6.5	.158	3
32	6.6	.152	5	7.0	.312	117	6.6	.168	12	6.6	.189	9
39	6.6	.148	4	7.0	.312	169	6.6	.148	5	6.6	.155	3
45	6.6	.158	5	6.8	.269	80	6.5	.158	4	6.6	.161	4
53	6.6	.158	6	7.0	.316	167	6.6	.155	10	6.6	.165	3
60	6.6	.144	13	7.0	.276	198	6.6	.141	13	6.6	.141	14
67	6.6	.176	17	6.9	.300	250	6.6	.158	10	6.6	.155	12
74	6.6	.161	22	7.0	.327	88	6.6	.152	25	6.6	.155	9
95	6.6	.155	23	6.8	.286	243	6.6	-------	6	6.6	.152	8
109	6.6	.182	2	7.0	.320	230	6.6	.176	14	6.6	.185	8
131	6.6	.165	3	6.9	.269	220	6.6	.165	3	6.6	.172	3
160	6.6	.152	1	7.0	.248	174	6.6	.152	13	6.6	.144	2
186	6.6	.161	5	6.9	.293	257	6.6	.168	5	6.6	.185	5
215	6.6	.168	22	6.9	.258	273	6.6	.165	5	6.6	.172	4
249	6.6	.175	44	6.8	.269	254	6.6	.148	11	6.6	.161	5
279	6.6	.158	21	6.6	.248	245	6.6	.158	0	6.6	.158	16
COW NO. 15												
0	6.2	−	172	6.0	−	192	6.2	−	162	6.1	−	121
7	6.3	−	97	6.3	−	74	6.2	+	79	6.2	+	80
14	6.4	+	41	6.6	−	73	6.6	−	43	6.6	+	23
21	6.5	+	47	6.6	+	28	6.5	+	28	6.5	+	15
27	6.2	−	20	6.8	+	20	6.6	+	23	6.4	−	27
34	6.6	+	35	6.8	+	20	6.6	+	16	6.4	−	23
48	6.6	+	46	6.8	+	26	6.8	+	54	6.7	+	23
53	6.6	+	24	6.8	+	15	6.8	+	21	6.6	−	9
60	6.9	+	24	6.8	+	13	6.9	+	18	6.6	−	9
68	6.8	+	26	6.8	+	11	6.8	+	17	6.7	−	0
74	6.7	+	30	6.8	+	11	6.8	+	15	6.5	−	3
81	6.9	0.221	21	6.9	0.200	10	6.9	0.217	12	6.6	−	7
88	6.7	.207	22	6.8	.221	31	6.8	.217	17	6.7	−	2
95	6.7	.197	19	6.8	.200	9	6.7	.178	9	6.5	−	30
103	6.8	.234	60	6.8	.217	17	6.8	.238	23	6.6	0.185	10
110	6.8	.197	20	6.8	.197	13	6.8	.200	10	6.6	.185	5
116	7.0	.234	53	6.9	.221	16	6.9	.221	16	6.6	.211	19
123	6.8	.227	47	6.9	.239	24	6.7	.217	20	6.6	.221	43
130	6.9	.221	44	6.8	.211	22	6.8	.217	12	6.6	.172	16
137	6.8	.207	29	6.9	.214	16	6.8	.202	15	6.8	.189	10
144	6.8	.202	22	6.7	.193	12	6.7	.207	29	6.6	.178	10
151	6.9	.207	40	6.8	.207	30	6.8	.202	33	6.6	.178	30
158	6.8	.211	70	6.8	.211	20	6.8	.211	29	6.6	.189	17
165	6.7	.193	55	6.8	.202	17	6.8	.197	9	6.7	.172	7
186	6.8	.234	67	6.8	.242	41	6.8	.231	36	6.7	.211	49
200	6.7	.234	33	6.8	.248	31	6.9	.231	27	6.6	.224	20
222	6.9	.262	72	6.9	.262	93	7.0	.276	59	6.6	.202	27
251	6.9	.282	147	7.0	.290	160	6.9	.303	215	6.6	.182	11
277	7.0	.320	230	7.0	.354	266	7.0	.330	235	6.8	.217	17
306	6.6	.279	158	6.7	.293	175	6.7	.262	88	6.6	.225	30
COW NO. 29												
1	6.3	−	110	6.2	−	79	6.0	−	46	6.2	−	156
7	6.5	−	8	6.5	−	10	6.4	−	18	6.5	−	25
15	6.5	−	11	6.5	−	9	6.5	−	12	6.5	−	19
22	6.4	−	6	6.4	−	7	6.4	−	8	6.4	−	19
30	6.4	0.144	16	6.4	0.148	6	6.4	0.158	6	6.6	0.197	58
37	6.6	.165	3	6.6	.172	9	6.5	.172	13	6.6	.193	51
43	6.6	.144	4	6.5	.138	3	6.6	.152	4	6.8	.200	57
50	6.6	.148	23	6.6	.165	13	6.5	.144	20	6.9	.282	206
57	6.4	.148	6	6.4	.152	6	6.4	.155	68	6.6	.193	40

[1] See note 2, table 3.

[2] +=0.15 percent or more; −=less than 0.15 percent.

[3] O_2 set free from a mixture of 2 parts milk and 1 part of 1.5-percent H_2O_2 solution in 24 hours at 20° C., expressed as percentage of the milk.

TABLE 4.—*The pH, chlorine content, and catalase value of the foremilk from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued.

