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# STUDIES ON THE ALCOHOL COAGULATION OF FRESH COW MILK

 $\mathbf{B}\mathbf{y}$ 

## KENTARO MITAMURA

# [With 21 Text-figures]

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#### Introduction

Milk which has been coagulated by mixing 2.0 cc of ethyl alcohol of 68–70 volume per cent into an equal volume of milk in test tube is generally treated in factory practice as heat-unstable milk. The alcohol test which is very convenient for judging the quality of milk especially for its acidity and heat stability, was originated by Eugling (13) who observed that the milk casein was coagulated by the addition of an equal volume of 95% ethyl alcohol. Following Eugling, Fleischmann and Martiny (15) perfected the present day method of this alcohol test.

This method for detecting the freshness and abnormality of milk is so simple and practical that it has been applied in almost every dairy in dealing with raw milk. This method is also practiced in almost all condensed milk factories and other dairies in Japan. The milk which shows a positive reaction to the alcohol test is usually graded as the second class and is used for butter making, not for condensed milk.

There are some investigations about the alcohol test already reported as to the reliability of the test for the detection of the heat stability and acidity of milk. Some of the important studies along this line may be reviewed.

SELIGMANN (77) and Morres (56) observed that the acidity of fresh milk does not run parallel with the alcohol stability, TILLMANS and OBERMEIER (90) obtained also the same experimental result. According to the study of RAUDNITZ (62) the susceptibility of milk is influenced by the pH value, and mainly depends upon the phosphorus content in it.

It is a well known fact that the colostrum, the strippings at the end of lactation, or milk secreted from the mastitis udder and from other diseased cows, each reacts positively to the alcohol test. Such results have already been reported by many investigators such as Henkel (30) Auzinger (3) Rullmann and Trommsdorf (68) and Rühm (67).

WEIMER (94) reported that milk secreted by a cow fed with mouldy ensilage showed positive reaction to the alcohol test.

The writer found that some times even a good fresh milk shows the positive reaction to the alcohol test, and as the results it is classed the second grade milk. In order to produce a high grade first class milk, care must be taken in the following points: milking from healthy cows, cleanliness at milking time, skilfulness of the milker, perfect cooling of the milk immediately after milking and omitting the colostrum, milk at the end of lactation, and milk secreted from unhealthy cows.

The question now arises as to why such a fresh milk often yields a positive reaction to the alcohol test in spite of paying sufficient attention in milking and handling to the points described above.

There have already been a good many investigations about this problem. It has been found to be mainly due to the salt balance in milk, and this phenomenon belongs to the field of colloid chemistry of milk, that is to say dehydration is caused in milk by the addition of alcohol, thereby increasing the coagulation of milk casein (63).

It has already been proven that the addition of salts to milk especially calcium, magnesium, phosphates, and citrates have a close relation to the alcohol coagulation of milk; calcium and magnesium promote, but on the contrary, citrates and phosphates discourage the alcohol coagulation of milk.

AUZINGER (3) reported that the alcohol coagulation of milk casein is due to the salt constituents, especially to the calcium content, not to the acidity of milk. He also found that by the addition of soluble calcium salt to milk, the tendency of its alcohol coagulation can be increased, and further that milk is more sensitive to alcohol coagulation by feeding 120 g. of calcium phosphate daily. Seno (78) has obtained a result that stronger the alcohol susceptibility of milk greater the calcium content in it.

Sommer and Binney (81) proved that the alcohol coagulation of milk can be encouraged by calcium feeding but did not find any changing of calcium content in the milk. They state further that there is a close relation between the ash constituents in milk and susceptibility to coagulation by alcohol, especially that the susceptibility to alcohol can be increased by the addition of calcium and magnesium salts, but is not changed by the addition of potassium and sodium salts, while on the contrary, it is decreased by the addition of citrates and phosphates. Also Kolthoff (39) and Ayer and Johnson (4) arrived at the conclusion that the salt constituents, particularly the content of calcium and phosphate, in milk have a close relation to its alcohol susceptibility.

It is a well known fact that the susceptibility of normal milk to coagulation by alcohol depends upon the ash constituents especially the balance of calcium and phosphate, as described above.

Then it is very important to investigate the percentage of positive reaction to the alcohol test in fresh milk among herds of milking cows, and it is also desirable to know the strength of alcohol solution that brings about the coagulation in fresh milk most. Again it is interesting to see if there is any relation of the alcohol susceptibility of milk to individuals or breeds of cow. Further, studies on the general alcohol reaction of milk throughout the whole lactation period, the interval after parturition when the alcohol reaction of colostrum will disappear and number of days before the end of lactation the alcohol coagulation of milk again appears, will be reported in this paper.

It is also interesting to learn the influence of neutral salts upon the alcohol coagulation of milk, and the relation between the ash constituents of milk and its alcohol susceptibility.

It is not yet determined whether or not the testing temperature has any influence on alcohol susceptibility of milk. It is still a question to be solved, what relation there is between the alcohol susceptibility of milk and the keeping quality of milk at a definite temperature.

Is the alcohol susceptibility of milk influenced by the oestrum of the cow, and are there any changes in the ash constituents in milk secreted at that time? Neither the influence of the freezing of milk upon its alcohol susceptibility nor the relation between the alcohol susceptibility of milk and the original enzyme, especially catalase content in milk which has an important meaning in physiological sense has yet been determined.

It is within the scope of the present paper to report studies regarding the influence of the feeding of various kinds of salts upon the alcohol coagulation of milk whether there is any close relation between the inorganic salts in milk and its alcohol susceptibility.

The feeding of cows is generally based on the feeding standard. But when cows are fed with an unbalanced ration such as one over or under supply in protein, a large or small amount of the ration itself, the question arises as to whether there is any influence upon the alcohol susceptibility of the milk.

It is also important to observe the influence of sudden change of the ration of the coarse fodder as well as concentrates, upon the alcohol susceptibility of milk. Another question is whether there is any relation between the fat content in milk and its susceptibility to coagulation by alcohol.

The author proposes to study whether or not the ash constituents in blood have any relation though it may be indirect, upon the alcohol susceptibility of milk, as it is clear that the blood takes part in the milk production.

The most important problem which the author has undertaken to report in this paper is the determination of the water soluble inorganic substances and undialyzed insoluble inorganic matters, especially Cl, Ca, Mg and P in milk, and the determination of the conditions under which these inorganic matters in milk take part in its alcohol susceptibility. Furthermore the author tried and succeeded in partially controlling the alcohol susceptibility of milk by mixing some water soluble salt prepared according to theoretical calculations.

As it has been known that the iso-electric point of milk casein has a very important meaning physiologically especially in respect to milk coagulation, the author studied the influence of the iso-electric point of milk upon the alcohol coagulation of milk.

The author hopes that this report may be of a help in promotion and enlightening of the question, on the alcohol coagulation of milk. This report contains most significant contributions on the feeding and management of dairy cows in respect to the alcohol coagulation of milk as mentioned above.

The authors wishes to express his sincere gratitude and hearty thanks to Dr. K. IGUCHI, Dr. A. MIYAWAKI and Dr. M. SATO, Professors of zootechny and also to Mr. K. OGURO, a former professor of medical chemistry of the Hokkaido Imperial University for their valuable suggestions and kind advices in his work. The author is also indebted to the employees in the First and Second Farms of the Hokkaido Imperial University who gave help during the carrying out of these investigations at the farms.

# I. Influence of the Breeds of Cow upon the Alcohol Susceptibility of Milk

#### 1. Experimental methods

The author determined the alcohol susceptibility of fresh milk from cows of various breeds, namely Holstein, Ayrshire, and Guernsey by mixing neutral alcohol of various percentages into the same volume of milk samples.

The milk samples were placed in 100 cc. glass bottles soon after milking, and cooled in cold water, especially in the summer season.

The percentages of alcohol used in the test vary generally from 68 to 70% by volume in practice, but in this experiment the author used the following percentages: 66, 70, 74, 78, 82, 86, 90 and 94%.

The determination of the alcohol susceptibility of milk was carried out in the test tube keeping the temperature at 15°C., by mixing 1.0 cc. each of the different percentages of alcohol into the same volume of each sample one to two hours after milking. The degrees of susceptibility are differentiated into following four classes: -,  $\pm$ , +, and ++ for the sake of easily noting the comparative degree of coagulation.

For the sake of convenience in the calculation of the experimental results the author converted the degrees of alcohol susceptibility of milk to one strength of alcohol to another as follows:

Table 1

Comparative degree of milk coagulation with converted alcohol percentages

Alcohol coagulation obtained from the experiment		Alcohol coagulation obtained from the conversion		
Alcohol %	hol % Degree of coagulation Alcohol %		Degree of coagulation	
66	++	65	+	
66	+	66	+	
66 .	±	67	+	
70	++ .	69	+	
70	+	70	+	
70	士	71	+	
74	++	73	+	
74	+	74	+	
74	士	75	+	
78	++	77	+	
78	+	78	4.	
78	土	79	+	

Table 1 (Continued)

A'cohol coagulation obtained from the experiment		Alcohol coagulation obtained from the conversion		
Alcohol %	Degree of coagulation Alcohol %		Degree of coagulation	
82	++	81	+	
82	+	82	+	
82	土	83	+	
86	++	85	+	
86	+	86	+	
86	±	87	+	
90	++	89	+	
90	+	90	+	
90	土	91	+	
94	++	93	+	
94	+	94	+	
94	±	95	+	

The degree of the alcohol coagulation of milk which is obtained by the conversion practically agrees with the experimental results as shown in the following table.

No. of milk sample	1	2	3	4
Percentages of	70—	74—	78—	73—
alcohol and	71 ±	75±	79 ±	<b>74</b> ±
degree of milk coagulation in	73-+	77+	81+	<b>75</b> +
the same sample	74++	78++	82++	· 77++

#### 2. Cows used in the experiment

Breeds of cows used in this experiment are Holstein, Ayrshire, and Guernsey. The Ayrshires belong to the First Farm, the Holsteins and the Guernseys to the Second Farm of the Hokkaido Imperial University. Particulars about these cows are given as follows.

TABLE 2
Particulars as to the Holstein cows

Cow No.	o. Date of parturition		Experimental period	
239	21/X	1930	19/IV - 9/VII	1931
274	10/X	,,	19/IV - 9/VII	,,
237	6/IV	. ,	19/IV - 9/VII	,,
243	10/I <b>I</b>	1931	19/IV -19/XII	,,
283	12/III	,,	19/IV - 8/VI	,,
284	3/X	1930	19/IV -26/X	,,
252	18/XI	,,	19/IV -19/IX	,,
270	20/XI	,,	19/IV -11/VIII	"
277	28/XII	,,	19/IV -10/IX	,,
254	10/I	1931	19/IV - 8/VI	,,
255	15/XI	1930	19/IV - 9/X	,,
230	20/XII	,,	11/VI -26/X	,,
279	17/IV	1931	11/VI -26/X	,,
250	27/V	,,	11/VI -19/XII	,,
294	1/V	,,	11/VI -19/XII	,,
260	29/VI	,,	6/VII -19/XII	,,
244	5/VI	,,	30/VII19/XII	,,
298	24/VIII	,,	2/X -19/XII	,,
300	15/VII	,,	14/VIII-19/XII	**
292	18/VII	,,	14/VIII-19/XII	,,
301	20/VII	,,	14/VIII19/XII	,,
287	1/VIII	,,	14/VIII-19/XII	,,
302	19/X	,,	2/XI -19/XII	,,
293	11/VII	,,	14/XI -19/XII	,,
297	7/VII	,,	17/XI19/XII	,,
239	24/VIII	,,	2/XI -19/XII	,,
254	6/XI	,,	7/XII -19/XII	,,

 $\begin{array}{c} \text{Table 3} \\ \text{Particulars as to the Ayrshire cows} \end{array}$ 

Cow No.	Date of parturition	Experimental period			
94	25/IX 1930	7/I -27/VII 1931			

TABLE 3 (	Continued)
-----------	------------

	ion	Experimental p	Experimental period		
	30	7/I -30/VII	1931		
	,,	7/I - 5/IV	,,		
	,,	7/I -19VI	,,		
	,,	7/I - 6/III	,,		
	,,	7/I -30/VII	,,		
	31	7/11I -15/VIII	,,		
	30	15/IV -18/XII	,,		
	,,	15/IV -19/VI	,,		
	31	15/IV -16/X	,,		
	,,	27/VII-18/XII	,,		
ĺ	,,	15/IV -18/XII	,,		
	,,	20/XI -18/XII	,,		
1	,,	21/XI -18/XII	,,		

TABLE 4
Particulars as to the Guernsey cows

Cow No.	Date of parturition		Experimental period	
205	23/I	1931	19/IV-11/VIII	1931
222	30/XII	1930	19/IV-20/XII	,,
217	20/IX	,,	19/IV-11/VII	,,
220	9/IX	,,	19/IV-20/XII	,,
223	20/VIII	,,	19/IV-14/VII	,,
223	7/IX	1931	2/X -20/XII	,,
225	28/VI	1930	19/IV- 7/IX	,,
214	$14/\mathrm{IV}$	"	19/IV-20/X1I	,,
226	15/VII	1931	13/X -20/XII	. ,,
227	18/VII	,,	14/IX-20/XII	,,
. 217	23/IX	,,	19/X -20/XII	,,

## 3. EXPERIMENTAL RESULTS

There were 28 Holstein cows, 14 Ayrshires and 11 Guernseys among the 53 heads of cows which were used in this experiment.

The experimental period for the Holstein cows was 19/IV-19/XII 1931, the alcohol test of milk was made every other day, and the total number of samples tested amounted to 1,195. The experimental period for the Ayrshire cows was 7/I-18/XII 1931, the alcohol test was made every day or every other day and the total number of samples tested amounted to 927. The alcohol susceptibility of milk secreted from the Guernsey cow was determined for the period 19/IV-20/XII 1931 every other day, and total tested samples came to 582. Therefore the number of tested samples of all breeds totalled 2,704.

The experimental results of the alcohol susceptibility of milk which have been secured in this experiment are as follows:

TABLE 5
Alcohol percentages and the degree of coagulation by alcohol of Holstein fresh milk

Cow No.	2	239 274 237				237
		nd degree pagulation	Alc. % and degree of milk coagulation			nd degree oagulation
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
19/IV	78++	77÷	82+	82+	86+	86+
21	78+	78+	82 +	82+	$82\pm$	83+ `
23	78+	78+	82+	82+	82++	81+
28	· 78±	79+	82 +	82+	82 + +	81+
30	$78\pm$	79+	82 +	82+	82++	81+
2/V	$78\pm$	79+	82 +	82+	82 + +	81+
4	78+	78+	$82\pm$	83+	82+	82+
6	78+	78+	$78\pm$	79+	82 +	82+
8	· 78±	79+	$78\pm$	79+	82±	83+
11	$74\pm$	75+	$78\pm$	79+	$82\pm$	83+
13	78++	77+	$78\pm$	79+	$82\pm$	83+
15	78++	77+	74+	74+	86++	85+
17	74+	74+	74+	74+	86+	86+
19	78+	78+	78++	77+	86++	85+
21	78+	78+	$74\pm$	75+	<b>3</b> 2+	82+
25	$78\pm$	79+	82++	81+	78++	77+

TABLE 5 (Continued)

Cow No.	2	39	2	274	. 2	237		
Data	Alc. % and degree of milk coagulation			Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted		
27/V	74±	75+	: 78±	79+	82++	81+		
29	78+	78+	78±	79+	$78\pm$	79+		
1/VI	78++	77+	78+	78+	86++	85+		
3	$74 \pm$	75+	78++	77+	$82 \pm$	83+		
5	$74\pm$	75+	<b>78</b> ±	79+	86++	85+		
8	74+	74+	78+	78+	86++	85+		
11	74 +	74+	78++	77+	86+	86+		
13	74+	74+	78++	77+	86+	86+		
15	74+	74+	78++	77+	$86\pm$	87+		
17	74+	74+	78++	77+	90++	89+		
19	78++	77+	78++	77+	$86\pm$	87+		
22	74±	75+	<b>7</b> 8+	78+	. 86±	87+		
24	74++	73+	74+	74+	86+	86-		
26	74+	74+	74+	74+	86+	86+		
29	$74\pm$	75+	74+	74+	$86\pm$ .	87+		
1/VII	$74\pm$	75+	74+	74+	90++	89+		
3	78++	77+	74+	74+	$86\pm$	87+		
6	74 +	74+	74+	74+	90++	89+		
9	74+	74+	74±	75+	<b>8</b> 6±	87+		
	243		283		284			
19/IV	86+	86+	74+	74+	86+	86+		
21	86++	85+	78++	77+	$86\pm$	87+		
23	$82 \pm$	83+	$74\pm$	75+	90++	89+		
28	82±	83+	<b>74</b> +	75+	86+	86+		
30	<b>8</b> 2±	83+	$74\pm$	75+	86±	87+		
2/V	82+	82+	78++	77+	86+	86+		
4	86++	85+	74±	75+	86±	87+		
6	82+	82+	74+	74+	$82\pm$	83+		
8	82+	82+	74+	74+	86+	86+		
11	82+	82+	74+	74+	86+	86+		

Table 5 (Continued)

243		283		284		
Alc. % and degree of milk coagulation			nd degree pagulation	Alc. % and degree of milk coagulation		
Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
82+	82+	74++	73+	82+	82+	
$78\pm$	79+	<b>7</b> 0+	70+	86+	86+	
82+	82+	74+	74+	$86\pm$	87+	
82++	81+	74±	75+	86++	85+	
78++	77+	70++	69+	86++	85+	
$78\pm$	79+	70+	70+	86+	86+	
78++	77+	$70\pm$	71+	86+	86+	
82++	81+	70+	70+	86+	86+	
	81+	70±	71+	86+	86+	
78+	78+	70±	71+	86+	86+	
78±	79+	74+	74+	$82\pm$	83+	
78+	78+	70+	70+	$\textbf{82}\pm$	83+	
<b>78</b> +	78+			82+	82+	
78+	78+		,	82±	83+	
$78\pm$	<b>79</b> +			82 +	82+	
78+	78+		}	$82\pm$	83+	
78+	78+			82+	82+	
,	78+		<u> </u>	86++	85+	
	77+			$82\pm$	83+	
78++	77+		1	82±	83+	
	78+			82+	82+	
·	1			82++	81+	
				78±	79+	
_	· ·		,	82+	82+	
	1		1	82+	82+	
•					85+	
	1				85+	
•				78++	77+	
•	1			82+	82+	
	1				83+	
					83÷	
				_	83+	
	82+ 78± 82+ 78+ 78+ 78+ 78+ 78+ 78+ 78+ 78+ 78+ 78	Experimented         Converted           82+         82+           78±         79+           82+         82+           82+         82+           82+         82+           82+         81+           78++         77+           78+         79+           78+         78+	Experimented         Converted         Experimented           82+         82+         74++           78±         79+         70+           82+         82+         74+           82+         82+         74+           82+         82+         74+           82+         81+         70+           78+         79+         70+           78+         70+         70+           82++         81+         70+           82++         81+         70+           82++         81+         70+           78+         78+         70+           78+         78+         70+           78+         78+         70+           78+         78+         70+           78+         78+         70+           78+         78+         70+           78+         78+         78+           78+         78+         78+           78+         78+         78+           78+         78+         78+           78+         78+         78+           78+         78+         78+           78+         78+ <td>of milk coagulation         Converted         Experimented         Converted           82+         82+         74++         73+           78±         79+         70+         70+           82+         82+         74+         74+           82+         82+         74+         74+           82+         82+         74+         74+           82+         82+         74+         74+           82+         82+         74+         74+           78+         78+         70+         70+           78+         79+         70+         70+           78+         78+         70+         70+           78+         78+         70+         70+           78+         78+         70+         70+           78+         78+         70+         70+           78+         78+         78+         70+         70+           78+         78+         78+         78+         78+         78+           78+         78+         78+         78+         78+         78+         78+         78+         78+         78+         78+         78+         78+         7</td> <td>of milk coagulation         of milk coagulation         of milk coagulation         of milk coagulation           Experimented         Converted mented         Experimented           82+         82+         74++         73+         82+           78±         79+         70+         70+         86+           82+         82+         74+         74+         86±           82+         82+         74+         74+         86±           82+         81+         70+         70+         86+           78++         77+         70+         70+         86+           78++         77+         70±         71+         86+           82++         81+         70+         70+         86+           82++         81+         70±         71+         86+           82++         81+         70±         71+         86+           82++         81+         70±         71+         86+           78+         78+         70±         70+         82±           78+         78+         70+         70+         82±           78+         78+         78+         82±           78+</td>	of milk coagulation         Converted         Experimented         Converted           82+         82+         74++         73+           78±         79+         70+         70+           82+         82+         74+         74+           82+         82+         74+         74+           82+         82+         74+         74+           82+         82+         74+         74+           82+         82+         74+         74+           78+         78+         70+         70+           78+         79+         70+         70+           78+         78+         70+         70+           78+         78+         70+         70+           78+         78+         70+         70+           78+         78+         70+         70+           78+         78+         78+         70+         70+           78+         78+         78+         78+         78+         78+           78+         78+         78+         78+         78+         78+         78+         78+         78+         78+         78+         78+         78+         7	of milk coagulation         of milk coagulation         of milk coagulation         of milk coagulation           Experimented         Converted mented         Experimented           82+         82+         74++         73+         82+           78±         79+         70+         70+         86+           82+         82+         74+         74+         86±           82+         82+         74+         74+         86±           82+         81+         70+         70+         86+           78++         77+         70+         70+         86+           78++         77+         70±         71+         86+           82++         81+         70+         70+         86+           82++         81+         70±         71+         86+           82++         81+         70±         71+         86+           82++         81+         70±         71+         86+           78+         78+         70±         70+         82±           78+         78+         70+         70+         82±           78+         78+         78+         82±           78+	

Table 5 (Continued)

Cow No.	2	43	2	244	2	284		
Date	Alc. % and degree of milk coagulation			Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted		
30/VII	78±	79+	$74\pm$	75+	82±	83+		
5/VIII	78++	77+	$78 \pm$	79+	$78\pm$	79+		
7	78+	78+	78+	78+	8 <b>2</b> +	82+		
11	74+	74+	$74\pm$	75+	82+	82+		
<u>-</u>	2	52	2	270	2	277		
19/IV	82+	82+	82++	81+	74±	75+		
21	$\textbf{82} \pm$	83+	82+	82+	78++	77+		
23	86++	85+	82+	82+	78++	77+		
28	$82\pm$	83+	82++	81+	78++	77+		
30	$82\pm$	83+	$78\pm$	79+	78++	77+		
2/V	82±	83+	78±	79+	78++	77+		
4	82+	82+	82++	81+	78++	77+		
6	82+	82+	$78 \pm$	79+	$74\pm$	75+		
8 .	$82\pm$	83+	$78\pm$	79+	78++	77+		
11	82+	82+	<b>78</b> +	78+	78++	77+		
13	82+	82+	78+	78+	$74\pm$	75+		
15	82+	82+	78+	78+	74+	74+		
17	82++	81+	$78\pm$	79+	74+	74+		
19	82 +	82+	78+	78+	74+	74+		
21	$78 \pm$	79+	78+	78+	78++	77+		
25	86++	85+	$78\pm$	79+	78++	77+		
27	82±	83+	78++	77+	78++	77+		
29	$82 \pm$	83+	78 + +	77+	$74\pm$	75+		
1/VI	82+	82+	$78\pm$	79+	74++	73+		
. 3	82+	82+	$78\pm$	79+	74 +	74+		
5	$78\pm$	79+	$74\pm$	75+	$70\pm$	71+		
8	74++	73+	· 78++	77+	$70\pm$	71+		
11	$78\pm$	79+	$74\pm$	75+	$70\pm$	71+		
13	82+	82+	$70\pm$	71+	74+	74+		
15	82+	82+	74±	75+	74++	73+		

Table 5 (Continued)

Cow No.	2	252	2	270	2	277	
7.	Alc. % and degree of milk coagulation			nd degree oagulation	Alc. % and degree of milk coagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
17/VI	82++	81+	78++	77+	70±	71+	
19	82+	82+	$78\pm$	79+	74+	74+	
<b>2</b> 2	82++	81+	78±	79+	74++	73+	
24	78+	78+	82+	82+	$70 \pm$	71+	
26	82++	81+	78+	78+	70+	70+	
29	78+	78+	$74 \pm$	75+	74 +	74+	
1/VII	78+	78+	70++	69+	74++	73+	
3	78++	77+	78++	77+	78++	77+	
6	$78\pm$	79+	<b>7</b> 8+	78+	$74\pm$	75+	
9	82++	81+	78++	77+	$74\pm$	75+	
11	82+	82+	78+	78+	$74\pm$	75+	
14	82+	82+	78+	78+	78++	77+	
17	78+	78+	$74\pm$	75+	$74\pm$	75+	
20	82+	82+	78++	77+	74++	73+	
22	82+	82+	$\textbf{74} \pm$	75+	$74\pm$	75+	
24	82 + +	81+	74++	73+	74++	73+	
28	78+	78+	70 $\pm$	71+	74++	73+	
30	$78\pm$	79+	70++	69+	74+	74+	
5/VIII	78+	78+	$66\pm$	67+	74++	73+	
7	78+	78+	70 +	70+	$70\pm$	71+	
11	78+	78+	70+	70+	$70\pm$	71+	
	2	54	255		279		
19/IV	74±	75+	82++	81+			
21	$74 \pm$	75+	86++	85+			
23.	74+	74+	$82\pm$	83+			
28	$74\pm$	<b>75</b> +	82+	82+			
30	74+	74+	$82\pm$	83+			
2/V	$74\pm$	<b>75</b> +	$82\pm$	83+			
4	74+	74+,	82±	83+			
6	74+	74+	70±	71+		1	

Table 5 (Continued)

Cow No.	254		2	55	279		
	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
8/V	74+	74+	70±	71+			
11	74 +	74+	78±	79+			
13	74++	73+	82+	82+			
15	74++	73+	78+	78+			
17	74 +	74+	$78\pm$	79+			
19	70±	71+	82++	81+			
21	74+	74+	$78\pm$	79+			
25	74++	73+	82+	82+		}	
27	70 +	70+	8 <b>2</b> +	82+			
29	70 +	70+	82+	82+			
1/VI	70++	<b>6</b> 9+	82++	81+			
3	$70\pm$	71+	78++	77+			
5	74++	73+	78+	78+			
8	74++	73+	78++	77+			
	2	30					
11	78+	78+	78+	78+	70++	69+	
13	82++	81+	82++	81+	70+	70+	
15	78++	77+	<b>78</b> +	78+	70+	70+	
17	$78\pm$	79+	. 82+	82+	70++	69+	
19	78++	77+	82++	81+	70+	70+	
22	82++	81+	86++	85+	70+	70+	
24	82++	81+	86++	85+	70++	69+	
26	<b>78</b> ±	79+	82+	82+	70++	69+	
29	78++	77+	$82 \pm$	83+	70++	69+	
1/VII	<b>78</b> +	78+	8 <b>2</b> +	82+	$70\pm$	71+	
3	$78\pm$	79+	86++	85+	$70\pm$	71+	
6	78+	78+	86++	85+	74++	73+	
9	82++	81+	86+	86+	74+	74+	
11	82++	81+	86++	85+	74++	73+	
14	78+	78+	86+	86+	74+	74+	

Table 5 (Continued)

Cow No.	2	30	2	55	2	279	
D	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
17/VII	78±	79+	86+	86+	74++	73+	
20	78±	79+	86++	85+	74 +	74+	
22	78±	79+	$82\pm$	83+	74++	73+	
24	82+	82+	82+	82+	74+	74+	
28	82+	82+	82+	82+	74++	73+	
30	$78\pm$	79+	82+	82+	74++	73+	
5/VIII	82 +	82+	82+	82+	70+	70+	
7	78+	78+	82 $\pm$	83+	$70\pm$	71+	
11	78++	77+	$\textbf{82}\pm$	83+	74+	74+	
	250		294		260		
11/VI	74±	75+	74++	73+			
13	78 +	78+	74+	74+			
15	78+	78+	74++	73+			
17	$78\pm$	79+	74+	74+			
19	82 +	82+	78++	77+			
22	82++	81+	78++	77+			
24	$78\pm$	79+	78++	77+			
26	82+	82+	78++	77+			
29	90 + +	89+	78++	77+			
1/VII	90++	89+	· 74±	73+			
3	90++	89+	78 +	78+			
6	$\textbf{82}\pm$	83+	$74\pm$	75+	78+	78+	
9	90+	90+	<b>78</b> +	78+	82++	81+	
11	$82\pm$	83+	$74\pm$	75+	82++	81+	
14	86++	85+	78++	77+	82+	82+	
17	$86\pm$	87+	74 $\pm$	75+	$82\pm$	83+	
20	90 + +	89+	$74\pm$	75+	86+	86+	
22	86++	85+	78++	77+	82 $\pm$	83+	
24	$82\pm$	83+	$74\pm$	75+	$\textbf{82}\pm$	83+	
28	86+	86+	74+	74+	78±	79+	

Table 5 (Continued)

Cow No.	2	250	2	94	260			
D-4	Alc. % and degree of milk coagulation			Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted		
30/VII	86+	86+	<b>7</b> 0±	71+	74+	74+		
5/VIII	90++	89+	74+	74+	78±	79+		
7	90+	90+	78+ <i>+</i>	77+	82 +	82+		
11	90+	90+	74 + +	73+	$82\pm$	83+		
14	90+	90+	74+	74+	86++	85+		
18	90+	90+	78++	77+	86+	86+		
21	90±	91+	74+	74+	90++	89+		
24	94++	93+	74++	.73+	$82\pm$	83+		
28	94+	94+	74++	73+	82 + +	81+		
1/IX	$\textbf{86} \pm$	87+	70±	71+	82 +	82+		
4	$86\pm$	87+	$\textbf{70}\pm$	71+	82++	81+		
7	90+	90+	74 +	74+	78+	78+		
10	90++	89+	74+	74+	82+	82+		
12	94++	93+	$70\pm$	71+	78+	78+		
14	90+	90+	74++	73+	78+	78+		
17	86+	86+	70 +	70+	78++	77+		
19	90+	90+	$70\pm$	71+	82++	81+		
25	90±	91+	74++	73+	82±	83+		
28	94++	93+	74+	74+	82+	82+		
30	90÷	90+	$70\pm$	71+	78++	77+		
2/X	$90\pm$	91+	70+	70+	82+	82+		
5	90++	89+	70+	70+	78+	78+		
8	90+	90+	70 +	70+	78+	78+		
10	90+	90+	<b>7</b> 0+	70+	82 +	82+		
13	90+	90+	74++	73+	78+	78+		
16	86++	85+	74++	73+	82++	81+		
19	$86\pm$	87+	74++	73+	78+	78+		
22	90++	89+	70±	71+	82++	81+		
26	90±	91+	$70\pm$	71+	78±	79+		
Ì	2	30		79	2	243		
14/VIII	74+	74+	74++	73+	74+	74+		

TABLE 5 (Continued)

Cow No.	2	30	2	79	2	243
Det		nd degree oagulation	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
18/VIII	78++	77+	70+	70+	74++	73+
21	$78\pm$	79+	70±	71+	74++	73+
24	82++	81+	70±	71+	74++	73+
23	82 + +	81+	70+	70+	78+	78+
1/IX	74+	74+	70±	. 71+	78++	77+
4	78++	77+	70±	71+	$74\pm$	75+
7	_		_		74+	74+
10	78+	· 78+	74+	74+	74+	74+
12	74+	74+	74++	73+	78++	77+
14	74++	73+	70±	71+	74 +	74+
17	74+	74+	70+	<b>7</b> 0+	78++	77+
19	78+	78+	70+	70+	$74\pm$	75+
25	$74\pm$	75+	70±	71+	· 74±	75+
28	74+	74+	74+	74+	78++	77+
30	74++	73+	<b>7</b> 0±	71+	$74\pm$	75+
2/X	74+	74+-	70±	71+	$\textbf{74}\pm$	75+
5	$74\pm$	75+	74+	74+	<b>78</b> +	78+
8	74+	74+	70±	71+	74±	75+
10	$74\pm$	75+	70±	71+	$74\pm$	75+
13	. 74++	73+	74++	73+		
16	$70\pm$	71+	70+	70+		
19	$74\pm$	75+	74+	74+		1
22	74++	73+	70+	70+		
26	74++	73+	70±	71+		
	250		2	94	2	260
29	90±	91+	70±	71+	78++	77+
31	90+	90+	74++	73+	78+	78+
2/XI	90+	90+	70±	71+	82++	81+
5	90+	90+	70±	71+	82++	81+
7	$86\pm$	87+	<b>7</b> 0+	70+	82+	82+

Table 5 (Continued)

Cow No.	2	50	2	94	2	60
<b>.</b>	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
9/XI	86±	87+	- 70+	70+	82+	82+
12	$^{-}_{86\pm}$	87+	70+	70+	82 +	82+
14	90++	89+	$70\pm$	71+	82++	81+
17	90+	90+	74++	73+	82 +	82+
20	<b>9</b> 0+	90+	74+	74+	$\textbf{82}\pm$	83+
23	$86\pm$	87+	74+	74+	$82\pm$	83+
26	$86\pm$	87+	74+	74+	82+	82+
28	$86 \pm$	87+	74+	74+	82++	81+
1/XII	$90\pm$	91+	74++	73+	82 + +	81+
4	90++	89+	$70\pm$	71+	82 + +	81+
7	90±	91+	74+	74+	$82\pm$	83+
9	90 $\pm$	91+	74+	74+	82 +	82+
11	90++	89+	74+	74+	$78 \pm$	79+
14	90±	91+	74+	74+	82++	81+
17	90±	91+ .	74+	74+	82 +	82+
19	90++	89+	74±	75+	82++	81+
	244		284		252	
14/VIII	74++	73+	78±	79+	78±	·79+
18	74+	74+	82+	82+	82+	82+
21	74++	73+	82++	81+	82+	82+
24	$\textbf{74}\pm$	75+	82+	82+	86++	85+
28	$74\pm$	75+	86++	85+	86++	85+
1/IX	74+	74+	86+	86+	82 +	82+
4	74+	74+	78±	79+	86 +	86+
7	74+	74+	78+	78+	86 +	86+
10	74+	74+	78+	78+	86 +	86+
12	78++	77+	78+	78+	86+	86+
14	78++	77+	74+	74+	90++	89+
17	$74\pm$	75+	$74 \pm$	75+	86+	86+
19	<b>7</b> 8++	77+	78++	77+	86 +	86+

Table 5 (Continued)

Cow No.	2	244	2	284	2	298
	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
25/1X	74±	<b>7</b> 5+	78±	79+		
28	74+	74+	82++	81+		
30	$74\pm$	75+	78+	78+		
2/X	78++	77+	78++	77+	74+	74+
5	78++	77+	78++	77+	74++	73+
8	74+	74+	74±	75+	74++	73+
10	78+	78+	$\textbf{74}\pm$	75+	$70\pm$	71+
13	82++	81+	74++	73+	74+	74+
16	78+	78+	74+	74+	$70\pm$	71+
19	82++	81+	78++	77+	74++	73+
22	78 <del>+</del>	78+	74++	73+	$70\pm$	71+
26	$78\pm$	79+	78+	78+	74+	74+
29	82+	82+			74+	74+
31	$78\pm$	79+		302	74±	75+
2/XI	$78\pm$	79+	$82\pm$	83+	$74\pm$	75+
5	82+	82+	82 +	82+	74+	74+
7	82+	82+	$78\pm$	79+	74+	74+
9	$78\pm$	79+	$78\pm$	79+	74 +	74+
12	$78\pm$	79+	78±	79+	$74\pm$	75+
14	$78\pm$	79+	$78\pm$	79+	$74\pm$	75+
17	82++	81+	$78\pm$	79+	$\textbf{74} \pm$	75+
20	82±	83+	78±	79+	74 $\pm$	75+
23	$82\pm$	83+	78 $\pm$	79+	78++	77+
26	82±	83+	<b>78</b> ±	79+	$74\pm$	75+
28	$\textbf{82}\pm$	83+	82+	82+	78+	78+
1/XII	82++	81+	78±	79+	$78\pm$	79+
4	82+	82+	78÷	78+	74±	75+
7	82±	83+	78++	77+	78++	77+
9	82±	83+	78±	79+	78+	78+
11	82++	81+	78++	77+	78+	78+
14	82++	81+	78±	79+	78+	78+
17	78±	79+	78±	78+	78+	78+

Table 5 (Continued)

Cow No.	2	44	8	302		298
Date	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
19/XII	82++	81+	82++	81+	82++	81+
	270		2	77	{	555
14/VIII	70±	71+	74++	73+	82±	83+
18	70+	70+	74++	73+	86 + +	85+
21	70 +	70+	74++	73+	86++	85+
24	70++	69+	74+	74+	86 +	86+
<b>2</b> 8	$70\pm$	71+	$70\pm$	71+	86 +	86+
1/IX	70++	69+	74+	74+	86++	85+
4	74++	73+	$70\pm$	71+	82+	82+
7	74+	74+	74+	74+	$82\pm$	83+
10	74+	74+	74++	73+	82+	82+
12	74++	73+		,	$82\pm$	83+
14	70+	70+			$82\pm$	83+
17	70++	69+			82 +	82+
19	70+	70+			86 + +	85+
25	74 +	74+		, ,	86++	85+
28	$\textbf{74}\pm$	75+			86++	85+
30	74 +	74+		<b>!</b>	$82\pm$	83+
2/X	74++	73+			$82\pm$	83+
5	74+	74+		1 1	$82\pm$	83+
8	78++	77+			86++	85+
10	$74\pm$	75+		1	82 +	82+
13	74++	73+				
	2	239				
2/XI	78+	78+				
5	82++	81+				
7	82+	82+				
9	$82 \pm$	83+				

TABLE 5 (Continued)

Cow No.	2	39	2	93	2	54
D-1		nd degree pagulation	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
12/XI	8 <b>2</b> ±	83+				
14	82+	82+	$82\pm$	83+		
17	82+	82+	$82\pm$	83+	74 +	74+
20	82++	81+	86++	85+	$74\pm$	75+
23	$78\pm$	79+	82±	83+	74±	75+
26	82++	81+	86++	85+	$74\pm$	75+
28	82+	82+	82+	82+	74+	74+
1/XII	82++	81+	82+	82+	$70\pm$	71+
4	$78\pm$	79+	78±	79+	74 +	74+
7	78±	79+	82+	82+	70 $\pm$	71+
9	82++	81+	82±	83+	$70 \pm$	71+
11	78±	79+	82+	82+	74++	73+
14	<b>78</b> +	78+	82+	82+	74+	74+
17	78++	77+	$78\pm$	79+	74 + +	73+
19	78±	79+	78±	79+	74++	73+
	. 3	00	292		301	
14/VIII	74++	73+	78+	78+	82+	82+
18	82+	82+	86++	85+	82+	82+
21	82+	82+	86++	85+	82++	81+
24	$78\pm$	79+	78+	78+	$78\pm$	79+
28	74±	75+	82±	83+	78±	79+
1/IX	78+	78+	78±	79+	$74 \pm$	75+
4	82++	81+	82+	82+	82+	82+
7	78+	78+	78+	78+	78+	78+
10	82+	82+	82++	81+	82+	82+
12	82+	82+	$74\pm$	75+	82+	82+
14	78+	78+	78+	78+	78+	78+
17	74+	74+	$74\pm$	75+	78++	77+
19	78+	78+	$78\pm$	79+	82++	81+
25	82++	81+	78+	78+	82++	81+