COW NO. 29—Continued

Days after lactation period	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	pH	Cl	Catalase	pH	Cl	Catalase	pH	Cl	Catalase	pH	Cl	Catalase
		Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
64	6.6	.158	7	6.6	.165	11	6.6	.172	5	6.8	.234	49
71	6.6	.148	2	7.0	.334	223	6.6	.158	6	6.6	.155	3
78	6.8	.141	32	6.6	.148	12	6.6	.148	17	7.2	.338	260
85	6.6	.148	26	6.6	.158	23	6.6	.148	15	6.6	.207	253
92	6.6	.134	14	6.6	.138	9	6.6	.138	10	6.6	.178	68
113	6.6	.148	11	6.6	.144	9	6.6	.144	85	6.6	.239	117
127	6.6	.165	4	6.6	.172	6	6.6	.161	6	7.0	.327	244
149	6.6	.161	10	6.6	.161	5	6.6	.152	3	6.8	.245	104
178	6.6	.138	8	6.6	.138	10	6.6	.148	10	6.9	.269	259

COW NO. 19

0	6.3	—	147	6.4	—	127	6.2	—	30	6.6	+	160
6	6.4	—	147	6.4	—	122	6.4	—	53	6.7	+	122
12	6.5	—	37	6.5	—	29	6.5	—	8	6.6	0.200	182
20	6.4	—	28	6.6	—	20	6.4	—	9	6.6	.207	204
27	6.6	—	8	6.6	—	19	6.5	—	8	7.0	.321	260
35	6.7	0.172	29	6.6	0.176	21	6.6	0.152	19	7.0	.392	265
42	6.6	.148	12	6.6	.148	12	6.4	.127	8	7.0	.330	198
48	6.6	.127	7	6.6	.141	11	6.4	.124	5	6.8	.245	195
55	6.6	.178	14	6.6	.168	15	6.6	.185	9	6.8	.258	190
62	6.5	.134	8	6.4	.131	9	6.4	.124	4	6.7	.189	180
69	6.8	.134	11	6.6	.144	5	6.6	.127	7	6.7	.193	81
76	6.6	.127	9	6.6	.124	10	6.6	.117	4	6.8	.221	152
83	6.6	.131	21	6.6	.124	27	6.4	.114	21	6.8	.211	260
90	6.7	.165	37	6.6	.155	18	6.6	.127	19	6.7	.234	59
97	6.9	.178	27	6.8	.148	26	6.8	.138	9	7.0	.310	300
118	6.8	.189	54	6.6	.168	23	6.6	.138	17	7.0	.372	282
132	6.8	.193	35	6.7	.168	18	6.6	.148	17	6.8	.234	232
154	6.6	.158	17	6.6	.144	14	6.6	.148	8	6.8	.211	161
183	6.6	.141	21	6.6	.138	8	6.6	.131	7	6.6	.152	145
209	6.9	.276	79	6.6	.172	17	6.6	.168	12	6.6	.158	10
238	6.6	.175	42	6.6	.148	45	6.6	.141	8	6.6	.172	168
272	6.6	.161	29	6.6	.158	6	6.6	.138	7	6.6	.155	104
302	6.6	.152	21	6.6	.124	3	6.6	.121	3	6.6	.141	139
328	6.6	.172	27	6.6	.144	4	6.6	.127	19	6.7	.161	210

COW NO. 21

0	6.1	+	232	6.2	+	214	6.3	+	162	6.3	—	243
7	6.3	—	56	6.4	+	162	6.4	—	97	6.3	—	44
13	6.5	—	64	6.5	—	212	6.4	—	105	6.4	—	23
20	6.6	—	19	6.5	—	24	6.4	—	31	6.4	—	16
29	6.8	—	31	6.6	+	29	6.6	—	15	6.6	—	12
34	6.6	—	75	6.7	+	69	6.7	—	53	6.7	—	64
41	6.6	—	6	6.8	+	51	6.8	—	16	6.6	—	4
49	6.5	—	4	6.6	—	79	6.6	—	5	6.6	—	4
55	6.6	+	23	7.0	+	197	6.6	+	25	6.5	—	17
61	6.6	—	4	7.0	0.313	183	6.6	—	11	6.5	—	6
69	6.7	0.165	15	7.0	.327	293	6.8	0.182	14	6.6	—	10
76	6.5	—	5	6.6	.165	222	6.6	—	23	6.5	—	5
84	6.7	.165	10	7.0	.348	226	6.6	.165	15	6.6	0.134	16
91	6.7	.161	19	7.0	.343	220	6.8	.211	33	6.6	.176	30
97	6.7	.158	13	7.0	.330	213	6.7	.197	22	6.6	.161	20
104	6.6	.178	17	7.0	.351	185	6.8	.197	26	6.7	.227	61
111	6.6	.165	24	7.0	.310	210	6.6	.152	16	6.6	.131	11
118	6.6	.155	10	6.8	.328	205	6.7	.185	18	6.6	.165	12
125	6.7	.172	23	6.9	.341	216	6.6	.178	29	6.7	.172	29
132	6.7	.161	12	6.9	.316	260	6.6	.176	26	6.8	.197	36
139	6.6	.158	26	7.0	.293	287	6.7	.178	28	6.8	.202	53
146	6.8	.144	13	7.0	.307	276	6.8	.165	21	6.7	.207	120
167	6.7	.165	20	6.9	.276	245	6.7	.178	37	6.7	.182	44
181	6.8	.178	31	7.0	.316	282	6.7	.211	46	6.7	.211	69
203	6.7	.172	11	7.0	.320	260	6.6	.200	25	6.7	.202	36
234	6.6	.158	17	6.8	.269	150	6.6	.138	9	6.6	.144	13
260	6.6	.148	2	6.9	.300	194	6.6	.168	8	6.7	.207	26

TABLE 4.—*The pH, chlorine content, and catalase value of the foremilk from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued.

COW NO. 5

Days after lactation period	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	pH	Cl	Catalase	pH	Cl	Catalase	pH	Cl	Catalase	pH	Cl	Catalase
		Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
4	6.3	—	35	6.2	+	190	6.6	—	65	6.2	—	30
11	6.4	—	17	7.0	+	197	6.5	—	43	6.4	—	13
17	6.6	—	26	7.0	+	183	6.6	—	66	6.7	—	27
24	6.7	—	32	7.1	+	142	6.8	+	56	6.6	—	14
31	6.6	—	15	7.1	+	157	6.4	—	10	6.0	—	8
38	6.5	—	7	7.0	+	170	6.6	—	23	6.4	—	10
45	6.5	—	20	7.0	+	131	6.6	—	31	6.6	—	16
51	6.6	—	31	7.0	+	171	6.6	—	51	6.5	—	23
58	6.6	—	13	6.8	+	58	6.7	—	34	6.6	—	11
67	6.6	—	8	6.8	+	29	6.6	—	14	6.6	—	8
72	6.5	—	68	6.6	—	33	6.4	+	56	6.5	—	54
79	6.6	—	11	6.8	+	29	6.8	+	28	6.5	—	14
87	6.4	—	9	6.5	—	21	6.6	+	31	6.4	—	5
93	6.5	—	21	6.6	—	22	6.6	+	64	6.4	—	9
99	6.6	0.197	14	6.7	0.200	30	6.7	0.207	34	6.5	—	11
107	6.6	—	13	6.6	—	12	6.6	—	33	6.6	—	17
114	6.7	.193	25	6.7	.165	15	6.8	.176	43	6.6	—	5
122	6.8	.248	64	6.6	.197	24	6.6	.224	75	6.5	0.185	46
129	6.9	.252	100	6.7	.197	19	6.8	.231	39	6.6	.175	25
135	6.8	-------	36	6.6	-------	8	6.9	-------	66	6.6	-------	10
142	6.8	.217	44	6.9	.182	23	6.7	.255	117	6.6	.178	17
149	6.7	.182	27	6.7	.172	31	6.8	.258	111	6.6	.158	25
156	6.7	.165	30	6.6	.168	9	6.6	.221	37	6.5	.172	10
163	6.6	.200	19	6.6	.178	24	6.8	.239	74	6.6	.207	16
170	6.8	.189	28	6.6	.165	23	6.9	.234	87	6.6	.189	33
177	6.6	.200	35	6.6	.168	28	6.7	.189	176	6.6	.134	52
184	6.6	.178	23	6.7	.161	15	6.8	.193	90	6.6	.165	30
205	6.6	.172	17	6.7	.168	46	6.8	.207	60	6.6	.168	21
219	6.6	.197	27	6.6	.176	19	6.8	.253	111	6.6	.202	33
240	6.6	.185	20	6.7	.176	22	6.9	.282	76	6.6	.176	21
270	6.7	.185	20	6.6	.185	11	6.8	.296	96	6.6	.172	14
296	6.6	.185	20	6.6	.207	26	6.8	.282	155	6.6	.172	18
325	6.6	.189	25	6.7	.197	20	6.9	.276	57	6.6	.182	40

COW NO. 31

79	6.6	—	16	6.6	—	28	6.6	—	41	6.6	—	16
86	6.6	—	3	6.5	—	6	6.5	—	6	6.5	—	5
94	6.7	—	21	6.8	—	46	6.6	—	95	6.7	—	25
101	6.6	—	3	6.7	—	21	6.6	—	13	6.6	—	0
108	6.4	—	0	6.6	—	21	6.6	—	14	6.5	—	5
115	6.6	—	0	6.6	—	7	6.7	—	18	6.6	—	0
123	6.6	—	15	6.5	—	6	6.6	—	7	6.5	—	34
130	6.6	—	9	6.6	—	6	6.6	—	22	6.6	—	0
137	6.5	—	0	6.6	—	8	6.6	—	16	6.7	—	33
143	6.6	—	0	6.6	—	4	6.6	—	10	6.5	—	4
150	6.6	—	6	6.6	—	6	6.7	—	23	6.6	—	31
159	6.6	—	2	6.6	—	6	6.6	+	22	6.6	—	0
164	6.7	—	26	6.6	—	34	6.8	—	88	6.6	—	38
171	6.6	—	7	6.6	—	7	6.8	+	65	6.6	—	9
179	6.5	—	4	6.5	—	3	6.6	—	24	6.5	—	4
185	6.6	—	2	6.6	—	11	6.6	—	37	6.6	—	6
191	6.6	—	14	6.6	—	10	6.7	0.172	46	6.6	—	19
199	6.5	—	9	6.5	—	7	6.6	.182	35	6.5	—	3
206	6.4	—	3	6.6	—	3	6.7	.185	38	6.6	—	6
214	6.6	0.165	17	6.8	0.234	94	6.8	.262	85	6.6	0.158	30
221	6.6	.141	15	6.7	.168	17	6.8	.193	40	6.6	.144	19
227	6.6	.152	9	6.7	.178	16	6.8	.202	65	6.5	.155	7
234	6.6	.161	20	6.6	.207	45	6.7	.221	68	6.6	.172	16
241	6.6	.134	7	6.7	.182	39	6.9	.234	90	6.6	.158	7
248	6.6	.141	9	6.6	.207	49	6.7	.224	145	6.6	.148	4
255	6.6	.127	0	6.8	.197	96	6.8	.202	88	6.4	.144	11
262	6.6	.141	52	6.6	.214	92	6.6	.239	32	6.6	.141	31
270	6.6	.141	23	6.6	.189	100	6.7	.224	50	6.6	.144	18
277	6.7	.148	6	6.9	.224	92	6.9	.211	164	6.7	.155	14
298	6.7	.158	29	6.8	.189	75	6.9	.224	208	6.6	.158	20
312	6.6	.148	12	6.8	.202	66	6.8	.224	215+	6.6	.161	14
334	6.6	.158	17	6.9	.217	78	6.8	.252	114	6.7	.172	16
353	6.6	.155	12	6.6	.217	82	6.7	.248	123	6.6	.189	21
379	6.6	.224	36	6.6	.227	60	6.6	.269	85	6.6	.265	45

All routine samples from animal 11 were cultured on various media and the cultures kept at 30° or at 37° C. None of the cultures made on agar slopes or in milk showed any significant result, either from the quantitative or qualitative point of view. Quarter 2 did not yield results materially different from those yielded by the other quarters. The quantitative results obtained with blood agar plates incubated 48 hours at 37° for both the fore milk and the whole milk are presented in table 5. It is to be noted that the foremilk was usually lower in bacteria than the remainder of the milk drawn from any quarter at any milking. It is also to be noted that the whole milk from quarter 2 was consistently higher in bacteria than that from the normal quarters. The plates seeded with the milk of the different quarters presented much the same appearance as to types of colonies. One gained the impression that the higher numbers in quarter 2 indicated an increase in the normal flora rather than an organism not usually present in the udder of cows. The milk from quarter 2 was never abnormal in appearance except that the fat-free milk had a watery appearance. Flakes were not noted. The quarter was not sore at any time and appeared normal when examined in a cursory manner, but a physical examination by a veterinarian skilled in such examinations revealed a fibrotic condition. The milk flow of the animal was well maintained. The maximum per week was during the first month after freshening—about 275 pounds. Eight months later about 150 pounds per week were produced.