TABLE 5 (Continued)

Cow No.	3	800	2	92	5	301
Dita	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
28/IX	78+	78+	82++	81+	82+	82+
30	78+	78+	78+	78+	82++	81+
2/X	78++	77+	82+	82+	78++	77+
5	78++	77+	$78\pm$	79+	78+	78+
8	78+	78+	$82\pm$	83+	82++	81+
10	82 + +	81+	78+	78+	82++	81+
13	$78 \pm$	79+	82+	82+	$82\pm$	83+
16	82++	81+	78+	78+	82+	82+
19	82+	82+	82+	82+	82++	81+
22	82+	82+	82++	81+	78+	78+
26	82++	81+	82+	82+	82+	82+
29	82+	82+	82+	82+	78±	79+
31	82++	81+	82+	82+	$78\pm$	79+
2/XI	$78\pm$	79+	$78\pm$	79+	$78\pm$	79+
5	$78\pm$	79+	82+	82+	78+	78+
7	$78\pm$	79+	82+	82+	78+	78+
9	82++	81+	82++	81+	82+	82+
12	$78\pm$	79+	82+	82+	82+	82+
14	82 +	82+	82+	82+	82+	82+
17	82±	83+	86++	85+	86++	85+
20	86++	85+	86++	85+	82+	82+
23	86++	85+	$82\pm$	83+	82±	83+
26	86++	85+	86++	85+	82±	83+
28	86 +	86+	86++	85+	82+	82+
1/XII	86 +	86+	86+	86+	82++	81+
4	86+	86+	86+	86+	82++	81+
7	86+	86+	86±	87+	82±	83+
9	86+	86+	86+	86+	82±	83+
11	86+	86+	82++	81+	82++	81+
14	86++	85+	82±	83+	82++	81+
17	86±	87+	82++	81+	78+	78+
19	86±	87+	82+	82+	82+	82+

Table 5 (Continued)

Cow No.	2	87	2	43	2	97
Date		nd degree pagulation	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
14/VIII 18 21 24 28	$78++\ 74\pm\ 74\pm\ 74\pm\ 78+$	77+ 75+ 75+ 75+ 75+ 78+				
1/IX 4 7 10 12 14 17 19 25 28 30	$78+78+74\pm78+74\pm78+74\pm82+74\pm74\pm74\pm74\pm74\pm74\pm74\pm$	78+ 77+ 75+ 78+ 75+ 75+ 81+ 79+ 75+ 75+				
2/X 5 8 10 13 16 19 22 26 29 31	$78+$ $78+$ $78+$ $78+$ $82++$ $82++$ $82++$ $82++$ $82++$ $82+$ $82+$ $82\pm$ $78\pm$	78+ 77+ 78+ 81+ 81+ 81+ 81+ 81+ 83+ 79+	$74 \pm 78 + 78 + 78 + 78 \pm 82 + 78 \pm 82$	75+ 77+ 77+ 78+ 79+ 82+ 79+		
2/XI 7 9 12 14 17 20 23 26 28	82± 82++ 82++ 82+ 82+ 82± 82± 82± 82± 82± 82± 82± 82±	83+ 81+ 82+ 82+ 82+ 83+ 82+ 83+ 83+ 83+	78± 78+ 78+ 78+ 78+ 78+ 78+ 78+ 78+ 78+ 78+	79+ 78+ 78+ 79+ 78+ 77+ 78+ 79+ 77+ 78+ 79+	74+ 78± 78± 78+ 78+	74+ 79+ 79+ 78+ 78+
1/XII 4 7 9 11 14 17	82± 82± 86++ 86++ 82++ 78± 78±	83+ 85+ 85+ 81+ 81+ 79+ 79+	82++ 78++ 82++ 82+ 82+ 78+ 78± 82++	81+ 77+ 81+ 82+ 81+ 78+ 79+ 81+	$78++74\pm$ $74++78++78+$ $74\pm$ $78+$ $74\pm$	77+ 75+ 73+ 77+ 78+ 75+ 78+ 75+

TABLE 6 Alcohol percentages and the degree of coagulation by alcohol of Ayrshire fresh milk

Cow No.	:	94	Ş	97	1	14
	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
7/I	86++	85+	90++	89+	86+	86+
8	82+	82+	$86\pm$	87+	$86\pm$	87+
9	$82 \pm$	83+	<b>86</b> ±	87+	86 +	86+
10	82 +	82+	90++	89+	86+	86+
12	82+	82+	90++	89+	86+	86+
13	82+	82+	$86\pm$	87+	90 + +	89+
14	82 +	82+	86±	87+	90 + +	89+
15	82±	83+	86 +	86+	$86\pm$	87+
16	82±	83+	86+	86+	$86\pm$	87+
17	$82\pm$	83+	86±	87+	86±	87+
19	82+	82+	90++	89-;-	86++	85+
20	82+	82+	90++	89+	$86\pm$	87+
21	82+	82+	86±	87+	86+	86+
22	82+	82+	86±	87+	86+	86+
23	82 +	82+	90++	89+	86++	85+
24	82+	82+	86±	87+	86++	85+
26	82+	82+	<b>86</b> ±	87+	86++	85+
27	82 +	82+	$86\pm$	87+	86++	85+
28	82++	81+	86±	87+	86++	85+
29	$82\pm$	83+	86±	87+	86±	87+
30	82+	82+	$86\pm$	87+	86++	85+
31	82 +	82+	86±	87+	86+	86+
2/II	82 +	82+	86±	87+	86±	87+
3	82 +	82+	86+	86+	$86\pm$	87+
4	82++	81+	86++	85+	86+ '	86+
5	82++	81+	86++	85+	86+	86+
6	82+	82+	86+	86+	86+	86+
7	8 <b>2</b> +	82+	86+	86+	86++	85+
9	82+	82+	86++	85+	86++	85+
10	78+	78+	86++	85+	88+	86+

TABLE 6 (Continued)

Cow No.	:	94	!	97	1	14
Data	Alc. % and degree of milk coagulation			nd degree pagulation	Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
12/II	78++	77÷	86+	86+	86++	85+
13	78+	78+	$\textbf{86}\pm$	87+	$86\pm$	87+
14	$78\pm$	79+	<b>86</b> +	86+	86++	85+
16	82++	81+	86+	86+	86++	85+
17	82++	81+	86+	86+	82+	82+
18	82 +	82+	86++	85+ -	82±	83+
19	82 +	82+	$82\pm$	83+	86++	85+
23	82 +	82+	$86\pm$	87+	82±	83+
24	82++	81+	86+	86+	86++	85+
25	82+	82+	$\pm 63$	87+	82+	82+
26	82++	81+	$86 \pm$	87+	86++	85+
27	82++	81+	$86\pm$	87+	86++	85+
28	$78\pm$	79+	$86\pm$	87+	$82\pm$	83+
2/III	82++	81+	86+	86+	82+	82+
3	82 + +	81+	$86\pm$	87+	78±	79+
4	82++	81 +	86+	86+	$78\pm$	79+
5	78±	79+	86±	87+	78+	78+
	1	15	86		128	
7/1	78++	77+	86+	86+	86+	86+
8	$74\pm$	75+	90++	89+	86±	87+
9	74+	74+	$86\pm$	87+	86+	86+
10	78++	77+	86++	85+	86±	87+
12	74+	74+	86++	85+	86++	85+
13	78++	77+	86+	86+	82+	82+
14	78+	78+	86++	85+	82+	82+
15	74++	73+	86±	87+	$82\pm$	83+
16	74 +	74+	86+	86+	$82\pm$	83+
17	74+	74+	86++	85+	$82\pm$	83+
19	74++	73+	86±	87+	82++	81+
20	78+	78+	$86\pm$	87+	$82\pm$	83+

Table 6 (Continued)

Cow No.	1	15	;	86	]	128
	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
21/I	78±	79+	<b>78</b> +	78+	86++	85+
22	78+	78+	<b>78</b> +	78+	86 + +	85+
23	78++	77+	78++	77+	86 +	86+
24	78++	77+	74+	74+	86++	85+
26	78++	77+	74+	74+	$82 \pm$	83+
27	78++	77+	78++	77+	86 +	86+
28	$74\pm$	<b>75</b> +	78++	77+	$82\pm$	83+
29	78++	77÷	$78\pm$	79+	86 +	86+
30	78++	77+	78++	77+	86++	85+
31	78++	77+	78++	77+	86+	86+
2/II	78+	78+	78++	77+	90 + +	89+
3	$78\pm$	79+	78++	77+	90++	89+
4	78+	78+	$74\pm$	75+	90++	89+
5	74++	73+	74±	75+	90 + +	89+
6	74+	74+	$74\pm$	75+	$86\pm$	87+
7	74++	73+	78+	78+	90++	89+
9	74+	74+	78+	78+	$\textbf{86}\pm$	87+
10	74+	74+	74+	74+	$86\pm$	87+
12	74+	74+	74+	74+	86+	86+
13	74++	73+	$74\pm$	75+	86 +	86+
14	74+	74+	<b>78</b> +	78+	86 +	86+
16	78++	77+	, 78++	77+	86++	85+
17	74+	74+	. 78±	79+	86±	87+
18	74+	74+	<b>7</b> 8+	78+	90 + +	89+
19	74+	74+	78±	79+	90++	89+
23	78++	77+	82++	81+	86++	85+
24	78++	77+	82++	81+	86 + +	85+
25	74+	74+	78±	79+	86++	85+
26	74++	73+	82++	81+	82±	83+
27	78++	77+	82++	81+	86++	85+
28	$74\pm$	<b>75</b> +	82++	81+ ·	82++	81+
2/III	74++	73+	78+	78+	82++	81+

Table 6 (Continued)

Cow No.	1	15	:	36	1	28
Date	Alc. % and degree of milk coagulation			nd degree oagulation	Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
3/111	74++	73+	78+	78+	82+	82+
4	74++	73+	78+	78+	82 +	82+
5	74+	74+	78+	78+	82+	82+
6	74++	73+	78+	78+	$82\pm$	83+
	94		97		]	114
6/III	82++	81+	8 <b>6</b> ±	87+	82++	81+
7	82+	82+	86+	86+	$82\pm$	83+
9	$82 \pm$	83+	86+	86+	86++	85+
10	82+	82+	$82\pm$	83+	86 + +	85+
11	$82 \pm$	83+	82±	83+	$86\pm$	87+
12	82+	82+	86+	86+	86 + +	85+
13	82++	81+	86++	85+	86+	86+
14	82++	81+	86++	85+	86++	85+
16	82++	81+	86++	85+	$82\pm$	83+
17	82++	81+	86+	86+	82 +	32+
18	82++	81+	86++	85+	$82 \pm$	83+
19	82++	81+	86++	85+	82++	81+
20	82++	81+	86++	85+	86 +	86+
22	82++	81+	86++	85+	86++	85+
23	82++	81+	86++	85+	86++	85+
24	82+	82+	86+	86+	86++	85+
25	82±	83+	86++	85+	86+	86+
26	82±	83+	86+	86+	86++	85+
27	82+	82+	86+	86+	86+	86+
28	82++	81+	86++	85+	86++	85+
30	82±	83+	86+	86+	86+	86+
1/IV	$82\pm$	83+	$86\pm$	87+	86++	85+
3	82±	83+	86+	86+	86++	85+
5	82+	82+	86 +	86+-	86++	85+
20	82++	81+	86+	86+	82+	82+

Table 6 (Continued)

Cow No.	!	94	!	97	1	.14
Dete	Alc. % and degree of milk coagulation		Alc. % a of milk c	nd degree oagulation	Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
22/IV	78±	79+	82+	82+	86++	85+
24	82++	81+	82+	82+	$86\pm$	87+
28	82++	81+	$82\pm$	83+	86++	85+
30	82++	81+	86++	85+	$82\pm$	83+
2/V	78+	78+	82±	83+	82+	82+
4	78+	78+	82+	82+	82±	83+
6	$74\pm$	75+	78++	77+	$82\pm$	83+
8	74++	73+	78 <del>+</del>	78+	82++	81+
11	74+	74+	$78\pm$	79+	78+	78+
13	74++	73+	78++	77+	82+	82+
15	$74\pm$	75+	82++	81+	82+	82+
18	74+	74+	82+	82+	82+	82+
20	74+	74+	$82\pm$	83+	86++	85+
25	78+	78+	82 +	82+	$82\pm$	83+
27	78 +	78+	82±	83+	82+	82+
29	78++	77+	$82\pm$	83+	$78\pm$	79+
1/VI	$74\pm$	75+	82 +	82+	82+	82+
3	74±	<b>7</b> 5+	82++	81+	$78\pm$	79+
5	$74\pm$	75+	82+	82+	82+	82+
9	$74\pm$	75+	82+	82+	82++	81+
11	78++	77+	78++	77+	$78\pm$	79+
13	$78\pm$	79+	82+	82+	82+	82+
15	78±	79+	$78\pm$	79+	82++	81+
17	74++	73+	82+	82+	82++	81+
19	74±	75+	82+	82+	<b>7</b> 8±	79+
	115		128		142	
7/111	74±	75+	82++	81+		
9	74++	73+	82±	83+	86++	85+
10	74++	73+	82+	82+	82±	83+
11	74+	74+	82±	83+	86++	85+

Table 6 (Continued)

Cow No.	115		1	28	128		
Data	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
12/III	74++	73+	86+	86+	86++	85+	
13	$\textbf{70}\pm$	71+	· 82±	83+	$\textbf{82}\pm$	83+	
14	74+	74+	<b>86</b> +	86+	86++	85+	
16	$74\pm$	75+	$82\pm$	83+	$\textbf{82}\pm$	83+	
17	74++	73+	$82\pm$	83+	86++	85+	
18	74++	73+	86++	85+	$82\pm$	83+	
19	$70\pm$	71+	82+	82+	$82\pm$	83+	
20	74 +	74+	86++	85+	82±	83+	
22	74+	74+	82+	82+	86++	85+	
23	74+	74+	82+	82+	86++	85+	
24	74++	73+	82±	83+	86+	86+	
25	74+	74+	86++	85+	86++	85+	
26	$74\pm$	75+	82±	83+	86++	85+	
27	$74 \pm$	75+	86+	86+	82 $\pm$	83+	
28	$\textbf{74}\pm$	75+	86+	86+	86+	86+	
30	$74\pm$	75+	86+	86+	86++	85+	
1/IV	78++	77+	86±	87+	86++	85+	
3	78++	77+	86++	85+	86+	86+	
5	$74\pm$	75+	86++	85+	86+	86+	
20			86++	85+	$82\pm$	83+	
22			82±	83+	82+	82+	
24			82++	81+	82++	81+	
28		j	82±	83+	82+	82+	
30			82±	83+	82+	82+	
2/V			82++	81+	82+	82+	
4			82++	81+	82++	81+	
6			74++	73+	82++	81+	
8			74++	73+	78±	79+	
11			74±	75+	82±	82+	
13			74±	75+	82++	81+	
15			78++	77+	82++	81+	
18			86++	85+	82+	82+	

TABLE 6 (Continued)

Cow No.	1	15	1	28	1	42
Data	Alc. % and degree of milk coagulation		Alc. % an	nd degree agulation	Alc. % and degree of milk coagulation	
D <b>at</b> e	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
20/V			78+	78+	86++	85+
25			$82\pm$	83+	86++	85-
27		1	$78\pm$	79+	86++	85+
29			$78\pm$	79+	$78\pm$	79+
1/VI			82 +	· 82+	$82\pm$	83+
3			82+	82+	$78\pm$	79±
5			82+	82+	82+	82+
9			82++	81+	82+	82+
11			82 +	82+	82 $\pm$	83+
13			82±	83+	82+	82+
15			82++	81+	82++	81+
17			$74\pm$	75∔	$78\pm$	79+
19	A		<b>74</b> ±	<b>75</b> +	78±	79+
	110		111		91	
15/IV	82+	82+	82++	81+	78+	78+
18	82++	81-+	82++	81+	78+	78+
20	82++	81+	82++	81+	<b>78</b> +	78+
22	78±	79+	78++	77+	78++	77+
24	78±	79+	$78\pm$	79+	$74\pm$	75+
28	78±	79+	78++	79+	78+÷	77+
30	82++	81+	82++	81+	74±	75+
2/V	78±	78+	82++	81+	78++	77+
4	78+	78+	82++	81+	$74\pm$	75+
6	78++	77+	78±	79+	$74\pm$	75+
8	78±	79+	82++	81+	$70\pm$	71+
11	82++	81+	82++	81+	$70\pm$	71+
13	82++	81+	$82\pm$	83+	$70\pm$	71+
15	82++	81+	82±	83+	74++	73+
18	78+	79+	82±	83+	74++	73+
20	82++	81+	86++	85+	74++	73+

TABLE 6 (Continued)

Cow No.	1	110	1	.11		91
	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
25	82+	82+	86++	85+	74++	73+
27	78+	78+	86++	85+	74++	73+
29	82++	81+	82+	82+	74 +	74+
1/VI	$78 \pm$	79+	82±	83+	74±	· 75+
3	$78 \pm$	79+	$78 \pm$	79+	$\textbf{74}\pm$	75+
5	82++	81+	82 +	82+	78 + +	77+
9	$78\pm$	79+	82++	81+	78++	77+
11	82++	81+	$\textbf{82}\pm$	83+	78 +	78+
13	78+	78+	$82\pm$	83+	78++	77+
15	82++	81+	86++	85+	78++	77+
17	78++	77+	82++	81+	74++	73+
19	78+	78+	$78 \pm$	79+	74+	74+
22	78+	78+	$74\pm$	75+		
24	78++	77+	78±	79+		1
30	$78\pm$	79+	82 +	82+		
3/VII	82++	· 81+	$78\pm$	79+		
6	78++	77+	82 +	82+		
9	82++	81+	82+	82+		
13	82 +	82+	$\textbf{82}\pm$	83+		
15	82++	81+	78++	77+		
18	78+	78+	82++	81+		
22	78++	77+	82++	81+		
24	74+	74+	82++	81+		
27	78++	77+	82 +	82+		
30	74++	73+	$78\pm$	79+		
7/VIII	74+	74+	78+	78+		
11	$\textbf{74} \pm$	75+	74++	73+		
15	$70\pm$	71+	$74\pm$	75+		
19	70++	69+	74+	74+		
29	74+	74+	78+	78+		
1/IX	74+	74+	78±	79+		,
4	$70\pm$	71+	78±	79+		

Table 6 (Continued)

Cow No.	1	10	1	11	1	91
	Alc. % and degree of milk coagulation		· Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
8/IX	74+	74+	78+	78+		
10	78++	77+	82 + +	81+		
14	$\textbf{74}\pm$	75+	78+	78+		
	127		97		128	
15/IV	82±	83÷				
18	86++	85+	ı	1		
20	86 +	86+				
22	$86\pm$	87+				
24	86++	85+				
28	<b>86</b> +	86+				
30	86+	86+		1		
2/V	$82\pm$	83+		1 1		
4	$86\pm$	87+				
6	$86\pm$	87+				
8	90++	89+				
11	90 + +	89+				
13	90 +	90+				
15	90+	90+		}		
18	90++	89+				
20	$90 \pm$	91+				
25	$90\pm$	91+				
27	90 +	90+		}		
29	$90 \pm$	91÷				-
1/VI	$90 \div +$	89+				1
3	90+	90+				
5	90 + +	89+	l :			
9	$90\pm$	91+				
11	90 + +	89+	ı			
13	90 +	90+				1
15	90+	90+				

Table 6 (Continued)

Cow No.	1	27	!	97	1	128
<b>.</b>	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
17/VI	90+	90+				
19	$90\pm$	91+				
22	$90 \pm$	91+	$82\pm$	83+	78++	77+
24	$90\pm$	91+	82±	83+	78+	78+
30	90+	90+	82++	81+	78+	78+
3/VII	90++	89+	82+	82+	74+	74+
6	94++	93+	$\textbf{82} \pm$	83+	78++	77+
9	94 + +	93+	86++	85+	82+	82+
13	94++	93+	82++	81+	82±	83+
15	94+	94+	82+	82+	$82\pm$	83+
18	$90\pm$	91+	82++	81+	78 +	78+
22	$90\pm$	91+	$78\pm$	79+	78 +	78+
24	90+	90+	$78\pm$	79+	78 +	78+
27	94++	93+	82++	81+	$74\pm$	75+
<b>3</b> 0	$90\pm$	91+	82++	81+	$74\pm$	85+
	•	94	142		128	
22/VI	74±	75+	78++	77+		
24	74+	74+	$78\pm$	79+		
30	74++	73+	82++	81+		
3/VII	74++	73+	78++	77+		
6	74++	73+	78+	78+		
9	78+	78+	82+	82+		
13	78±	79+	82±	83+		l l
15	78+	78+	$82\pm$	83+		
18	74+	74+	82++	81+		
22	74+	74+	78++	77+		
24	$74\pm$	75+	$74\pm$	<b>7</b> 5+		
27	74+	74+	78 <del>+</del>	78+		
30			78++	77+		
7/VIII			78++	77+		

Table 6 (Continued)

Cow No.	1	11	1	42	` 1	.28	
-	Alc. % and degree of milk coagulation		Alc. % an of milk co	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
11/VIII 15			$74++\ 74++$	73+ 73+			
16/IX 18 25 28	82++ $82++$ $82++$ $82++$	81+ 81+ 81+ 81+					
1/X 5 10	$78\pm \\ 74\pm \\ 78\pm \\ 68++$	79+ 75+ 79+ 77+					
14 16	78+ 74±	78+ 75+	1	15			
20 22 26 29			$74\pm \\ 74+ \\ 74\pm \\ 74\pm \\ 74\pm$	75+ 74+ 75+ 75+			
2/XI 5 9			$74\pm \\ 78++ \\ 74\pm \\ 74++$	75+ 77+ 75+ 73+			
17 19 21			74++ 74+ 78+	73+ 74+ 78+	<b>78</b> +	78+	
24 27			78++ 78±	77+ 79+	82± 82+	83+ 82+	
3/XII 7			78++ 78+	77+ 78+ 78+	78± 32++	79+ 81+ 79+	
9 11 14			78+ 78+ 78++	78+ 78+ 77+	$78\pm\ 82++\ 78\pm$	81+ 79+	
18			$74\pm$	75+	78++	77+	

Table 6 (Continued)

Cow No.	1	127		44	1	10
<b>D</b> .	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
27/VII			86++	85+		
30			86++	85+		
7/VIII	$90\pm$	91+	86++	85+		
11	90++	89+	82++	81+		
15	82±	83+	82++	81+		
19	86+	86+	√ 78÷	78+		
29	86+	86+	82±	83+		
1/IX	86++	85+	82+	82+		
4	86++	85+	82+	82+		
8	86±	87+	82±	83+		
10	90++	89+	82±	83+		
14	86+	86+	$82\pm$	83+		
16	$86 \pm$	87+	86++	85+	78+	78+
18	90++	89+	$\textbf{82}\pm$	83+	$78\pm$	79+
25	$86\pm$	87+	86++	85+	78+	78+
28	90++	89+	86+	86+	$78 \pm$	79+
1/X	90++	89+	86+	86+	78+	78+
5	$86 \pm$	87+	86++	85+	78++	77+
10	82+	82+	78+	78+	78++	77+
12	$82\pm$	83+	<b>7</b> 8+	78+	78+	78+
14	$82 \pm$	83+	$78\pm$	79+	78++	77+
16	86++	85+	$82\pm$	83+	$74\pm$	75+
20	86++	85+	82++	81+	$74 \pm$	75+
22	$86\pm$	87+	78±	79+	$74\pm$	75+
26	$\textbf{86} \pm$	87+	82+	82+	78 +	78+
29	86 +	86+	$82\pm$	83+	78++	77+
2/XI	$86\pm$	87+	82±	83+	74+	74+
5	90++	89+	$82\pm$	83+	$74 \pm$	75+
9	86+	86+	$82\pm$	83+	$74 \pm$	75+
12	86+	86+	82+	82+	$74\pm$	75+
17	$86\pm$	87+	82+	82+	74+	74+
19	<b>86</b> ±	87+	82±	83+	70±	71+

Table 6 (Continued)

Cow No.	127		144		110	
	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
21/XI	$86\pm$	87+	86++	85+	$\textbf{74}\pm$	75+
24	90 + +	89+	82 +	82+	74+	74+
27	$86\pm$	87+	82+	82+	$\textbf{74}\pm$	75+
3/XII	$\textbf{86}\pm$	87+	82++	81+	$74\pm$	75+
7	$86\pm$	87+	$78\pm$	79+	74±	75+
9	86 +	86+	78+	78+	$\textbf{74}\pm$	75+
11	$86\pm$	87+	78+	78+	$74\pm$	75+
14	$86\pm$	87+	$78\pm$	79+	74 $\pm$	75+
18	90 + +	89+	78+	78+	78++	77+

TABLE 7

Alcohol percentages and the degree of coagulation by alcohol of Guernsey fresh milk

Cow No.	225		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
	Alc. % and degree of milk coagulation					
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
19/IV	86++	85+	82±	83+	90+	90+
21	86++	85+	82 +	82+	86±	87+
23	82 +	82+	82±	83+	90++	89+
28	86++	85+	74+	74+	90 + +	89+
30	$82 \pm$	83+	74+	74+	90++	89+
2/V	$82 \pm$	83+	74++	73+	86±	87-
4	$\textbf{82}\pm$	83+	78++	77+	90++	89+
6	$78\pm$	79+	74+	74+	90 +	90+
8	82 +	82+	$74\pm$	75+	86 $\pm$	87+

TABLE 7 (Continued)

Cow No.	2	25	2	05	2	22
	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
11/V	82+	82+	$\textbf{74} \pm$	75+	$86\pm$	87+
13	86++	85+	$74 \pm$	75+	86 +	86+
15	78+	78+	74++	73+	86+	86+
17	78±	79+	78++	77+	$86\pm$	87+
19	82++	81+	$74\pm$	75+	86++	85+
21	82++	81+	$\textbf{74}\pm$	75+	$86\pm$	87+
25	82 +	82+	78+	78+	$86\pm$	87+
27	$82\pm$	83+	$74 \pm$	75+	86+	86+
29	$\textbf{82}\pm$	83+	$74 \pm$	75+	86 +	86+
1/VI	86++	85+	74++	73+	90++	89+
3	82+	82+	70 +	70+	90++	89+
5	86++	85+	70 $\pm$	71+	86++	85+
8	82 +	82+	$70\pm$	71+	$86\pm$	87+
11	$78\pm$	79+	$\textbf{70}\pm$	71+	90 + +	89+
13	<b>78</b> +	78+	74 + +	73+	$86\pm$	87+
15	$78 \pm$	79+	70±	71+	86++	85+
17	$78\pm$	79+	70 +	70+	90 + +	89+
19	78±	79-	70++	69+	90 + +	89+
22	82++	81+	70 +	70+	90++	89+
24	82++	81+	$\textbf{70}\pm$	71+	90++	89+
26	82++	81+	$\textbf{70}\pm$	71+	90+	90+
29	82 +	82+	<b>70++</b>	69+	90+	90+
1/VII	78+	78+	$\textbf{70}\pm$	71+	$\textbf{86}\pm$	87+
3	$78\pm$	79+	<b>7</b> 0+	70+	90++	89+
6	78++	77+	$70 \pm$	71+	$\textbf{86}\pm$	87+
9	$78\pm$	79+	<b>√7</b> 0±	71+	90++	89+
11	78++	77-+	70+	70+	90++	89+
14	78++	77+	70 +	70+	90++	89+
17	78++	77+	70 +	70+	$86\pm$	87+
20	<b>78</b> +	78+	70±	71+	90 + +	89+
22	82++	81+	$70\pm$	71+	$86\pm$	87+
24	82++	81+	$70\pm$	71+	86±	87+

Table 7 (Continued)

Cow No.	2	225	2	205	2	<b>222</b> .
Data	Alc. % and degree of milk coagulation		Alc. % a of milk c	nd degree oagulation	Alc. % and degree of milk coagulation	
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
28/VII	78±	79+	70+	70+	8 <b>6</b> ±	87+
30	78++	77+	70+	70+	90++	89+
5/VIII	$74\pm$	75+	$66 \pm$	67-+	86 +	86+
7	$70\pm$	71+	$70\pm$	71+	86 +	87+
11	78++	77+	$70\pm$	71+	86+	86+
	217		214		2	220
19/IV	78+	78+	74++	73+	86+	86+
21	78++	77+	70±	71+	86+	86+
23	78++	77+	$70\pm$	71+	$86\pm$	87+
28	78++	77+	$74\pm$	75+	86+	86+
30	78++	77+	74+	74+	82±	83+
2/V	74+	74+	74+	74+	82+	82+
4	$74\pm$	75+	74 +	74+	82 $\pm$	83+
6	78++	77+	74 +	74+	$82\pm$	83+
8	78++	77+	74++	73+	82±	83+
11	78++	77+	78+	78+	74 $\pm$	75+
13	78++	77+	$74\pm$	75+	$78\pm$	79+
15	74 +	74+	78+	78+	78+	78+
17	74++	73+	74 +	74+	$78\pm$	79+
19	$74\pm$	75+	74++	73+	82++	81+
21	74 +	74+	74+	74+	82++	81+
25	78 +	78+	74+	74+	82±	83+
27	$74\pm$	75+	74++	73+	82++	81+
79	78++	77+	74 ±	75+	$82\pm$	83+
1/VI	78+	78+	74+	74+	82 +	82+
3	78 +	78+	$74\pm$	75+	82++	81+
5	78+	78+	70 +	70+	78+	78+
8	$74\pm$	75+	70+	70+	74 $\pm$	75+
11	$74\pm$	75+	70 +	70+	82++	81+
13	78++	77+	74 +	74+	82 +	82+

TABLE 7 (Continued)

Cow No.	2	17	2	214		220	
	Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		Alc. % and degree of milk coagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
15/VI	78+	78+	74++	73+	82++	81+	
17	<b>78</b> +	78+	$70\pm$	71+	$78\pm$	79+	
19	74++	73+	70 +	70+	78++	77+	
22	74+	74+	70±	71+	78++	77+	
24	78±	79+	70+	70+	78+干	77+	
26	<b>78</b> +	78+	70++	69+	78++	77+	
29	74+	74+	70+	70+	78++	77+	
1/VII	70±	71+	74+	74+	78+	78+	
3	74士	75+	$70\pm$	71+	78++	77+	
6	78++	77+	$70\pm$	71+	78++	77+	
9	74+	74+	74+	74+	78++	77+	
11	78+	78+	78++	77+	78+	78+	
14	78++	77+	<b>74</b> ±	75+	78++	77+	
17	<b>74</b> ±	75+	74±	75+	78+	78+	
20	78+	78+	$74\pm$	75+	78 +	78+	
22	$78\pm$	79+	$74\pm$	75+	78+	78+	
24	78++	77+	74+	74+	78+	78+	
28	74+	74+	74+	74+	<b>' 74</b> +	74+	
30	74+	74+	74++	73+	78++	77+	
5/VIII	74++	73+	74+	74+	74++	73+	
7	74 +	74+	74++	73+	70+	70+	
11	74+	74+	74+	74+	$70\pm$	71+	
	2	23	2	220		227	
19/IV	86++	85+					
21	82 +	82+					
23	82 +	82+					
28	82+	82+					
30	82+	82+					
2/V	82+	82+					
4	82+	82+					

Table 7 (Continued)

	223	2	20	2	227
Experimented  6/V 82± 8 82+ 11 82++ 13 78+ 15 78++ 17 78+ 19 78± 21 78+ 25 82+ 27 82++ 29 82+ 1/VI 82± 3 82+ 1/VI 82± 15 82+ 11 82++ 11 82++ 11 82++ 12 78+ 15 82+ 17 82+ 17 82+ 19 82+ 21 78+ 22 78+ 24 78++ 26 78++ 29 78++ 21 78++ 29 78++ 29 78++ 21 78+ 21 78+ 22 78+ 24 78++ 25 78++ 26 78++ 27 78++ 28 78++ 29 78++ 29 78++ 29 78++ 29 78++ 29 78++ 20 78++ 21 78+ 22 78+ 23 78+ 24 78++ 25 78++ 26 78++ 27 78++ 27 78++ 28 78++ 29 78++ 29 78++ 29 78++ 21 78++ 21 78++ 22 78++ 23 78++ 24 78++ 25 78++ 26 78++ 27 78++ 27 78++ 28 78++ 29 78++ 29 78++ 21 78++ 21 78++ 22 78++ 23 78++ 24 78++ 25 78++ 26 78++ 27 78++ 27 78++ 28 78++ 29 78++ 29 78++ 29 78++ 20 78++ 20 78++ 21 78++ 22 78++ 23 78++ 24 78++ 25 78++ 26 78++ 27 78++ 27 78++ 28 78++ 29 78++ 29 78++ 20 78++	and degree coagulation	Alc. % an	nd degree pagulation	Alc. % a of milk c	nd degree oagulation
8 82+ 11 82++ 13 78+ 15 78++ 17 78+ 19 78± 21 78+ 25 82+ 27 82++ 29 82+ 1/VI 82± 3 82++ 5 82++ 11 82++ 13 82± 15 82++ 17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	Converted	Experi- mented	Converted	Experi- mented	Converted
8 82+ 11 82++ 13 78+ 15 78++ 17 78+ 19 78± 21 78+ 25 82+ 27 82++ 29 82+ 1/VI 82± 3 82+ 5 82+ 1 82++ 11 82++ 13 82± 15 82++ 17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 26 78++ 29 78++ 29 78++ 21 78+ 29 78+ 21 78+ 22 78+ 24 78++ 25 78+ 27 78+ 28 78+ 29 78+ 29 78+ 20 78+ 21 78+ 22 78+ 23 78+ 24 78++ 25 78+ 26 78++ 27 78+ 27 78+ 28 78+ 29 78+ 29 78+ 20 78+ 20 78+ 21 78+	83+				
13	82+				
15	81+				
17	78+				
19	77+				
21	78+				
25 82+ 27 82++ 29 82+ 1/VI 82± 3 82+ 5 82+ 8 82++ 11 82++ 13 82± 15 82++ 17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	79+				
27	78+				
29 82+ 1/VI 82± 3 82+ 5 82+ 8 82++ 11 82++ 13 82± 15 82++ 17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	82+				κ.
29 82+ 1/VI 82± 3 82+ 5 82+ 8 82++ 11 82++ 13 82± 15 82++ 17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	81+				
1/VI 82± 3 82+ 5 82+ 8 82++ 11 82++ 13 82± 15 82++ 17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	82+				
3     82+       5     82+       8     82++       11     82++       13     82±       15     82++       17     82+       19     82+       22     78+       24     78++       26     78++       29     78++       1/VII     74++       3     78+       6     78+       9     78+       11     78+	83+				
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	82+				
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	82+				
13 82± 15 82++ 17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	81+				
15 82++ 17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	81+				
17 82+ 19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	83+				
19 82+ 22 78+ 24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	81+				
22 78+ 24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	82+				
24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	82+				
24 78++ 26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	78+				
26 78++ 29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	77+				
29 78++ 1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	77+				
1/VII 74++ 3 78+ 6 78+ 9 78+ 11 78+	77+				
3 78+ 6 78+ 9 78+ 11 78+	73+				
6 78+ 9 78+ 11 78+	78+				
9 78+ 11 78+	78+				
11 78+	78+		]		
	78+		1		
	77+				
14/VIII	'''	74+	74+		
18		78++	77+		

Table 7 (Continued)

Cow No.	2	23	2	20	227			
D		nd degree pagulation		nd degree pagulation		nd degree oagulation		
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted		
21/VIII			$74\pm$	75+				
24			$74\pm$	75+				
28			74++	73+				
1/IX			$70\pm$	71+				
4			74+	74+				
7			74+	74+				
10			74+	74+				
12			74+	74+				
14			70+	70+	82+	82+		
17			70++	69+	<b>8</b> 2+	82+		
19			74+	74+	82+	82+		
	225		2	22	2	214		
14/VIII	82++	81+	90++	89+	78++	77+		
18	$78\pm$	79+	90±	91+	$74\pm$	75+		
21	$78\pm$	79+	94++	93+	<b>74</b> ±	75+		
24	78++	77+	94++	93+	78++	77+		
28	$74\pm$	75+	94+	94+	$74\pm$	75+		
1/IX	<b>78</b> +	78+	94++	93+	74+	74+		
4	78++	77+	<b>9</b> 0±	91+	74++	73+		
7	78±	79+	94++	93+	74++	73+		
10		·	94++	93+	74+	74+		
12		[	90±	91+	$74\pm$	75+		
14			94++	93+	78+	78+		
17			94++	93+	74++	73+		
19			<b>9</b> 0+	90+	78++	77+		
25		.	94++	93+	78+	78+		
28			94+	94+	<b>78</b> +	78+		
30			90+	91+	78++	77+		
2/X			90++	89+	78+	78+		
		ı i	1	'		1		

Table 7 (Continued)

Cow No.	217		2	22	214			
		nd degree oagulation		nd degree pagulation	Alc. % and degree of milk coagulation			
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted		
8/X			<b>9</b> 0+	90+	82++	81+		
10			86±	87+	78+	78+		
13			86±	87+	74±	75+		
16			86++	85+	74+	74+		
19	78 +	<b>79</b> +	86++	85+	78++	77+		
22	82++	81+	82+	83+	74±	75+		
26	78+	78+	86++	85+	70±	70+		
29	78±	79+	82+	83+	74++	73+		
31	78+	78+	82+	83+	70+	71+		
2/XI	78++	77+	86++	85+	70 ±	71+		
5	$74\pm$	75+	82+	83+	$70\pm$	71+		
7	$74\pm$	75+	82±	83+	$70\pm$	71+		
9	78++	77+	82±	83+	74++	73+		
12	78++	77+	86++	85+	74+	74+		
14	78+	78+	86++	85+	74 + +	73÷		
17	82++	81+	86++	85+	74+	74+		
20	78±	79+	82±	83+	$74\pm$	75+		
23	78+	78+	$82\pm$	83+	78++	77+		
26	78±	79+	<b>8</b> 2±	83+	74+	74+		
28	82++	81+	86++	85+	78 + +	77+		
1/XII	$78\pm$	79+	86++	85+	74+	74+		
4	82++	81+	$82\pm$	83+	82++	81+		
7	82++	81+	$82\pm$	83+	82++	81+		
9	82++	81+	$82\pm$	83+	$78\pm$	79+		
11	78 +	78+	82+	82+	82++	81+		
14	$78\pm$	79+	$82\pm$	83+	78±	79+		
17	78+	78+	82+	82+	$78\pm$	79+		
19	$78\pm$	79+	$82\pm$ .	83+	$78\pm$	79+		
20	$78\pm$	79+	$82\pm$	83+	$78\pm$	79+		

Table 7 (Continued)

Cow No.	2	20	2	23	2	27
Data		nd degree pagulation	Alc. % ar	nd degree pagulation	Alc. % a of milk c	nd degree oagulation
Date	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
25/IX	74+	74+			86++	85+
28	78±	79+			86 +	86+
30	78++	77+	1		86++	85+
2/X	$78\pm$	79+	82++	81+	$82\pm$	83+
5	$78\pm$	79+	82+	82+	$82\pm$	83+
8	82++	81+	78±	79+	82±	83+
10	$78\pm$	79+	82+	82+	86++	85+
13	$78\pm$	79+	82++	81+	86+	86+
16	78+	78+	78±	79+	86++	85+
19	82++	81+	82++	81+	86++	85+
22	78±	79+	82+	82+	86+	86+
26	$78\pm$	79+	78±	79+	82+	82+
29	82++	+18	82++	81+	78±	79+
31	78+	78+	78±	79+	78+	78+
2/XI	78++	77+	78+	78+	<b>78</b> +	78+
5	78++	77+	78+	78∔	78+	78+
7	78++	77+	78±	79+	78±	79+
9	$78\pm$	79+	82++	81+	82++	81+
12	$78\pm$	79+	82++	81+	82++	81+
14	82++	81+	82+	82+	82+	82+
17	82±	83+	82±	83+	82+	82+
20	86++	85+	82±	83+	82++	81+
23	82±	83+	82±	83+	78±	79+
26	82+	82+	82+	82+	78+	78+
28	86++	85+	$82\pm$	83+	82++	81+
1/XII	82+	82+	82+	82+	82++	81+
4	82±	83+	82+	82+	82++	81+
7	82+	82+	82++	81+	82+	82+
9	86++	85+	82+	82+	82+	82+
11	86++	85+	82+	82+	78±	79+
14	78±	79+	78±	79+	82++	81+
17	82±	83+	82++	81+	82++	81+
19	82++	81+	78±	79+	78±	79+
20	82+	82+	78+	79+	78±	79+

Table 7 (Continued)

Cow No.	2	26	Cow No.	Alc. % and degree of milk coagulation			
		nd degree oagulation					
Date	Experi- mented	Converted	Date	Experi- mented	Converted		
13/X	82++	81+	20/XI	86++	85+		
16	82+	82+	23	86++	85+		
19	82+	82+	26	86++	85+		
22	82 +	82+	28	82+	82+		
26	78±	78+	1/XII	82++	81+		
29	82 + +	81+	4	82±	83+		
31	82++	81+	7	82++	81+		
2/XI	8 <b>2</b> ++	81+	9	82+	82+		
5	$78\pm$	79+	11	82+	82+		
7	$78\pm$	<b>79</b> +	14	82+	82+		
9	82++	81+	17	82+	82+		
12	82++	81+	19	78±	79+		
14	82+	82+	20	78±	79+		
17	$82\pm$	83+					

## 4. DISCUSSION

The experimental results shown in the above tables regarding the coagulation of various kinds of milk by alcohol may be arranged in various ways in order to make the interpretation of the results easier.