TABLE 5.—*Yield, pH, acidity, chlorine content, catalase value, and bacterial count, of both the foremilk and the remainder of the milk (whole milk) from the different quarters*[1] *of the udders of the definitely abnormal cows at various times after their lactation periods began*

COW NO. 11, FRESHENED JUNE 7, 1935

Date	Quarter	Yield		Catalase		pH		Acidity		Chlorine		Bacteria per cubic centimeter in—	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Lb.	*Per cent*[2]	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Hundreds*	*Hundreds*
Aug. 29	1	4.1	27	7	14	6.6	6.6	-------	0.157	0.144	0.152	1	16
	2	1.0	7	242	236	6.8	6.6	-------	.067	.231	.248	1	35
	3	6.0	39	17	68	6.6	6.6	-------	.139	.138	.148	1	13
	4	4.2	27	0	16	6.6	6.6	-------	.139	-------	.148	1	12
Total		15.3											
Sept. 5	1	4.5	28	2	9	6.6	6.6	-------	.157	.155	.141	7	9
	2	1.0	6	164	156	6.9	6.9	-------	.067	.258	.255	2	40
	3	6.1	38	9	7	6.6	6.6	-------	.057	.148	.138	1	5
	4	4.4	28	4	9	6.6	6.6	-------	.057	.148	.148	1	5
Total		16.0											
Oct. 2	1	3.7	37	19	10	6.6	6.6	0.121	.156	.172	.168	1	3
	2	.3	3	273	111	6.8	6.8	.040	.045	.312	.242	1	16
	3	3.6	37	14	8	6.6	6.6	.135	.156	.172	.158	1	11
	4	2.3	23	9	15	6.6	6.6	.144	.156	.172	.161	1	11
Total		9.9											
Dec. 17	1	5.5	4	8	9	6.6	6.6	.135	.144	.172	.155	40	9
	2	.07	1	214	252+	6.8	7.0	.045	.063	.303	.258	29	74
	3	4.0	29	13	12	6.6	6.6	.144	.144	.155	.152	2	20
	4	4.0	29	35	30	6.6	6.6	.139	.135	.158	.168	14	72
Total		13.57											
Jan. 22	1	3.8	29	6	11	6.6	6.6	.135	.135	.168	.161	88	92
	2	.1	1	266	145	6.8	6.8	-------	.126	-------	.172	200	106
	3	5.3	40	11	6	6.6	6.6	.144	.153	.155	.158	76	3
	4	4.0	30	19	9	6.6	6.6	.144	.144	.168	.168	4	84
Total		13.2											

[1] See note 2, table 3.
[2] Of total.

TABLE 5.—*Yield, pH, acidity, chlorine content, catalase value, and bacterial count, of both the foremilk and the remainder of the milk (whole milk) from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued

COW NO. 15, FRESHENED MARCH 13, 1935

Date	Quarter	Yield		Catalase		pH		Acidity		Chlorine		Bacteria per cubic centimeter in—	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Lb.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Hundreds*	*Hundreds*
Sept. 18	1	1.8	19	40	30	6.9	6.8	0.072	0.096	0.231	0.202	110	172
	2	3.1	33	54	32	6.8	6.8	.072	.090	.239	.214	1	90
	3	2.4	26	28	19	6.9	6.8	.063	.099	.239	.202	57	34
	4	2.1	22	20	13	6.7	6.6	.081	.117	.214	.185	1	568
Total		9.4											
Oct. 24	1	1.8	19	105	93	6.7	6.6	.081	.117	.245	.197	115	20
	2	2.8	29	37	89	6.7	6.7	-------	.099	.217	.214	2	28
	3	2.5	26	50	122	6.8	6.7	-------	.108	.207	.227	2	48
	4	2.5	26	17	17	6.7	6.6	.135	.126	.172	.178	15	14
Total		9.6											
Nov. 8	1	1.4	17	243	170	7.0	6.8	.063	.117	.282	.200	111	73
	2	2.1	25	187	227	7.0	6.8	-------	.099	.290	.224	20	61
	3	2.5	29	208	199	7.0	6.9	.054	.081	.310	.258	7	92
	4	2.5	29	18	81	6.8	6.6	.135	.135	.172	.168	56	23
Total		8.5											
Dec. 19	1	1.0	14	224	192	6.9	6.7	.063	.103	.310	.224	216	26
	2	1.5	21	260	282	7.0	6.8	.036	.072	.344	.310	3	20
	3	2.2	30	240	223	7.0	7.0	.045	.049	.324	.282	12	24
	4	2.6	35	50	44	6.6	6.6	.126	.126	.182	.168	3	39
Total		7.3											
Jan. 23	1	.3	13	117	43	6.8	6.8	.126	.144	.234	.182	228	260
	2	.1	4	132	139	6.8	6.8	.126	-------	.224	.217	34	400
	3	.7	29	47	58	6.6	6.7	.135	.135	.197	.203	35	146
	4	1.3	54	14	29	6.8	6.8	.153	.144	.158	.182	11	200
Total		2.4											

COW NO. 29, FRESHENED MAY 20, 1935

Sept. 17	1	2.1	18	10	14	6.5	6.5	0.126	0.162	0.155	0.144	1	5
	2	3.7	32	9	19	6.6	6.5	.144	.166	.155	.147	42	5
	3	4.1	35	10	12	6.6	6.5	.153	.171	.155	.147	2	33
	4	1.8	15	199	233	7.0	6.8	.058	.112	.303	.214	35	21
Total		11.7											
Oct. 23	1	2.3	18	32	50	6.7	6.6	.135	.162	.134	.134	1	5
	2	4.2	34	26	11	6.6	6.6	-------	.153	.138	.131	1	4
	3	4.5	36	27	39	6.6	6.6	.135	.162	.141	.134	1	7
	4	1.5	12	267	313	7.0	6.9	.063	.090	.282	.224	5	13
Total		12.5											
Nov. 20	1	1.1	17	25	50	6.6	6.6	-------	.157	.161	.161	2	40
	2	2.2	35	24	40	6.5	6.6	.162	.153	.155	.161	1	2
	3	2.4	38	23	41	6.5	6.6	.171	.162	.152	.155	33	6
	4	.6	10	77	143	6.7	6.6	.153	.139	.197	.178	20	32
Total		6.3											
Dec. 12	1	2.1	20	20	27	6.6	6.6	.162	.162	.155	.152	4	69
	2	3.4	32	14	20	6.6	6.6	.162	.162	.158	.155	29	34
	3	4.0	38	14	11	6.6	6.6	.189	.171	.144	.148	4	3
	4	1.0	10	39	90	6.6	6.6	.175	.149	.148	.189	63	96
Total		10.5											