TABLE 8
Frequency distribution of the alcohol susceptibility of milk secreted from three different breeds of cow

Alc. % a of milk c	nd degree oagulation		Frequency of					
Experi- mented	Converted	Holstein	Ayrshire	Guernsey	Total			
66++	65+	_	_ :	_	-			
66+	66+	_	-	-	_			

Table 8 (Continued)

Alc. % a of milk c	nd degree oagulation		Freque	ncy of		
Experi- mented	Converted	Holstein	Ayrshire	Guernsey	Tota	
66±	67+	1	_	1	2	
70 + +	69+	12	1	4	17	
<b>70</b> +	70+	32	_	18	50	
$70\pm$	71+	<b>54</b>	8	29	91	
74++	73+	69	36	25	130	
74+	74+	107 51		43	201	
$74\pm$	75+	94	60 35		189	
<b>7</b> 8++	77+	98	64	55	217	
78+	78+	125	71	52	248	
$78\pm$	79+	113	73 ,	67	253 255 298	
82++	81+	99	, 98	58 61		
82+	82+	141	96			
$82\pm$	83+	85	88	38	211	
86 + +	85+	<b>52</b>	94	24	170	
86+	86+	43	67	11	121	
$86\pm$	87+	22	64	21	107	
90 + +	89+	17	31	21	69	
90+	90+	17	10	6	33	
$90\pm$	91+	10	10	4	24	
94++	93+	4	4	7	15	
<b>94</b> +	94+	_	- 1 1		3	
<b>64</b> ±	95+		_	_	_	
T	otal	1,195	927	582	2,704	

There is only one example of milk coagulated by 67% alcohol to the degree of + among 1,195 tests of Holstein milk samples, only 12 samples coagulated by 69% alcohol, 32 samples by 70% alcohol; the number of the samples coagulated by stronger alcohol increased more and more with some fluctuations. There were 107 examples coagulated by 74%, 125 examples by 78% and 141 by 82% alcohol. It is interesting to know that the milk samples coagulated by the alcohol of higher concentrations than 82% decreased gradually in number

to the highest concentration: there were 43 examples of milk coagulated by 86%, 17 examples by 90% and only 4 by 93% alcohol.

In case of the Ayrshire breed there were 927 samples of milk tested in this experiment. There was only one case of milk coagulated by 69% alcohol and with this breed also there existed the same tendency that the more concentrated the alcohol the larger became the number of samples coagulated showing the maximum with 82% alcohol. There were 51 cases by 74% alcohol, 71 by 78% and 96 by 82%. But there are 94 cases by 85%, 10 by 90% alcohol and only 4 coagulated by 93% alcohol.

The same phenomena were found in the Guernsey milk. That is, there was only one case of milk being coagulated by 67% alcohol among a total 582 samples, 4 by 69% alcohol, 43 by 74%, 52 by 78% 61 by 82%, but the cases of coagulation by the stronger alcohol than 82% decreased as follows: 24 samples by 85%, 21 by 89% and only 2 cases by 94% alcohol.

In the next table are shown the calculated means of the alcohol percentages which coagulated the most of milk samples from these breeds.

Table 9

Type and variability of coagulation by alcohol for three different kinds of milk

	Mean (alcohol %)	Standard deviation (alcohol %)	Coefficient of variability (%)
Holstein	78.82±0.10	4.92±0.07	$6.24 \pm 0.09$
Ayrshire	81.17±0.10	4.68±0.07	5.77±0.09
Guernsey	79.26±0.15	5.24±0.10	6.61±0.10
Total	79.72±0.07	5.03±0.05	<b>6.31</b> ±0.06

The mean percentage of alcohol which coagulated the most samples of milk is almost the same in spite of the different kinds of milk, namely 78.82% for the Holstein milk, 81.17% for the Ayrshire milk and 79.26% alcohol for the Guernsey milk. The number of samples coagulated by higher or lower percentages of alcohol than these decreased proportionately. Therefore it may be recognized that the alcohol susceptibility of milk is not influenced materially by the breeds of cow.

Next the author will arrange the entire tested samples of milk including all breeds of cow and determine the average experimental result of alcohol susceptibility of milk as follows. There are only 2 samples of milk which were coagulated by 67% alcohol, 17 by 69%, 201, 248, 298, samples by each 74, 78 and 82% alcohol among 2,704 samples, but as the alcohol percentage became higher than 80 the number of samples coagulated decreased gradually, viz. 121, 33 and 3 by 86, 90 and 94% of alcohol respectively. The mean alcohol percentage which coagulated the most samples of milk was 79.72% as shown in the above table.

By observing the above table thoroughly, the author recognized that the alcohol susceptibility of each milk was differed with the individual cows, and in the same individuals it was almost constant through the normal lactation period. Each of the Holstein milk from cow Nos. 239, 274, 237, 243, 283, 284, 252, 270, 277, 254, 255, 230, 279, 250, 294, 260, 244, 300, 292, 301, 287, 298, 302, 297, 293 and 254 was coagulated by alcohol of the following percentages in order: 76, 78, 85, 77, 72, 79, 80, 75, 74, 74, 82, 78, 72, 89, 73, 82, 75, 78, 82, 82, 81, 79, 75, 75, 82 and 73 to the degree of +.

Of the Ayrshire cows, the milk from cow Nos. 94, 97, 114, 115, 86, 128, 142, 110, 111, 91, 127 and 144 coagulated by alcohol of the following percentages to the degree of +: 82, 86, 86, 74, 76, 85, 81, 80, 81, 74, 89 and 82.

The Guernsey milk, cow Nos. 225, 205, 222, 217, 214, 220, 223, 227, 217 and 226 each was coagulated by alcohol of the following percentages: 79, 73, 88, 74, 76, 78, 82, 81, 78 and 81.

Finally the author calculated the percentages of the samples by breeds of cows which were coagulated by alcohol of different percentages.

Table 10  $\begin{tabular}{ll} \label{table 10} Frequency percentage of milk samples coagulated by the different <math>\%$  of alcohol

Alc. % a of milk c	nd degree oagulation		Frequency pe	ercentage of	
Experi- mented	Converted	Holstein	Ayrshire	Guernsey	Total
66++ $66++$ $66++$ $70++$ $70+$	65+ $66+$ $67+$ $69+$ $70+$ $71+$ $73+$ $74+$ $75+$ $77+$ $78+$ $79+$ $81+$ $82+$ $83+$ $85+$ $86+$ $87+$ $89+$ $90+$ $91+$ $93+$ $94+$ $95+$				
Tota	al %	99.99	99.98	99.99	100.00

By observing this table it can be recognized that a greater number of the Holstein milk was coagulated by alcohol ranging in percentage from 74 to 83, and mostly by 78–82% alcohol. The most part of the Ayrshire milk was coagulated by 75–87%, especially by 81–85% alcohol. Also in the Guernsey milk just the same phenomena can be observed, i.e., there were many cases coagulated by 74–83% and the most of them by 77–82% alcohol.

Considering the whole of the results the author comes to a conclusion that the higher the percentage of alcohol the greater the number of milk samples coagulated, reaching to a maximum at 82% of alcohol, and gradually decreasing in number as the percentage of alcohol increased than this point.

The frequency curves of the coagulation of the tested milk samples by alcohol in each breed are as follows.

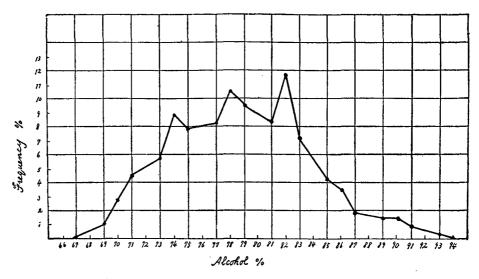


Fig. 1. Frequency curve of Holstein milk coagulated by different percents of alcohol.

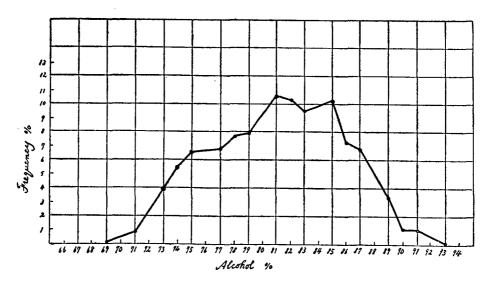


Fig. 2. Frequency curve of Ayrshire milk coagulated by different percents of alcohol.

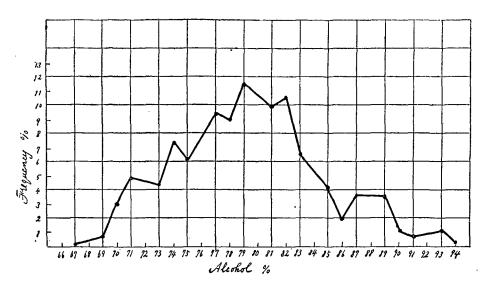


Fig. 3. Frequency curve of Guernsey milk coagulated by different percents of alcohol.

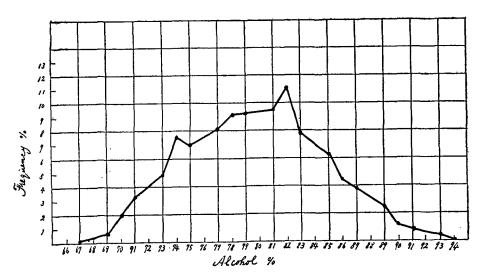


Fig. 4. Frequency curve of normal milk coagulated by different percents of alcohol.

## II. General Reaction to the Alcohol Test of Milk Throughout the Whole Lactation Period

It has already been explained by Henkel (29), Metzger (53) and Auzinger (3) that the colostrum reacts positively to the alcohol test. Rogers (65) showed that the milk at the end stage of lactation very often reacts positively to the test. But there are no reports of experiments about the general reaction to the alcohol test of milk throughout the whole lactation period in the same herd continuing at least two years using many milking cows.

The author has observed how many days the positive reaction to the alcohol test is continued in the colostrum after parturition, whether there is any case of the positive reaction even in the stage of normal milk, further whether a positive reaction to the test is usual in the end stage of lactation without exception, and from how many days before the drying of milk that positive reaction to the test appeared.

## 1. Experimental methods

Fifteen heads of Ayrshire cows belonging to the First Farm in the Hokkaido Imperial University were used in this experiment. The experimental period was 2 years and 3 months from 6/VII 1925 to 5/X 1927.

There were 27 samples of colostrum and 25 of the milk at the end of lactation period. The alcohol which was used in this experiment was diluted to 70% volumetrically and kept neutral.

The determination of the coagulation of milk by alcohol was carried out in the test tube keeping the testing temperature at  $15-20^{\circ}$ C. by adding 2.0 cc. of 70% alcohol into the same volume of milk sample milked within 1-2 hours. The degree of its susceptibility was differentiated into the following five classes, viz.,  $-\pm + ++ +++$ ; the sign "-" means no casein flakes on the wall of test tube by shaking, and other signs represent the respective amount of casein flakes coagulated by the alcohol.

## 2. Experimental results

In the following table are shown the experimental result of the general reaction to the alcohol test of milk in each individual during the whole lactation period.

Table 11

General reaction to the alcohol test of Ayrshire milk through the successive whole lactation periods

(1925)

Cow No.	77	82	85	86	91	94	97	110	112	113	114
Date											
6/VII 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31	++++++++++++++++++++++++++++++++++++++		calved ++++++++++++++++++++++++++++++++++++			111++#1111111111111111111111					
1/VIII 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16	+++++++++++++++++++++++++++++++++++++++		+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++							calved ++++++++++++++++++++++++++++++++++++

TABLE 11 (Continued)

(1925)

												(192	(D)
Cow No.	77	82	85	86	91	94	97	99	110	111	112	113	114
17/VIII 18 19 20 21 22 23 24 25 26 27 28 29 30 31	+++++++++++++++++++++++++++++++++++++++		+++++++++++++++	++++++++++++++++++++++++++++++++++++++			1 #+++++++++++	calved +++ +++ +++ +++	111111111111				± ±
1/IX 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30	+++++++++++++++++++++++++++++++++++++++		+++++++++++++++++++++++++++++++++++++++				++ ++ ++ ++ ++ ++ ++ dried	+++++++++++++++++++++++++++++++++++++++	111++++++++++++++++++++++++++++++++++++	calved ++++++++++++++++++++++++++++++++++++			

Table 11 (Continued)

(1925)

										(19	25)
Cow No.	77	82	85	91	94	99	110	111	112	113	114
1/X 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 23 24 25 26 27 28 29 30 31	+++++++++++++++++++++++++++++++++++++++		+++++++++++++++++++++++++++++++++++++++				++ ++ ++ ++ ++ ++ ++ ++ ++ dried				
1/XI 2 3 4 5 6 10 11 12 13 14 15 16 17 18 19 20	+++++++++++++++++++++++++++++++++++++++		++++++++++++++++		    dried						

TABLE 11 (Continued)

(1925-1926)

								(1925-1	1926)
Cow No.	77	82	85	91	99	111	112	113	114
21/XI 22 24 25 26 27 28 30	++ ++ ++ ++ ++ ++ ++	111111	+ + + + + + + + + + + + + + + + + + + +				- - - - - - -		- - - - - - -
1/XII 2 3 4 5 7 8 9 10 11 12 14 15 16 17 18 19 20 21 22 23 24 6/I 7 8 9 10 11 12 13 14 15 16 17 18	+++++++++++++++++++++++++++++++++++++++		+++++++++++++++++++++++++++++++++++++++						

TABLE 11 (Continued)

													(102	
Cow No.	77	82	85	86	91	94	99	110	111	112	113	114	115	116
21/I 22 23 24 25 26 27 28 30 1/II 2 3 4 5 6 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 1/III 2 3 4 5 6 7 8 9 10	++++++++++++++++++++++++++++++++++++++		+++++++++++++++++++++++++++++++++++++++	calved ++++++++++++++++++++++++++++++++++++	dried	calved ++++++++++++++++++++++++++++++++++++		calved ++++ +++++++++++++++++++++++++++++++					calved ++++++++++++++++++++++++++++++++++++	calved ++++ ++++ +++ +++ ++ +- 

Table 11 (Continued)

·				_							(192	26)
Cow No.	85	86	94	97	99	110	111	112	113	114	115	116
11/III 12 13 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1/IV 2 3 4 5 6 7 8 9 10 12 13 14 15 16 17 19 20 21 22 23 24 26 27 28 29 30	++++++++++++++++++++++++++++++++++++++			calved +++ +++ +++ +++				++++++++++++++++++++++++++++++++++++++				

Table 11 (Continued)

													(102	
Cow No.	77	82	85	86	91	94	97	99	110	111	113	114	115	116
1/V 3 4 5 6 7 8 10 11 12 13 14 15 17 18 19 20 21 22 24 25 26 27 28 29 30 31	calved ++++ +++ +++ +++ +++ +++ +++	,	+++++++++++++++++++++++++++++++++++++++		calved ++++ ++++ +++ ++-			dried			dried			
1/VI 2 3 4 5 7 8 9 10 11 12 14 15 16 17 18	+++++++++++++++++++++++++++++++++++++++	calved ++++ +++	+++++++++++++++++++++++++++++++++++++++			111111111111111						     dried		

Date

Cow No.

77

82

+++±---

85

++ ++ ++ ++ dried

Table 11 (Continued)

91

86

calved +++

±---++-±--

97 94 110 113 115 116 calved +++ +++ +++ ++ 

Table 11 (Continued)

Cow No.	77	82	85	86	91	94	97	99	110	111	113	114	115	116
16/VIII 17 18 19 20 21 23 24 25 26 27 28 30 31 1/IX 2 3 4 6 7 8 9 10 11 13 14 15 16 17 18 20 21 22 23 24 25 27 28 29 1/X 2 4 5 6 7 8	+++++++++++++++++++++++++++++++++++++++		+++++++++++++++++++++++++++++++++++++++		#	+-±-±±		calved ++++++++++++++++++++++++++++++++++++		calved ++++ +++ ++ ++ 		calved ++++ +++ +++ +++ +±		

TABLE 11 (Continued)

Table 11 (Continued)

(1926-1927)

									_		(1926-1	941)
Cow No.	77	82	85	86	91	97	99	110	111	114	115	116
2/XII 3 4 5 6 7 8 9 10 11 13 14 15 16 17 18 20 21 22 23 24 25 7 8 10 11 12 13 14 15 17 18 19 20 21 22 23 24 25 27 5/I 6 7 8 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 27 18 19 20 21 22 23 24 25 27 28 29 20 21 22 23 24 25 27 28 29 20 21 22 23 24 25 27 28 29 20 21 22 23 24 25 27 28 29 20 21 22 23 24 25 27 28 29 20 21 22 23 24 25 27 28 29 20 21 22 23 24 25 27 28 29 20 21 22 23 24 25 26 27 28 29 20 20 21 22 23 24 25 26 27 28 29 20 20 21 22 23 24 25 26 27 28 29 20 20 21 22 28 28 29 20 20 20 20 20 20 20 20 20 20	+++++++++++++++++++++++++++++++++++++++		++++++++++++++++++++++++++++++++++++++		1 # # #			++++++++++++++++++++++++++++++++++++++				+ + + + + + + dried

Table 11 (Continued)

(1927)

										.541)
Cow No.	77	82	85	86	91	94	97	99	111	114
1/II 2 3 4 5 9 10 12 14 15 16 17 18 19 21 22 23 24 25 26 28 1/III 2 3 4 5 7 8 9 10 11 12 13 14 15 16 17 18 19 19 10 11 11 11 12 13 14 15 16 17 18 19 10 11 11 11 12 13 14 15 16 17 18 19 10 11 11 11 12 13 14 15 16 17 18 19 10 11 11 12 13 14 15 16 17 18 18 19 10 11 11 12 13 14 15 16 17 18 18 19 10 10 10 10 10 10 10 10 10 10	++++++++++++++++++++++++++++++++++++++		++++++++++++++++++++++++++++++++++++++			calved ++++++++++++++++++++++++++++++++++++	++++++			

Table 11 (Continued)

(1927)

											(1	1927)
Cow No.	77	82	85	86	91	94	97	99	110	111	114	115
1/IV 2 4 5 6 7 8 9 11 12 13 14 15 16 18 19 20 21 22 23 25 26 27 28 30	calved +++ +++ +++ +++	+ + + + + + + + + + + + + + + + + + +	dried		dried		++ ++ ++ ++ ++ ++ dried		calved ++++++++++++++++++++++++++++++++++++			calved +++ ++
1/V 2 3 4 5 6 7 9 10 11 12 13 14 16 17 18 19 20 21 23	+++++++++++++++++++++++++++++++++++++++											+++++++++++ ++

Table 11 (Continued)

(1927)

				•				(1	.927)
Cow No.	77	85	86	94	97	99	110	111	115
24/V 25 26 27 28 30 31 1/VI 2 3 4 6 7 8 9 10 11 13 14 15 16 17 18 20 21 22 23 24 25 27 28 29 30 1/VII 2 4 5 6 7 8 9 11 12 13 14 15	+++++++         ++++++++++++++++++++++++++++++++++++	calved ++++++++++++++++++++++++++++++++++++			calved ++++ +++++++++++++++++++++++++++++++			dried	

Table 11 (Continued)

(1927)

					·					(132	
Cow No.	77	82	85	86	91	94	97	99	110	111	115
10/5715			,								_
16/VII	++		++	_		-	-	_			
18 19	++		<del> </del> +	-		_ 					_
19 20	++		++	_				_			_
20 21	++		++						_		_
21 22	++		++	_	calved	_	_		-		
25	1		++	土	+++		_		_		
26 26	++		+		+++	_	_	_	_		_
20 27	++		++	±	++	_	_	_	_		_
28	++	1	+	_	++	_	_		l		
29	++		+	_	++	_	_		l _		
30	1		++	_	++	_	_				_
	++	}	' '		+	1	1	}	t		
1/VIII	++		+		}	—		-	<u> </u>		_
2	++		++	_	+	_	-		-		_
3	+-		+	-	+			_	_		_
4	++		+		±	_	-	_	-		
5	++		+	_	-	-	-	_	-		_
6	++		++		_	-	-	_	-		_
8	++		+	-	_		-		-		_
9	++		++		_	-	-	_	-		_
10	++		+	-	_	-	-	_	-		
11	++		++		_	—	-	_	-		_
12	++	١	++	—	_ _ _ _	_	-	_			_
13	++	cavled	++	—	1	-	-	_	-		_
15	++	+++	++	\ —	_			_	] —		_
16	++	+++	++	-		-	_	_		calved	_
17	++	++	+	—	_		-	_	<u> </u>	+++	
18	++	++	++	—	_			-	_	+++	_
19	++	++	+	-		_	_		- ,	++	
20	++	+	++	—	_		_	-	-	++	
21	++	+	++		_	—	-	_	-	+	_
22	++	+	++	—	_	—	-	-	-	+	_
23	++	+	+	±	_	土	-	-	_	+	_
24	++	±	+	-		_	-	_	_	土	
25	++		++	-	_			-	-		_
		[	l	L		į.	: !		l		

Table 11 (Continued)

(1927)

			,			_				(13	<del></del>
Cow No.	77	82	85	86	91	94	97	99	110	111	115
27	++	_	++			_	l _	_		_	_
28	++		+	±		_		_	·_	_	
29		_	+			_	l _		_		
30	++	_	++	_	_	-		_	_		
31	++		+	-	_	-	_	_	_	_	_
1/IX	++		+	_	_	_	_	_			_
2	++		++		_	-	-	-		_	
3	++	_	+	-		-	-	-	_		-
5	++		+		_	-	<u> </u>	—		_	_
6	++		+	-		-	-	_		_	_
7	++		++	-		-	<del>-</del>	-		_	-
8	++		+		_		—			-	-
9	++		+	_		-		-	_	_	-
10	++	_	+	-	_	-		-	_	_	-
12	++	-	++	-	_		_	_ I	_	_	
13	++		++	-	_			-	_	_	—
14	++	_	++	-	_			_	_	_	—
15	++		+	±		-	-	-	_	_	_
16	++		+	±						_	<b>–</b>
17	++	_	+	-	-	-		-		_	_
20	++	_	+	-	-	-	<b> </b>				-
21	++		++	±		-		-		_	- ·
22	++	_	+	±	_	-	—	-	_	_	±
23	++	-	+	±	_	-	—	dried		. —	-
24	++		+	±	_	-	_			-	l –
26	++		++	±			<u> </u>				<u> </u>
27	++		+	±	_					_	-
28	++	_	++	+		-	_			-	±
29	++		++	+	_	-	-		_	_	l –
30	++	_	++	+		-	_		_		-
1/X	++	_	++	+	_	_	_		_	<u>·</u>	_
3	++		+	+	_		—		_	_	±
4	++	_	+	+	-	-			_		_
5	++	<u> </u>	++	+		-	_			_	±

## 3. DISCUSSION

For easy observation these experimental results of each individual are arranged in the following table.

 ${\bf TABLE~12}$  General experimental results of the alcohol test of milk in each individual

Çow	Ped	igree	Date	Date of	Number of	Date of drying	Date of diminishing posit.	Number of days con-	Date of appearance of	Number of days con-	Remarks
No.	Sire	Dam	birth	rition	gestation	of milk	alc. reaction	tinuance of it	posit. alc. reaction		
				5/IV 1925	10th	15/II 1926	Unknown	Unknown	+	+	Always positive re-
77	Lord George Eth	Sakura 2nd	25/XII 1910	21/V 1926	11th	1/III 1927	+	+	+	+	action to the alcohol test through the whole
	·			25/IV 1927	12th	Milking at end of expt.	+	+	Unknown	Unknown	lactation period
	_			6/V 1925	7th	15/II 1926	Unknown	Unknown	2/II 1926	14	
82	Lord George 8th	Crown Rose	11/IX 1914	17/VI 1926	8th	26/IV 1927	26/VI 1926	9	16/III 1927	42	
				15/VIII 1927	9th	Milking at end of expt.	25/VIII 1927	10	Unknown	Unknown	
	_			15/VII 1925	6th	25/VI 1926	+	+	+	+	Dam of this cow is
85	Lord G∈orge 8th	Sakura 4th	27/XII 1915	31/VII 1926	7th	31/III 1927	+	+	+	+	Cow No. 77. Always showed positive re-
				3/VII 1927	8th	Milking at end of expt.	Ŧ	+	Unknown	Unknown	action to the test

TABLE 12 (Continued)

Cow No.	Ped Sire	digree Dam	Date of birth	Date of parturition	Number of gestation	Date of drying of Milk	Date of diminishing posit, alc.	tinuance	Date of appearance of posit. alc.	Number of days con- tinuance	Rem arks
	Site	Dam	_				reaction	of it	reaction	of it	
	Snow-		30/VIII	29/VI 1924	5th	30/VIII 1925	Unknown	Unknown	28/VII 1925	34	Non pregnancy after 6th gestation, and sometimes positive
86	ball	Hariett	1916	1/II 1926	(th	Milking at end of expt.	11/II 1926	10	Unknown	Unknown	reaction to the test during 12-20 months after of it
				14/I 1925	4th	23/I 1926	Unknown	Unknown	_		Sometimes weak positive reaction to
91	Intoto	Yustan- noble	5/III 1918	21/V 1926	5th	1/IV 1927	31/V 1926	10	_	_	the test during 25/X-21/XI 1925 Sometimes weak
			1010	25/VII 1927	6th	Milking at end of expt	5/VIII 1927	11	Unknown	Unknown	positive reaction to the test during 12/VIII-4/IX 1926
				1/II 1925	4th	11/XI 1925	Unknown	Unknown		_	Sometimes weak positive reaction to the test during 22/VII-26/VIII 1926
94	Intoto	Shi <b>ra</b> giku	23/1V 1918	10/II 1926	5th	25/XI 1926	28/II 1926	18	_		Sometimes weak positive reaction to the test during 7/X-30/X 1926
				12/II 1227		Milking at end of expt.	27/II 1927	15	Unknown	Unknown	Sometimes weak positive reaction to the test during 31/V-19/VII 1927

Table 12 (Continued)

Cow	Pe	ligree	Date of	Date of partu-	Number of	Date of drying	Date of diminishing posit.	Number of days con-	Date of appearance of	Number of days con-	Remarks
No.	Sire	Dam	birth	rition	gestation	of Milk	alc. reaction	tinuance of it	posit. alc. reaction	tinuance of it	Itemarks
				11/IX 1924	4th	10/IX 1925	Unknown	Unknown	18/VIII 1925	24	
97	Intoto	Koyanagi	20/XI 1918	20/IV 1926	5th	8/IV 1927	27/IV 1926	7	5/III 1927	35	
				7/VII 1927	6 <b>t</b> h	Milking at end of expt.	15/VII 1927	8	Unknown	Unknown	
99	Intoto	Hariett	28/II	27/VIII 1925	4th	30/IV 1926	21/IX 1925	25		_	
33	111000	Harlett	1919	19/VIII 1926	5th	22/IX 1927	16/IX 1926	28			
				21/VI 1925	1st	12/X 1925	Unknown	Unknown	4/IX 1925	38	
110	Intoto	Yustan- noble	28/VI 1921	9/II 1926	2nd	17/XII 1926	21/II 1926	12	10/XI 1926	<b>3</b> 8	,
				8/IV 1927	3rd	Milking at end of expt.	22/IV 1927	14	Unknown	Unknown	
				2/JX 1925	2nd	16/VI 1926	11/IX 1925	9	7/VI <b>1</b> 926	10	
111	Intoto	Aya	25/VIII 1921	6/IX 1926	3rd	24/V 1927	14/IX 1926	8		_	
				16/VII 1927	4th	Milking at end of expt.	24/VIII 1927	8	Unknown	Unknown	

TABLE 12 (Continued)

Cow No.	Pec Sire	digree Dam	Date of birth	Date of partu- rition	Number of gestation	Date of drying of milk	Date of diminishing posit. alc. reaction	Number of days con- tinuance of it	Date of appear- ance of posit. alc. reaction	Number of days con- tinuance of it	Remarks
112	Intoto	Joias	5/I 1922	4/III 1925	1st	23/III 1926	Unknown	Unknown	17/II 1926	35	
113	Ofu	Northern Noble	7/II 1922	15/VI 1925 26/VI 1926	1st 2nd	3/V 1926 Unknown	Unknown 11/VII 1926	Unknown 15	- Unknown	– Unknown	Slaughtered on 18/X 1926
114	Ofu	Insite 4th	29/III 1922	7/VIII 1925 27/IX 1926	1st 2nd	15/VI 1926 20/V 1927	15/VIII 1925 7/X 1926	12 10	_		
115	Ofu	Hariett	28/IX 1921	1/II 1926 28/IV 1927	1st 2nd	26/I 1927 Milking at end of expt.	11/II 1926 12/V 1927	10 14	9/XII 1926 Unknown	41 Unknown	Sometimes weak positive reaction to the test during 22/IX-5/X 1927
116	Ofu	Sakura 4th	1/V 1923	26/II 1926	lst	13/XII 1926	7/III 1927	9	29/XI 1926	15	

Note: Sign "-" shows negative reaction to the alcohol test, and sign "+" shows always positive, therefore there are no records. And sign of "unknown" shows out of the experiment.

Fifteen heads of Ayrshire cows were used in this experiment for a period of 2 years and 3 months from 6/VII 1925 to 5/X 1927 continuing over 2 lactation periods on an average.

Included are 27 samples of colostrum and 25 of the milk at the end of lactation period.

Observations on the experimental results in each individual may be reported as follows.

Cow No. 86 calved on 1/II 1926 and the milk reacted strongly positive to the alcohol test soon after parturition but the degree of the coagulation weakened gradually and quite disappeared after 11/II 1926, therefore the days of the continuance were 10. Other cows manifested just the same phenomena as cow No. 86 in respect to the alcohol test for the colostrum.

Cow No. 97 calved on 20/IV 1926 and for following 7 days the colostrum reacted positively to the test. This cow again calved on 7/VII 1927 and the colostrum showed positive reaction to the test for 8 days after parturition.

Cow No. 94 bore her 5th calf on 10/II 1926 and the milk reacted positively to the test for 18 days, and when she calved again on 12/II 1927 the milk showed positive reaction for 15 days after parturition.

The colostrum secreted from cow No. 91 showed positive reaction to the alcohol test for 10 days after her 5th calving and for 11 days after her 6th.

The milk secreted from cow No. 110 reacted positively to the test for 12 days after her 2nd parturition, and for 14 days following the 3rd.

The colostrum of cow No. 82 showed positive reaction for 9 days from the birth of her 8th calf, and 10 days from her 9th. In case of cow No. 113 the milk showed a positive reaction for 15 days after her 2nd parturition. With cow No. 111 a positive reaction was observed for 9 days after the 2nd and 8 days after the 3rd and 4th.

With cow No. 99 the colostrum reacted positively for 25 days after parturition, which is much longer than other colostrums but she again showed nearly the same phenomenon giving the positive reaction for 28 days after her next parturition. At the 1st and 2nd calvings of cow No. 114 her colostrums showed positive reaction for 12 and 10 days respectively. With cow No. 115, the colostrum showed a positive reaction for 10 days after her first calving and 14 days after her 2nd; with cow No. 116 the same observation were made for 9 days after her first calving.

It is an interesting fact that the milk secreted from both cow Nos. 77 and 85 always showed positive reaction to the alcohol test throughout their whole lactation periods. Physiologically they are very healthy. They secreted a large amount of milk in comparison with other milking cows. The author found that they were related very closely, for their sire was just the same and the dam of cow No. 85 is cow No. 77 herself. From these facts it can easily be imagined that such a quality in milk may be inherited as to cause the appearance of the positive reaction to the alcohol test throughout the whole lactation period.

From the table it may be seen that the milk secreted by cow No. 77 after the 11th gestation always showed positive reaction, and also that the milk of cow No. 85 always reacted positively during her 6th, 7th, and 8th lactation periods.

There are 22 samples of colostrum which showed positive reactions to the test for from 7 to 28 days after parturition, with an average of 12.4 days excepting 5 samples which always showed positive.

Perhaps it may be stated conclusively that the colostrum reacts positively for an average of 12 days after the parturition. Generally the normal milk does not show the positive reaction, but there are some cows whose normal milk may react positively. Just such a pertinent example is found here in the milk from cows 77 and 85 whose milk always reacted positively to the test during the whole lactation period as described above. Furthermore when cow No. 94 calved on 10/II 1926 the milk reacted positively to the test for 18 days after her parturition. After that time she secreted normal milk which sometimes during the period 22/VII-26/VIII or 7/X-30/X 1926 reacted positively to the test though the degree was very weak.

This cow's milk has shown, however, negative reaction to the test when she dried up in the last stage of lactation. When this cow No. 94 calved next on 12/II 1927 she secreted the milk which reacted positively to the test occasionally during 31/V-19/VII; of course the degree of coagulation was not so strong. From this the author can state that such a cow habitually secrete the same quality of milk in spite of the change of her lactation.

The alcohol susceptibility of milk varies with different individual cows, namely some milk reacts to strong alcohol, but other reacts to weak alcohol. And further there may be observed that the alcohol

reaction of milk is almost constant during their normal lactation period in the same individuals. Examples of animals whose milk exhibits such phenomena during their normal lactation period are cow Nos. 91 and 115.

Next consideration will be the problem of the relation between alcohol and the milk at the end of lactation period. There are 25 samples of milk at the end of lactation period, 4 of them being of two cows No. 77 and 85 which doubled the lactation always showed positive reaction to the test during their whole lactation period, but 10 of them always reacted negatively till the end of lactation. Thus the milk, which shows negative reaction for the test in the end of lactation period amounts to 48% of all milk excepting 4 samples of cows No. 77 and 85.

These ten cases are the milk of cow Nos. 94 and 91 each at their 4th and 5th lactation periods, milk from No. 113 at her 1st lactation, from cow No. 99 at her 4th and 5th lactations, from cow No. 114 at the 1st and 2nd lactations, and from cow No. 111 at her 3rd lactation.

From this it may be said that the milk at the end of lactation period does not necessarily show the positive reaction to the alcohol test, and it can be seen that these phenomena vary with different individuals and are specific to individuals, in spite of change in lactation periods.

The number of samples which showed positive reactions are eleven out of twenty one, being 52% of all.

The milk secreted by cow No. 86 after her 5th calving reacted positively to the test during 34 days preceding to her drying of milk, and cow No. 97 after her 4th and 5th calvings produced milk showing positive reaction during 24 days and 35 days respectively preceding to the drying of milk.

Cow No. 110, after her 1st and 2nd calving produced milk which reacted positively during 38 days preceding the drying.

Cow No. 112 after her 1st gestation during 35 days, and cow No. 82 after her 7th and 8th gestations during 14 and 42 days respectively, cow No. 111 for 10 days after her 2nd calving, and cows No. 115 and 116 for 41 and 15 days respectively after their first calving produced milk exhibiting the positive reaction.

The period of continuance of the positive reaction to the alcohol test at the end of lactation period was 10 days in shortest and 42 days in longest, with an average of 29.6 days in these 11 samples.

Hence it may be concluded that the alcohol susceptibility of milk differs with the individuals and it is almost constant for the same individuals not only in the case of colostrum but also of the normal milk and of the milk at the end of lactation period.

# III. Influence of Neutral Salts upon the Alcohol Susceptibility of Milk

It has been reported by Auzinger (3) Sommer and Binney (81) and also by Ayer and Johnson (4) that the alcohol susceptibility of milk mainly depends upon its salt balance, especially that by the addition of calcium and magnesium salts, the alcohol susceptibility is increased but on the contrary phosphates and citrates have the opposite effect. Moreover Sommer and Binney incidentally remarked that potassium and sodium salts did not influence the alcohol susceptibility of milk.

It was the purpose of the following experiments to find the influence of the addition of various neutral salts in milk upon the alcohol coagulation.

#### 1. EXPERIMENTAL METHODS

The alcohol which was used in this experiment is anhydrous chemically pure alcohol; it was diluted to 90% volumetrically and then again diluted to the following concentrations by adding distilled water keeping neutral.

TABLE 13
Alcohol Dilution

Alc. No.	I	II	III	IV	v	VI	VII	VIII	IX	X	XI	XII	XIII
90% alc.	100	90	81	73	66	60	54	49	44	40	36	32	29
H <sub>2</sub> O	0	10	19	27	34	40	46	51	56	60	64	<b>6</b> 8	71

The neutral salts used in this experiment were chemically pure, made in Germany, marked as "Merk".

The milk samples for testing were milked from Ayrshire cows at the First Farm of the Hokkaido Imperial University and were used in the experiment 2-3 hours after milking, the temperature being kept within 15-20°C in cold running water.

#### 2. EXPERIMENTAL RESULTS

## A. Relation between the Alcohol Susceptibility and pH Value of Milk

SELIGMANN (77) and TILLMANS and OBERMEIER (90) reported that there is no relation between the alcohol susceptibility and the hydrogen ion concentration of milk, yet RAUDNITZ (62) obtained the opposite result.

The author presents here the results of his experiment in detail on the matter in consideration. The determination of pH value was made by the chain method of hydrogen gas.

TABLE 14

Relation between the alcohol susceptibility and pH-value of milk

Date		1/X	1925		2/	X		5/	X
Milk Sample No.	рH	Alc.	Alcohol Number to coagulate the milk	pН	Alc.	Alcohol Number to coagulate the milk	pН	Alc.	Alcohol Number to coagulate the milk
1	6.66	_	III	6.59	_	11	6.59		II
2	6.75	++	VI	6.76	++	VI	6.94	++	VI
3	6.77	+	III	6.69	_	III	6.78	_	III
4	6.73	+++	VII	6.65	++	VII	6.68	+++	VIII
5	6.78	+++	II	6.63	_	III	6.61	_	IV
6	6.70	+	v	6.65	+	v	6.73	±	VI
7	6.61	_	II	6.60	_	III	6.66		III
8	6.68	_	11	6.60	_	III	6.70	_	III

Date	<u> </u>	7/	X		8/	X		9/	X
Milk Sample No.	рН	Alc.	Alcohol Number to coagulate the milk	рН	Alc.	Alcohol Number to coagulate the milk	pH	Alc.	Alcohol Number to coagulate the milk
1	6.68		III	6.78 —		II	6.75	_	III
2	7.00	++	VI	6.99	++	VI	6.81	++	v
3	6.81	±	1V	6.81	土	III	6.74	±	III
4	6.74	+++	VIII	7.00	+++	VIII	6.81	+++	VII
5	6.65	_	1V	6.67	士	IV	6.67	土	IV
6	6.68	+	VI	6.65	+	VI	6.72	+	VI
7	6.62	_	III	6.63	_	II	6.63	_	111
8	6.68	_	III	6.72		III	6.63	_	III

TABLE 14 (Continued)

There are some differences in the pH values of milk with the different cows, but the author can recognize that the value is almost constant in the same individual. It may be said that there is no relation between pH value and the alcohol susceptibility of milk from the observation of the data for the milk of No. 4 which showed a strong positive reaction to the alcohol test in spite of low pH value compared with the milk of No. 7 which showed a negative reaction in spite of a rather high pH value. Other conflicting result may be seen in samples of No. 6 having a high pH value and reacting positively to the test, and of No. 2 which reacted positively in spite of a low pH value.

# B. Influences of Dialysis upon the Alcohol Susceptibility of Milk

The author experimented on the influence of dialysis upon the coagulation of milk, knowing that the milk sugar and soluble salts in milk will pass through a collodion membrane.

One hundred and fifty cc of milk were put into a sack of collodion membrane and dialyzed in a beaker containing 1,000 cc of distilled water keeping the volume of milk constant. The changing of the alcohol susceptibility of the dialyzed milk was tested at successive hourly intervals.

The experimental results are shown in the following table.

Milk		Alc.			ed to coa s of each		Heat test hours in ro	
sample No.	pН	test	0	1	2	3	whole milk	dialyzed milk
1	6.75	_	III	II	I	· I	++++	_
2	6.81	++	v	VI	III	III	++++	_
3	6.74	±	III	II	II	I	++++	_
4	6.81	+++	VII	v	IV	IV	++++	_
5	6.69	±	IV	III	III	II	++++	_
6	6.72	+	VI	v	III	II	++++	_
7	6.63	_	III	II	II	II	++++	_
8	6.63	_	III	II	II	I	++++	

By observing the above table it will be recognized that the alcohol susceptibility of dialyzed milk decreased and its degree became more marked each passing hour. For instance, milk sample No. 1 was coagulated by alcohol No. III before dialysis but its degree of coagulation decreased, and it was coagulated by the stronger alcohol No. II after dialysis for 1 hour, and after two hours dialysis the degree was more decreased and it was coagulated only by the strong No. I alcohol.

The degree of alcohol susceptibility of dialysed milk is governed by the degree of its susceptibility of whole milk. There is a pertinent example such as milk sample No. 4, coagulated by the weak alcohol No. VII before dialysis, but after 1 hour dialysis the degree of susceptibility became more strong, being coagulated by alcohol No. V. It is somewhat different comparing with milk No. 1 in being a stronger alcohol susceptibility in original whole milk.

The author can explain these facts as due to the marked change in the salt constituents of milk by dialysis, the soluble salts passing through the membrane. It may probably due also to the lactic fermentation taken place during 24 hours of standing at the room temperature that the whole milk was coagulated strongly by the heat test in spite of the negative reaction to the test in the case of dialyzed milk.

## C. Influence of Various Valencies of Salts upon the Alcohol Susceptibility of Whole and Dialyzed Milk

Experiments were performed for the purpose of observing what change would appear in the alcohol coagulation of whole and dialyzed milk by addition of various valencies of salts independently.

The salts having cation of one, two, and three valencies were used. The salts solutions were made in the following ten concentrations viz.: N/1, 7N/10, 5N/10, 3.2N/10, 2N/10, N/10, 3.2N/100, N/100, 3.2N/1,000, N/1,000. 10 cc each of these solution were added to 40 cc of whole or dialyzed milk, then 0.7 cc each of the samples was taken into a test tubes adding to it 0.3 cc of various concentrations of alcohol and the coagulating degree of the mixture was determined.

Dialyzed milk was obtained from the sack of collodion membrane containing 150 cc of whole milk left for 2 hours in 1000 cc of distilled water keeping the contents as constant as possible in the room temperature. The experimental results are as follows.

#### a. Monovalent cation of neutral salts (NaCl, KCl, and NH<sub>4</sub>Cl)

i. Milk showing negative reaction to the alcohol test

Table 16

Influence of monovalent cation of neutral salt upon the alcohol susceptibility of milk

Salt	Concen- tra- tion Milk	N/1	7N/10	6N/10	3.2N/10	2N/10	N/10	3.2N/100	N/100	3.2N/1000	N/1000	Milk No.
NaCl	Whole Dialyzed	ĬII III	III	III	III II	II	II I	<u> </u>	· I	<u>I</u>	<u>I</u>	8 8
KCI	Whole Dialyzed	IV III	IV III	IV III	III	II	II I	II I	I —	I -	I	8
NH <sub>4</sub> Cl	Whole Dialyzed	IV III	IV	IV III	III	III	II II	I		I -	I -	8 8

Notice: The numbers III, IV, II or I in the above and succeeding tables in this chapter represent the strength of the alcohol necessary to coagulate the mixture in the degree of + (cf. Table 13) and the sign "+" shows the coagulation of the mixture without adding alcohol while sign "-" shows non-coagulation of the mixture in spite of the addition of alcohol.

ii. Milk showing positive reaction to the alcohol test

Table 17

Influence of monovalent cation of neutral salt upon the alcohol susceptibility of milk

Salt	Concen- tra- tion Milk	N/1	7N/10	5N/10	8.2N/10	2N/10	N/10	3.2N/100	N/100	3.2N/1000	N/1000	Milk No.
NaCl	Whole Dialyzed	IV IV	IV IV	IV IV	IV III	IV III	IV II	III	III	III	III	4
KCI	Whole Dialyzed	v IV	V	V	V IV	V	V	V	V	IV III	IV III	4 4
NH <sub>4</sub> Cl	Whole Dialyzed	V IV	V IV	V IV	V IV	V IV	V III	V III	IV III	IV III	IV III	4 4

- b. Divalent cation of neutral salts (CaCl<sub>2</sub>, MgCl<sub>2</sub>, and BaCl<sub>2</sub>)
  - i. Milk showing negative reaction to the alcohol test

Table 18

Influence of divalent cation of neutral salt upon the alcohol susceptibility of milk

Salt	Concen- tra- tion Milk	N/1	7N/10	6N/10	3.2N/10	2N/10	N/10	3.2N/100	N/100	3.2N/1000	N/1000	Milk No.
$\operatorname{CaCl}_2$	Whole Dialyzed	X IX	XI X	XI X	XI X	X IX	IX VIII	V V	II ———	I I	_ 	8
MgCl <sub>2</sub>	Whole Dialyzed	XI XI	XII	XII	XII	XI XI	XI IX	V II	II I	<u>I</u>	_	8 8
BaCl <sub>2</sub>	Whole Dialyzed	IX IX	X X	X X	X X	IX IX	1V IIV	IV II	II I	<u>I</u>	_	8 8

ii. Milk showing positive reaction to the alcohol test

Table 19
Influence of divalent cation of neutral salt upon the alcohol susceptibility of milk

Salt	Concen- tra- tion Milk	N/1	7N/10	5N/10	3.2N/10	2N/10	N/10	3.2N/100	N/100	3.2N/1000	N/1000	Milk No.
CaCl <sub>2</sub>	Whole Dialyzed	IX IX	X IX	X X	X	1X 1X	IX VIII	VI V	III	III	III II	4 4
$\mathrm{MgCl}_2$	Whole Dialyzed	IX IX	X X	X X	X X	IX IX	IX	VI VI	V III	IV II	IV II	44
BaCl <sub>2</sub>	Whole Dialyzed	IX IX	IX IX	X	X X	IX IX	VIII	VI VI	V	IV II	IV II	4 4

- c. Trivalent cation of neutral salt (FeCl<sub>3</sub>)
- i. Milk showing negative reaction to the alcohol test

Table 20
Influence of trivalent cation of neutral salt upon the alcohol susceptibility of milk

Salt	Concen- tra- tion Milk	N/1	7N/10	5N/10	3.2N/10	2N/10	N/10	3.2N/1.00	N/100	3.2N/1000	N/1000	Milk No.
$\mathrm{FeCl}_3$	Whole Dialyzed	+++	++	+++	XIII +	VIII +	VI XI	III IV	I	_	_	8 8

ii. Milk showing positive reaction to the alcohol test

Table 21
Influence of trivalent cation of neutral salt upon the alcohol susceptibility of milk

Salt	Concentra- tion Milk	N/1	7N/10	5N/10	3.2N/10	2N/10	N/10	3.2N/100	N/100	3.2N/1000	N/1000	Milk No.
FeCl <sub>3</sub>	Whole Dialyzed	++	++	++	XIII	1X +	VIII X	VI	IV II	IV II	IV II	4 4

It will be observed that monovalent cation of neutral salt has no special influence upon the alcohol susceptibility of milk. But this property was accelerated by the addition of divalent cation of salt and the degree of coagulation varies with the concentration of the salt solution, above all the milk samples were most sensitive at the concentrations of 3.2N/10-7N/10 of these salts.

Both whole milk and dialyzed milk were coagulated by addition of trivalent cation of salt only having concentrations of over 5N/10-2N/10.

Therefore it may be concluded that the alcohol susceptibility of milk is rendered sensitive by the addition of neutral salts having cations of the higher valencies.

# D. Influence of Monovalent Cation of Neutral Salt upon the Alcohol Susceptibility of Milk Containing a Definite amount of Divalent Cation of Neutral Salt

a. Cases of NaCl, KCl, or NH<sub>4</sub>Cl added independently to milk containing a definite amount of CaCl<sub>2</sub>

Milk  $(40 \text{ cc}) + \text{N/10 CaCl}_2$  (10 cc) = A solution

i. NaCl

Table 22

Influence of NaCl upon the alcohol susceptibility of milk containing a definite amount of CaCl<sub>2</sub>

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	A sol.	рН	Alc. No. to coag. mixture
	2.0 cc					8.0 c <b>c</b>	6.33	v
		2.0 cc				8.0 cc	6.34	VII
NaCl			2.0 cc			8.0 cc	6.37	VII
				2.0 cc		8.0 cc	6.37	VII
					2.0 cc	8.0 cc	6.51	VII

ii. KCl Table 23

Influence of KCl upon the alcohol susceptibility of milk containing a definite amount of CaCl<sub>2</sub>

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	A sol.	pН	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	6.35	VI
		2.0 cc				8.0 ec	6.36	VII
KCl ·			2.0 cc			8.0 cc	6.40	VII
				2.0 cc		8.0 cc	<b>6.4</b> 0	VII
					2.0 cc	8.0 ec	6.51	VII

iii. NH<sub>4</sub>Cl

TABLE 24

Influence of NH<sub>4</sub>Cl upon the alcohol susceptibility of milk containing a definite amount of CaCl<sub>2</sub>

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	A sol.	pН	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	6.32	v
		2.0 cc				8.0 cc	6.34	VI
NH₄Cl	:		2.0 cc			8.0 cc	6.36	VII
				2.0 cc		8.0 cc	6.36	VII
					2.0 cc	8.0 cc	6.51	VII

b. Cases of NaCl, KCl or NH<sub>4</sub>Cl added independently to milk containing a definite amount of BaCl<sub>2</sub>

Milk  $(40 \text{ cc}) + \text{N/10 BaCl}_2$  (10 cc) = B solution

#### i. NaCl

Table 25  $\label{eq:table 25} \mbox{Influence of NaCl upon the alcohol susceptibility of milk containing a definite amount of BaCl_2 }$ 

Concentration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	B sol.	pН	Alc. No. to coag. mixture
NaCl	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	6.30 6.32 6.36 6.36 6.31	V VI VII VII VII				

ii. KCl

 $\begin{array}{c} \text{Table 26} \\ \text{Influence of KCl upon the alcohol susceptibility of milk} \\ \text{containing a definite amount of } BaCl_2 \end{array}$ 

Concentration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	B sol.	pН	Alc. No. to coag. mixture
KCl	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	6.26 6.28 6.34 6.34 6.31	V VI VII VIII VII				

#### iii. NH<sub>4</sub>Cl

. Table 27 Influence of  $NH_4Cl$  upon the alcohol susceptibility of milk

containing a definite amount of  $BaCl_2$ oncentration N/1 N/10 N/100 N/1000  $H_2O$  B sol. pH to co

Concentration Salt	N/1	<b>N</b> /10	N/100	N/1000	${ m H_2O}$	B sol.	pН	Alc. No. to coag. mixture
NH <sub>4</sub> Cl	2.0 cc	2.0 cc	2.0 cc	2.0 çc	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	6.27 6.30 6.34 6.34 6.31	V VI VII VII VII

c. Cases of NaCl, KCl or NH<sub>4</sub>Cl added independently to milk containing a definite amount of MgCl<sub>2</sub>

Milk  $(40 \text{ cc}) + \text{N/10 MgCl}_2$  (10 cc) = C solution

i. NaCl

TABLE 28

Influence of NaCl upon the alcohol susceptibility of milk containing a definite amount of MgCl<sub>2</sub>

Concen- tration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	C sol.	pН	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	6.16	v
		2.0 cc				8.0 cc	6.19	VI
NaCl			2.0 cc			8.0 cc	6.21	VII
				2.0 cc		8.0 cc	6.21	VII
					2.0 cc	8.0 cc	<b>6.</b> 18	VII

ii. KCl

Table 29

Influence of KCl upon the alcohol susceptibility of milk containing a definite amount of  $MgCl_2$ 

Concentration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	C sol.	pН	Alc. No. to coag. mixture
1	2.0 cc					8.0 cc	6.16	IV
		2.0 сс				8.0 cc	6.18	VI
KCl			2.0 cc			8.0 cc	6.25	VII
				2.0 cc		8.0 cc	6.25	VII
		1			2.0 cc	8.0 cc	6.18	VII

iii. NH<sub>4</sub>Cl

TABLE 30

Influence of NH<sub>4</sub>Cl upon the alcohol susceptibility of milk containing a definite amount of MgCl<sub>2</sub>

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	C sol.	pH .	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	6.18	IV
		2.0 cc				8.0 cc	6.20	VI
NH <sub>4</sub> Cl			2.0 cc			8.0 cc	6.23	VII
				2.0 cc		8.0 cc	6.23	VII
					2.0 <b>c</b> e	8.0 cc	6.18	VII

By observing the above tables it will be recognized in each case that the alcohol susceptibility of milk containing a definite amount of divalent cation of neutral salt is decreased by the addition of more concentrated monovalent cation of salt solution though the degree is very slight, and that the pH value of the mixture increased a little by the addition of monovalent cation of salt.

This is very interesting, because it is just the opposite in comparison to the case of the experimental results showing that the addition of more concentrated mono-, di- or trivalent cation of neutral salt independently to milk renders alcohol susceptibility of milk more sensitive.

### E. Influence of Divalent Cation of Neutral Salt upon the Alcohol Susceptibility of Milk Containing a Definite amount of Divalent Cation of Neutral Salt

a. Cases of MgCl<sub>2</sub> or BaCl<sub>2</sub> added independently to milk containing a definite amount of CaCl<sub>2</sub>

Milk 
$$(40 \text{ cc}) + \text{N/10 CaCl}_2$$
  $(10 \text{ cc}) = \text{A solution}$ 

#### i. MgCl<sub>2</sub>

TABLE 31

Influence of MgCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of CaCl<sub>2</sub>

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	A sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	ΧI
		2.0 сс				8.0 cc	ïx
$MgCl_2$			2.0 cc			8.0 cc	VII
				2.0 cc		8.0 cc	VI
					2.0 cc	8.0 cc	VI

#### ii. BaCl<sub>2</sub>

TABLE 32

Influence of BaCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of CaCl<sub>2</sub>

Concentration Salt	N/1	N/10	N/100	N/1 <b>0</b> 00	H <sub>2</sub> O	A sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	X
		2.0 cc				8.0 cc	IX
BaCl <sub>2</sub>			2.0 cc			8.0 cc	VII
				2.0 cc		8.0 cc	VI
					2.0 cc	8.0 cc	VI

b. Cases of  $CaCl_2$  or  $BaCl_2$  added independently to milk containing a definite amount of  $MgCl_2$ 

Milk 
$$(40 \text{ cc}) + \text{N/10 MgCl}_2$$
  $(10 \text{ cc}) = \text{B solution}$ 

## $i. \quad CaCl_2 \\$

TABLE 33

Influence of CaCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of MgCl<sub>2</sub>

Concentration Salt	N/1	<b>N</b> /10	N/100	N/1000	$ m H_2O$	B sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	X
		2.0 cc				8.0 cc	IX
$CaCl_2$			2.0 cc			8.0 cc	VI
				2.0 cc		8.0 cc	VI
					2.0 cc	8.0 cc	VI

ii. BaCl<sub>2</sub>

TABLE 34

Influence of BaCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of MgCl<sub>2</sub>

Concentration Salt	N/1	N/10	· N/100	N/1000	H <sub>2</sub> O	B sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	XI
		2.0 cc				8.0 cc	X
BaCl <sub>2</sub>		ļ	2.0 cc			8.0 cc	VII
				2.0 cc		8.0 cc	VI
					2.0 cc	8.0 <b>c</b> c	VI

.c. Cases of CaCl<sub>2</sub> or MgCl<sub>2</sub> added independently to milk containing a definite amount of BaCl<sub>2</sub>

Milk  $(40 \text{ cc}) + \text{N/10 BaCl}_2$  (10 cc) = C solution

#### i. CaCl<sub>2</sub>

#### Table 35

Influence of CaCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of BaCl<sub>2</sub>

Concentration Salt	N/1	N/10	N/100	N/1000	$\mathrm{H}_2\mathrm{O}$	C sol.	Alc. No. to coag. mixture
CaCl <sub>2</sub>	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	X IX VII VI VI				

### ii. MgCl<sub>2</sub>

#### TABLE 36

Influence of MgCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of BaCl<sub>2</sub>

Concen- tration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	C sol.	Alc. No. to coag. mixture
$\mathrm{MgCl}_2$	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	X XI VIII VI VI				

By observing the above tables the author recognizes that the alcohol susceptibility of milk containing a definite amount of divalent cation of neutral salt is much increased by the addition of more concentrated solution of the neutral divalent salt.

# F. Influence of Trivalent Cation of Neutral Salt upon the Alcohol Susceptibility of Milk Containing a Difinite amount of Divalent Cation of Neutral Salt

a. Case of FeCl<sub>3</sub> added to milk containing a definite amount of CaCl<sub>2</sub>

Milk  $(40 \text{ cc}) + \text{N/10 CaCl}_2$  (10 cc) = A solution

Table 37  $\label{eq:table 37} \mbox{Influence of FeCl}_{3} \mbox{ upon the alcohol susceptibility of milk containing a definite amount of $CaCl_{2}$ }$ 

Concen- tration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	A sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	+
		2.0 cc				8.0 cc	XI
$FeCl_3$			2.0 cc			8.0 cc	VIII
				2.0 cc		8.0 cc	VII
					2.0 cc	8.0 cc	VI

b. Case of  $FeCl_3$  added to milk containing a definite amount of  $MgCl_2$ 

Milk  $(40 cc) + N/10 MgCl_2 (10 cc) = B$  solution

Table 38

Influence of FeCl<sub>3</sub> upon the alcohol susceptibility of milk containing a definite amount of MgCl<sub>2</sub>

Concen- tration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	B sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	+
		2.0 cc				8.0 cc	XI
$\mathrm{FeCl}_3$			2.0 <b>c</b> c			8.0 cc	VIII
				2.0 cc		8 0 cc	VII
			:		2.0 cc	8.0 cc	VI

c. Case of FeCl<sub>3</sub> added to milk containing a definite amount of BaCl<sub>2</sub>

Milk  $(40 \text{ cc}) + \text{N}/10 \text{ BaCl}_2$  (10 cc) = C solution

TABLE 39

Influence of FeCl<sub>3</sub> upon the alcohol susceptibility of milk containing a definite amount of BaCl<sub>2</sub>

Concen- tration Salt	N/1	N/10	N/100	N/1000	${ m H_2O}$	C sol.	Alc. No. to coag. mixture
${ m FeCl}_3$	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	+ XI VIII VII VI				

By observing the above tables the author recognizes that the alcohol susceptibility of milk containing a definite amount of divalent cation of neutral salt is increased when the more concentrated trivalent cation of neutral salt is added to these mixtures. It was also observed in this experiment that the coagulation occurred immediately after the addition of N/1 FeCl<sub>3</sub> solution without the addition of alcohol.

# G. Influence of Monovalent Cation of Neutral Salt upon the Alcohol Susceptibility of Milk Containing a Definite amount of Monovalent Cation of Neutral Salt

a. Cases of KCl or NH<sub>4</sub>Cl added independently to milk containing a definite amount of NaCl

Milk (40 cc) + N/1 NaCl (10 cc) = D solution

### i. KCl

containing a definite amount of NaCl

TABLE 40
Influence of KCl upon the alcohol susceptibility of milk

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	D sol.	Alc. No. to coag. mixture
KCl	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	IV III II II II				

ii. NH<sub>4</sub>Cl

TABLE 41

Influence of NH<sub>4</sub>Cl upon the alcohol susceptibility of milk containing a definite amount of NaCl

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	D sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	IV
		2.0 <b>c</b> c				8.0 cc	IV
NH,Cl			2.0 cc			8.0 cc	III
				2.0 cc		8.0 cc	II
					2.0 cc	8.0 cc	II

b. Cases of NaCl or NH<sub>4</sub>Cl added independently to milk containing a definite amount of KCl

Milk (40 cc) + N/1 KCl (10 cc) = E solution

i. NaCl

Table 42

Influence of NaCl upon the alcohol susceptibility of milk containing a definite amount of KCl

Concentration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	E sol.	Alc. No. to coag. mixture
	2.0 ec					8.0 cc	IV
		2.0 cc				8.0 <b>cc</b>	IV
NaCl			2.0 cc			8.0 cc	III
				2.0 cc		8.0 cc	II
					2.0 cc	8.0 cc	II

ii. NH<sub>4</sub>Cl

Table 43

Influence of NH<sub>4</sub>Cl upon the alcohol susceptibility of milk containing a definite amount of KCl

Concentration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	E sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	IV
		2.0 <b>c</b> c		1		8.0 cc	IV
NH₄Cl			2.0 ce			8.0 <b>c</b> c	III
				2.0 cc		8.0 cc	11
					2.0 cc	8.0 cc	II

c. Cases of KCl or NaCl added independently to milk containing a definite amount of NH<sub>4</sub>Cl

Milk  $(40 \text{ cc}) + \text{N/1 NH}_4\text{Cl }(10 \text{ cc}) = \text{F solution}$ 

i. KCl

TABLE 44

Influence of KCl upon the alcohol susceptibility of milk containing a definite amount of NH<sub>4</sub>Cl

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	F sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	IV
		2.0 cc	-			8.0 cc	III
KCI			2.0 cc			8.0 cc	III
				2.0 cc		8.0 cc	II
					2.0 cc	8.0 cc	11

ii. NaCl

Table 45

Influence of NaCl upon the alcohol susceptibility of milk containing a definite amount of  $NH_4Cl$ 

Concen- tration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	F sol.	Alc. No. to coag. mixture
NaCl	2.0 cc	2.0 c <b>c</b>	2.0 cc	2.0 cc	2.0 <b>c</b> c	8.0 cc 8.0 cc 8 0 cc 8.0 cc 8.0 cc	VI III III II II

It is to be recognized that the alcohol susceptibility of milk containing a definite amount of monovalent cation of neutral salt is slightly increased when the more concentrated solution of the same salt added.

# H. Influence of Divalent Cation of Neutral Salt upon the Alcohol Susceptibility of Milk Containing a Definite amount of Monovalent Cation of Neutral Salt

a. Cases of CaCl<sub>2</sub>, MgCl<sub>2</sub> or BaCl<sub>2</sub> added independently to milk containing a definite amount of NaCl

Milk (40 cc) + N/1 NaCl (10 cc) = D solution

#### i. CaCl<sub>2</sub>

TABLE 46

Influence of CaCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of NaCl

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	D sol.	Alc. No. to coag. mixture
CaCl <sub>2</sub>	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	IX VII III II				

ii.  $MgCl_2$   $TABLE\ 47$  Influence of  $MgCl_2$  upon the alcohol susceptibility of milk

Concen- tration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	D sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 <b>c</b> c	IX
		2.0 cc				8.0 cc	VII
$\mathbf{MgCl}_2$			2.0 cc			8.0 cc	III
				2.0 ec		8.0 cc	II
					2.0 c <b>c</b>	8.0 cc	II

containing a definite amount of NaCl

 $\label{eq:Table 48} {\small \mbox{Table 48}}$  Influence of BaCl2 upon the alcohol susceptibility of milk containing a definite amount of NaCl

Concentration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	D sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	VIII
		2.0 cc				8.0 <b>c</b> c	VII
BaCl <sub>2</sub>			2.0 cc			8.0 cc	III
				2.0 cc	Į.	8.0 cc	II
					2.0 cc	8.0 cc	II

b. Cases of  $CaCl_2$ ,  $MgCl_2$  or  $BaCl_2$  added independently to milk containing a definite amount of KCl

Milk (40 cc) + N/1 KCl (10 cc) = E solution

#### i. CaCl<sub>2</sub>

containing a definite amount of KCl

 $\label{eq:table 49} \textbf{Influence of } CaCl_2 \ \textbf{upon the alcohol susceptibility of milk}$ 

Concentration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	E sol.	Alc. No. to coag. mixture
CaCl <sub>2</sub>	2.0 сс	2.0 cc	2.0 cc	2.0 cc	2.0 cc	8.0 cc 8.0 cc 8 0 cc 8.0 cc 8.0 cc	IX VII III II

### ii. $MgCl_2$

Table 50

Influence of MgCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of KCl

Concen- tration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	E sol.	Alc. No. to coag. mixture
$\mathrm{MgCl}_2$	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	IX VI III II II				

## iii. $BaCl_2$

Table 51

Influence of BaCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of KCl

Concen- tration Salt	<b>N</b> /1	<b>N</b> /10	N/100	N/1000	H₂O	E sol.	Alc. No. to coag. mixture
BaCl <sub>2</sub>	2.0 cc	2.0 cc	2.0 cc	2.0 cc	2.0 cc	8 0 cc 8 0 cc 8.0 cc 8.0 cc 8.0 cc	VIII VI III II

c. Cases of  $CaCl_2$ ,  $MgCl_2$  or  $BaCl_2$  added independently to milk containing a definite amount of  $NH_4Cl$ 

Milk  $(40 cc) + N/1 NH_4Cl (10 cc) = F$  solution

i. CaCl<sub>2</sub>TABLE 52

Influence of  $CaCl_2$  upon the alcohol susceptibility of milk containing a definite amount of  $NH_4Cl$ 

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	F sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	J <b>X</b>
		2.0 cc				8.0 cc	VI
$\operatorname{CaCl}_2$			2.0 cc			8.0 cc	III
				2.0 cc		8.0 cc	II
					2.0 cc	8 0 cc	II

ii. MgCl<sub>2</sub>

Table 53

Influence of  $MgCl_2$  upon the alcohol susceptibility of milk containing a definite amount of  $NH_4Cl$ 

Concen- tration Salt	N/1	N/10	N/100	N/1000	H₂O	F sol.	Alc. No. to coag. mixture
	2.0 cc					8.0 cc	IX
		2.0 cc				8.0 cc	VI
$\mathrm{MgCl}_2$			2.0 cc			8.0 cc	III
				2.0 cc	,	8.0 cc	II
					2.0 cc	8.0 cc	II

iii. BaCl<sub>2</sub>

Table 54

Influence of BaCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of NH<sub>4</sub>Cl

Concen- tration Salt	N/1	N/10	N/100	N/1000	${ m H_2O}$	F sol.	Alc. No. to coag. mixture
BaC!2	2.0 ec	2.0 cc	2.0 cc	2.0 cc	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	VIII VI III II

It may be recognized that the alcohol susceptibility of milk containing a definite amount of monovalent cation of neutral salt is increased upon the addition to the mixture of the divalent cation of salt. The degree of the influence of these salts upon the alcohol coagulation is Ca>Mg>Ba though the different is very small.

# I. Influence of Trivalent Cation of Neutral Salt upon the Alcohol Susceptibility of Milk Containing a Definite amount of Monovalent Cation of Neutral Salt

a. Case of FeCl<sub>3</sub> added to milk containing a definite amount of NaCl

Milk 
$$(40 \text{ cc}) + \text{N/1}$$
 NaCl  $(10 \text{ cc}) = \text{D}$  solution

Table 55

Influence of FeCl<sub>3</sub> upon the alcohol susceptibility of milk containing a definite amount of NaCl

Concentration Salt	N/1	N/10	N/100	N/1000	${ m H_2O}$	D sol.	Alc. No. to coag. mixture
$\mathrm{FeCl}_3$	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	+ IV II II II				

b. Case of FeCl<sub>3</sub> added to milk containing a definite amount of KCl

Milk (40 cc) + N/1 KCl (10 cc) = E solution

TABLE 56

Influence of FeCl<sub>3</sub> upon the alcohol susceptibility of milk containing a definite amount of KCl

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	E sol.	Alc. No. to coag. mixture
$\mathbf{FeCl}_3$	2.0 cc	2.0 cc	2.0 <b>c</b> c	2.0 cc	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	H III II II II

c. Case of FeCl<sub>3</sub> added to milk containing a definite amount of NH<sub>4</sub>Cl

Milk  $(40 \text{ cc}) + \text{N/1 NH}_4\text{Cl }(10 \text{ cc}) = \text{F solution}$ 

Table 57  $\label{eq:table 57} \mbox{Influence of FeCl}_3 \mbox{ upon the alcohol susceptibility of milk containing a definite amount of $NH_4$Cl}$ 

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	F sol.	Alc. No. to coag. mixture
$\mathbf{FeCl}_3$	2.0 cc	2.0 cc	2.0 ec	2.0 cc	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	III II II II

The author recognizes that the alcohol susceptibility of milk containing a definite amount of monovalent cation of neutral salt is increased when the more concentrated trivalent cation of neutral salt is added to the mixture.

J. Influence of Monovalent Cation of Neutral Salt upon the Alcohol Susceptibility of Milk Containing a Definite amount of Trivalent Cation of Neutral Salt

Milk  $(40 \text{ cc}) + \text{N/10 FeCl}_3$  (10 cc) = G solution

a. Case of NaCl added to milk containing a definite amount of FeCl<sub>3</sub>

Table 58

Influence of NaCl upon the alcohol susceptibility of milk containing a definite amount of FeCl<sub>3</sub>

Concentration Salt	N/1	N/10	N/100	N/1000	$ m H_2O$	G sol.	Alc. No. to coag. mixture
	2.0 cc	2.0 cc				8.0 cc 8.0 cc	III IV
NaCl		9	2.0 <b>c</b> c			8.0 cc	IV
				2.0 cc		8.0 cc	v
					2.0 cc	8.0 cc	v

b. Case of KCl added to milk containing a definite amount of  $FeCl_3$ 

Table 59  $\label{eq:table 59}$  Influence of KCl upon the alcohol susceptibility of milk containing a definite amount of FeCl $_3$ 

Concentration Salt	N/1	N/10	N/100	N/1000	H₂O	G sol.	Alc. No. to coag. mixture
	2.0 cc	2.0 cc				8.0 cc 8.0 cc	III IV
KCl	·		2.0 cc			8.0 cc	v
				2.0 cc		8.0 cc	v
					2.0 cc	8.0 cc	v

c. Case of NH<sub>4</sub>Cl added to milk containing a definite amount of FeCl<sub>3</sub>

 $\begin{array}{c} \text{Table 60} \\ \text{Influence of NH}_4\text{Cl upon the alcohol susceptibility of milk} \\ \text{containing a definite amount of FeCl}_3 \end{array}$ 

Concen- tration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	G sol.	Alc. No. to coag. mixture
NH <sub>4</sub> Cl	2.0 cc	2.0 c <b>c</b>	2.0 cc	2.0 cc	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	III III IV V V

It may be recognized that the alcohol susceptibility of milk containing a definite amount of trivalent cation of neutral salt is decreased a little when the more concentrated monovalent cation of neutral salt is added to the mixture. This phenomena is practically the same as in the case of milk containing a definite amount of divalent cation of neutral salt admixed with various concentrations of monovalent cation of neutral salt. The author concluded from these results that the so-called antagonistic ion action has occurred in these combinations of salts in milk.

# K. Influence of Divalent Cation of Neutral Salt upon the Alcohol Susceptibility of Milk Containing a Definite amount of Trivalent Cation of Salt

Milk  $(40 \text{ cc}) + \text{N/10 FeCl}_3$  (10 cc) = G solution

a. Case of CaCl<sub>2</sub> added to milk containing a definite amount of FeCl<sub>3</sub>

 $\begin{array}{c} \text{Table 61} \\ \text{Influence of } \text{CaCl}_2 \text{ upon the alcohol susceptibility of milk} \\ \text{containing a definite amount of } \text{FeCl}_3 \end{array}$ 

Concen- tration Salt	N/1	N/10	N/100	N/1000	H <sub>2</sub> O	G sol.	Alc. No. to coag. mixture
CaCl <sub>2</sub>	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	X IX V V V				

b. Case of MgCl<sub>2</sub> added to milk containing a definite amount of FeCl<sub>3</sub>

Table 62
Influence of MgCl<sub>2</sub> upon the alcohol susceptibility of milk containing a definite amount of FeCl<sub>3</sub>

Concentration Salt	N/1	N/10	N/100	N/1000	$_{\rm H_2O}$	G sol.	Alc. No. to coag. mixture
$\mathbf{MgCl}_2$	2.0 ce	2.0 cc	.2.0 cc	2.0 cc	2.0 cc	8.0 cc 8.0 cc 8.0 cc 8.0 cc 8.0 cc	X IX VI V

c. Case of BaCl<sub>2</sub> added to milk containing a definite amount of FeCl<sub>3</sub>

Table 63 Influence of  $BaCl_2$  upon the alcohol susceptibility of milk containing a definite amount of  $FeCl_3$ 

Concentration Salt	N/1	N/10	N/100	N/1000	${ m H}_2{ m O}$	G sol.	Alc. No. to coag. mixture
BaCl₂	2.0 cc	8.0 ec 8.0 ec 8.0 ec 8.0 ec 8.0 ec	X IX VI V V				

It may be observed that the alcohol susceptibility of milk containing a definite amount of trivalent cation of neutral salt is increased when the more concentrated divalent cation of salt is added to the mixture.

### 3. Discussion

There is no correlation between the hydrogen ion concentration and the alcohol susceptibility of fresh normal milk.

The alcohol susceptibility of dialyzed milk is not more sensitive than of whole milk perhaps for the reason of dialyzation of water soluble salt in milk out of the membrane. There will be described a more detailed experiment concerning the quantitative determination of salt in the dialyzed milk in a later chapter.

On the other hand it is recognized that monovalent cation of neutral salt has no special influence upon the alcohol susceptibility of milk but this property is accelerated by the addition of di- or trivalent cation of salt, and the degree of the coagulation varies with the concentration of salt solutions, above all it is most sensitive at the concentration of 3.2N/10-7N/10 of these salts. Whole and dialyzed milk were coagulated only by the addition of trivalent cation of neutral salt having the concentrations of 5N/10-2N/10 before the mixing of alcohol.

The alcohol susceptibility of milk containing a definite amount of mono-, di- or trivalent cation of neutral salt is increased when the more concentrated di- or trivalent cation of salt is added to the mixture, but the susceptibility of the alcohol coagulation of a milk mixture containing a definite amount of di- or trivalent cation of salt is decreased by the addition of more concentrated monovalent cation of salt though the degree is not great. This may be explained by the theory of the antagonistic ion action of these salts in milk. Therefore the author recognizes that the alcohol susceptibility of milk is controlled by the salt balance and the most stable condition for the alcohol coagulation of milk is at the time when a definite salt balance is being kept in the milk.