TABLE 5.—*Yield, pH, acidity, chlorine content, catalase value, and bacterial count, of both the foremilk and the remainder of the milk (whole milk) from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued

COW NO. 19, FRESHENED MAY 15, 1935

Date	Quarter	Yield		Catalase		pH		Acidity		Chlorine		Bacteria per cubic centimeter in—	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Lb.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Hundreds*	*Hundreds*
Oct. 3	1	3.3	23	22	34	6.6	6.6	0.162	0.144	0.144	0.158	-------	-------
	2	4.4	31	10	21	6.6	6.6	.171	.153	.138	.148	-------	-------
	3	5.6	39	17	12	6.6	6.6	.178	.162	.134	.134	-------	-------
	4	1.0	7	107	246	6.7	6.7	.144	.144	.161	.165	-------	-------
Total		14.3											
Oct. 22	1	2.6	21	33	34	6.6	6.6	-------	.144	.185	.148	50	21
	2	4.2	34	34	17	6.6	6.6	-------	.144	.155	.131	40	17
	3	4.6	38	7	11	6.6	6.6	.135	.162	.144	.131	1	11
	4	.8	7	183	232	6.9	6.8	.126	.135	.176	.168	734	68
Total		12.2											
Nov. 19	1	3.2	22	40	44	6.9	6.6	.099	.162	.197	.141	135	21
	2	4.6	32	23	12	6.7	6.6	.108	.162	.172	.138	10	18
	3	5.7	39	5	10	6.6	6.6	.144	.171	.141	.124	2	24
	4	1.0	7	159	232	6.7	6.7	.153	.144	.178	.134	464	176
Total		14.5											
Jan. 30	1	2.7	20	13	63	6.7	6.7	.144	.144	.182	.158	58	28
	2	4.0	30	22	14	6.6	6.6	-------	.162	.155	.148	5	36
	3	5.7	43	10	13	6.6	6.6	.162	.180	.138	.138	15	33
	4	.9	7	114	180	6.6	6.7	.182	.162	.144	.155	90	108
Total		13.3											

COW NO. 21, FRESHENED MAR. 29, 1935

Aug. 28	1	3.7	28	21	27	6.6	6.4	-------	0.157	0.158	0.121	2	60
	2	2.3	18	255	240	6.8	6.6	-------	.126	.316	.161	244	140
	3	3.5	27	43	50	6.6	6.6	-------	.144	.161	.134	3	3
	4	3.6	27	55	70	6.6	6.6	-------	.162	.134	.127	232	28
Total		13.1											
Sept. 3	1	3.4	29	28	14	6.7	6.6	-------	.162	.158	.121	1	2
	2	2.0	18	171+	178	6.8	6.7	-------	.121	.276	.172	238	12
	3	2.8	24	27	29	6.6	6.6	-------	.157	.176	.127	8	10
	4	3.4	29	60	24	6.6	6.6	-------	.153	.172	.121	94	34
Total		11.6											
Nov. 8	1	1.4	17	54	13	6.6	6.6	0.130	.153	.155	.131	5	44
	2	2.6	31	174	106	6.7	6.6	.081	.126	.217	.148	680	43
	3	2.0	24	34	6	6.6	6.6	.126	.148	.165	.127	2	14
	4	2.4	28	17	63	6.6	6.6	.139	.139	.144	.138	130	14
Total		8.4											
Dec. 20	1	2.2	30	43	55	6.6	6.6	.139	.144	.172	.138	12	19
	2	1.2	16	252	230	6.8	6.6	.063	.126	.269	.161	992	40
	3	2.2	30	26	53	6.6	6.6	-------	.153	.155	.138	6	12
	4	1.8	24	28	50	6.6	6.6	-------	.135	.155	.148	72	36
Total		7.4											

TABLE 5.—*Yield, pH, acidity, chlorine content, catalase value, and bacterial count, of both the foremilk and the remainder of the milk (whole milk) from the different quarters of the udders of the definitely abnormal cows at various times after their lactation periods began*—Continued

COW NO. 5, FRESHENED FEB. 17, 1935

Date	Quarter	Yield		Catalase		pH		Acidity		Chlorine		Bacteria per cubic centimeter in—	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Lb.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Hundreds*	*Hundreds*
Aug. 27	1	2.0	24	49	54	6.6	6.6	-------	0.144	0.168	0.127	2	6
	2	2.0	25	37	67	6.6	6.5	-------	.148	.168	.124	1	23
	3	2.0	25	87	92	6.8	6.6	-------	.135	.207	.148	42	88
	4	2.1	26	60	62	6.6	6.6	-------	.144	.144	.127	10	31
Total		8.1											
Sept. 3	1	2.3	24	28	15	6.8	6.7	-------	.148	.165	.127	2	12
	2	2.3	24	26	10	6.7	6.6	-------	.157	.158	.124	20	71
	3	2.6	27	83	43	6.8	6.6	-------	.148	.207	.138	107	52
	4	2.4	25	24	15	6.6	6.6	-------	.153	.155	.124	800	35
Total		9.6											
Nov. 1	1	1.8	25	30	17	6.6	6.6	0.099	.130	.178	.155	50	21
	2	2.1	30	22	8	6.6	6.6	-------	.144	.189	.141	29	68
	3	1.2	17	259	140	6.8	6.8	.054	.103	.300	.207	164	42
	4	2.0	28	22	17	6.6	6.6	.108	.135	.176	.148	2	30
Total		7.1											
Dec. 19	1	1.2	19	44	19	6.9	6.8	.090	.135	.197	.138	63	4
	2	2.1	34	35	27	6.7	6.6	.108	.135	.197	.144	3	3
	3	1.3	21	170	167	6.9	6.8	.054	.090	.282	.193	8	35
	4	1.6	26	29	16	6.7	6.6	.108	.144	.182	.138	6	13
Total		6.2											