## IV. Relation between the Alcohol Susceptibility of Milk and the Ash Constituents in it

The alcohol susceptibility of milk is clearly influenced by the lactation period. From the preceding experiments it has been learned that the colostrum, very often the milk at the end stage of lactation, and sometimes even the milk in normal condition have shown the positive reaction to the alcohol test, and also that the alcohol coagulation of these classes of milk are almost certainly due to the salt balance in the samples.

The author undertook next a determination of the quantitative analysis of ash of milk at different stages of the lactation period.

Comparative observations were made on these experimental results to discover whether there may or may not be found any relation to the alcohol coagulation.

#### 1. Experimental methods

Milk which was used in this experiment was produced from Ayrshire, Holstein, and Guernsey cows in the Hokkaido Imperial University farms.

Milk was filtered through cotton cloth after mixing the sample to homogeneity, filled up to 500 cc sample bottle, and then used for the analysis.

The estimation of total solid and ash (28):— Placed about 3 grams of fine asbestos fibres in a small platinum basin, and ignited strongly.

The asbestos is soaked in hydrochloric acid, and thoroughly washed before use; when ignited and shaken with water containing a few drops of phenol-phthalein no red colour produced. After weighing, added about 5 grams of milk, and weighed again as quickly as possible to the nearest milligram. Placed the basin for an hour or two on a water bath, and dried in a electric oven till constant in weight.

The residue of total solids served excellently for the determination of the ash. By igniting over a small BUNSEN flame, a white ash can be obtained. The temperature must not be allowed to rise above a barely perceptible red heat.

The material for the determination of ash constituents:— Evaporated 400 cc of milk and igniting gently till thoroughly charred, the mass is extracted with hot water and filtered, the insoluble portion and the filter being (after washing) ignited at a red heat till white; this will give the insoluble ash.

By evaporating the filtrate and igniting cautiously at a low temperature, the soluble ash is obtained. The sum of the soluble and insoluble ash gives the total ash. This well mixed material was used for the determination of ash constituents. The analysis was performed twice in each sample and the experimental result obtained by averaging them.

The estimation of Cl (87): Two-tenths gram of the sample is dissolved in a small portion of distilled water and the insoluble por-

tion washed well after being filtered out. The filtrate is made up to 100 cc. Cl is determined by titration with standard nitrate of silver (dissolve 29.06 g of silver nitrate (AgNH<sub>3</sub>) in 1000 cc and took 1 cc of this solution as corresponding to 0.006064 g of Cl), using potassium chromate as the indicator.

The estimation of CaO (31):— Two-tenths gram of sample is dissolved in a slight excess of dilute hydrochloric acid, and the solution heated to boiling; a cold saturated solution of ammonium oxalate is dropped in slowly till the addition of a further drop gives no more precipitate. After standing at least three hours the precipitate is filtered off, washed, and ignited at a low temperature to convert the oxalate into carbonate; it is best to moisten the ignited precipitate with ammonium carbonate solution and reignite at a very low temperature. The precipitate, after weighing, is dissolved in dilute hydrochloric acid, keeping the bulk small: ammonia is added to alkaline reaction, and the small precipitate of calcium phosphate collected, ignited, and weighed. Its weight is subtracted from the previous weight, and the difference gives the weight of the calcium carbonate, which multiplied by 0.56 gives the lime contained in it; the weight of the calcium phosphate multiplied by 0.5419 gives the lime, contained The total lime is the sum of the two.

The estimation of MgO (31):— The filtrate which was obtained from the filtration of calcium oxalate by the addition of ammonium oxalate is made strongly ammoniacal by the addition of 0.880 ammonia and allowed to stand twenty-four hours. The precipitated magnesium-ammonium phosphate is filtered off, washed with dilute ammonia, ignited and the magnesium pyrophosphate weighed. Its weight multiplied by 0.36036 yields the magnesia contained in it.

The estimation of  $P_2O_5(31)$ :— To the filtrate from the above, magnesia mixture is added. The precipitate of magnesium-ammonium phosphate is filtered off after twenty-four hours and treated as above.

From the total weight of the two quantities of magnesium pyrophosphate, the phosphoric anhydride is calculated by multiplying by 0.63946; to this is added the phosphoric anhydride in the calcium phosphate calculated by multiplying the weight by 0.4581.

The estimation of  $SO_3(31)$ :— Two-tenths gram of the sample is dissolved in dilute hydrochloric acid and boiled; 0.5 cc of normal solution of barium chloride is added, and boilng is continued for some

time. After some hours the precipitate of barium sulphate is filtered off, ignited, and weighed; its weight multiplied by 0.34335 will give the sulphuric anhydride in the milk.

The estimation of  $Na_2O$  and  $K_2O(31)$ :— A little phosphoric acid dropped into the filtrate. A quantity of ferric chloride solution sufficient to colour the solution brown is added, and the filtrate made alkaline with ammonia. The precipitate is washed well, and the filtrate evaporated and ignited very cautiously; the weight will give the alkaline chlorides. The residue is dissolved in water, and the solution should be quite clear; if it is not so, a little ammonium carbonate is added, the liquid evaporated to dryness, and the residue ignited cautiously and weighed.

The potassium is estimated directly by evaporating the solution of alkaline chlorides with an excess of platinum tetrachloride solution almost to dryness; the pasty residue is treated with 80% alcohol containing about 5% of ether and washed repeatedly with this; the alcohol is passed through a weighed filter, and the precipitate is finally transfered to this and washed with ether. It is then dried at 100°C. and weighed; the weight multiplied by 0.3056 will give the potassium chloride; this subtracted from the weight of the alkaline chlorides is the sodium chloride.

The potassium chloride multiplied by 0.6341 is potash. The sodium chloride multiplied by 0.5299 is soda.

The estimation of  $Fe_2O_3(12)$ :— Three-tenths gram of the sample is taken up in about 10 cc of water and 5 cc of conc. HCl and allowed to stand for several hours. The residue is filtered off and the phosphorus removed from the filtrate in the usual manner which consists of adding conc.  $NH_4OH$  until the solution becomes cloudy. Cleaning up with conc.  $HNO_3$  and adding 10 drops of  $HNO_3$  in excess, 30 cc of ammonium molybdate solution is added, digested on a water bath at 65°C. for 1/2 hour, and the yellow precipitate of ammonium phosphomolybdate filtered off. The precipitate is carefully washed with dilute  $HNO_3$  (9 cc  $HNO_3$  in 100 cc  $H_2O$ ) to insure the removal of the last traces of iron to the filtrate. The solution is brought almost to boil and 40% KOH (iron free) is added until no further precipitate forms, usually about 20 cc are required. The solution is boiled for

several minutes to remove the ammonia present. The solution is allowed to cool and if the hydroxides do not settle properly a few cc of KOH are added and heated further. The precipitate is filtered off on an asbestos gooch crucible, which has been carefully washed with HCl, by decanting the clear liquid first, and finally adding the precipitate to the gooch crucible. The precipitate is washed with very dilute KOH solution (1%). Best results are obtained if only a low pressure is maintained on a suction flask during filtering. The precipitate is dissolved from the gooch crucible with 1-2 cc of conc. HCl, which is added in several portions (a few drops at a time) washing with water after each addition of acid. In this way the iron may be dissolved off completely and the total filtrate kept below 30-35 cc. The best method of handling this small amount of solution is to introduce a test tube into the suction flask, allowing the end of the funnel to reach into the test tube so that the solution will be caught in the test tube instead of the suction flask. The solution in the test tube is then washed into the original beaker and the iron determined colorimetrically by adding enough N/5 KMnO<sub>4</sub> to produce a faint pinkish colour then adding 5 cc of 10% solution of potassium thiocyanate and making up to 50 cc volume in a volumetric flask, the colour produced is compared in a DUBOSCQ colorimeter with a standard colour developed by taking 1 cc of the standard iron solution, adding 1 to 2 drops of KMnO<sub>4</sub> and 5 cc of the 10% solution of potassium thiocyanate and making up to 50 cc volume. The standard solution is set at 20 mm in the Duboscq and the unknown adjusted until the colours are equal. The standard iron solution will be obtained by the following prescription: Dissolve 0.7 grams of ferrous ammonium sulfate (dried to constant weight) in 100 cc of distilled water and add 5 cc of concentrated sulfuric acid. Warm the solution slightly and add potassium permanganate until the iron is completely oxidised. Dilute the solution to 1 liter, 1 cc of the standard iron solution equals 0.1 mg Fe.

### 2. Experimental results

i. Results of analysis of the ash constituents in colostrum which shows positive reaction to alcohol test.

Cow No.	Date of parturition	Date of sample taken	Alcohol test	Total solids	Ash	Cl	CaO	MgO	$P_2O_5$	$SO_3$	$\mathrm{Fe_2O_3}$	Na <sub>2</sub> O	K <sub>2</sub> O
H. 255 H. 244 H. 215 A. 85 H. 249 A. 97	28/VI 1927 1/VII 1927	15/VI 1927 22/VI 1927 39/VI 1927 3/VII 1927 5/VII 1927 9/VII 1927	++++++++++	14.041 13.870 13.780 12.271 14.407 12.€47	0.917 0.820 0.850 0.814 0.820 0.842	11.777 14.550 11.918 13.640 10.612 13.340	24.413 26.717 25.012 23.575 24.730 25.605	3.146 3 677 2.923 2.625 3.645 3.356	30.389 30.965 31.449 30.745 33.233 29.687	2.627 2.030 2.051 1.965 2.150 2.750	0.438 0.457 0.315 0.401 0.390 0.296	6.515 7.800 9.150 10.260 8.020 7.627	22.590 15.760 18.865 19.855 20.205 19.100
	Average	•	+++	13.503	0.844	12.640	25.009	3.229	31.078	2.262	0.283	8.229	19.296

ii. Results of analysis of the ash constituents in normal milk which shows negative reaction to alcohol test.

Table 65

Determination of the ash constituents in normal milk which shows negative reaction to alcohol test

Cow No.	Date of parturition	Date of sample taken	Alcohol test	Total solids	Ash	Cl	CaO	MgO	$P_2O_5$	$SO_3$	Fe <sub>2</sub> O <sub>3</sub>	Na <sub>2</sub> O	K <sub>2</sub> O
A. 97 A. 82 A. 97 A. 82 H. 254 H. 169 H. 204	20/IX 1926 17/VI 1926 29/VII 1926	8/X 1926 8/X 1926 11/X 1926 11/X 1926 29/XI 1926 29/XI 1926 29/XI 1929	-	13.530 12.920 13.160 13.260 13.350 14.421 13.118	0.710 0.690 0.680 0.710 0.675 0.635 0.602	15.244 15.038 17.949 15.911 14.796 13.462 14.129	20.140 20.632 20.025 20.615 19.985 17.884 20.800	2.295 2.234 2.503 2.162 2.315 3.023 2.758	28.816 28.014 28.703 29.145 30.783 33.714 31.140	2.654 2.717 2.153 2.287 2.262 2.873 2.110	0.350 0.291 0.381 0.290 0.408 0.435 0.251	6.650 7.523 6.952 5.125 5.560 7.205 6.112	25.618 26.090 23.100 27.212 25.008 23.606 24.050

6.700

7.075

23.512

24.724

Total solids	Ash	Cl	CaO	MgO	$P_2O_5$	$SO_3$	$\mathrm{Fe_2O_3}$	Na₂O	K <sub>2</sub> O
11.939	0.595	13.947	17.953	2.180	30.947	2.050	0.281	8.870	24.892
15.110	0.798	13.917	20.953	2.789	34.173	2.296	0.324	5.905	22.200
12.816	0.665	13.340	18.876	2.450	29.395	2.356	0.316	9.190	24.956
14.280	0.719	11.521	21.740	2.800	28.204	2.579	0.415	8.265	24.325
10.705	0.655	11.964	21.776	2.555	29.935	2.425	0.330	6.107	26.050
11.417	0.637 $0.690$ $0.728$	15.689	19.422	1.881	28.600	2.150	0.290	7.200	25.520
13.125		17.464	20.683	2.459	26.277	2.437	0.315	8.251	24.000
12.018		13.098	20.365	2.656	28.701	2.695	0.296	7.596	25.450

30.411

29.810

2.860

2.432

0.360

0.338

2.363

2.463

Table 65 (Continued)

Date of

sample taken

29/XI 1926

9/XII 1926 9/XII 1926

9/XII 1926

23/XI 1926

1926

1927

1927

1927

23/XI

18/X 18/X

Alcohol

test

12.795

12.998

0.682

0.679

Cow

No.

H.S.M. H.D.S.

H. 238 21/IX H. 178 29/III

H. 242 23/III 1926

H. 249 30/VII 1926

A. 91 25/VII 1927 A. 114 26/VIII 1927

Date of

parturition

21/IX 1926

15/VIII 1926

27/VI 1926

A. 85 | 14/VIII 1927 | 18/X

Average

1926

iii. Results of analysis of the ash constituents in normal milk which shows positive reaction to alcohol test.

12.855

14.358

23.463

20.332

Table 66 Determination of the ash constituents in normal milk which shows positive reaction to alcohol test

Cow No.	Date of parturition		Alcohol test	Total solids	Ash	Cl	CaO	MgO	$P_2O_5$	SO <sub>3</sub>	$\mathrm{Fe_2O_3}$	Na <sub>2</sub> O	K <sub>2</sub> O
A. 77 A. 94 A. 77 A. 94 H. 171 H. 193 G. 183		8/VIII 1926 11/VIII 1926 11/VIII 1926 9/VII 1926 9/VII 1926	++. + ++ + ++ ++ ++	13.719 13.900 13.842 14.398 15.132 12.340 13.444	0.729 0.780 0.715 0.745 0.807 0.684 0.711	18.587 20.375 20.132 18.919 16.980 18.435 16.009	25.841 24.688 25.577 25.918 23.692 26.276 23.231	2.522 2.652 2.984 2.638 3.358 5.568 3.344	27.401 30.950 29.411 30.746 31.944 34.317 25.712	2.018 2.082 1.803 1.751 1.454 1.939 1.282	0.402 0.395 0.380 0.410 0.490 0.365 0.215	8.254 6.655 7.765 6.590 8.021 6.960 10.225	17.362 15.960 14.100 16.051 15.050 13.100 19.190

Cow No.	Date of parturition	Date of sample taken	Alcohol test	Total solids	Ash	Cl	CaO	MgO	$P_2O_5$	$SO_3$	Fe <sub>2</sub> O <sub>3</sub>	Na <sub>2</sub> O	K <sub>2</sub> O
G. 176 H.H.R. H.D.C. H. 239 A. 85	17/IV 1926 12/V 1926	9/VII 1926 24/IX 1926 24/IX 1926 9/VIII 1927 23/VIII 1927	+++++	16.432 11.406 11.216 16.664 14.126	0.824 0.668 0.638 0.847 0.785	18.192 14.432 13.855 15.160 16.995	25.231 23.492 29.485 30.504 27.021	2.905 2.868 2.990 3.052 3.860	30.910 31.180 31.200 30.298 26.295	1.751 1.695 1.369 1.476 2.005	0.333 0.290 0.315 0.421 0.218	7.890 8.724 8.790 6.265 8.560	15.600 18 918 13.107 15.700 19.236
	Average	)	++	13.885	0.744	17.339	25.913	3.062	30.030	1.719	0.353	7.892	19.115

iv. Results of analysis of the ash constituents of milk at the end stage of lactation.

TABLE 67

Determination of the ash constituents in milk at the end stage of lactation

Cow No.	Date of parturiti		Date sam take	ple	Alcohol test	Total solids	Ash	Cl	CaO	MgO	$P_2O_5$	$\mathrm{SO}_3$	$Fe_2O_3$	Na <sub>2</sub> O	K <sub>2</sub> O
G. 170 H. 193 A. 111 H. 204 H. 249 H. 169 H. 243	14/II	)26   1 )26   2 )26   )26   )26	18/V 18/V 23/V 6/VI 6/VI 8/VI 8/VI	1927 1927 1927 1927 1927 1927 1927	++ ++ - - ++ ++ +	15.670 14.274 14.715 11.893 17.275 15.228 15.083	0.871 0.712 0.955 0.692 0.920 0.896 0.838	18.919 18.434 21.102 24.983 19.128 22.558 20.155	25.875 22.870 24.300 19.464 25.803 19.960 24.413	4.381 4.936 2.240 2.140 3.675 2.560 2.890	27.560 30.543 29.192 24.505 30.848 27.727 30.172	2.351 2.660 2.407 1.287 1.802 1.541 1.888	0.316 0.405 0.390 0.270 0.321 0.412 0.356	6.250 7.562 8.020 10.127 8.250 8.456 7.095	13.660 14.965 13.000 18.882 13.215 19.050 16.152
	. Aver	age		-	+	14.877	0.811	20.754	23.241	3,260	28.650	1.992	0.352	7.966	15.561

Notice: In the above tables the number value of total solids and ash represent the % in milk by weight, and also the number value of ash constituents represent the % in ash by weight.

The capital letters H, A and G represent the Holstein, Ayrshire and Guernsey breeds of cows respectively.

#### 3. Discussion

Under this heading will be discussed the relation between the alcohol susceptibility and the ash constituents of milk which have been secreted at the different stages of the lactation by considering the experimental results in respect to the ash constituents in milk.

TABLE 68

Average number values of the ash constituents in milk secreted at different stages of the lactation period

Milk stage	Colostrum	Normal milk	Normal milk	Milk on the stage of last lactation
Alcohol test	+++		++	+
Total solids	13.503	12.998	13.885	14.877
Ash	0.844	0.678	0.744	0.841
Cl	12,640	14.358	17.339	20.754
CaO	25.009	20.332	<b>2</b> 5.913	23.241
MgO	3.229	2.563	3.062	3.260
$P_2O_5$	31.078	29.810	30.030	28.650
$SO_3$	2.262	2.432	1.719	1.992
$Fe_2O_3$	0.383	0.338	0.353	0.352
Na <sub>2</sub> O	8.229	7.075	7.892	7.966
K <sub>2</sub> O	19,396	24.724	16.115	15.561
No. of samples	6	16	12	7

It has already been reported that the ash constituents of milk are influenced by the period of lactation, feeding, or health condition of cow.

TRUNZ (91) found experimentally that the contents of Na<sub>2</sub>O, CaO and MgO in the colostrum and also the milk at the end stage of lactation are more than those of normal lactation period, but on the contrary the K<sub>2</sub>O content is less than these, and the chlorine content in the milk has a tendency to increase with the progress of the lactation period. There is however no remarkable change in the contents of Fe<sub>2</sub>O<sub>3</sub>, P<sub>2</sub>O<sub>5</sub> and SO<sub>3</sub> in these experimental milk throughout the whole lactation period.

According to the experiment of SCHRODT and HANSEN (75) the content of CaO and  $P_2O_5$  in milk is increased a little by the pasture

feeding over that by the stall feeding, but on the contrary the chlorine content is increased by the stall feeding.

On the other hand, according to the studies of Bögold and Stein (9) Steinegger and Allemann (85) Hashimoto (27) and Sato (69) the contents of chlorine and  $Na_2O$  are much increased but on the contrary the contents of CaO,  $P_2O_5$  and  $K_2O$  in milk are much decreased under the condition of the sickness of the cow.

The author proposes to observe the relation between the alcohol susceptibility and ash constituents of milk secreted at the different stages within the lactation period.

It is usual that the alcohol test changes to negative reaction after passing the colostrum stage which reacted positively to the test. The milk at the end stage of lactation usually reacted positively to the test though the degree of coagulation was not very marked.

There are some cases presenting the positive reaction to the alcohol test even in the normal stage of lactation though as a rule the milk at that stage of lactation is negative to the test. The author recognized that the total ash content in the normal milk reacting positively to the alcohol test is a little greater than that of normal milk reacting negatively to the test although it is less than that of colostrum or the milk at the end stage of lactation. The same can not be observed so definitely in relation of total solids to the alcohol susceptibility as seen in the total ash.

It is interesting to know that the contents of CaO, MgO and Cl in the normal milk reacting positively to the test are increased in comparison with the normal milk reacting negatively, but on the contrary the contents of  $K_2O$  and  $SO_3$  are decreased.

The explanation of these phenomena is easily deduced from the experimental results in respect to the alcohol coagulation of milk containing various kinds of neutral salts which are reported in the preceding chapter: the facts that the higher the content of divalent cation of neutral salt such as Ca or Mg in milk the greater the increase of alcohol susceptibility and that the alcohol susceptibility decreased more when the more concentrated monovalent cation of neutral salt such as Na or K was found in milk containing a definite amount of divalent cation of salt. The same can be said rather more emphatically to the alcohol susceptibility of milk at the colostrum as well as drying stage of lactation.

In the next table the comparative values of the ash constituents of colostrum, normal milk, and the milk at the end stage of lactation considering CaO as 100 in each sample. The ratio of the alkali values  $K_2O/Na_2O$  of these milk ash samples are also given.

Table 69

Comparative number values of ash constituents in milk when the content of CaO is assumed as 100

Milk stage	Colostrum	Normal milk	Normal milk	Milk at the end stage of lactation
Alcohol Con- test stituents	+++	_	++	+
Cl	50.54	70.62	66.91	89.30
CaO	100.00	100.00	100.00	100.00
MgO	12.91	12.11	11.83	14.03
$P_2O_5$	124.26	144.62	115.88	123.27
SO <sub>3</sub>	9.04	11.96	6.63	8.57
$Fe_2O_3$	1.53	1.66	1.36	1.51
Na <sub>2</sub> O	32.90	34.80	30.46	34.28
K <sub>2</sub> O	77.56	121.60	62.19	66.95
K <sub>2</sub> O/Na <sub>2</sub> O	2.36	3.49	2.04	1.95

There will be found a marked decrease in the contents of  $P_2O_5$ ,  $K_2O$  and  $SO_3$  in the milk reacting positively to the alcohol test in comparison with the corresponding values of milk reacting negatively to the test, when the content of CaO is assumed as 100. The value of the ratio of  $K_2O/Na_2O$  in this case is very low, but it coincides well with that of the colostrum and the milk at the end stage of lactation.

## V. Influence of the Testing Temperature upon the Alcohol Susceptibility of Milk

It has already been reported by SOXHLET (84) that the viscosity of milk is increased by a descent in the temperature. WHITAKER, SHERMAN and SHARP (96) recognized that the viscosity of skimmed milk is changed remarkably at the temperature of 5°-60°C., which

is perhaps due to the physical changing of the casein quality. On the other hand lab susceptibility of milk is influence by the temperature; Fleischmann (18) found that the optimum temperature for the lab coagulation of milk is 41°C.

In this chapter is reported a determination of the influence of testing temperature upon the alcohol susceptibility of milk.

#### 1. EXPERIMENTAL METHODS

The temperature range from 0°C to 70°C was divided into fifteen different stages and the alcohol susceptibility of milk was determined at each of those divisions, a low temperature such as 0°-10°C was obtained by mixing ice and salt into the water. Eight grades of alcohol solutions were prepared having the following different concentrations: 98, 94, 90, 86, 82, 78, 74 and 70% volumetrically.

Two cc of milk samples were taken into the test tube and the coagulating point observed by mixing the same volume of the different alcohol solution of percentages.

The author converted the degree of alcohol susceptibility of milk into the same degree of coagulation in each case by changing the alcohol % for convenience in order to calculate the experimental results mathematically: for example, the coagulation of milk by 70–74% alcohol is arranged as follows.

Alcohol coagulation obtained from the experiment		Alcohol coagulation obtained from the conversion		
Alcohol %	Degree of coagulation	Alcohol %	Degree of coagulation	
74	±	74	±	
74	+	73	土	
74	++	72	· ±	
70		71	±	
70	±	70	±	

#### 2. Experimental results

The experimental results obtained here are as follows.

TABLE 70

Influence of testing temperature upon the alcohol susceptibility of milk

Cow No.	. н.	252	А.	A. 114		. 97
Tested date	24/X 1929		25/X	1929	28/X	1929
Temp.	Alc. % and milk coa	l degree of gulation	Alc. % and milk coa	l degree of gulation	Alc. % and milk coa	d degree of gulation
°C.	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted
70 65 60 55 50 45 40 35 30 25 20 15 10						
Cow No.	Α.	85	Α.	94	Α.	. 85
Tested date	4/XI	1929	5/XI	1929	6/XI	1929
70 65 60 55 50 45 40 35 30 25 20 15 10 5	98± 86± 86+ 82++ 78++ 78++ 74± 82+ 86± 98±		98± 86± 86++ 82± 82± 82+ 86+ 90+ 98±			94± 90± 82± 78± 73± 73± 73± 73± 74± 84± 90±

Table 70 (Continued)

A. 85		A.	94	H. 277		
13/XI 1929		13/X	I 1929	28/III 1931		
			Alc. % and degree of milk coagulation  Alc. % and deg milk coagulation		d degree of agulation	
Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
_	_	_	-	-	-	
_		_	} -	_		
98±	98±	_		_	-	
$90\pm$	90±	_		-	_	
90+	89±	98±	98±		_	
86+	85±	86±	86±	98±	98±	
74+	73±	82+	81±	90+	89±	
74++	72±	82++	80±	90+	89±	
74++	72±	82++	80±	<b>82</b> +	81 ±	
74++	72±	82++	80±	82+	81 ±	
74++	72±		80±	78±	78±	
74++	72±		80±	$78\pm$	78±	
82 +	81±	86++	84±	82+	81 ±	
94++	92±	98++	96±	<b>9</b> 0±	90±	
$94\pm$	94±	98+	97±	94++	92±	
A.	142	A. 97		A.	115	
28/II	I 1931	29/III 1931		29/11	I 1931	
_	_	_	-	_	_	
	_		-		_	
	· -			-	_	
_					_	
_	_	_			-	
_	- 1				_	
	_	_	] - ]	_		
98±	98±	_	-	98±	98±	
94±	94±	98±	98±	90+	89±	
90±	90±	94++	92±	82+	81 ±	
86++	84±	86++	84±	$74\pm$	74±	
86++	84±	86++	84±	74±	$74\pm$	
90++	83±	90++	88±	82++	80±	
	1			, ,	, —	
90+	89±	· 90±	90士	$82\pm$	82±	
	Alc. % and milk coamilk coamily coamil	Alc. % and degree of milk coagulation  Experimented   Converted	13/XI 1929       13/X         Alc. % and degree of milk coagulation       Alc. % and milk coagulation         Experimented       Converted       Experimented         —       —       —         98±       98±       —         90+       89±       98±         90+       89±       98±         86+       85±       86±         74+       73±       82+         74++       72±       82++         74++       72±       82++         74++       72±       82++         74++       72±       82++         74++       72±       82++         82+       81±       86++         94++       92±       98++         94±       94±       98+         -       -       -         -       -       -         -       -       -         -       -       -         -       -       -         -       -       -         -       -       -         -       -       -         -       -       -         -       -       -<	13/XI 1929       13/XI 1929         Alc. % and degree of milk coagulation       Alc. % and degree of milk coagulation         Experimented       Converted       Experimented       Converted         —       —       —       —         98±       98±       —       —         90±       90±       —       —         90+       89±       98±       98±         86+       85±       86±       86±         74+       73±       82+       81±         74++       72±       82++       80±         74++       72±       82++       80±         74++       72±       82++       80±         82+       81±       86++       84±         94++       92±       98++       96±         94±       94±       98+       97±         A. 142       A. 97         28/III 1931       29/III 1931         —       —       —         —       —       —         —       —       —         —       —       —         94±       94±       98±       98±         90±       94++       92±	13/XI 1929         13/XI 1929         28/II           Alc. % and degree of milk coagulation         Alc. % and milk coagulation         Alc. % and milk coagulation           Experimented         Converted         Experimented           —         —         —           98±         98±         —           90±         90±         —           90+         89±         98±           86+         85±         86±         86±           74+         73±         82+         81±         90+           74++         72±         82++         80±         92+           74++         72±         82++         80±         82+           74++         72±         82++         80±         82+           74++         72±         82++         80±         78±           74++         72±         82++         80±         78±           82+         81±         86++         84±         82+           74++         72±         82++         80±         78±           82+         81±         86++         84±         82+           94++         92±         98++         96±         90±	

Table 70 (Continued)

Cow No.	A	94	A.	142	A. 115		
Tested date	30/III 1931		30/II	30/III 1931		I 1931	
Temp.		d degree of gulation		d degree of gulation	Alc. % and milk coa	d degree of gulation	
°C.	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	
70	<del>-</del>	_				_	
65		_	-	-			
60	_	- 1	_				
55	-			-	_	_	
50		_		_	-		
45		- 1	_	-	_		
40	-	_		_	98±	98±	
35		_	98±	98±	$90\pm$	90±	
30	$94\pm$	94±	90±	90±	$86\pm$	86±	
25	$90\pm$	90±	86±	86±	$82\pm$	82±	
20	$86\pm$	86±	86++	84±	74++	72±	
15	86++	84±	86++	84±	74++	$72\pm$	
10	90 + +	88±	86+	85±	78+	$77\pm$	
5	94+	93±	86±	86±	82++	80±	
0 ·		_	98±	98±	$82\pm$	82±	
Cow No.	A.	114	A.	A. 128		. 94	
Tested date	30/II	I 1931	2/IV	1931	2/IV	1931	
70	_	_	_	_	-	_	
65	_	_	_	_	_		
60	-	_		_	_		
55			—	_	<del></del>	_	
50			-	-	_	_	
45	_	-		_	_	_	
40	-	-	_	_	98±	98±	
35	-	_	_		98++	96±	
30	$98\pm$	98±	_	_	94++	92±	
25	94+	93±	90±	90±	82±	82±	
20	90++	88±	86+	85±	82++	0±	
15	90++	88±	86++	84±	78±	78±	
10	90+	89±	90++	88±	78±	78±	
5	94+	93±	94+	93±	90+	89±	
0			•		90±	90±	

TABLE	70 -	(Continued)	)

Cow No.	A. 94		Cow No. A. 94		Average of 19
Tested date	2/IV	1931	tested samples		
Temp. °C.	Alc. % and milk coas	Alc. % and degree of milk coagulation			
-	Experimented	Converted	Converted		
70	_	_			
65	· _ ]	- 1	<b></b>		
60	-	-	<del>-</del>		
55	! -		$99.2\pm$		
50	i – I	;	$98.8\pm$		
45	-		$97.3 \pm$		
40	_	<del></del>	$94.3 \pm$		
<b>3</b> 5	98+	$97\pm$	$92.2\pm$		
30	$94\pm$	$94 \pm$	$88.6\pm$		
<b>2</b> 5	86+	$85\pm$	$85.5\pm$		
20	82+	$81\pm$	$81.3\pm$		
15	82+	81 ±	81.1±		
10	$86\pm$	$86\pm$	$84.7\pm$		
5	94+	$93\pm$	$90.2 \pm$		
U	. 98±	$98\pm$	$94.3\pm$		

Notes: The sign "-" in the above tables is taken as 100 for convenience of calculating the average.

The following curve was obtained in connection with the alcohol coagulation of milk at the different testing temperatures.

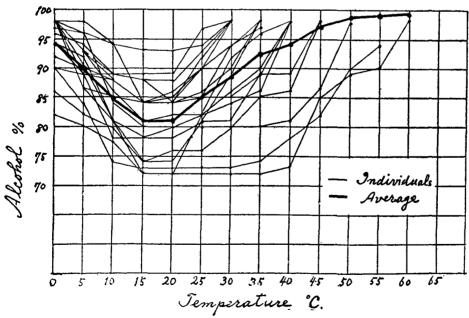


Fig. 5. Alcohol coagulating curve of milk at the different temperatures in the degree of  $\pm \mbox{.}$ 

#### 3. Discussion

The alcohol susceptibility of milk is influenced by the testing temperature. It is decreased not only at the higher but also at the lower temperature, with the optimum coagulating temperature lying between  $15^{\circ}-20^{\circ}$ C.

It is usual that the higher the temperature of a material the more active its physico-chemical reaction, but in this case the alcohol coagulation of milk is decreased at a higher temperature. The explanation for this is very complicated, yet it seems to the author that it is perhaps due to the decrease of the surface tension of the milk casein by the ascending of the temperature. Furthermore the reason for the decrease in the alcohol susceptibility of milk at a temperatures lower than 15°C is due to the increase of its viscosity with the descending temperature.

## VI. Relation Between the Passage of Time and the Alcohol Susceptibility of Milk kept at a Definite Temperature

It is a well known fact that the acidity and also the alcohol susceptibility of milk are increased by standing for a long time owing to the bacterial fermentation. On the other hand the alcohol susceptibility of milk varies with the individuals even though it is fresh and normal.

The author tried next to observe whether or not the degree of alcohol coagulation of milk at the beginning of the experiment has any influence upon the change in the alcohol susceptibility during fifteen hours keeping at a definite temperature such as 30°C.

#### 1. EXPERIMENTAL METHODS

Milk used in this experiment were milked from cows at the same time and under the same conditions as clean as possible. Eighthundred cc milk samples were brought to the laboratory, filtered, and put into the electric incubator kept at a temperature of 30°C. Determinations were made of the alcohol susceptibility, acidity and heat stability each time after 1, 3, 5, 7, 9, 11, 13 and 15 hours at a temperature between 15–18°C. The acidity of milk was determined

by titration with N/10 NaOH using phenol-phthalein as indicator. The author took 17.6 cc of milk sample to a beaker adding two drops of phenol-phthalein solution (0.5 g per 100 cc of 50% of alcohol) and titrated with N/10 NaOH till a faint pink colour is noted. The acidity number was obtained by usual calculation.

#### 2. EXPERIMENTAL RESULTS

The experimental results obtained for each cow are as follows.

Table 71

Relation between the alcohol susceptibility and the lapse of time keeping milk at the temperature of 30°C

Cow No.	H. 250			H. 284				
Hours after	1	Alc. % and degree of milk coagulation		Heat	Acidity	Alc. % and degree of milk coagulation		Heat
milking		Experi- mented	Con- verted	test		Experi- mented	Con- verted	test
1	0.215	98±	99+		0.185	86+	86+	_
3	0.220	98+++	96+	_	0.190	86++	85+	_
5	0.220	$94\pm$	95 +	_	0.190	$82\pm$	83+	-
7	0.220	$94\pm$	95+	_	0.190	82±	83+	_
9	0.220	$94\pm$	95+	_	0.190	82+++	80+	
11	0.225	94++	93 +	_	0.200	70+	<b>7</b> 0+	_
13	0.250	82++	81+	_	0.230	54±	5 <b>5</b> +	±
15	0.295	46++	45+	++	0.275	38+++	<b>3</b> 6+	++++
Cow No.		H. 316			H. 283			
1	0.220	90+	90+		0.170	74+	<b>74</b> +	<del></del>
3	0.220	90 + +	89+	_	0.170	74+	74+	_
5	0.220	86±	87 +	-	0.175	$70\pm$	71 +	_
7	0.220	86±	87+	-	0.180	70+	<b>7</b> 0+	_
9	0.220	86+	86+	-	0.180	70+	<b>7</b> 0+	
11	0.225	82++	81+	-	0.185	$66\pm$	67 +	_
13	0.235	$70\pm$	71 +	-	0.200	62++	61+	_
15	0.250	42++	41 +	+++	0.210	<b>5</b> 0+	50+	±

Cow No.

Hours

after

milking

1

3

5

7

9

11

13

15

Acidity

0.180

0.175

0.175

0.185

0.185

0.185

0.195

0.200

Н. 279		Н. 287
Alc. % and degree of milk	-	Alc. % and degree of milk

Acidity

0.200

0.195

0.200

0.200

0.200

0.200

0.220

0.235

Heat

test

coagulation

Con-

verted

87 +

86+

85 +

83+

83 +

82 +

78 +

67 +

Experi-

mented

 $86 \pm$ 

86 +

 $82\pm$ 

 $82\pm$ 

82 +

78 +

 $66 \pm$ 

86 + +

Table 71 (Continued)

Heat

test

Experimented

 $78 \pm$ 

78+

 $74 \pm$ 

74++

74 + +

 $70\pm$ 

 $66 \pm$ 

 $54 \pm$ 

coagulation

Con-

verted

79 +

78 +

75 +

73 +

73+

71 +

67 +

55 +

#### 3. DISCUSSION

Six Holstein milk were taken as samples. Three samples of milk from cows No. 250, 284 and 316 were tested at the same time. The acidity of these milk was each very high already at the beginning of the experiment, but on the contrary the alcohol susceptibility was weak in each case. For example the milk of cow No. 250 was scarcely coagulated by 98% alcohol. The acidity of these milk samples kept at 30°C showed almost no change during 9 hours but with the lapse of 11 hours after milking, it was increased gradually and suddenly changed at 15 hours.

The alcohol susceptibility of milk did not show any remarkable change during 7-9 hours after milking. For instance the milk of cow No. 250 is coagulated barely by 95% alcohol in spite of the lapse of 9 hours after milking.

The milk of cow No. 284 which was coagulated by 86% alcohol at the beginning of its test was scarcely coagulated by 83% alcohol after standing for 7 hours.

Almost the same phenomena were recognized in the milk of cow However the alcohol susceptibility of these milk samples markedly increased after the lapse of 11 hours after milking.

Observing the above experimental results the author recognizes that the susceptibility of a milk which coagulates only with a strong concentrated alcohol at the beginning of the test, is not greatly accelerated by the lapse of time. For example, the milk of cow No. 250, coagulated by 98% alcohol at the beginning of the experiment, coagulated with the following concentrations of alcohol: 96, 95, 95, 95, 93, 81 and 45% after the passing of 3, 5, 7, 9, 11, 13 and 15 hours respectively.

On the other hand, mik of cow No. 284 which was coagulated by 86% alcohol at the beginning of the experiment was coagulated by a more dilute alcohol after the lapse of time, i.e., by 36% alcohol after 15 hours. The alcohol susceptibility of a third sample, milk from cow No. 316 was in just between the above two throughout the experiment.

It is of interest to know that the heat stability of milk is almost proportional to the alcohol susceptibility. This was shown by the milk of cow No. 284 which was coagulated by 86% alcohol at 1 hour after milking, coagulated by the heat to the degree of  $\pm$  at 13 hours after milking and also coagulated by the heat to the degree of +++ at 15 hours after milking. On the other hand, milk of cow No. 250 which was coagulated by 98% alcohol at the beginning of the experiment had barely coagulated by heat to the degree of ++ at 15 hours after milking.

The heat stability of the milk of cow No. 316 was in just between them.

Just the same phenomena are recognized with the milk samples of cow Nos. 283, 279 and 287 in these characters. This experiment was performed again by keeping the temperature at 30°C for 15 hours in an incubator. The alcohol susceptibility of milk did not occur until 9 to 11 hours after milking, but suddenly increased in each sample 13–15 hours after milking. The final degree of the progress in alcohol susceptibility was proportional to that at the beginning.

There was only one example, of milk of cow No. 283, which was coagulated by heat at 15 hours after milking. The alcohol susceptibility of this milk was strongest amongst all the samples.

Then it may be concluded that the alcohol susceptibility of milk kept at a definite temperature such as 30°C is increased suddenly at 13-15 hours after milking, and that it is proportional to the heat stability.

## VII. Influence of Oestrum of Cow upon the Alcohol Susceptibility of Milk and its Ash Constituents

It has already been reported by the author and a senior collaborator (34) that the first occurrence of oestrum will appeared on an average at 75 days after the parturition and it will be repeated in each 3-4 weeks provided the cow is not bred or fails to become pregnant.

No report has yet been available on the influence of oestrum upon the alcohol coagulation of milk nor upon the ash constituents of milk, although the subject has been studied in respect to other properties of milk.

ECKLES (11) reported in his book that there is no special change chemically in the milk and fat production during the period of heat. According to the study of STEUERT (86) it was recognized that in the oestrum period the milk production decreased and corpuscles of colostrum appeared in the milk, moreover the milk had a purgative action upon young calves and also it was more sensitive to the heat coagulation.

Weber (93) found that the milk and fat decreased during the period of heat. On the other hand there are some opinions at variance to the above experimental results. Pusch (61) Scharffer (73) Albrecht (1) concluded that the fat test increased during the period of oestrum. McCandlish (48) reported that the decrease in milk production during the period of heat is more than made up for by the increase in milk secreted for the 2 days before oestrum. Further he explained that in some animals there is no effect upon the milk production while in others the reverse may be true, and also that there is a close relation to the degree of oestrum strength.

The questions about the influence of heat upon the acidity of milk have been studied by a number of investigators. Jörg (37) Baummeister (7) Schmaltz (74) Weber (93) and Metzger (53) found by experiment that the acidity is increased during the period of oestrum, but Henkel (30) concluded that the acidity of milk produced during the period of heat is rather decreased.

FLEISCHMANN (18) reported that the milk production during the period of heat is influenced by the individuals but the opinion is commonly held that the milk and fat production and also the acidity of milk are decreased during the period of heat. There is only one report by Weber (93) concerning the influence of heat upon the alcohol coagulation of milk that the milk produced during the period of heat is coagulated by 68% alcohol. But there have been reported no systematic investigations about this subject such as the detailed determination of the alcohol susceptibility of milk and its ash constituents especially in CaO and  $P_2O_5$  produced during the period of heat.

In the chapter the author will report his observations on these questions in detail.

## A. Influence of Oestrum upon the Alcohol Susceptibility of Milk

#### 1. EXPERIMENTAL METHODS

Ten heads of Holstein and Guernsey cows belonging to the Second Farm of our university were used in this experiment. The alcohol susceptibility was determined of milk which was milked each evening throughout the whole experimental period.

Some accounts on the cows used in this experiment are as follows.

TABLE 72
Some accounts of the cows in the experiment

Breed	Cow No.	Date of birth		Date of parturitio		
Holstein	244	11/VIII	1923	6/XI	1928	
Guernsey	204	11/II	1919	1/XII	,,	
17	211	21/IV	1926	14/I	1929	
Holstein	M.Y.K.	12/II	1921	6/I	,,	
,,	272	20/VII	1926	23/XII	1928	
,,	250	21/V	1924	8/III	1929	
,,	254	9/VIII	,,	8/III	,,	
,,	259	26/IV	1925	12/II	,,	
,,	261	18/VI	. ,,	1/I	,, .	
Guernsey	193	<b>3</b> 0/III	1923	29/XI	1928	

The alcohol susceptibility of milk was determined by mixing 1 cc each of various alcohol percentages such as 98, 94, 90, 86, 82, 78,

74 and 70 into the same volume of milk keeping the contents of the test tube at the temperature of  $15^{\circ}$ C. The degree of casein coagulation was differentiated in three classes signified by the following signs ++, +,  $\pm$  according to the degree of coagulation. Boiling test was performed on the Bunsen flame using in each case 2 cc of milk in a test tube. If the coagulation occurred it is indicated by sign "+", if not by sign "-".

"Stall feeding" in the remark's column in the following tables means fed all day long in the stall; "exercise" means that the cows were allowed to take exercise about 2 hours after noon milking; "pasture" means that the cows were kept in pasture and allowed to consume green grass freely. Four herdsmen took charge of milking during the whole experimental period.

The experimental period continued for 75 days from 18/III 1929 to 31/V 1929.

#### 2. Experimental results

In the following tables will be recorded the experimental results on the alcohol susceptibility, acidity, and the heat stability of the milk of each cow produced daily during the whole experimental period.

TABLE 73

Influence of oestrum upon the alcohol susceptibility of milk

Cow No.	H. 244						
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks		
18/III	90++	0.163	_	+	Stall feeding		
19	90-	0.163			,,		
20	94-+	0.163	_	_	,,		
21	90±	0.170	_	_	,,		
22	90± '	0.170	-	_	,,		
23	94++	0.166	_	_	,,		
24	94 ±	0.165		-	,,		
25	94+	0.166	_	-	,,		

Table 73 (Continued)

Cow No.	H 244							
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks			
26/111	90±	0.172	_	_	Stall feeding			
27	90±	0.171	_	-	,,			
28	90±	0.172	_	-	,,			
30	90 ±	0.177	-	_	**			
31	94++	0.176	-	-	,,			
1/ <b>IV</b> ·	90±	0.177	_	-	,,			
2	90±	0.166	_	-	,,			
4	94++	0.172	_		,,			
5	90 ±	0.160	_	~	,,			
6	90±	0.158	_	-	,,			
7	90++	0.159	<u> </u>	++	,,			
8	86++	0.163	_	++	$\mathbf{Bred}$			
9	86±	0.167	-	-	,,			
10	90++	0.172	-		,,			
11	90+	0.169	<u> </u>		,,			
12	90++	0.174	_		,,			
13	86±	0.176	_	_	,,			
14	90++	0.176			,,			
15	90++	0.174	_	-	,,			
16	86+	0.163	_		**			
17	86±	0.169	! _	-	,,			
18	86±	0.169	-	_	Exercise			
19	86++	0.172	i —	_	,,			
20	82±	0.176	: -	_	Stall feeding			
21	90++	0.166	<u> </u>		,,			
22	86±	0.172			Exercise			
23	82±	0.169	<u> </u>		,,			
24	86++	0.165	i –		,,			
25	86++	0.165	_	_	**			
26	86++	0.163		_	,,			
27	82±	0.163		_	,,			
28	86++	0.165	_	_	,,			
29	86++	0.172	_	_	,,			
30	86++	0.165	-		,,			

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Table 73 (Continued)

Cow No.		H	244				
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks		
1/V	86++	0.167		_	Exercise		
2	86±	0.169	-	_	,,		
3	82±	0.165	_	_	,,		
4	86±	0.163	_	_	Stall feeding		
5	86±	0.162	_	-	,,		
6	86±	0.161	_	_	Exercise		
7	82±	0.161	_	_	,,		
8	86++	0.158	_	-	,,		
9	86++	0.161	_		,,		
Cow No.	G 204						
18/ <b>II</b> I	86±	0.187	_	_	Stall feeding		
19	86±	0.187	_	++	,,		
20	86±	0.187		+	,,		
21	86±	0.189	_	_	,,		
22	86±	0.194		\	,,		
23	86±	0.194			,,		
24	90+	0.190			,,		
25	90+	0.194	_	_	,,		
26	86±	0.194		_	"		
27	90+	0.190			,,		
28	90+	0.190	_	_			
<b>3</b> 0	90+	0.184	_	_	,,		
31	90±	0.189	_	_			
1/IV	90+	0.194		_	"		
2	90±	0.189	_	_	,,		
4	90+	0.187	_	_	"		
5	86±	0.187	_		,,		
6	90+	0.187	_		,,		
7	90+	0.185	_	• _	,,		
8	90++	0.178	_	++	,,		
9	80++	0.176		++	,,		
10	90++	0.110		++	,,		

Table 73 (Continued)

Cow No.	G 204						
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks		
11/IV	90++	0.183	_		Stall feeding		
12	90+	0.185		-	,,		
13	90+	0.185	-	-	,,		
14	90+	0.179		_	,,		
15	90- -	0.178	_	_	,,		
16	86±	0.180	_	-	,,		
17	86±	0.179	_	- 1	,,		
18	86±	0.180		_	,,		
19	82±	0.178	_	- 1	Exercise		
20	86++	0.176	_		,,		
21	86++	0.180	_	-	Stall feeding		
<b>2</b> 2	86±	0.180		_	,,		
23	86++	0.180	_	- 1	Exercise		
24	82++	0.178	_		,,		
25	82++	0.172	_	++	,,		
26	82+	0.173	_	+	,,		
27	86+	0.176	_	- 1	,,		
28	86±	0.178	_	_	, ,,		
29	86+	0.176	_	_	,,		
30	82±	0.180	_	_	,,		
1/V	86++	0.183	_		,,		
2	86+	0.176	_		,,		
3	86+	0.176		_	,,		
4	86+	0.178		_	Stall feeding		
5	90++	0.176	_	_	••		
6	86++	0.176	_	_	Exercise		
7	82±	0.176	_	_	,,		
8	86++	0.176	_	_	, ,,		
9	86+	0.178	_	_	,,		
Cow No.	1	(	÷ 211	, J			
18/III	94±	0.154	·		Stall feeding		
19	94±	0.158	_	_	,,		
10				]	**		

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Table 73 (Continued)

Cow No.		G	211		
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks
20/III	94±	0.153	_	_	Stall feeding
21	94±	0.142	-		,,
22	94±	0.150	_		,,
23	98++	0.143	_	- :	**
24	98++	0.150	-		**
25	98++	0.150	-	- '	,,
26	98++	0.147		- `i	,,
27	98++	0.150	_	-	**
28	98++	0.150	_	-	,,
30	98++	0.143	_	_ {	,,
31	98++	0.150	_	_	,,,
1/I V	98++	0.151	_	_	**
2	98++	0.142	_		,,
4	98++	0.154	_	_	**
5	98++	0.147	_		**
6	98++	0.156		_	. ,,
7	98++	0.151		_	**
8	98++	0.156	_		71
9	98++	0.154	_	_	,,
10	98++	0.150	_	_	
11	98++	0.156	_	_	**
12	98++	0.152	_	_	"
13	98++	0.151	_	_	"
14	98++	0.152	_	_	
15	98++	0.150	_	_	"
16	98++	0.150			
17	98++	0.150	_		,,
18	98++	0.150	_	_	**
19	94±	0.153		_	Exercise
20	94±	0.154			
21	98++	0.154	_		Stall feeding
22	98++	0.154	_	_	_
23	98++	0.154			,, Exercise
24	98++	0.152		_	-
7.3	30++	0.102	_	-	**

Table 73 (Continued)

Cow No.		(	G 211		
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks
25/IV	98++	0.150	_	_	Exercise
26	\$8++	0.143	_	_	,,
27	98++	0.147	_	-	39
28	98++	0.149	_	-	**
29	98++	0.147	_	-	**
30	98++	0.147	-	-	,,
1/V	98+	0.141	-	-	,,
2	98++	0.136	_	_	•,
3	94++	0.136		+	,,
4	94++	0.125	ļ. <u> </u>	++	Stall feeding
5	94 ±	0.134	_	-	,,
6	£4±	0.136	_		,,
7	94 ±	0.136	_	-	,,
8	98++	0.139	-	_	,,
9	98+	0.139	l. –	_	,,
11	98+	0.139	_	_	Exercise
13	98+	0.141	_	-	,,
14	98+	0.141	_	_	,,
15	98++	0.141	_		,,
16	98++	0.141	-	_	,,
17	98++	0.141	_	_	,,
	98+	0.143	_	-	,,
19	98+ .	0.139	_		**
20	98+	0.141	_	<u> </u>	,,
21	98 ±	0.145	_		,,
22	98±	0.154	_	_	Pasture
23	98+	0.150		_	**
24	98++	0.145	-	_	,,
25	94±	0.147	-	_	**
26	94±	0.147	_	-	,,
27	94±	0.142		+	,,
28	94+	0.136		++	,,
29	94±	0.141	_	_	,,
30	94±	0.143	-	_ ·	,,
31	94±	0.143	_	1 _ 1	,,

Table 73 (Continued)

Cow No.		M	.Y.K.		
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks
18/III	86+	0.159	_	_	Stall feeding
19	86+	0.160	_	-	,,
20	86+	0.162	_	-	**
21	90++	0.163	-	-	,,
22	90++	0.161	-	· -	,,
23	86±	0.160	-		,,
24	86±	0.158	_	-	**
<b>2</b> 5	86++	0.158	_	-	,,
26	86++	0.150	_	++	,,
27	86++	0.162		+	,,
<b>2</b> 8	86++	0.163		_	"
30	86++	0.164	_	-	**
31	86±	0.166	-	-	,,
1/IV	86+	0.167	_	_	,,
2	86+	0.167		_	,,
4	86+	0.166		_ '	,,
5	86++	0.164	_	-	,,
6	82±	0.167	_		,,
7	86++	0.166	_		,,
8	82±	0.163	_	_	,,
9	82±	0.162	(		,,
10	82±	0.158		_	,,
11	82++	0.158	_		,,
12	82++	0.158	_	_	,,
13	82±	0.157	_	_	,,
14	82±	0.147	_		,,
15	82++	0.147	_	++	,,
16	82++	0.147	_	++	,,
17	82+	0.154	_	_	,,
18	82±	0.156	_	_	,,
19	82±	0.156	_	_	Exercise
20	82±	0.153	_	] _ ]	,,
21	82±	0.155	_		Stall feeding
22	82±	0.156			

Table 73 (Continued)

Cow No.	! 	M	Y.K.		
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks
23/IV	82±	0.156	_	_	Exercise
24	82±	0.154	_	-	,,
25	82±	0.150	_		,,
26	82±	0.170	_	·	,,
27	82±	0.154	_	-	,,
28	82±	0.150	_		,,
29	82±	0.150	_		,,
30	82 ±	0.150	<u> </u>	-	,,
1/V	82±	0.150	_	l –	,,
2	82±	0.154	<del></del>	-	,,
3	82±	0.152	_ <del>_</del>	_	,,
4	82±	0.154	_	-	,,
5	82±	0.150	_	_	,,
6	82±	0.152	<b>\</b>	l – l	,,
7	82±	0.151	_	-	,,
8	82±	0.152	-		,,
9	82±	0.152		-	,,
11	82±	0.152		_	,,
13	82±	0.154	_	_ [	,,
Cow No.		F	272	<u> </u>	
18/III	86±	0.158	_	_	Stall feedin
19	86±	0.153	_	_	,,
20	86±	0.160			,,
21	90+	0.159	_	_	,,
22	86±	0.153	-	-	,,
<b>2</b> 3	86±	0.160	-		,,
24	90+	0.158	_	-	,,
25	. 90+	0.158	_	-	,,
26	90+	0.159			,,
27	86±	0.158		-	,,
28	86±	0.156	-		,,
30	86±	0.158	ĺ	1 _	,,

Table 73 (Continued)

Cow No.		H	272		
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks
31/III	86+	0.162		_	Stall feeding
1/IV	86+	0.159	-	-	,,
2	86±	0.158	_	_	,,
4	86±	0.160	_	_	,,
5	86±	0.161	-	-	,,
6	86±	0.159	<u> </u>	- 1	,,
7	86±	0.165	_	-	1,
8	86±	0.164		-	,.
9	86±	0.163		] - ]	,,
10	86±	0.161			**
11	86±	0.161			**
12	86±	0.163	_	-	,,
13	86±	0.165	_	_	,,
14	86±	0.165	<u> </u>	_	,,
15	86±	0.161		- 1	,,
16	86±	0.159	_	_	,,
17	86±	0.156	_	_	,,
18	86++	0.154	_	++	,,
19	86++	0.157		+	Exercise
20	86++	0.158	_	_	,,
21	90++	0.158	_	_	Stall feeding
22	90++	0.159		_	,,
23	85±	0.163	_	_	Exercise
24	86±	0.161	_	_	,,
25	86±	0.163	_	-	,,
26	86±	0.159	_		,,
27	86±	0.161	_	-	,,,
28	86±	0.163	_	_	,,
29	86±	0.161	_	_	,,
30	86±	0.154		·	,,
1/V	86±	0.154		_	,,
2	86±	0.156	_	_	, ,,
3	86 ±	0.154	_	-	,, ,,
4	86±	0.152	_	_	Stall feeding

Table 73 (Continued)

Cow No.		F	I 272					
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks			
5/V	86±	0.150	_	_	Stall feeding			
6	82+	0.150	_	-	Exercise			
7	82++	0.150	_	++	,,			
8	82+	0.143	<del>-</del>	+	,,			
9	86++	0.143			,,			
11	86+	0.150	_		,,			
13	86+	0.150	_	_	,,			
Cow No.	H 250							
18/III	94±	0.169	_	_	Stall feeding			
19	94 ±	0.170	_		,,			
20	94±	0.159	_		,,			
21	94±	0.164			,,			
22	98++	0.168	_	-	,,			
23	98++	0.167	<b>-</b> .	~ '	,,			
24	94±	0.163	_	-	,,			
25	94±	0.165	_	_	,,			
26	94±	0.159	_	_	,,			
27	94±	0.159		_	,,			
28	94±	0.161	_		,,			
30	98++	0.161	_		,,			
31	94±	0.162	_		,,			
1/IV	98++	0.164	_	-	,,			
2	98++	0.166		-	,,			
4	98++	0.167		-	,,			
5	98++	0.172			,,			
6	98++-	0.172	_		,,,			
7	98++	0.173	_		,,			
8 .	98++	0.164	_		,,			
9	98++	0.169		_	,,			
10	98++	0.168	_	~	. ,,			
11	98++	0.167	-	-	,,			
12	98++	0.172	_		,,			

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Table 73 (Continued)

Cow No.	H 250						
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks		
13/IV	98++	0.168	_	-	Stall feeding		
14	98++	0.167		-	,,		
15	98++	0.163	_	-	,,		
16	98++	0.161		_	,,		
17	98++	0.163	<u> </u>	-	,,		
18	98++	0.161	–	+	,,		
19	94+	0.158	_	_	,,		
20	98++	0.161	_	_	,,		
21	98±	0.163	<b>)</b> _	_	,,		
22	98±	0.172	_	-	,,		
23	98±	0.166	_	_	Exercise		
24	98±	0.172	_	_	,,		
25	98±	0.169	_	_	,,		
2 <b>6</b> .	98±	0.169	_	_	,,		
27	98±	0.172	_	- '	,,		
28	98±	0.170	_	_	,,		
29	98±	0.167		_	,,		
30	98±	0.163	_		,,		
1/ <b>V</b>	98+	0.163		_	,,		
2	98±	0.165	_	_	,,,		
3	98±	0.160		_ !	,,		
4	98±	0.160	_		Stall feeding		
5	98±	0.163	_	_	,,		
6	98±	0.169			Exercise		
7	98±	0.163	· -	_	,,		
8	98++	0.158	_	++	,,		
9	98+	0.161	_	_	"		
11	98±	0.172	_	_	,,		
13	98±	0.176		_	••		
Cow No.		Н	254				
18/III	86+	0.191		[	Stall feeding		
19	86+	0.190	_	_	,,		

Table 73 (Continued)

Cow No.	H 254						
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks		
20/III	86++	0.194	_		Stall feeding		
21	90++	0.194	_		,,		
22	86+	0.190	_	_	"		
23	86++	0.190	_	_	,,		
24	. 90++	0.187	_		,,		
25	86+	0.188	_	· _			
26	82±	0.189	_	_	,,		
27	86+	0.181			,,		
28	86+	0.178		_	**		
30	86±	0.180	_		,,		
31	90++	0.184	_		**		
					,,		
1/IV	- 86+	0.187	_	-	,,		
2	86+	0.186	_	-	"		
4	82±	0.183	_	-	"		
5	82±	0.180		-	,,		
6	82±	0.187	_	-	,,		
7	82±	0.183	_	-	,,		
8	82±	0.179	-	-	**		
9	82±	0.180	-	-	,,		
10	82±	0.176	-	-	,,		
11	82±	0.176		-	,,		
12	82±	0.178		-	,,		
13	82±	0.180	-	-	**		
14	82±	0.178	_	_	,,		
15	82±	0.178	_	_	,,		
16	82±	0.179	_	_	,,		
17	82±	0.178	_	_	,,		
18	82++	0.163	_	++	,,		
19	82++	0.161	_	++	"		
20	82±	0.162	_		,,		
21	86++	0.172	_	_	"		
22	82±	0.172	_	_	,,		

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Table 73 (Continued)

Cow No.		G	193		
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks
27/IV	74+	0.130	_	++	Exercise
28	82±	0.134	_	-	,,
29	86++	0.154			,,
30	86++	0.154	_	-	,,
1/V	86++	0.158	_	_	,,
2	86++	0.158	_	_	,,
3	82±	0.156	_	- '	,,
4	86++	0.156		_	Stall feeding
5	86+	0.154	_		,,
6	86++	0.151	_	_	Exercise
7	86+	0.150	_	_	,,
8	86+	0.152		_	,,
9	86+	0.154		_	,,
11	86++	0.154	-		,,
13	82±	0.150	_	_	,,
14	86++	0.154	-	_	,,
15	86++	0.141	_	_	,,
16	86++	0.141	_	_	,.
17	82+	0.110	_	+	,,,
18	78++	0.112	_	++	,,,
19	82+	0.136	_	_	,,
20	86++	0.147	_	_	,,
21	86++	0.154	_	_	,,
22	86++	0.150	_	-	Pasture
23	86++	0.150	_	-	,,
Cow No.		Н	259	<del>,, , , , , , , , , , , , , , , , </del>	
18/III	90++	0.169		_	Stall feeding
19	90++	0.174	_		,,
20	90++	0.168	_	_	,,
21	90+	0.170		_	,,
22	94++	0.165	_	_	,,
23	90+	0.176	_		,,

Table 73 (Continued)

Cow No.	,	H	259		
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks
24/III	90±	0.162	_		Stall feeding
25	90±	0.165	_	_	,,
26	94++	0.167	_	-	,,
<b>27</b> .	94+	0.162	_	-	,,
28	94+	0.161	_	-	,,
30	90±	0.159	-	-	,,
31	90±	0.171	_		,,
1/IV	90±	0.163	<u> </u>	- 1	,,
2	90±	0.163	_	_	,,
4	90±	0.162	_	_ {	,,
5	90±	0.159	_	-	,,
6	90±	0.161	_	-	17
7	90±	0.167	_	_	,,
8	94++	0.163	-	-	,,
9	90±	0.165	-	-	,,
10	86±	0.159		-	,,
11	90±	0.169	_	-	,,
12	86±	0.169		_	,,
13	90 ±	0.168		_	,,
14	90±	0.163	_	_	,,
15	90±	0.159	-		,,
16	90±	0.159		-	,,
17	90±	0.158		_	,,
_ 18	90±	0.158	-	_	,,
19	90++	0.161	_		Exercise
20	86±	0.162	_	_	,,
21	90++	0.163		_	Stall feeding
22	90++	0.163		]	,,
23	86±	0.167	_	_	Exercise
24	86±	0.163	_		,,
25	86+	0.159			,,
26	86±	0.158	_	_	, ,, ,,
27	86±	0.161			,,
28	86±	0.158		_	,,

Table 73 (Continued)

Cow No.	H 259											
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks							
29/IV	86±	0.158	-	-	Exercise							
30	86++	0.158	-	-	,,							
1/V	86++	0.154	_	++	,,							
2	86+	0.158	-	-	,,							
3	86+	0.161	_	_	,,							
4	90++	0.159	_	( - 1	Stall feeding							
5.	90++	0.158	_	_	,,							
6 .	86±.	0.158	_	_	Exercise							
7	86±	0.156	_	- 1	,,							
8	86±	0.158	_	_	,,							
9	86±	0.158	-	-	,,							
11	86±	0.158	_	-	,,							
13	86±	0.162		_	17							
14	86±	0.163	-	-	, ,,							
15	86±	0.163	_		,,							
16	86±	0.161	_	_	,,							
17	86±	0.165	_	_	**							
18	86±	0.161	-	_	,,							
19	86±	0.163	_	_	,,							
20	86±	0.161	-	_	,,							
21	86±	0.163	_	_	,,							
22	86±	0.167	_	-	Pasture							
23	86±	0.163	-	-	,,							
24	86±	0.167	_	_	,,							
25	86±	0.168	_	_	,,							
Cow No.		H	261	· · · · · · · · · · · · · · · · · · ·								
18/III	90±	0.148		++	Stall feeding							
19	90±	0.149	_	_	,,							
20	94++	0.150	_	_	,,							
21	94++	0.150	_	_	,,							
<b>2</b> 2	94+	0.146	_	_	,,							
23	94++	0.152		1	,,							

Table 73 (Continued)

Cow No.		Н 261											
Date 1929	Alc. % and degree of milk coagulation	Acidity	Boiling test	Oestrum	Remarks								
24/III	94++	0.145	_	_	Stall feeding								
25	94+	0.147	_		,,								
26	94 ±	0.150	_	_	**								
27	94±	0.149		_ '	,,								
28	94±	0.149	_	_	,,								
30	94±	0.151	_		,,								
31	94++	0.148	·_		,,								
-		0.147											
1/IV	94±	0.147	_	_	**								
2	94±	0.150			"								
4	94+	0.145	_	_	**								
5	94+	0.143	-	_	,,								
6	94±	0.146 $0.149$	_		,,								
7	90±	0.149		+	,,								
8	90±		_	++	"								
9	94+	0.144	_	_	**								
10	94+	0.154	_	-	,,								
11	94+	0.158	_	_	"								
12	94±	0.158		_	"								
13	98++	0.152	_	_	"								
14	98++	0.154	_	_	"								
15	98++	0.154	-	-	,,								
16	98++	0.152	-	-	**								
17	98-+	0.153	_	-	,,								
18	98+	0.153	_	-	,,								
19	98++	0.147			Exercise								
20	94±	0.156	-	-	,,								
21	94±	0.150	_	-	Stall feeding								
22	98++	0.154	_	-	,,								
23	94±	0.150			Exercise								
24	98++	0.152	-		,,								
25	94±	0.148	- '	1 – 1	,,								

The author will observe here whether or not the oestrum does exert an influence not only on the alcohol susceptibility and heat stability but also on the acidity of milk.

Cow No. 244 came to heat on 18/III and the milk was coagulated by the addition of 90% alcohol in a degree of ++, the acidity was 0.163%, but the alcohol susceptibility decreased showing coagulation by 94% alcohol in the degree of  $\pm$  after the passing of the oestrum period but on the contrary the acidity increased a little to 0.169%.

There was no positive reaction to the boiling test observed during the oestrum period.

The same cow came in heat again on 7-8/IV, the milk was coagulated by 90% alcohol to a degree of ++ or by 86% alcohol to a degree of  $\pm$ , and the acidity decreased a little because of oestrum.

Cow No. 204 was in heat on 19/III and the alcohol susceptibility of milk increased a little and also the acidity decreased during the period of oestrum. The same cow came in heat again on 8–9/IV and the alcohol susceptibility of milk increased showing the coagulation by 90% alcohol to a degree of ++ although it showed coagulation by 90% alcohol to a degree of  $\pm$  before the oestrum. Also the acidity of milk produced during the period of the oestrum decreased to 0.176% from 0.185%. This cow came in heat again on 25/IV, and there appeared just the same phenomena as before: the milk which reacted to 86% alcohol to a degree of ++ in the normal condition was coagulated by 82% alcohol to a degree of ++ on account of the oestrum, and also the acidity decreased to 0.172% from 0.180%. But these characteristics were restored to the former condition as soon as the oestrum diminished.

Guernsey cow No. 211 showed a sign of heat very distinctly on 4/V and the alcohol susceptibility of the milk increased to such a degree that the coagulation has taken place ever with 94% alcohol, and the acidity decreased to 0.125% from 0.147%. This cow again heated on 28/V and both these properties changed a little though the degree was not so marked as before.

Holstein cow M. Y. K. heated on 26/III and the alcohol susceptibility of milk increased to coagulation by 86% alcohol in a degree of  $\pm$  but the acidity decreased to 0.154% from 0.158%. On 15-16/IV this cow was again in heat and the milk which had been coagulated by 82% alcohol in degree of  $\pm$  was coagulated by 82% alcohol in degree of ++; the acidity was lowered to 0.147% from 0.158%.

Cow No. 272 came in heat on 18/IV, the alcohol susceptibility of milk increased to degree of ++ from a degree of  $\pm$  though the alcohol percentage was the same in both cases; the acidity decreased to 0.154% from 0.161%. This cow came in heat again on 7/V and the alcohol susceptibility of milk increased to coagulation by 82% alcohol in a degree of + or  $\pm$  and the acidity of milk decreased to 0.150-0.143% from 0.154%. The same tendency was observed on the milk produced during the periods of oestrum by cow No. 250 in both cases of 19/IV and 8/V.

In cases of Holsteins Nos. 254, 259, and 261, each of them came in heat once or twice during the experimental period, and in each case the milk produced during the period of the oestrum increased a little in alcohol susceptibility but the acidity decreased in comparison with the normal condition.

Representative examples may be pointed out as follows: the milk of Guernsey cow No. 193 was caused to coagulate by 74% alcohol in degree of + on account of oestrum but this characteristics decrease markedly as soon as the oestrum diminished when the milk was scarcely coagulated by 86% alcohol in degree of ++. Also the acidity of milk produced during the period of oestrum dropped to 0.130% from 0.158%. When this cow came in heat again on 18/V the acidity of milk decreased to 0.112% from 0.154% and also the alcohol susceptibility of milk increased being coagulated by 78% alcohol instead of 86%.

The result of the boiling test in connection with above tests was apparently negative.

There were 10 heads of cows used in this experiment and 19 samples of milk produced during the period of oestrum. By observing each sample the author recognizes clearly that the alcohol sus-

ceptibility of milk increased but the acidity decreased a little on account of oestrum. The heat stability of milk is not changed at all by the occurrence of oestrum at least in so far as can be found by employing the boiling test. Moreover there has been no recognizable influence upon the alcohol susceptibility of milk by the changing of the management such as stall feeding, exercising, or pasturing respectively though these problems will be considered in more detail in a later chapter.

# B. Influence of Oestrum upon the Ash Constituents of Milk

In the preceding section it has been shown that the alcohol susceptibility of milk is influenced by the oestrum. The author next tried to ascertain whether or not the ash constituents of milk are influenced by the oestrum.

# a. Ash constituents of milk secreted at the time of heat determined by the general analytical methods

#### 1. Experimental methods

Milk samples used in this experiment came from the Second Farm of the university. Determinations were made of specific gravity, and fat percentages. Specific gravity was determined by using the lactometer, fat percentage by BABCOCK method. The general analytical method applied in this experiment has already been described in chapter IV.

#### 2. Experimental results

The experimental results in regard to this subject are as follows.

Table 74 Influence of oestrum upon the ash constituents of milk

Sample No.	Date o	of sample	Cow No.	Oest- rum	Sp. gr.	Fat	Total solids	Ash	CaO	MgO	$P_2O_5$	Cl	$SO_3$	Na₂O	K <sub>2</sub> O	$\mathrm{Fe_2O_3}$	Re- marks
1	Noon	11/VI '29	260		1.0316	4.15	13.012	0.842	18.390	2.023	29.949	12.915	2.480	7.926	25.807	0.428	
2	Noon	15/VI '29	,,	++	1.0316	3.65	12.875	0.766	19.939	<b>2.13</b> 0	30.119	13.522	2.526	7.668	26.154	0.431	
3	Noon	20/VI '29	,,	_	1.0318	<b>3.</b> 90	12.874	0.834	19.853	2.023	29.949	12.915	<b>2.4</b> 80	<b>7.92</b> 6	25.807	0.428	
Αv	erage of	1 and 3	,,	_	1.0317	4.03	12.943	0.838	19.122	2.023	29.949	12.915	2.480	7.926	25.807	0.428	
	Balan	ce	,,		-0.0001	-0.38	-0.068	-0.072	+0.817	+0.107	+0.170	+0.607	+0.046	-0.258	+0.347	+0.003	
4	Noon	12/VI '29	237		1.0329	3.30	11.768	0.687	20.555	1.912	30.527	13.522	2.530	7.690	24.384	0.352	'
5	Noon	16/VI '29	,,	++	1.0326	<b>2</b> .95	11.703	0.740	20.802	1.912	30.738	13.829	2.631	7.754	24.283	0.348	i
6	Noon	21/VI '29	,,		1.0324	3.40	11.901	0.655	19.911	1.946	29.991	13.643	2.626	8.126	24.256	0.350	
Ave	erage of	4 and 6	,,	_	1.0327	3.35	11.835	0.671	20.233	1.929	30.259	13.589	2.578	7.908	24.320	0.351	
	Balan	<b>c</b> e	,,		-0.0001	<b>-0.4</b> 0	-0.132	+0.069	+0.569	-0.017	+0.479	+0.242	+0.053	-0.154	-0.037	-0.003	
7	Evenin	g 10/X '29	252	++	1.0321	4.00	12.137	0.688	21.687	2.173	29.593	14.859	2.641	7.298	23.968	0.333	
8	Evenin	g 15/X '29	,,	_	1.0326	4.20	12.287	0.702	19.802	1.946	28.817	13.644	2.758	7.642	24.164	0.342	
	Balan	ce	,,		-0.0005	-0.20	-0.150	_0.014	+1.885	+0.227	+0.776	+1.215	-0.117	-0.344	-0.196	-0.009	

Sample No.		of sample aken	Cow No.	Oest- rum	Sp. gr.	Fat %	Total solids	Ash	CaO	MgO	P <sub>2</sub> O <sub>5</sub>	Cl	SO <sub>3</sub>	Na <sub>2</sub> O	K <sub>2</sub> O	$Fe_2O_3$	Re- marks	
9	Evenin	g 19/IX '3	0 287	++	1.0328	3.60	13.626	0.771	19.736	2.177	29.776	12.930	2.586	7.333	24.908	0.346		
10	Evenin	g 23/IX '3	0 ,,	-	1.0330	3.90	<b>13.96</b> 0	0.756	19.493	2.184	30.748	12.607	2.626	7.658	23.985	0.350		
	Balan	ce	,,		-0.0002	-0.30	-0.334	-0.015	+0.243	-0.007	-0.972	+0.323	-0.038	-0.325	-0.923	-0.004		
11	Noon	8/X '3	0 287	++	1.0326	3.55	12.813	0.763	20.361	2.350	28.777	13.825	2.652	8.164	24.130	0.402		KEN
12	Noon	14/X '3	,,	_	1.0325	3.75	12.871	0.775	19.214	2.209	29.161	13.331	2.695	8.028	25.245	0.402		KENTARO
	Balan	ce	,,		+0.0001	-0.20	-0.058	-0.012	+0.147	+0.141	-0.384	+0.494	-0.043	-0.136	-1.115	0		_
13	Noon	10/X '3	283	++	1.0305	3.35	12.128	0.745	20.880	2.241	29.769	12.703	2.780	7.116	23.806	0.374		MITAMURA
14	Noon	14/X '3	,,	_	1.0305	3.50	12.227	0.730	20.198	2.249	31.387	12.423	2.752	7.082	24.612	0.402		JRA
	Balanc	ce	,,		0	-0.15	-0.095	+0.015	+0.682	-0.008	-1.618	+0.280	+0.028	-0.034	-0.806	· 0.028		
A	erage o	f normal	nilk	_	1.0321	3.79	12.687	0.745	19.677	2.093	30.054	13.084	2.648	7.707	24.689	0.379	8 samples	
Av	erage of	f oestrum	milk	++	1.0320	3.52	12.547	0.746	20.567	2.164	29.795	13.611	2.636	7.556	24.542	0.372	6 samples	
	Bal	lance			-0.0001	-0.27	-0.140	+0.001	+0.891	+0.671	+0.259	+0.527	-0.012	-0.151	-0.127	-0.007		

# 3. DISCUSSION

The values of the specific gravity, fat percent and total ash in the milk of cow No. 260 produced during the period of oestrum are 1.0316, 3.65% and 0.766% respectively, but these values under normal condition for this cow were on the averages 1.0317, 4.03% and 0.838%. The percentages of the ash constituents were as follows: 19.939% CaO, 2.130% MgO, 30.119%  $P_2O_5$ , 13.522% Cl, 2.526%  $SO_3$ , 7.668%  $Na_2O$ , 26.154%  $K_2O$  and 0.431%  $Fe_2O_3$ . But the average ash composition of the normal milk obtained before and after the oestrum period were as follows: 19.122% CaO, 2.023% MgO, 29.949%  $P_2O_5$ , 12.915% Cl, 2.480%  $SO_3$ , 7.926%  $Na_2O$ , 25.807%  $K_2O$  and 0.428%  $Fe_2O_3$ .

There may be found some difference in individuals, but most ash constituents except Na<sub>2</sub>O in the oestrum milk increased a little in comparison with the normal milk.

With the milk from cow No. 237, the specific gravity is almost the same in both cases, but the fat percent and total solid decreased on account of oestrum, while total ash increased a little. On the other hand there are increases of CaO,  $P_2O_5$ , Cl and  $SO_3$  but slight decreases in MgO, Na<sub>2</sub>O, K<sub>2</sub>O and Fe<sub>2</sub>O<sub>3</sub> in the total ash.

The specific gravities are almost the same in both cases in cow Nos. 252, 287, and 283 but the fat percent and total solid of the oestrum milk decreased in comparison with normal. The ash constituents in milk produced by cow No. 252, especially CaO, MgO,  $P_2O_5$ , Cl increased a little but  $SO_3$ ,  $Na_2O$ ,  $K_2O$  and  $Fe_2O_3$  decreased in the oestrum period though the degree is very slight. The amounts of CaO, Cl and  $Na_2O$  increased in the oestrum milk of cow No. 283, but MgO,  $P_2O_5$ ,  $K_2O$  and  $Fe_2O_3$  contents decreased.

By averaging the above 6 samples it will be recognized that the specific gravity and total ash are almost the same in both cases but fat percent and total solid decreased on account of oestrum. The ash constituents in the oestrum milk, especially CaO, MgO and Cl are increased but on the contrary  $P_2O_5$ ,  $SO_3$ ,  $Na_2O$ ,  $K_2O$  and  $Fe_2O_3$  are decreased a little in comparison with normal.

# b. Ash constituents especially Ca, Mg, Cl and P content in milk secreted at the heat period determined by the microanalytical methods

From the above it may be recognized that there are close relations between the ash constituents especially CaO, MgO, Cl and  $P_2O_5$  and the alcohol susceptibility of milk produced during the period of oestrum. In this connection it will be very interesting to make a microchemical study, aside from the general analytical one, on the ash constituents during the oestrum period.

#### 1. Experimental methods

Cows used in this experiment belonged to the Second Farm of our university. The milk samples were taken at the time of strong oestrum and 4-7 days after the oestrum for the same cows.