COW NO. 31, FRESHENED NOV. 22, 1934

Sept. 9	1	2.4	31	3	17	6.5	6.6	0.153	0.153	0.141	0.127	2	20
	2	1.9	25	26	65	6.7	6.8	.117	.144	.189	.161	70	49
	3	1.4	18	52	72	6.9	6.6	.081	.117	.221	.168	164	25
	4	2.0	26	9	40	6.6	6.6	.144	.153	.155	.138	10	5
Total		7.7											
Oct. 25	1	1.8	39	13	37	6.6	6.6	.144	.162	.172	.158	1	39
	2	1.1	24	113	100	6.8	6.7	.099	.153	.224	.172	41	22
	3	.6	13	129	100	6.8	6.7	.117	.126	.211	.200	25	57
	4	1.1	24	13	17	6.6	6.6	.162	.171	.161	.152	1	10
Total		4.6											
Dec. 20	1	0.5	26	125	62	6.6	6.6	.135	.144	.258	.161	5	14
	2	.6	32	108	76	6.6	6.6	.135	.162	.245	.155	208	56
	3	.4	21	253	144	6.8	6.6	-------	.162	.279	.152	296	144
	4	.4	21	96	54	6.6	6.6	.135	.126	.242	.189	2	28
Total		1.9											

Quarter 2 of cow 11, secreting abnormal milk, never produced over 7 percent instead of the theoretical yield of about 28 percent of the total production. The inference is that the total yield was decreased because of the abnormality of the quarter. The correctness of such an inference is uncertain since one cannot judge the degree of compensation by the other quarters. Quarter 1, supplied by the same

blood stream as no. 2, showed no sympathetic response to the condition in no. 2. The cause of the physiological abnormalities must have been resident in the quarter. It may have been a congenital defect, which may disappear by the second lactation period, as Turner [4] has shown that the secreting tissue of the caprine udder largely disappears at the end of each lactation period and new tissue is formed at the beginning of each succeeding period. It is hoped that the animal can be observed during the second lactation period.

Cow 15 presents a different picture in that during the first 3 weeks the milk of each quarter was normal. Abnormal milk was noted first in quarter 2, 2 weeks later in quarter 3, and in quarter 1, 3 weeks later than it appeared in quarter 2, while quarter 4 yielded no abnormal sample until the one hundred and forty-second day, and not again until the two hundred and forty-fifth day.

The results of the various tests on the routine samples from cow 15 are presented in table 4. It is to be noted that the chlorine content is consistently high in all quarters and that the pH in quarter 4 exceeded 6.7 but twice—on the one hundred and thirty-seventh and on the two hundred and seventy-seventh days. The catalase content in quarter 4 did not reach 50 after the colostral period. In the case of the other quarters the abnormalities were in chlorine and in pH rather than in catalase.

The detailed data of the special samples from cow 15 are presented in table 5. It is to be noted that quarter 4 maintained its yield better than the others until on January 23, the three hundred and seventeenth day, it produced 54 percent, there being a constant increase from 22 percent on the one hundred and nintieth day. The animal produced about 300 pounds per week during the first 8 weeks of the lactation period, and showed a more rapid decrease in production than did the normal animals of the herd. It seems safe to assume that the animal produced a smaller yield of milk and fat than would have been true if the condition of each quarter at the beginning of the period had been maintained. The physical examination revealed nothing of significance.

The abnormalities were not confined to the foremilk, but were present to much the same extent in the entire amount of milk produced by each quarter.

Very low total acidity was noted; for example, the whole milk sample of quarter 3 on December 19 had an acidity of 0.049 percent, a pH of 7.0, a chlorine content of 0.28 percent, and a catalase content of 223.

The cause in the case of this animal must have increased in effect about 1 month after the lactation period began. Some disturbance must have been present from the beginning, as is evidenced by the high chlorine content constantly exceeding 0.15 percent.

The numbers of bacteria bore little relation to the extent of chemical change. This is evident in the case of the samples of December 19 which were markedly abnormal, while the bacterial content was lower than at the other periods. As with cow 11, the abnormality in bacteria seemed to be confined to an increase in the normal udder types rather than to the invasion by a new form.

Cow 29 presents a picture of an abnormal condition in the fourth quarter beginning about 1 month after freshening, while the other

[4] TURNER, C. W., and REINEKE, E. P. A STUDY OF THE INVOLUTION OF THE MAMMARY GLAND OF THE GOAT. Mo. Agri. Ex. Sta. Research Bull. 235, 23 pp., illus. 1936.

three quarters were normal with one exception, 2 on the seventieth day. The general picture is presented in table 4 and the character of the special samples in table 5. The abnormalities in the case of quarter 4 are chiefly in chlorine and in catalase, the pH being less consistently abnormal. The quarter apparently improved in condition, as is shown by the sample drawn therefrom on December 12, which was more nearly normal than any of the three special samples previously examined. The data gathered from the various types of cultures made from the special samples indicated neither such numbers nor such kinds of bacteria as to prove that they were the cause of the disturbance. Quarter 4 usually showed more bacteria than the others, table 5.

Cow 19 is comparable to cow 11 in that the samples yielded by quarter 4 were quite consistently abnormal (table 3). Quarter 1 yielded abnormal samples on the ninety-eighth, one hundred and twenty-first, one hundred and thirty-fifth, one hundred and eighty-eighth, and two hundred and tenth days. Other data on this cow are recorded in table 4.

It is to be noted from table 5 that quarter 4 of cow 19 produced only a small percentage of the milk, and the percentage did not change during the 4 months covered by the data of the special samples. The foremilk of quarter 4 was no more abnormal than the entire quantity of milk drawn from the quarter at any period of observation, a condition differing from that noted in some other animals.

The bacterial content of quarter 4 was high as revealed by the agar slopes inoculated from the routine samples, during the entire period of lactation except the first 2 months. These data were confirmed by the examination of the special samples as are presented in table 5.