The author determined the contents of Cl, Ca, P and Mg in milk samples by applying the microanalytical method (80) which will be given in detail in later chapters XIII and XIV. He also determined the acidity and alcohol susceptibility of these milk samples.

#### 2. Experimental results

The experimental results concerning to the oestrum and normal milk are as follows.

Table 75

Experimental results with oestrum milk (mg/dl)

Date		Cow	Oest-	Acidity	Alc. % degree coagul	of milk	Cl	P	Ca	Mg	
		No.	rum		Experi- mented	Con- verted					
19/I	1933	260	+	0.180	86±	87+	187.20	62.50	109	16.45	
19/I	1933	274	+	0.155	82±	83+	195.98	47.62	116	15.35	
19/I	1933	301	++	0.140	90±	91+	209.20	51.28	121	14.25	
2 <b>3/I</b>	1933	244	++	0.180	86±	87+	157.95	52.63	112	16.98	
30/I	1933	310	++	0.165	90±	91+	140.04	57.69	111	14.51	

Table 75 (Continued)

Da	Date Co		Oest-	Acidity	Alc. % degree o coagul	f milk	Cl	P	Ca	Mg	
Negotian delina en		No.	rum		Experi- mented	Con- verted					
7/II	1933	301	+	0.150	82±	83+	197.33	62.50	119	14.34	
13/II	1933	304	+	0.160	86+++	84+	186.62	57.15	100	13.89	
6/III	1933	254	+	0.175	$74\pm$	75+	157.95	52.63	110	15.00	
8/III	1933	310	+	0.160	94+++	92+	180.77	64.52	111	15.62	
9/111	1933	260	++	0.185	78+	78+	<b>163.8</b> 0	58.82	112	15.95	
	Averag	;e	+	0.165		85.1+	177.68	56.73	112.1	15.23	

Next are shown the experimental results with normal milk produced after the oestrum in the same cows.

 $TABLE\ 76$  Experimental results with normal milk (mg/dl)

T).		Cow	A '11'4		nd degree agulation	G)		a	
Da	i <b>c</b> e	No.	Acidity	Experi- mented	Con- verted	Cl	P	Ca	Mg
23/I	1933	260	0.200	86±	87+	160.88	63.29	98	16.64
23/I	1933	274	0.155	86+	86+	181.35	47.62	106	15.43
30/I	1933	244	0.185	86±	87+	145.08	62.50	109	15.91
7/II	1933	310	0.160	94++	93+	137.15	60.00	103	15.91
13/II	1933	301	0.160	86+	86+	180.18	62.50	109	13.39
10/III	1933	254	0.180	78++	77+	164.97	54.05	104	15.62
10/III	1933	260	0.185	82+	82+	162.05	58.82	106	16.30
12/III	1933	310	0.165	94+	94+	167.90	62.50	108	15.95
	Average		0.171		87.2+	166.26	58.90	106.0	15.48

In the next table are set down the average experimental results in two cases.

Table 77
Comparison of the average experimental results with oestrum and normal milk (mg/dl)

Number of samples	Oestrum	Acidity	Alc. % and degree of milk coagulation Converted	Cl	P	Ca	Mg
10	+	0.165	85.1+	177.68	56.73	112.1	15.23
9	_	0.171	87.2+	166.26	58.90	106.0	15.48
Balance		-0.006	-2.1+	+11.42	-2.17	+6.1	-0.25

There are 10 samples of milk produced during the period of oestrum and 9 samples of normal milk each from the same cow in this experiment. The author will discuss the experimental results obtained and tabulated in table 77.

The acidity in the two cases is 0.165 and 0.171% respectively and also they are respectively coagulated by 85.1 and 87.2% alcohol to a degree of +.

It will be recognized that the acidity of oestrum milk is lower than that of normal milk, and the alcohol susceptibility is a little higher in comparison with normal which agrees well with the experimental results described above in section "a" of this chapter.

The average contents of Cl, Ca, Mg, and P in the two cases are respectively 177.68 and 166.28 mg/dl of Cl, 112.1 and 106.0 mg/dl of Ca, 15.23 and 15.48 mg/dl of Mg and 56.73 and 58.90 mg/dl of P. There is a little increase of Cl (11.42 mg/dl) and Ca (6.1 mg/dl) and a little decrease of P (2.17 mg/dl) and Mg (0.25 mg/dl).

These experimental results are almost the same as reported in the preceding section of this chapter except Mg content.

Therefore it may be concluded that the oestrum has practically no influence on specific gravity and total ash of milk but has tendency to increase the alcohol susceptibility a little and to lower the acidity slightly. It is also indicated that the alcohol susceptibility of milk is influenced by the ash constituents of the oestrum milk especially by the increase of Ca and Cl, and the decrease of P.

# VIII. Influence of Salts Dosage upon the Alcohol Susceptibility of Milk

It has just been ascertained in the foregoing chapter that the alcohol susceptibility of milk is influenced by the ash balance especially by the content of calcium, magnesium and phosphorus. Consequently it becomes necessary to investigate whether or not the ash balance and the alcohol susceptibility of milk are influenced by certain salts dosage for the cow. Therefore the author experimented as described in this chapter to ascertain the influence of a high dose of salts upon the alcohol susceptibility of milk. The procedure was to mix the following salts in ration singly: bone meal, calcium lactate, calcium carbonate, magnesium carbonate, di-potassium phosphate, sodium bicarbonate, sodium citrate, di-sodium phosphate and phoscalpin.

It may be reviewed here some of the studies on this subject already reported by many investigators.

Lander and Fagon (41) found that a plentiful dosage of calcium phosphate for a cow does not exert any influence on the ash constituents of milk. Jordan, Hart and Patten (36) also obtained a similar result concerning the phosphates. According to the experiments of Schrott and Hansen (75) there is a slight increasing tendency of calcium and phosphorus in milk by changing from stall to pasture feeding. Jensen (35) reported that the plentiful giving of each of iron lactate, sodium phosphate, calcium chloride or sodium chloride to dairy cows did not influence the ash constituents of milk.

FINGERLING (14) as a result of his experiments concluded that a plentiful calcium feeding does not influence on the milk constituents, but on the contrary that the milk secretion will be depressed gradually by a deficient feeding of calcium and later a decrease of calcium content in milk will be brought about.

It is generally recognized that the process of assimilating calcium and phosphorus in the organic form is more efficient than those in the inorganic form as shown in the experiments of MEIGS, TURNER and HARDING (51).

HART and STEENBOCK (26) found that when cows are fed with green alfalfa the assimilation of calcium and phosphorus is better than when hay is fed.

ORR and CRICHTON (58) reported a good influence of calcium carbonate, iron oxide, and potassium iodide upon the milk produc-

tion. KRAUE (40) found that the addition of calcium chloride efficiently helps the calcium assimilation of dairy cow.

From the study of AUZINGER (3) one learns the alcohol susceptibility of milk has been increased by the addition of plentiful calcium materials such as calcium phosphate at a rate of 120 g. per cow per day. Sommer and Binney (81) also obtained the result that the plentiful calcium dosage for dairy cow exerted an influence upon the alcohol susceptibility of milk, but did not change the calcium content in the milk ash.

Wendt (95) reported that there was no influence of a plentiful feeding of sodium chloride, sodium phosphate, magnesium salt or calcium phosphate upon the ash constituents of milk.

MILLER, BRANDT and JONES (55) proved that the deposition of calcium and phophorus in the body becomes positive by the mixing of plentiful bone meal in the ration. Moreover MAEOMBER (44) found that the cow which has been fed with a ration deficient in calcium for a long time will be unhealthy and that at last a bad influence upon the gestation of the cow will appear.

SOMMER and LERCH (82) obtained the experimental result that the alcohol susceptibility, acidity, and the content of calcium and phosphorus in milk are not influenced by feeding of a plentiful amount of precipitated calcium carbonate to the dairy cow. According to the study of McDonald (50) there is no influence upon the calcium and phosphorus content in the milk and blood of a cow in spite of a plentiful dosage of calcium phosphate.

On the other hand HUFFMAN and his coworkers (32) reported recently that giving a phosphorus ration to young calves, for about 90 days after birth, has resulted in decreasing the phosphorus content in the blood; but on the contrary there was an increasing of the calcium content.

There are many other similar investigations but after all they come to conclusion that the milking cow be supplied with sufficient supply of calcium and phosphorus in the ration mainly in the form of bone meal, calcium carbonate, and at the same time the animal must receive the vitamin D or the ultra-violet ray on the body.

The author will describe here the results of his experiments on the influence of feeding various kinds of mineral salts upon the alcohol susceptibility of milk. Each salt will be dealt independently.

#### A. Bone Meal

#### 1. EXPERIMENTAL METHODS

The author carried the experiments with this salt twice. In the first experiment 3 Ayrshire cows from the First Farm of our university were used. One complete experimental period of 36 days were divided into 3 periods of 12 days each. The 1st and 3rd periods were controls and the 2nd was the experimental period during which the cows are given 150 g. of bone meal in the ration. There were fed 22 kg. of corn silage, 3.0 kg. of timothy hay, and 1.9 kg. of the mixture of wheat bran and soybean cake per day for each cow throughout the whole experimental period.

Some accounts of the experimental cows are as follows.

Cow No.	86		85		110	0	
Date of birth	30/VIII	1916	27/XII	1915	28/VI	1921	
Date of parturition	20/II	1929	24/VII	1929	13/VI	1929	
Date of next parturition	14/VII	1930	6/V	1930	22/XII	1930	
Daily milk production kg.	4.0	)	3.0	0	3.0		

In the second experiment, four Holstein cows from the Second Farm of the university were used for 45 days, divided into 3 periods. Cows No. 255 and 252 were fed 300 g. of bone meal during the 1st and 3rd periods but cows No. 239 and 275 were given these materials in the 2nd period only.

Some accounts of these cows are as follows.

Cow No.	25	5	23	9	255	2	275	
Date of birth	15/VII	1924	23/VI	1923	6/VII	1924	19/I	1927
Date of parturition	15/VII	1929	24/VII	[ 1929	16/IX	1929	31/VII	1929
Date of next parturition	15/XI	1930	21/X	1930	18/XI	1930	13/V	1931
Daily milk production kg.	12.0		8.	0	15.	0	14.0	

The daily ration consisted 9 kg. of timothy hay, 5–7 kg. of mixture of candy-cake, green peas, copra meal, crushed oats and 100 g. of salt in addition to which 40 g. of cod liver oil were added daily throughout the whole experimental period.

The acidity and the alcohol susceptibility of each milk were determined throughout the experimental period.

#### 2. Experimental results

The experimental results with the Ayrshire cows are as follows.

Table 78

Influence of bone meal dosage upon the alcohol susceptibility of milk

	Cow No.	1	86			· 85			110		A.	verage
Experi-	Date	Acidity	of milk co	nd degree agulation	Acidity	Alc. % ar of milk co		Acidity	Alc. % an of milk co		Acidity	Alc. % and degree of milk coa-
period	Date	Acidity	Experi- mented	Con- verted	Actuity	Experi- mented	Con- verted	Actuity	Experi- mented	Con- verted	Acturty	gulation Converted
	20/XI	0.158	82+	82+	0.135	78+	78+	0.138	74±	75+	0.137	78.3+
	21	0.140	82+	82+	0.135	78+	78+	0.140	$74\pm$	<b>75</b> +	0.138	78.3+
	22	0.130	78±	<b>79</b> +	0.135	78+	78 +	0.140	$74\pm$	<b>75</b> +	0.133	77.3+
	23	0.130	77±	<b>79</b> +	0.135	78+	78+	0.140	$74\pm$	<b>7</b> 5+	0.135	77.3+
1st.	24	0.136	78±	79+	0.130	73+	78+	0.140	78+	78+	0.135	78.3+
•	25	0.140	78 ±	79+	0.130	78+	<b>7</b> 8+	0.140	78±	79+	0.137	78.3+
Con-	26	0.130	82++	81+	0.120	78+	78+	0.148	78+	<b>78</b> +	0.133	79.0+
trol	27	0.135	78±	<b>79</b> +	0.120	78+	78+	0.150	78+	<b>7</b> 8+	0.135	78.3+
	28	0 132	78±	<b>79</b> +	0.120	78+	<b>7</b> 8+	0.150	78+	78+	0.134	78.3 +
	29	0.130	78±	<b>79</b> +	0.115	<b>78</b> +	78+	0.150	78+	78+	0.132	78.3+
	30	0.1 2	82++	81+	0.118	78 +	78+	0.148	78++	77+	0.133	78.7+
1	1/XII	-	_	_		_	_	_	_	_	_	_
	2	0.130	82++	81+	0.115	78+	78+	0.148	78++	77+	0.130	78.7+
	3	0.135	78±	79+	0.110	78++	77 +	0.148	78++	77+	0.131	77.7+
2nd.	4	0.132	78+	<b>78</b> +	0.125	78+	78+	0.150	74±	75+	0.136	77.0+
Bone	5	0.135	78+	<b>7</b> 8+	0.117	78+	78+	0.140	74±	<b>75</b> +	0.131	77.0+
meal	6	0.135	78+	<b>78</b> +	0.110	<b>7</b> 8+	<b>7</b> 8+	0.140	74±	<b>75</b> +	0.128	77.0+
	7	0.125	78+	78+	0.115	78±	79 +	0.138	$74\pm$	75+	0.126	77.3+

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Table 78 (Continued)

	Cow No.		86			85			110		A	verage
Experi- mental	Date	Acidity	Alc. % an of milk co	d degree agulation	A -: 3:4	Alc. % ar	nd degree agulation	A -: 3:4	Alc. % ar	nd degree pagulation		Alc. % and degree of
period	Date	Actuity	Experi- mented	Con- verted	Acidity	Experi- mented	Con- verted	Acidity	Experi- mented	Con- verted	Acidity	miik coa- gulation Converted
	8/XII	0.124	78+	78+	0.110	78±	79+	0.140	74±	75 +	0.125	77.3+
	9	0.120	78+	78+	0.115	78±	79+	0.140	$74\pm$	<b>75</b> +	0.125	77.3+
2nd.	10	0.117	78+	78 +	0.120	78++	77+	0.140	$74\pm$	75 <i>+</i>	0.126	76.7+
Bone	11	0.120	78++	77+	0.117	78++	77 +	0.145	$74\pm$	75+	0.127	76.3 +
meal	12	0.110	78++	77 +	0.115	78++	77 +	0.140	74±	75+	0.122	76.3+
	13	0.112	78++	77+	0.114	78++	77+	0.143	$74\pm$	75+	0.123	76.3 +
	14	0.120	74+	74+	0.105	78++	77+	0.142	74±		0.122	75.3+
	15	_	_	_	<b> </b>	_		_		_	_	_
	16	0.110	74±	75+	0.120	74+	74 +	0.140	78++	77+	0.123	75.3 +
	17	0.120	74+	74 +	0.105	74+	74+	0.140	78++	77+	0.122	75.0+
3rd.	18	0.120	74++	73 +	0.113	74+	74+	0.148	$74\pm$	75+	0.127	75.0 +
	19	0.115	74+	74+	0.112	78++	77+	0.150	74+	74+	0.126	75.0+
Con-	20	0.110	78++	77 +	0.105	78++	77+	0.142	74+	74+	0.119	76.0+
trol	21	0.110	$74\pm$	75+	0.110	74±	75+	0.145	78++	774	0.122	75.7+
	22	0.120	74+	74+	0.115	74+	74 +	0.145	78++	77+	0.127	75.0 +
	23	0.120	74±	75+	0.115	74±	75+	0.140	74±	<b>75</b> +	0.125	<b>75.</b> 0+
	24	0.110	74±	75 +	0.110	78++	77+	0.136	74+	<b>74</b> +	0.119	75.3 +
	25	_	-	-	_	_	-	-	_	****		

The experimental results in regard to the Holstein cows are as follows.

 ${\bf TABLE~79}$  Influence of bone meal dosage upon the alcohol susceptibility of milk

	Cow No.		255			239			252			275	
Experi- mental	Date	Acidity	Alc. % degree coagul	of milk	Acidity	Alc. % degree coagul	of milk	Acidity	Alc. % degree coagul	of milk	Acidity	Alc. % degree coagul	ot milk
period			Experi- mented	Con- verted		Experi- mented	Con- verted		Experi- mented	Con- verted		Experi- mented	Con- verted
lst	5/VI 6 7 8 9 10 11 12 13 14 15 16 17 18	0.175 0.180 0.177 0.180 0.180 0.180 0.180 0.175 0.175 0.175 0.175 0.175 0.175 0.175	$82 + \\ 82 + \\ 82 + \\ 78 \pm \\ 78 \pm \\ 82 + \\ $	82+ 81+ 81+ 79+ 79+ 81+ 81+ 82+ 82+ 82+ 82+ 81+ 81+	0.170 0.175 0.165 0.175 0.160 0.156 0.152 0.160 0.160 0.162 0.160 0.156 0.155	$78++\ 78++\ 74++\ 74++\ 74\pm\ 78+\ 74\pm\ 74\pm\ 74\pm\ 74\pm\ 78++\ 74\pm\ 78++\ 74\pm\ 78++\ 74\pm\ 78++\ 74\pm$	77+ $77+$ $73+$ $73+$ $75+$ $75+$ $78+$ $75+$ $75+$ $75+$ $75+$ $75+$ $75+$ $75+$ $75+$	0.135 0.145 0.150 0.150 0.140 0.159 0.145 0.145 0.146 0.140 0.145 0.146 0.140 0.140	$\begin{array}{c} 82 \pm \\ 82 + \\ 82 \pm \\ 82 + \\ 82 + \\ 82 + \\ 82 + \\ 86 + + \\ 86 + \\ 86 + \\ 86 + \\ 86 + + $	83+ $82+$ $83+$ $82+$ $82+$ $82+$ $85+$ $85+$ $85+$ $85+$ $85+$ $85+$	0.172 0.175 0.165 0.163 0.160 0.165 0.150 0.150 0.154 0.160 0.154 0.162 0.154	$74++\ 78++\ 78++\ 78++\ 78++\ 78++\ 78++\ 78++\ 78++\ 74+\ 78++\ 78++\ 78++\ 78++\ 78++\ 78+$	73+ 77+ 78+ 77+ 77+ 77+ 77+ 77+ 77+ 77+ 77
2nd	20 21 22 23 24 25	0.170 0.170 0.165 0.170 0.173 0.165	82++ 82++ 82++ 82++ 82± 86++	81+ 81+ 81+ 81+ 83+ 85+	0.145 0.140 0.150 0.145 0.140 0.140	78+ 78++ 82++ 78± 82++ 82+	78+ 77+ 81+ 79+ 81+ 83+	0.135 0.140 0.143 0.140 0.130 0.130	82± 82+ 82++ 82++ 82+ 82±	83+ 82+ 81+ 81+ 82+ 83+	0.158 0.170 0.160 0.163 0.160 0.158	$74++\ 74++\ 74+\ 74+\ 74++$	73+ 73+ 74+ 74+ 74+ 73+

	Cow No.		255		<u> </u>	239			252			275	
Experi- mental	Date	Acidity	Alc. % degree coagula	of milk	Acidity	Alc. % degree coagul	of milk	Acidity	Alc. % degree coagul	of milk	Acidity	Alc. % degree c	f milk
period			Experi- mented	Con- verted		Experi- mented	Con- verted		Experi- mented	Con- verted		Experi- mented	Con- verted
2nd	26/VI 27 28 29 30 1/VII 2 3	0.170 0.165 0.175 0.170 0.165 0.175 0.170 0.175 0.170	82± 82± 86++ 82± 82± 82± 82+ 82+ 82++	83+ 83+ 85+ 83+ 83+ 82+ 81+ 83+	0.150 0.145 0.145 0.145 0.147 0.140 0.135 0.140 0.140	82± 82++ 82++ 82++ 78++ 78++ 78++ 78±	83+ 81+ 82+ 81+ 77+ 77+ 77+ 79+ 79+	0.132 0.140 0.135 0.132 0.130 0.130 0.135 0.140 0.138	82 ± 86++ 86++ 86+ 86+ 86++ 82++ 82± 82±	83+ 85+ 85+ 86+ 86+ 85+ 81+ 83+ 83+	0.155 0.152 0.155 0.155 0.150 0.140 0.144 0.148 0.150	$74 \pm \\ 74 \pm \\ 78 + + \\ 74 \pm \\ 74 \pm \\ 82 + + \\ 82 + + \\ 82 + + \\ 82 + + \\ 82 + + \\$	75+ 75+ 77+ 75+ 75+ 81+ 81+ 79+ 81+
3rd	5 6 7 8 9 10 11 12 13 14 15 16 17 18	0.170 0.165 0.170 0.160 0.170 0.175 0.170 0.170 0.170 0.170 0.170 0.168 0.165 0.165	$\begin{array}{c} 82 + \\ 82 + + \\ 78 \pm \\ 82 + + \\ 82 + + \\ 82 \pm \\ 82 + \\ 82 \pm \\ 82 + + \\ 82 + + \\ 82 + + \\ 82 + + \\ 78 \pm \\ 78 \pm \\ 78 \pm \\ 78 \pm \\ \end{array}$	82+ 81+ 79+ 81+ 81+ 83+ 82+ 83+ 81+ 82+ 81+ 79+ 79+ 79+	0.140 0.137 0.150 0.148 0.140 0.145 0.140 0.137 0.145 0.145 0.145 0.140 0.140	82 ± 82 + 78 ± 78 + 78 + 78 + 82 + 82 + 82 + 78 ± 82 + 78 ± 78 + 78 ± 78 +	83+ 83+ 79+ 78+ 77+ 78+ 81+ 81+ 81+ 79+ 81+ 79+ 77+ 79+ 78+	0.140 0.129 0.130 0.130 0.130 0.129 0.133 0.134 0.130 0.130 0.140 0.132 0.130	$86++$ $86\pm$ $86++$ $86++$ $86++$ $86++$ $82\pm$ $86++$ $82\pm$ $86++$ $82\pm$ $86++$ $82\pm$ $86++$	85+ 87+ 85+ 85+ 85+ 85+ 85+ 83+ 85+ 85+ 85+ 85+ 85+ 85+	0.150 0.150 0.140 0.142 0.145 0.145 0.150 0.150 0.150 0.150 0.150 0.150	$78++\ 78++\ 78++\ 78++\ 78++\ 74\pm\ 78++\ 74\pm\ 74\pm\ 74\pm\ 74\pm\ 74\pm\ 78+$	77+ 77+ 79+ 78+ 77+ 75+ 75+ 75+ 75+ 75+ 75+ 75+ 75+ 75

Table 79 (Continued)

	Cow No.	Aver	age of 255 and 252	Aver	age of 239 and 275
Experi- mental period	Date	Acidity	Alc. % and degree of milk coagulation Converted	Acidity	Alc. % and degree of milk coagulation Converted
	5/VI	0.155	82.5+	0.171	75.0+
	6	0.165	81.5+	0.175	77.0+
	7	0.164	82.0+	0.165	75.5 +
	8	0.165	80.5+	0.169	75.0+
	9	0.160	80.5+	0.160	76.0+
	10	0.165	81.5+	0.161	76.0+
	11	0.163	81.0+	0.151	77.5+
1st	12	0.160	81.5+	0.160	77.5+
	13	0.156	82.0+	0.155	76.0+
	14	0.158	83.5+	0.155	74.5 +
	15	0.160	82.0+	0.158	76.0+
	16	0.163	83.5+	0.160	77.0+
	17	0.158	82.5+	0.154	75.0+
	18	0.158	83.0+	0.155	77.0+
	19	0.155	83.0+	0.153	76 5+
	2)	0.153	82.0+	0.152	75.5+
	21	0.155	81.5+	0.155	75.0 +
	22	0.154	81.0+	0.155	77.5+
	23	0.155	81.0+	0.154	76.5+
	24	0.152	82.5+	0.150	77.5 +
	25	0.148	84.0+	0.149	78 <b>.</b> 0+
	26	0.151	83.0+	0.153	79.0+
2nd	27	0.153	84.0+	0.149	78.0+
	28	0.155	85.0+	0.15)	79.5 +
	29	0.152	84.5+	0.149	78.0+
	30	0.148	84.5+	0.149	76.0+
	1/VII	0.153	84.0+	0.140	79.0+
	2	0.153	81.5+	0.138	79.0+
	3	0.158	82.0+	0.144	79.0+
		0.154	83.0+	0.145	80.0+
	5	0.155	83.5+	0.145	80.0+
3rd	6	0.143	84.0+	0.144	79.5 +
	. 7	0.150	82.0+	0.145	79.0 +

	Cow No.	Avera	age of 255 and 252	Average of 239 and 275			
Experi- mental period	Date	Acidity	Alc. % and degree of milk coagulation Converted	Acidity	Alc. % and degree of milk coagulation Converted		
3rd	8/VII 9 10 11 12 13 14 15 16 17 18	0.145 0.150 0.143 0.152 0.162 0.162 0.150 0.150 0.150 0.148 0.150	83.0+ $84.0+$ $84.0+$ $82.5+$ $84.0+$ $82.5+$ $82.0+$ $82.0+$ $82.0+$ $82.0+$ $82.0+$ $82.0+$	0.145 0.140 0.145 0.143 0.144 0.143 0.144 0.148 0.143 0.145 0.145 0.145	78.0+ $77.0+$ $79.5+$ $79.0+$ $78.0+$ $77.0+$ $79.0+$ $78.0+$ $79.0+$ $78.0+$ $76.0+$ $77.0+$ $78.0+$		

Table 79 (Continued)

It will be considered here the influence of a high dosage of bone meal to the cow upon the acidity and alcohol susceptibility of milk in the light of the above experimental results.

Graphs based upon the results which were obtained in the two series of experiments are as follows.

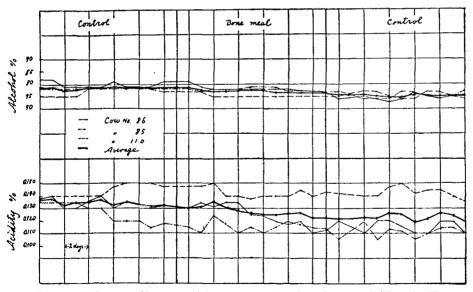


Fig. 6. Influence of bone meal dosage upon the alcohol susceptibility of milk.

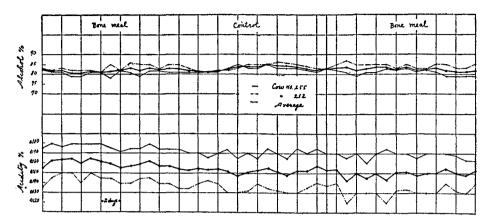


Fig. 7. Influence of bone meal dosage upon the alcohol susceptibility of milk.

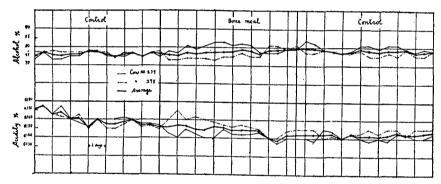


Fig. 8. Influence of bone meal dosage upon the alcohol susceptibility of milk.

In the first experiment there were some differences when considered individually in the acidity and alcohol susceptibility of milk secreted from the three Ayrshire cows during the whole experimental period. But the average experimental results of these three cows in respect to these properties were almost constat throughout the whole experimental period though the each animal obtained a plentiful dosage of bone meal in the second experimental periods.

There were no special changes in the acidity and alcohol susceptibility of milk which had been secreted from each of the four Holstein cows though they were specially given some cod liver oil in the bone meal period. In the 2nd series of the experiment, cows No. 252 and 255 obtained a plentiful dosage of bone meal in the 1st

and 3rd periods while the 2nd served as control. On the other hand cows No. 239 and 275 obtained a plentiful dosage of bone meal in the second period only while 1st and 3rd were periods of controls. By looking at Figures 7 and 8 it will be recognized that the acidity and the alcohol susceptibility of these milks were not influenced at all by the plentiful dosage of bone meal to the cow.

#### B. Calcium Lactate

#### 1. EXPERIMENTAL METHODS

Three Ayrshire cows were used in this experiment. There were 3 experimental periods of which the 1st and 3rd served as controls, each consisting of 10 days. The 2nd was the experimental, consisting of 24 days which were further divided into 3 subperiods, during each of which different amounts of calcium lactate were given: 30 g. of calcium lactate daily for the first 10 days, 40 g. for the next 4 days and 50 g. for the last 10 days.

The ration for the cows consisted of 3 kg. of hay, 18 kg. of corn silage, and 2 kg. of a mixture of soybean cake, wheat bran and malt sprouts. Fifty g. of salt were added to the ration.

Some accounts of the cows used in this experiment are as follows.

Cow No.	114	115	128		
Date of birth	29/III 1922	28/IX 1921	15/VII 1924		
Date of parturition	19/XI 19 <b>3</b> 0	9/VI 1930	10/V 1930		
Date of next parturition	15/XI 1931	10/VI 1931	13/XI 1931		
Daily milk production kg.	4.0	3.0	3.5		

The acidity and the alcohol susceptibility of each milk were determined throughout the whole experimental period.

#### 2. Experimental results

The experimental results obtained with each cow are set down in the following tables.

Table 80

Influence of calcium lactate dosage upon the alcohol susceptibility of milk

	Cow No.		114			115			128		Averag	e of 3 cows
Experi- mental	Date	Acidity	Alc. % a of milk c	nd degree oagulation	Acidity	Alc. % a of milk c	nd degree pagulation	4		nd degree oagulation	A . 111	Alc. % and degree of milk
period	Date	Acturty	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	coagulation Converted
1st Control	7/I 8 9 10 11 12 13 14 15 16	0.138 0.138 0.135 0.135 0.130 0.123 0.138 0.140 0.140	86+ 86± 86+ 86+ 90++ 90++ 86±+ 86±	86+ 86+ 86+ 86+ 89+ 89+ 87+ 87+	0.160 0.150 0.145 0.150 	$ \begin{array}{c} 78 + + \\ 74 \pm \\ 78 + + \\ 74 + \\ - \\ 74 + \\ 58 + + \\ 78 + \\ 74 + + \\ 74 + \\ \end{array} $	77+ 75+ 77+ 74+ - 74+ 77+ 78+ 73+ 74+	0.185 0.186 0.180 0.175 	86+ 86± 86++ 86± - 86++ 82+ 82+ 82± 82±	86+ 87+ 85+ 87+ - 85+ 82+ 82+ 83+ 83+	0.161 0.158 0.153 0.153  0.150 0.154 0.154 0.154 0.153	83.0+ 83.0+ 82.7+ 82.3+  81.7+ 82.7+ 83.0+ 81.0+ 81.3+
2nd Calcium lactate 30 g	17 18 19 20 21 22 23 24 25 26	0.140 	86± 	87+  85+ 87+ 86+ 86+ 85+ 85+  85+	0.140 	74+ - 74± 78+ 74± 78+ 78++ 74± - 78++	74+  75+ 78+ 75+ 78+ 77+ 75+  77+	0.168 	82± 	83+ 85+ 85+ 85+ 86+ 86+ 85+	0.149 	81.3+ 81.7+ 82.7+ 82.0+ 83.0+ 82.7+ 82.0+ - 82.3+

Table 80 (Continued)

	Cow No.		114			115			128	i	Averag	ge of 3 cows
Experi-	Ditt	A -: 3:4		nd degree agulation	A . 17.		nd degree pagulation		of milk c	nd degree oagulation	A .: 1:4	Alc. % and degree of milk
mental period	Date	Acidity	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	coagulation Converted
2nd Calcium lactate 40 g	27 28 29 30	0.150 0.140 0.140 0.135	86++ 86++ 86± 86++	85+ 85+ 87+ 85+	0.145 0.138 0.143 0.140	78++ 74± 78++ 78++	77+ 75+ 77+ 77+	0.160 0.170 0.170 0.170	86++ 82± 86+ 86++	85+ 83+ 86+ 85+	0.152 0.149 0.151 0.148	81.7+ 81.0+ 83.3+ 82.3+
,, 50 g	31 1/II 2 3 4 5 6 7 8	0.130 0.150 0.150 0.150 0.152 0.152 0.145 0.145	86+ 	86+ 85+ 85+ 86+ 86+ 86+ 85+ 85+	0.140 	78++ 74+ 74± 74++ 74± 74++ 74+ 74+	77+  74+ 75+ 75+ 75+ 75+ 75+ 74+	0.170 0.150 0.155 0.155 0.155 0.155 0.155 0.155	86+ 90++ 90++ 90++ 90++ 86± 90++ 96±	86+  89+ 89+ 89+ 89+ 87+ 89+  87+	0.147 	83.0+  82.7+ 83.0+ 83.3+ 82.7+ 82.7+ 82.3+  82.0+
3rd Control	10 11 12 13 14 15 16 17 18	0.143 0.145 0.150 0.140 0.143 	86+ 86++ 86++ 86± 86++ 86++ 86++ 86++ 82± 86++	86+ 85+ 85+ 87+ 85+ - 85+ 85+ 85+	0.150 0.150 0.150 0.150 0.143 0.145 0.145 0.140 0.143 0.148	74+ 74+ 74+ 74+ 74+ 74+ 78++ 74+ 74+	74+ 74+ 74+ 73+ 74+ - 77+ 73+ 74+ 74+	0.160 0.155 0.160 0.165 0.155 	86± 86± 86± 86± 86± 86± 90++ 86+	87+ 87+ 87+ 87+ 87+ 87+ 87+ 87+ 89+ 85+	0.151 0.150 0.153 0.149 0.148 	82.3+ 82.0+ 82.0+ 82.3+ 82.0+ - 82.7+ 82.0+ 82.0+ 81.3+

Before any comment is made in respect to the experimental results of each cow and the average of them, the graphs drawn from the data may well be observed.

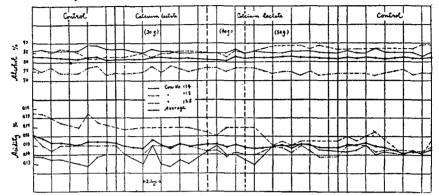


Fig. 9. Influence of calcium lactate dosage upon the alcohol susceptibility of milk.

By considering the above figures it will be recognized that there is no special influence of calcium lactate in the ration upon the acidity and the alcohol susceptibility of milk. The curve platted for the average of these cows represents almost a straight line which shows the indifference of the calcium lactate dosage to the milk properties under consideration.

# C. Di-potassium Phosphate

# 1. EXPERIMENTAL METHODS

Three Ayrshire cows used in the former experiment were again used in this experiment. There were 3 experimental periods of which the 1st and 3rd were control each continuing for 6 days, while the 2nd was the experimental, consisting of 14 days. This period was divided into 2 sub-periods, during each of which different weights of di-potassium phosphate ( $K_2HPO_4$ ) were given: 30 g. daily for the first 8 days and 40 g. for the last 6 days.

The feeding and management of these cows were just the same as in the former experiment.

Determinations were made of the acidity and alcohol susceptibility of these milk every days throughout the whole experimental period.

#### 2. Experimental results

The following experimental results were obtained.

Table 81

Influence of di-potassium phosphate dosage upon the alcohol susceptibility of milk

	Cow No.		114			115			128		Averag	ge of 3 cows
Experi	Late	Acidity	Alc. % a	nd degree pagulation	Acidity		ind degree oagulation	Acidity	Alc. % a of milk c	nd degree oagulation	Acidity	Alc. % aud degree of milk
mental period	Late	Acidity	Experi- mented	Converted		Experi- mented	Converted	Actury	Experi- mented	Converted	Acidity	coagulation Converted
1st Control	19/II 20 21 22 23 24	0.140 0.138 0.138 0.135 0.140 0.138	86++ 86++ 82+ 82+ 82± 82++	85+ 85+ 82+ 82+ 82+ 83+ 81+	0.148 0.143 0.140 0.144 0.143 0.143	74+ 74+ 74++ 74± 74++ 74++	74+ 74+ 73+ 75+ 73+ 73+	0.155 0.150 0.148 0.150 0.150 0.155	86++ 86++ 86++ 82± 82± 86++	85+ 85+ 85+ 83+ 83+ 85+	0.148 0.144 0.142 0.143 0.144 0.145	81.3+ 81.3+ 80.0+ 80.0+ 79.8+ 79.8+
2nd K₂HPO₄ 30 g	25 26 27 23 1/III · 2 3 4	0 140 0.148 0.145 0.145 0.140 0.130 0.140 0.135	82+ 86++ 86++ 82± 82± 82+ 78± 78±	82+ 85+ 85+ 83+ 83+ 82+ 79+ 79+	0.146 0.145 0.144 0.140 0.140 0.130 0.135 0.135	74+ 74++ 78++ 74± 74± 74++ 74++	74+ 73+ 77+ 75+ 75+ 73+ 73+	0.150 0.150 0.153 0.153 0.153 0.155 0.155	86++ 82± 86++ 82± 82± 82± 82± 82±	85+ 3+ 85+ 83+ 83+ 83+ 83+ 83+	0.145 0.147 0.147 0.146 0.146 0.138 0.143 0.140	80.3+ 80.3+ 82.0+ 80.3+ 80.3+ 79.3+ 78.3+
K <sub>2</sub> HPO <sub>4</sub> 40 g	5 6 7 8 9	0.140 0.133 0.142 0.150 0.155 0.145	82± 82++ 82± 82± 86++ 86++	83+ 81+ 83+ 83+ 85+ 85+	0.127 0.135 0.132 0.140 0.150 0.140	74± 74++ 74± 74± 74++ 74++	75+ 73+ 75+ 75+ 75+ 73+	0.150 0.147 0.150 0.150 0.150 0.150	82± 82± 82± 82± 82± 82± 82±	. 83+ 83+ 83+ 83+ 83+ 83+	0.139 0.138 0.141 0.147 0.1£2 0.148	80.3+ 79.0+ 80.3+ 80.3+ 80.3+ 80.3+
3rd Control	11 12 13 14 15 16	0.135 0.130 0.138 0.140 0.140 0.142	86++ 86++ 86++ 82± 82± 86++	85+ 85+ 85+ 83+ 83+ 81+	0.140 0.148 0.140 0.148 0.145 0.140	74+ 74++ 70± 74+ 74+ 74+	74+ 73+ 71+ 74+ 74+ 73+	0.145 0.150 0.152 0.160 0.155 0.161	82± 86++ 82± 86+± 82± 82±	83+ 85+ 83+ 85+ 83+ 83+	0.140 0.142 0.143 0.149 0.145 0.148	80.7+ 81.0+ 79.7+ 80.7+ 80.0+ 79.0+

A graph representing the data in the above table and the averages is given below.

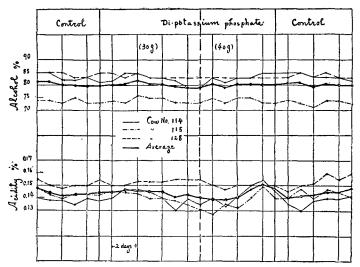


Fig. 10. Influence of di-potassium phosphate dosage upon the alcohol susceptibility of milk.