Cow 21 was judged as normal for the first 4 weeks of the lactation period; subsequent thereto, quarter 2 was abnormal, as is shown in table 3. Evidences of abnormality were present early in the period, as is shown by the high chlorine content (table 4). Four special samples were taken in the last 4 months of the lactation period (table 5). Quarter 2 produced less than its normal part of the milk; however, its yield did not decline more rapidly than that of the other quarters. The catalase content of the foremilk and of the remainder of the milk from quarter 2 was much the same, while as regards acidity and chlorine the foremilk was more abnormal than the remainder. Such irregularities in abnormalities cannot be explained. The bacterial content of the routine samples was relatively high. This was confirmed by the results of the plate cultures made from the special samples.

The milk from quarters 2 and 4 did not differ significantly in numbers of bacteria but did differ in composition. The cultures of the milk of quarters 2 and 4 showed sufficient differences to attract attention. The plates seeded with the milk of quarter 2 showed many yellow colonies, while those from quarter 4 were almost completely nonchromogenic. This difference persisted in a marked degree during a number of months and to the end of the lactation period. The varying action of the two types of organisms on red blood cells and on casein showed that they were actually different, not simply different in pigment production. Whether the pigment-forming organism is the cause of the abnormal secretion or has any relation to it cannot be stated.

Cow 5 yielded from quarter 2 only abnormal samples until the samples taken on the seventieth day of lactation; thereafter this quarter yielded but two abnormal samples on the seventy-fifth and one hundred and forty-second days. Quarter 3 was quite consistently abnormal (table 4).

The detailed data of the samples are presented in tables 4 and 5.

Cow 31 produced but one abnormal sample during the first 198 days of the lactation period. On the one hundred and sixty-first day, quarter 3 yielded an abnormal sample, and again on the two hundred and third day. During the remaining 175 days, the quarter yielded 1 normal sample and 12 abnormal. Quarter 2 yielded its first abnormal sample on the two hundred and third day, its second on the two hundred and forty-fifth. Beginning then for 133 days 10 abnormal samples were collected. Quarters 1 and 4 remained normal, as is shown by table 3 to the three hundred and seventy-eighth day. On the three hundred and ninety-third day, all quarters yielded abnormal milk (table 5, samples of Dec. 20).

The detailed data of the 36 routine samples taken from each quarter of cow 31 are presented in table 4, from which it is to be noted that the abnormality in the milk from quarters 2 and 3 is marked by a high chlorine and catalase more than by an increase in pH.

The special samples were not collected until the eleventh month of the lactation period. The difference between quarters 1 and 4 as compared with 2 and 3 is evident in the data recorded in table 5 relating to production, catalase, acidity, pH, and chlorine. The bacterial data indicate a higher number of bacteria in quarters 2 and 3 than in 1 and 4 during the period in which the special samples were taken. The increase seems to be in the normal flora rather than in the presence of unusual types of bacteria.

PRODUCTION

The influence of the abnormality on the amount of milk produced cannot be ascertained with certainty from the data. Indeed, the question of the influence of chronic mastitis on milk production is one almost impossible of definite answer, for the yield of milk in one lactation period is governed by a number of factors. In any subsequent period, some change in one or more factors may occur. Such a change may not be apparent and yet may be very significant. No one can estimate the significance of compensation by the normal quarters. One must admit that statements as to the influence of chronic mastitis on milk production have little factual basis. They are surmises, and probably one may safely surmise that an animal which has a disturbed physiological function cannot produce milk with the same degree of efficiency as though the function were normal. The fact that the abnormal quarters produce less milk than the corresponding normal quarters has been presented in the detailed data of a number of the animals. The only satisfactory way of studying the influence of a chronic inflammatory process in a quarter on the milk production thereof is to determine the yield of each quarter of a number of animals at frequent intervals throughout an entire lactation period, then, to compare the condition of the quarters and the amount of milk therefrom, and especially to note whether a quarter which became abnormal at some time in the lactation

period decreased in flow more rapidly than did the normal quarters of the same animal. The milking of the abnormal animals in the herd by quarters was not begun until late in the lactation period. The data are not sufficient to be conclusive. The records of two of the animals are presented in table 6.

TABLE 6.—*Milk production of the 4 quarters*[1] *of the udders of cows 15 and 29 during periods of approximately 3 months*

COW 15

Date of observation	Quarter 1		Quarter 2		Quarter 3		Quarter 4	
	Pounds	Percentage of total	Pounds	Percentage of total	Pounds	Percentage of total	Pounds	Percentage of total
Sept. 18	1.8	19	3.1	34	2.4	25	2.1	22
Oct. 24	1.8	18	2.8	30	2.5	26	2.5	26
Nov. 8	1.4	17	2.1	25	2.5	29	2.5	29
Dec. 19	1.0	14	1.5	20	2.2	30	2.6	36
COW 29								
Sept. 17	2.1	18	3.7	32	4.1	35	1.8	15
Oct. 23	2.3	18	4.2	34	4.5	36	1.5	12
Nov. 20	1.1	17	2.2	35	2.4	38	.6	10
Dec. 12	2.1	20	3.4	32	4.0	38	1.0	10

[1] See note 2, table 3.

Cow 15 freshened on March 13; quarters 1, 2, and 3 had been abnormal about 5 months previous to the first observation. Quarter 4, which had been normal throughout, showed a persistence in production not shown by the other quarters. When making comparisons one should keep in mind that quarters 1 and 4 are forequarters and 2 and 3 are hindquarters.

Cow 29 freshened on May 17; quarter 4 became abnormal about 4 months previous to the first observation.

Cows 5, 19, 21, and 31 showed the same tendency toward a greater decrease of production in the abnormal quarters than in the normal. The data which have been studied in other ways are too few to indicate other than probabilities. It may be said that no matter how the data are handled, the result is to indicate that the abnormal condition caused a more rapid reduction in yield than was noted in the normal quarters.

TRANSMISSION

There has been no reason to believe that the condition has spread from one quarter to another of the same animal or from one animal to another; especially does this seem a safe conclusion in view of the fact that no type of bacteria could be found to be causally related to the abnormality.

PERSISTENCE

The disturbance of function, once established, seems very persistent. Later observations will reveal whether the disturbance will again appear in the next lactation period.

In the case of cows 5, 11, 19, and 35, the condition was present very early in the period and persisted in a relatively consistent manner

throughout the period. In the case of cows 10, 15, 21, 28, 29, 31, 34, and 40, it appeared later and persisted, while in the case of cow 13 it was present early in the period and disappeared later.

SIGNIFICANCE TO PRODUCER

The significance of the disturbance to the producer cannot now be stated. If milk of abnormal composition from quarters free from udder streptococci should prove to persist throughout a number of lactation periods in the same quarter or in the same animal, or if it should prove to be a transmissible trouble, its significance would be greater than the present data indicate. It would seem unwise, considering the present paucity of knowledge regarding this condition, for a farmer to remove from his herd an animal in her first lactation period because of this type of abnormality of the mammary gland. No one is in a position to advise the farmer as to means of prevention or to suggest changes in management that might possibly correct it.

SIGNIFICANCE TO THE CONSUMER OF MILK

As has been stated, no definite agent has been found which seems to stand in causal relation to the abnormality other than possibly a growth of normal udder bacteria beyond the usual number. There is no reason to believe that the trouble has other than an aesthetic significance.

PRESENCE IN OTHER GROUPS OF CATTLE

The same abnormality has been noted in an entirely separate group of animals in the first lactation period. The incidence is much the same as in the herd discussed herein. A review of the data collected in a survey of farm herds made in 1934, using much the same methods, shows a lack of correlation between an abnormal secretion and the presence of *Streptococcus agalactiae* or other streptococci in the udder. Since but one examination of each animal was made, or at most two, the lack of correlation was considered at the time to be due to the inadequacies of the samples and of the tests used for the detection of streptococci. Thirty-nine herds, containing 711 animals, were examined, with the following result:

	Percent
Normal milk	61
Abnormal milk and streptococci	27
Abnormal milk and no streptococci	12

THE CAUSE OF THE ABNORMAL MILK

The detailed examinations as to kind and number of bacteria in the abnormal milk as compared with those in normal milk have supplied little significant information. In general, the total bacterial content of the abnormal milk is higher than that of the normal. In most instances it does not seem high enough to account for the physiological disturbance. Again, the types of bacteria impress one as being the normal udder forms. It should be remembered that nothing is known of the products produced by these bacteria, especially as they grow in the udder, and hence nothing of their pathological effect. It may be that some form of bacteria is present in the quarters supplying abnormal milk that is not present in the other quarters.

Again, the difference may be in the varying susceptibility of the tissue of different quarters to some agent of bacterial origin. It scarcely seems possible that such inequality of response to such a factor would be present without previous tissue injury. Such injury seems improbable in such a large part of a group of young animals.

It is difficult to think that similar tissues in an animal will be unlike in quality; as for example, the different quarters of the mammary gland of the cow. It is supposed that the different quarters have the same supply of nutrients and are controlled by the same supply of identical hormones, therefore, variation in quality of secretion from different quarters is difficult to conceive. Such reasoning indicates that the cause of the trouble must be an invading agent, bacterial or otherwise. On the other hand, there is plenty of evidence to show that variations in quality of milk from the quarters of an udder are present in the case of all cows. The magnitude of variation may not be great, but some variation can be detected at any milking. Physiologists consider the animal as a unit. In the present approach to chronic mastitis, the tendency is to examine the product of the different quarters, and to draw conclusions with little knowledge of the variation which may be considered normal. So far as the authors are aware, no study including the milking of a number of normal animals by quarters over a period of time, followed by a detailed study of the milks, has been carried out. Such a study is essential to any wise approach to the problem of chronic mastitis.

Many attempts have been made to produce chronic mastitis by the introduction of cultures of *Streptococcus agalactiae* into the teat duct, or by bringing them in contact with the end of the teat. Milk containing the same organism has likewise been used. The results have been disappointingly negative in spite of the commonly accepted idea that the organism slowly passes from animal to animal under natural conditions. The failure to produce the disease artificially has led many to invoke a special condition in the udder necessary for the semipermanent or permanent establishment of *Streptococcus agalactiae*. The absence of this condition at the moment the artificial infection was attempted prevents the organism from gaining a foothold, while under natural conditions, the organism obtains its opportunity by being constantly present. Because of the limitations under which the observations herein presented were made, there has been no opportunity to see whether a quarter producing an abnormal milk provides such an acceptable environment for *Streptococcus agalactiae* as to permit it to establish itself with ease and consistency.

SUMMARY

The production of milk of abnormal composition in a herd of 31 cows which had had no contact with other cattle either during or for 1 year before the period of observation was studied during the first lactation period.

Three thousand samples of foremilk from separate quarters were analyzed for chlorine, for catalase, and for pH value. Each sample was cultured on glucose agar and in milk. Additional samples of foremilk and of the entire product of each quarter from animals producing abnormal milk were examined in greater detail as to composition and bacterial content.

The abnormalities noted were such as to indicate a chronic inflammation of low intensity in the involved quarters. No streptococci were found in any sample. It is thus evident that the pathological condition was not caused by the organism, *Streptococcus agalactiae*, admittedly the most frequent cause of chronic mastitis. The abnormal condition is apparently related to higher numbers of the usual types of bacteria found in the udder. It has not been possible to make a detailed differential study of the types of bacteria found in quarters producing normal milk and of those producing abnormal milk, hence the conclusion that the disturbance is due to an increased development in the udder flora over that found in the normal quarter is a purely tentative one. The factors that restrain the growth of the usual udder forms are not known. If these factors are decreased in effect, the udder flora would grow more freely. The growth of any type of organism in the udder may elicit a response, which alters the composition of the milk.

Observations do not indicate that the cause of the condition passes from animal to animal.

In some of the animals the abnormality in a quarter was present at the beginning of the lactation period and persisted throughout the period. In other cases the abnormality appeared at some time during the lactation period and continued to the end thereof, and in still other cases it disappeared.

One or more samples of milk which satisfied the criteria of abnormality used, namely, a pH value above 6.8, a chlorine value above 0.15 percent, and a catalase value in excess of 50 percent, were obtained from 23 of the 31 cows. The detailed study of the records indicates that 17 of the animals may be considered as normal; 14 as abnormal. The detailed data of some of the abnormal animals are presented.

The same condition, that is, abnormal milk in the absence of any definite bacterial cause, is being studied in a second group of first-lactation-period animals. The significance of the abnormality to the herd owner and to the milk consumer is discussed.