There were found no marked influences of di-potassium phosphate given daily amounting 30-40 g. per cow upon the acidity and alcohol susceptibility of milk. It is quite clear by observing the above figures however that the average acidity of these milk was a little increased at the end of the 2nd experimental period.

## D. Sodium Citrate

# 1. EXPERIMENTAL METHODS

The three Ayrshire cows used in the preceding experiment were again used. There were 3 experimental periods the 1st and 3rd being controls each continuing for 6 days and the 2nd was the experimental, consisting of 9 days, when 40 g. of sodium citrate were given with the daily ration.

The feeding and management of these cows were same as in the former experiment. The author determined the acidity, and alcohol

Table 82 Influence of sodium cirtrate dosage upon the alcohol susceptibility of milk

	Cow No.		٠114			115			128		Avera	ge of 3 cows
Experi- mental	Date	Acidity	Alc. % a of milk co	nd degree pagulation	Acidity		nd degree pagulation	A oiditu		nd degree oagulation	A	Alc. % and degree of milk
period	Date	Acturey	Experi- mented	Converted	, .	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	coagulation Converted
1st Control	11/III 12 13 14 15 16	0.135 0.130 0.138 0.140 0.140 0.142	86++ 86++ 86++ 82± 82± 82++	85+ 85+ 85+ 83+ 83+ 81+	0.140 0.148 0.140 0.148 0.145 0.145	$74+\ 74++\ 70\pm\ 74+\ 74+\ 74+$	74+73+71+74+74+73+	0.145 0.150 0.152 0.160 0.155 0.161	$82\pm 86++82\pm 86++82\pm 82\pm 82\pm$	83+ 85+ 83+ 85+ 83+ 83+	0.140 0.142 0.143 0.149 0.145 0.143	80.7+ 81.0+ 79.7+ 80.7+ 80.0+ 79.0+
2nd Sodium citrate	17 18 19 20 21 22 23 24 25	0.143 0.145 0.140 0.138 0,133 0.138 0.138 0.142 0.142	$82 + \\ 82 \pm \\ 82 + + \\ 86 + \\ 86 + \\ 86 + + \\ 86 + + \\ 86 + + \\ 86 + $	82+ 83+ 81+ 86+ 86+ 85+ 85+ 85+ 86+	0.133 0.138 0.138 0.135 0.155 0.142 0.142 0.132 0.150	74++ 74++ 74+ 74+ 74+ 74+ 74+ 74+ 74+ 74	73+ 73+ 75+ 74+ 74+ 74+ 74+ 73+ 74+	0.153 0.162 0.158 0.160 0.160 0.155 0.155 0.160 0.161	82 ± 86 + + 82 ± 86 + + 86 + + 82 ± 82 ± 82 ± 82 +	83+ 85+ 85+ 85+ 85+ 83+ 83+ 85+	0.145 0.145 0.145 0.144 0.144 0.145 0.145 0.145	79.3+ 80.3+ 79.7+ 81.7+ 81.7+ 80.7+ 80.7+ 80.3+ 81.7+
3rd Control	26 27 28 29 30 1/IV	0.150 0.150 0.145 0.145 0.148 0.145	86++ 86++ 86++ 86++ 86++ 86++	85+ 85+ 85+ 85+ 85+ 85+	4.145 0.140 0.140 0.145 0.140 0.140	74± 74± 74± 74+ 74+ 74+	75+ 75+ 75+ 74+ 75+ 74+	0.160 0.155 0.160 0.155 0.155 0.160	82± 86++ 86++ 86++ 86++ 86++	83+ 85+ 85+ 85+ 85+ 85+	0.152 0.148 0.148 0.148 0.148 0.148	81.0+ 81.7+ 81.7+ 81.3+ 81.7+ 81.3+

susceptibility of these milk every day during the whole experimental period.

#### 2. Experimental results

The following experimental results were obtained in this experiment.

# 3. DISCUSSION

Graphs embodying the experimental results of individual cow and the average were drawn as follows.

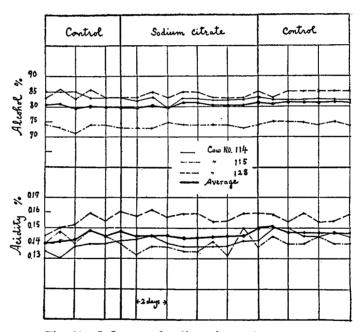


Fig. 11. Influence of sodium citrate dosage upon the alcohol susceptibility of milk.

There was not a marked influence of giving about 40 g. of sodium citrate daily mixed in the ration upon the acidity and the alcohol susceptibility of milk. It is, however, quite clear by observing the above figure obtained from the average of 3 cows, that these properties are

a little changeable in each individual throughout the whole experimental period.

#### E. Sodium Bicarbonate

#### 1. EXPERIMENTAL METHODS

Three Ayrshire cows were used in this experiment which was divided into 3 experimental periods the 1st and 3rd being controls. The 2nd period was experimental. Each consisted of 14 days. Forty g. of sodium bicarbonate were added to the ration daily during the 2nd experimental period.

The cows were fed daily 3 kg. of hay, 23 kg. of corn silage and 2-3 kg. of the concentrates; mixture of soybean cake, wheat bran and malt sprouts, with 40 g. of salt added.

Some accounts of the experimental cows are as follows.

Cow No.	110	91	111
Date of birth	28/VI 1921	5/111 1916	25/VIII 1921
Date of parturition	22/XII ·1930	11/VIII 1930	22/III 1931
Date of next parturition	16/VI 1932	23/V 1932	27/V 1932
Daily milk production kg.	3.5	3.0	4.5

The acidity and the alcohol susceptibility of each milk were determined daily throughout the whole experimental period.

## 2. EXPERIMENTAL RESULTS

The experimental results obtained in this experiment are as follows.

Table 83

Influence of sodium bicarbonate dosage upon the alcohol susceptibility of milk

	Cow No.	110			91			111			Average of 3 cows	
Experi- mental period	Date	Acidity	Alc. % and degree of milk coagulation		A 1. /	Alc. % and degree of milk coagulation		A	Alc. % and degree of milk coagulation		, .,,,	Alc. % and degree of milk
			Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	coagulation Converted
1st Control	20/IV 21 22 23 24 25 26 27 28 29 30 1/V 2	0.145 0.150 0.147 0.138 0.140 0.145 0.140 0.142 0.1440 0.145 0.140 0.147 0.143	$82++\\78\pm\\78\pm\\78\pm\\78\pm\\82++\\82++\\82++\\78\pm\\78\pm\\82++\\78\pm\\82++\\78+\\82++\\78+\\78+\\78+$	81+ 79+ 79+ 79+ 81+ 81+ 79+ 79+ 81+ 81+ 78+ 78+	0.140 0.145 0.135 0.137 0.140 0.130 0.140 0.140 0.140 0.144 0.145	$78+$ $78+$ $78+$ $78+$ $78+$ $74\pm$ $74\pm$ $74+$ $74\pm$ $74\pm$ $74\pm$ $74\pm$ $74\pm$ $74+$ $74+$ $74+$ $74+$ $74+$ $74+$	78+ 77+ 77+ 75+ 75+ 75+ 75+ 75+ 75+ 75+ 77+ 77	0.145 0.145 0.150 0.148 0.145 0.142 0.138 0.140 0.147 0.145 0.145 0.145 0.145 0.150	$82++$ $82++$ $78\pm$ $78\pm$ $78\pm$ $82++$ $78\pm$ $82++$ $82++$ $82++$ $82++$ $82++$ $82++$	81+ 81+ 79+ 79+ 79+ 81+ 79+ 81+ 81+ 81+ 81+ 81+	0.143 0.147 0.141 0.139 0.141 0.142 0.136 0.141 0.142 0.142 0.142 0.145 0.146	80.0+ 79.0+ 78.3+ 78.3+ 77.7+ 79.0+ 78.3+ 78.3+ 78.3+ 78.3+ 79.0+ 78.7+
2nd Sodium bicar- bonate	4 5 6 7 8 9	0.145 0.142 0.145 0.145 0.140 0.143 0.140	78+ 78+ 78++ 82++ 78± 78± 78±	78+ 78+ 77+ 81+ 79+ 79+	0.140 0.140 0.144 0.135 0.130 0.130	74± 74+ 74± 74± 74± 74± 74± 74+	75+ 74+ 75+ 75+ 75+ 75+ 75+ 74+	0.148 0.144 0.145 0.156 0.148 0.148 0.145	82++ 78± 78± 78± 82++ 82± 82++	81+ 79+ 79+ 79+ 81+ 83+ 81+	0.144 0.142 0.145 0.145 0.139 0.140 0.188	78.0+ 77.0+ 77.0+ 78.3+ 78.3+ 79.0+ 78.0+

Table 83 (Continued)

	Cow No.	110			91				111	Average of 3 cows		
Experi- mental period	Date	Acidity	Alc. % and degree of milk coagulation			Alc. % and degree of milk coagulation			Alc. % and degree of milk coagulation			Alc. % and degree of milk
			Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	coagulation Converted
2nd	11 12 13	0.145 0.148 0.140	82++ 82++ 82++	81+ 81+ 81+	0.125 0.130 0.130	74± 74± 74±	75+ 75+ 75+	0.145 0.145 0.142	82++ 78± 82±	81+ 79+ 83+	0.138 0.141 0.137	79.0+ 78.3+ 79.7+
Sodium bicar- bonate	14 15 16 17	0.145 0.140 0.140 0.145	78± 82++ 78± 78±	79+ 81+ 79+ 79+	0.132 0.135 0.130 0.135	74+ 74++ 74± 74++	74+ 73+ 75+ 73+	0.147 0.148 0.140 0.150	82 + + 82 ± 82 + 82 ±	81+ 83+ 82+ 83+	0.141 0.141 0.137 0.143	78.0+ 79.0+ 78.7+ 78.3+
3rd Control	18 19 20 21 22 23 24 25 26 27 28 29 30 31	0.148 0.135 0.135 0.139 0.145 0.140 0.135 0.145 0.140 0.137 0.140 0.137	78± 82++ 82++ 82++ 82++ 78+ 78+ 78± 78± 82++ 82++ 78± 82++ 82++	79+ 81+ 81+ 81+ 82+ 81+ 82+ 79+ 78+ 79+ 81+ 81+ 79+	0.125 0.130 0.130 0.130 0.125 0.130 0.145 0.145 0.135 0.135 0.135 0.135 0.135	74++ 74++ 74++ 74++ 74++ 74++ 74++ 74++	73+ 74+ 73+ 74+ 73+ 74+ 74+ 74+ 73+ 74+ 74+ 74+ 74+ 74+ 73+	0.145 0.145 0.150 0.143 0.150 0.145 0.140 0.143 0.141 0.140 0.140 0.135 0.140	$\begin{array}{c} 82 \pm \\ 82 \pm \\ 86 + + \\ 82 + \\ 86 + + \\ 82 + \\ 86 + + \\ 82 \pm \\ 82 $	83+ 83+ 85+ 82+ 81+ 85+ 82+ 85+ 82+ 83+ 83+ 83+ 82+	0.139 0.137 0.133 0.137 0.140 0.138 0.140 0.143 0.139 0.137 0.137 0.136 0.140	78.3+ 79.3+ 79.7+ 79.0+ 78.3+ 80.0+ 79.0+ 80.0+ 78.3+ 78.7+ 78.7+ 79.3+ 78.3+

The graphs based upon the above data will be given below.

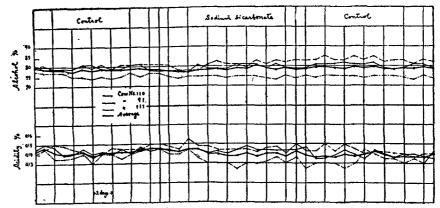


Fig. 12. Influence of sodium bicarbonate desage upon the alcohol susceptibility of milk.

No influence can be observed on the acidity and the alcohol susceptibility of milk by mixing about 40 g. sodium bicarbonate daily in the ration. It is quite clear however by observing the figure closely that these properties are a little changeable in each individual throughout the whole experimental period.

# F. Di-sodium Phosphate, Calcium Carbonate or Magnesium Carbonate

#### 1. Experimental methods

Three Holstein cows were used in this experiment, and the experiment was divided into 4 periods, each consisting 10 days. The 1st was control; in the 2nd 80 g. of di-sodium phosphate were given, in the 3rd 120 g. of calcium carbonate, and in the 4th 50 g. of magnesium carbonate were added to each ration daily.

The author determined the acidity and alcohol susceptibility of each milk from these three cows throughout the whole experimental period. The contents of Cl, P, Ca and Mg in milk from each cow were analyzed quantitatively twice in each period by using the microanalytical method which will be described in detail later in chapter XIV.

TABLE 84

Influence of di-sodium phosphate, calcium carbonate and magnesium carbonate dosage upon the alcohol susceptibility of milk

	Cow No.	277			284				308	Average of 3 cows		
Experi- mental period	Date		Alc. % and degree of milk coagulation			Alc. % and degree of milk coagulation			Alc. % and degree of milk coagulation			Alc. % and degree of milk
		Acidity	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	Experi- mented	Converted	Acidity	coagulation Converted
1st Control	19/XI 20 21 22 23 24 25 26 27 28	0.160 	78± 78± 82++ 78± 78± 78± 82++ 82++	79+  79+ 81+ 79+ 79+ 79+ 81+  81+	0.180 	86 + 86 ± 86 ± 86 ± 86 ± 86 ± 86 ± 86 ±	86+  85+ 87+ 87+ 87+ 87+ 87+ 87+	0.190 	82± 	83+ 85+ 83+ 85+ 85+ 85+ 83+ 83+	0.177 	82.7+ 83.3+ 83.7+ 83.7+ 83.7+ 83.7+ 83.7+ 83.7+
2nd Di- sodium phos- phate	29 30 1/XII 2 3 4 5 6 7 8	0.160 0.170 0.160 0.175 0.175 - 0.165 0.165 0.170 0.170	73± 82++ 82++ 78± 82+ 78± 78± 78± 78± 78±	79+ 81+ 81+ 79+ 82+ - 79+ 79+ 79+ 79+	0.175 0.180 0.180 0.175 0.175 	86+ 86+ 86± 86± 90++ 86± 86± 86± 86±	86 + 86 + 87 + 87 + 89 + - 87 + 87 + 87 +	0.190 (.205 0.200 0.200 0.235 	82± 86+ 82± 86+ 82±  86++ \$2± 82± 86++	83+ 86+ 83+ 86+ 83+ - 85+ 83+ 85+	0.175 0.185 0.180 0.183 0.185 - 0.182 0.178 0.177 0.182	82.7+ 84.3+ 83.7+ 84.0+ 84.7+ 

Table 84 (Continued)

	Cow No.	277				284			308		Average of 3 cows		
Experi-				nd degree pagulation			nd degree pagulation			nd degree oagulation		Alc. % and degree of milk	
mental period	Date	te Acidity Experimented Converted Experimented Converted Converted	Acidity	Experi- mented	i- Converted	Acidity	coagulation Converted						
	9 10	0.175 0.160	78± 78±	79+ 79+	0.170 0.170	86± 90++	87+ 89+	0.200 0.195	86++ 82±	85+ 83+	0.182 0.175	83.7+ 83.7+	
3rd Calcium car- bonate	11 12 13 14 15 16 17 18	0.170 0.175 0.170 0.170 0.170 0.170 0 165	78± 78± 78± 78± 78± 78± 78±	79+ 79+ 79+ 79+ 79+ 79+	0.165 0.165 0.160 0.165 0.160 0.170	86 ± 86 ± 86 ± 86 ± 90 + +	86+ 87+ 87+ 87+ 89+ 89+	0.200 0.195 6.195 0.190 0.190 0.195	82± 86++ 82+ 82± 86++ 86++	83+ 85+ 82+ 83+ 85+ 85+	0.178 0.178 0.175 0.175 0.173 0.177	82.7+ 83.7+ 82.7+ 82.0+ 84.3+ 83.0+	
4th Magne- sium car- bonate	19 20 21 22 23 24 25 26 27 28	0.170 0.160 0.170 0.170 0.165 0.170 - 0.160 0.170 0.170	78++ 78+ 78++ 78++ 78++ 78+ 78++ 78+	77+ 78+ 79+ 77+ 77+ 79+ 77+ 79+ 78+	0.165 0.170 0.170 0.170 0.165 0.165 	90++ 86+ 86± 90++ 86± 90++ 90++ 90++ 86±	89 + 86 + 87 + 89 + 89 + 89 + 89 + 89 + 87 +	0.200 0.200 0.190 0.200 0 195 0.200 	86++ 82± 86++ 86++ 82+ 86++ 86+ 86+ 86+	85+ 83+ 85+ 85+ 82+ 85+ 86+ 86+ 86+	0.178 0.178 0.180 0.180 0.175 0.178 	83.7+ 82.3+ 83.3+ 83.7+ 82.8+ 84.3+ 84.0+ 84.7+ 83.7+	

There were 4 kg. of hay, 25 kg. of corn silage and 3-4 kg. of the concentrates consisting of crushed oats, wheat bran and soybean cake, in the daily ration to which were added 70 g. of salt.

Some accounts of the experimental cows are as follows.

Cow No.	277		· 284		308	
Date of birth	1/V	1927	23/IX	1927	3/XII	192 <b>9</b>
Date of parturition	10/II	1932	13/I	1932	29/I	1932
Daily milk production kg.	9.0		8.0		8.0	

## 2. Experimental results

In the following table the results are tabulated.

The content of Cl, Mg, P and Ca obtained are given in the following tables.

TABLE 85
Content of Cl, P, Ca and Mg in the milk of cow no. 277 (mg/dl)

		Cow No. 277								
Experi- mental period	Date of sample taken	Cl	P	Ca	Mg	Acidity	Alc. % and degree of milk coagulation Converted			
1st	22/XI	235.22	51.28	135.00	15.31	0.170	81+			
	26/XI	234.15	53.19	135.00	13.64	0.170	81+			
2nd	2/XII	232.83	51.28	135.00	15.62	0.175	79+			
	7/XII	236.34	52.63	135.00	15.62	0.170	79+			
3rd	12/XII	234.15	53.19	137.00	15.31	0.170	79+			
	17/XII	230.00	52.63	135.00	13.64	0.165	78+			
4th	21/XII	234.15	52.63	132.00	15.31	0.170	79+			
	26/XII	236.93	54.05	140.00	13.94	0.160	77+			

TABLE 86
Content of Cl, P, Ca and Mg in milk of cow No. 284 (mg/dl)

		Cow No. 284								
Experi- mental period	Date of sample taken	Cl	Р	Ca	Mg	Acidity	Alc. % and degree of milk coagulation Converted			
1st ·	22/XI	201.24	50.00	117.00	16.30	0.175	87+			
	26/XI	201.24	51.28	110.00	15.31	0.170	87+			
2nd	2/XII	184.28	51.28	110.00	16.30	0.175	87+			
	7/XII	201.24	50.00	117.00	16.30	0.165	87+			
3rd	12/XII	198.32	47.62	114.00	15.63	0.165	86+			
	17/XII	201.41	50.00	117.00	13.64	0.170	85+			
4th	21/XII	211.19	47.62	110.00	15.31	0.170	87+			
	26/VII	210.02	55.56	117.00	14.71	0.160	89+			

TABLE 87
Content of Cl, P, Ca and Mg in milk of cow No. 308 (mg/dl)

		Cow No. 308							
Experi- mental period	Date of sample taken	CI	P	Ca	Mg	Acidity	Alc. % and degree of milk coagulation Converted		
1st	22/XI	169.65	64.52	113.00	16.59	0.195	83+		
	26/XI	159.71	61.87	115.00	15.61	0.210	83+		
2nd	2/XII	159.71	60.61	110.00	16.59	0.210	86+		
	7/XII	175.50	66.67	118.00	17.44	0.195	83+		
3rd	12/XII	170.24	64.52	112.00	16.59	0.200	83+		
	17/XII	169.65	62.50	113.00	15.61	0.195	85+		
4th	21/XII	169.65	61.84	116.00	17.44	0.190	84+		
	26/XII	169.65	64.52	117.00	15.61	0.190	86+		

What the influence of di-sodium phosphate, calcium carbonate and magnesium carbonate dosage for cow has on the acidity and the alcohol susceptibility of milk may be studied by graphing the above experimental results as follows.

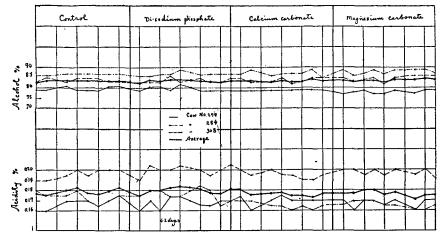


Fig. 13. Influence of di-sodium phosphate, calcium carbonate or magnesium carbonate dosage upon the alcohol susceptibility of milk.

By studying the above graphs it will be seen that the acidity and the alcohol susceptibility of milk are almost constant in each cow throughout the whole experimental period in spite of the continuous feeding of each of these materials. Especially one can see it clearly by noting the average figures of these cows which show as almost a straight line during the whole experimental period.

The average contents of Cl, P, Ca and Mg in milk having been analyzed twice in each period are also nearly constant as shown in the following tables.

TABLE 88

Average contents of Cl, P, Ca and Mg in the milk of 3 cows (mg/dl)

Experimental period	1st					
Constituents Cow No.	Cl	P	Cá	Mg		
277	234.74	52.24	135.00	14.48		
284	201.24	50.64	113.50	15.81		
308	164.68	63.20	114.00	16.10		
Average of 3 cows	200.22	55.36	120.83	15.46		

Table 88 (Continued)

Experimental period		21	nd		
Cow No.	Cl	Р	Ca	Mg	
277	234.59	51.96	135.00	15.62	
284	192.72	50.64	113.50	16.30	
308	167.61	63 64	114.00	17.02	
Average of 3 cows	198.31	55.41	120.83	16.31	
Experimental period		31	·d		
Constituents Cow No.	Cl	P	Ca	Mg	
277	232.08	52.91	136.00	14.48	
284	199.87	48.81	115.00	14.64	
308	169.95	63.51	112.50	16.10	
Average of 3 cows	200.95	54.97	121.30	15.07	
Experimental period	4th				
Constituents Cow No.	Cl	P	Са	Mg	
277	235.54	53.34	136.00	14.48	
284	210.61	51.58	113.50	15.01	
304	169.65	63.18	116.50	16.53	
Average of 3 cows	205.27	56.03	122.00	15.34	

Taking all in consideration the author can say that the di-sodium phosphate, calcium carbonate and magnesium carbonate dosage for

cow did not exert any influence on the acidity or alcohol susceptibility of milk, nor on the content of Cl, P, Ca and Mg in these milk.

# G. Phoscalpin

## 1. EXPERIMENTAL METHODS

In Japan phoscalpin as a source of calcium in the cattle feed has recently come into use. It is made of oyster shell consisting mainly of about 86% of calcium carbonate and 1.8% of calcium phosphate.

It will be a worth while to observe here what influence the plentiful dosage of this material to a cow exerts upon the alcohol susceptibility of milk.

The experiment was divided into three periods of which the 1st and 3rd were controls each consisting of 8 days. In the 2nd period 140 g. of phoscalpin were added to the ration daily during 14 days.

4 Holstein cows were placed under the same feeding and management as in the former experiments throughout the whole experimental period.

Some accounts of the experimental cows are as follows.

Cow No.	27	77	283		29	94	272	
Date of birth	1/V	1927	12/VI1I	1927	1/I	1929	20/VII	1926
Date of parturition	24/V	1933	6/VI	1933	6/111	1933	18/IV	1933
Daily milk production kg.	18	3.0	25.	0	28	5.0	27	.0

### 2. EXPERIMENTAL RUSULTS

The data obtained for each cow are tabulated in the following table.

. Table 89
Influence of Phoscalpin dosage upon the alcohol susceptibility of milk

	Cow No.		277			283			294			272		Avera	ge of 4 cows
Experi- mental period	Date	Acidity	Alc. % degree coagu	of milk	Acidity	Alc degree coagu	lation	Acidity	Alc. 9 degree coagu Experi-		Acidity	degree	lation	Acidity	Alc. % and degree of milk coagulation
	<u>]</u>		mented	verted		mented			mented			mented		<u> </u>	Converted
1st Control	10/X 11 12 13 14 15	0.160 0.160 0.155 0.165 0.160	78++ 78++ 78++ 78+ 78+	77+ 77+ 77+ 78+ 78+	0.180 0.180 0.175 0.180 0.185	70± 70± 74++ 70± 70±	71+ 71+ 73+ 71+ 71+	0.180 0.180 0.190 0.190 0.190	78+ 78++ 78± 78± 78±	78+ 77+ 79+ 79+ 79+	0.190 0.190 0.185 0.190 0.185	82+ 82+ 82++ 78+ 82+	82+ 82+ 81+ 78+ 82+	0.178 0.178 0.176 0.181 0.180	77.0+ 76.8+ 77.5+ 76.5+ 77.5+
	16 17	$\begin{array}{c} 0.155 \\ 0.165 \end{array}$	82++ 82++	81+ 81+	$0.175 \\ 0.180$	70± 70±	71+ 71+	$0.180 \\ 0.180$	78± 78±	79+ 79+	0.180 0.185	82± 82++	83+ 81+	$0.173 \\ 0.178$	78.5+ 78.0+
	18 19 20 21	0.165 0.165 0.155 0.160	82++ 82++ 82+ 78±	81+ 81+ 82+ 79+	0.185 0.180 0.175 0.180	70± 74++ 70± 70±	71+ 73+ 71+ 71+	0.190 0.185 0.185 0.185	78+ 78+ 78+ 78+	78+ 78+ 78+ 78+	0.180 0.170 0.180 0.190	82++ 78+ 78+ 78±	81+ 78+ 78+ 79+	0.180 0.175 0.174 0.179	77.8+ 77.5+ 77.3+ 76.8+
2nd Phos- calpin	22 23 24 25 26 27 28 29	0.155 0.160 0.160 0.165 0.160 0.160	82++ 78± 82+ 78± 82++ 78+	81+ 79+ 82+ 79+ 81+ 78+	0.180 0.180 0.175 0.175 0.175 0.180	70± 74++ 74++ 74++ 74± 74+	71+ 73+ 73+ 73+ 75+ 74+	0.190 0.190 0.190 0.185 0.190 0 185 - 0.185	78± 74± 78± 78+ 78+ 78+ 78+	79+ 75+ 79+ 79+ 78+ 77+ -	0.190 0.185 0.180 0.180 0.180 0.180	82++ 78± 78++ 78++ 78+ 78+ 82++	81+ 79+ 77+ 77+ 78+ 81+ 82+	0.179 0.179 0.176 0.176 0.176 0.176	78.0+ 76.5+ 77.8+ 17.0+ 77.8+ 77.5+ 78.5+
	30 31	0.165 0.165	82+ 82++	82+ 81+	0.175	74++ 74++	73+ 73+	0.185	78++ 78+	78+	0.180	82++	81+	0.179	78.3+
3rd	1/XI 2 3 4	0.170 0.170 0.170 0.175	82++ 78++ 78+ 78+	81+ 77+ 78+ 78+	0.180 0.180 0.180 0.180	74++ 74++ 74++ 74++	73+ 73+ 73+ 73+	0.180 0.185 0.185 0.180	78± 78+ 78+ 78+	79+ 78+ 78+ 78+	0.185 0.180 0.180 0.170	78± 82++ 82++ 82++	79+ 81+ 81+ 81+	0.179 0.179 0.179 0.176	78.3+ 77.3+ 77.5+
Control	5 6 7 8	$0.170 \\ 0.175 \\ 0.170$	78± 82++ 78+	79+ 81+ 78+	0.180 0.175 0.180	$74++\ 74++\ 74++$	73+ 73+ 73+	0.185 0.190 0.185	78+ 78+ 78+	78+ 78+ 78+	0.175 0.170 0.175	82+ 82+ 82++	82+ 82+ 81+	$0.178 \\ 0.178 \\ 0.178$	78.0+ 77.5+ 77.5+

Graphs embodying the experimental results of each cow and also the average of them are as follows:

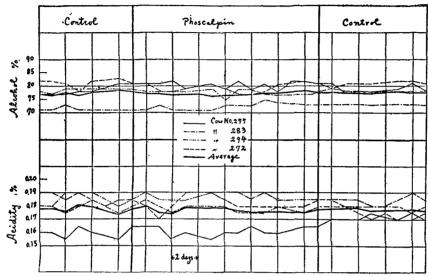


Fig. 14. Influence of Phoscalpin dosage upon the alcohol susceptibility of milk.

There were no influences upon the acidity and alcohol susceptibility of milk by mixing about 140 g. of phoscalpin daily in the ration. It is quite obvious however by observing the graph that these properties were slightly variable in each individual throughout the whole experimental period.

## H. Calcium

Although there seems sometimes to be found a positive reaction to the alcohol test in milk when the milking cow is kept under an unrational feeding for a long time, such as rice straw only as the coarse fodder and rice bran as concentrates, this alcohol positive reaction of the milk will be corrected to negative by changing to a rational feeding or giving calcium materials in the ration. Generally speaking this phenomenon can not be considered definite being dependent upon the condition of health or upon the individual of cow.

The author made an experiment by the following method in order to gain some light on these facts.

## 1. EXPERIMENTAL METHODS

Four Ayrshire cows were used in this experiment, two of them were used rice straw, rice bran and calcium feeding experiment, and to the other two for an experiment in which calcium was added to the ration for milking cows which secreted milk showing positive reaction always in the alcohol test in spite of their healthy condition.

The alcohol susceptibility and acidity of milk were examined daily throughout the experimental period. Some accounts of the experimental cows, experimental period and feeding conditions are as follows.

Table 90 Some particulars as to the experimental cow

Cow No.	154	146	115	110	
Date of parturition	22/IX 1933	5/I 1933	19/X 1933	26/XII 1933	
Date of copulation	8/VIII 1934	7/VI 1934	7/VI 1934	9/VIII 1933	
Daily milk production kg.	6–3	6–3	8-6	9–6	

TABLE 91
Feeding table

Experimental period	, Cows No. 154 and 146	Cows No. 115 and 110		
1st (25/VII-3/VIII)	Hay, and mixture of soybean cake, beet pulp, rice bran, and malt sprouts	Hay, and mixture of soy- bean cake, beet pulp, rice bran and malt sprouts		
2nd (4/VIII-15/VIII)	Rice straw, 50 % of rice bran in total concentrates, others are the same as in 1st period	Same feed as in 1st period, added green dent corn sometimes		

Table 91 (Continued)

Experimental period	Cows No. 154 and 146	Cows No. 115 and 110			
3rd (16/VIII-4/IX)	Same feed as in 2nd period and added 150 g. of "calcium" daily.	Same feed as in 1st period, added green red clover and 150g. of "calcium" daily			
4th (5/IX-14/IX)	Same feed as in 1st period	Same feed as in 1st period and some times given green red clover			

Notice: "Calcium" consists mainly of ground oyster shell and partially of ground whale bone.

Cows No. 154 and 146 were hitched in the stall all day long but cows No. 115 and 110 were kept in the exercise enclosure except at the milking time. Fifty g. of salt were added to each daily ration during the experimental period.

## 2. Experimental results

The experimental results with each cow throughout the experimental period are as follows.

TABLE 92

Influence of "Calcium" dosage upon the alcohol susceptibility of milk

	Cow No.		154			146		Average of 154 and 146		
Experimental period	Date	Acidity	Alc. 9 degree coagu		Acidity	Alc. 9 degree coagu		Acidity	Alc. % and degree of milk	
		———	Experi- mented	Con- verted	¥	Experi- mented	Con- verted	AC	coagulation Converted	
1st	25/VII 26 27 28 29 30 31 1/VIII 2 3	0.195 0.190 0.190 0.190 0.190 0.190 0.195 0.190 0.190	78± 82+ 82++ 82++ 82+ 82+ 82+ 82++ 82++ 78±	79+ 82+ 81+ 81+ - 82+ 82+ 81+ 81+ 79+	0.210 0.215 0.215 0.210 	78+ 78+ 78+ 78++ - 78++ 78++ 78++ 78++	78+ 78+ 78+ 77+ - 77+ 77+ 78+ 77+	0.203 0.203 0.203 0.200 	78.5÷ 80.0÷ 79.5÷ 79.0÷ - 79.5÷ 79.5÷ 79.5÷ 79.0÷ 78.0÷	

Table 92 (Continued)

	Cow No		154		<del></del>	146			rage of 154 and 146
Experi-	Date	Acidity	degree	% and of milk lation	Acidity	degree	% and of milk lation	Acidity	Alc. % and degree of milk
period		Aci	Experi- mented	Con- verted	Aci	Experi- mented	Con- verted	Aci	coagulation   Converted
	4 5	0.185	78+	78+	0.200	78+	78+	0.198	78.0+
	6 7 8 9 10 11	0.185 0.190 0.190 0.185 0.190 0.185	78+ 82++ 78+ 78± 78± 78+	78+ 81+ 78+ 79+ 79+ 78+	0.215 0.210 0.210 0.215 6.230 0.230	78++ 78+ 78+ 78++ 78+	77+ 78+ 78+ 77+ 78+ 77+	0.200 9.200 0.200 0.200 0.210 0.200	77.5+ 80.0+ 78.0+ 78.0+ 78.5+ 77.5+
	12 13 14 15	0.185 0.185 0.185	82++ 82++ 82++	81+ 81+ 81+	0.230 0.235 0.230	78+ 78++ 78+	78+ 77+ 78+	0.208 0.210 0.238	79.5+ 79.0+ 79.5+
	16 17 18 19	0.190 0.190 0.190	82++ 82++ 78±	81+ 81+ 79+	0.220 0.215 0.210	78+ 78+ 78+	78+ 78+ 78+	0 205 0.203 0.200	79.5+ 79.5+ 78.5+
3rd	20 21 22 23 24 25 26	0.185 0.170 0.180 0.180 0.180 0.185	$\begin{array}{c} 78 \pm \\ 82 + + \\ 82 + + \\ 82 + + \\ 82 + + \\ 82 + + \\ - \end{array}$	79+ 81+ 81+ 81+ 81+ 81+	0.210 0.210 0.210 0.210 0.210 0.220 0.205	78++ 78± 78+ 78+ 78+ 78+	77+ 79+ 78+ 78+ 78+ 78+	0.198 0.190 0.190 0.190 0.200 0.195	78.0+80.0+79.5+79.5+79.5+79.5+
	27 28 29 30 31 1/IX	0.195 0.190 0.180 0.180 0.185 0.175	78± 82++ 82++ 78+ 82++ 82++	79+ 81+ 81+ 78+ 81+ 81+	0.225 0.220 0.220 0.235 0.240 0.235	$78\pm \\ 78++\\ 78++\\ 78\pm \\ 78++\\ 78\pm \\ 78++$	79+ 77+ 79+ 77+ 79+ 77+	0.210 0.205 0.200 0.210 0.213 0.205	79.0+ 79.0+ 80.0+ 77.5+ 80.0+ 79.0+
	2 3 4	0.180 0.175	82++ 78±	81+ 79+	0.225 0.225	78± 78±	79+ 79+	0.203 0.200	80.0+ 79.0+
	5 6 7 8 9	0.175 0.175 0.180 0.180	78+ 82++ 82++ 78±	78+ 81+ 81+ 79+	0.225 0.225 0.225 0.225	78++ 78++ 78+ 78+	77+ 77+ 78+ 78+	0.200 0.200 0.205 0.205	77.5+ 79.0+ 79.5+ 78.5+
4th	10 11 12 13 14	0.175 0.180 0.185 0.170 0.175	78+ 78+ 82++ 82++ 78±	78+ 78+ 81+ 81+ 79+	0.230 0.235 0.220 0.230 0.230	78+ 78+ 78+ 78++ 78++	78+ 78+ 77+ 77+ 78+	0.203 0.208 0.203 0.200 0.203	78.0+ 78.0+ 79.0+ 79.0+ 78.5+

Table 92 (Continued)

	Cow No.		115			110			age of 115 and 146
Experi- mental	Date	Acidity	degree	% and of milk lation	Acidity	Alc. 9 degree coagu	% and of milk lation	Acidity	Alc. % and degree of milk
period		Aci	Experi- mented	Con- verted	Aci	Experi- mented	Con- verted	Aci	coagulation Converted
	25/VII 26 27 28 29	0.165 0.160 0.165 0.165	66+ 66++ 66+ 70++	66+ 65+ 66+ 69+	0.155 0.155 0.160 0.150	70++ 70++ 66± 66±	69+ 69+ 67+ 67+	0.160 0.158 0.163 0.158	67.5+ 67.0+ 66.5+ 68.0+
1st	30 31 1/VIII 2 3	0.160 0.165 0.165 0.155 0.165	66+ 66+ 66+ 66+ 66±	66+ 66+ 65+ 66+ 67+	0.160 0.155 0.155 0.155 0.155	70++ 66+ 66+ 66+ 66±	69+ 66+ 66+ 66+ 67+	5.160 0.160 0.160 0.165 0.160	67.5+ 66.0+ 65.5+ 66.0+ 67.0+
2nd	4 5 6 7 8 9 10 11 12 13 14	0.165 0.165 0.165 0.160 0.165 0.165 0.165 0.165 0.165	66+ 70++ 70++ 66++ 66++ 66+ 66+ 66+ 66+ 66+	66+ 	0.155 	66+ 66++ 66++ 66+ 66+ 66+ 66+ 66+ 66+	66+ 66+ 65+ 66+ 66+ 66+ 67+ 67+ 66+	0.160 	66.0+  67.5+ 67.0+ 66.0+ 65.5+ 66.0+  66.5+ 66.5+ 66.5+
3rd	16 17 18 19 20 21 22 23 24 25 26 27 28 29 30	0.165 0.165 0.165 0.175 0.160 0.165 0.170 0.165 0.160 0.160 0.160 0.160 0.155	66+ 66+ 66+ 70++ 70++ 70++ 70++ 70++ 70+	66+ 66+ 66+ 69+ 69+ 69+ 69+ 69+ 66+ 65+	0.155 0.150 0.155 	66± 66± 70++ 70++ 66+ 66+ 70++ 70++ 66± 66± 66± 70++	67+ 67+ 69+ 69+ 66+ 66+ 69+ 67+ 67+ 69+	0.160 0.158 0.160 	66.5 + 66.5 + 66.5 + 67.5 + 69.0 + 69.0 + 69.0 + 69.0 + 68.0 + 66.5 + 67.0 +
·	31 1/IX 2 3 4	0.165 0.165 0.165	66++ 66++ 66++ 66++	65+ 65+ 65+ 65+	0.155 	66± 66+	$^{69+}_{67+}_{-}_{66+}$	0.158 0.158 - 0.160 0.160	66.0+ 66.0+ 66.0+ 65.5+

Table 92 (Continued)

	Cow No.		115			110			age of 115 nd 110
Experi-	Date	ity	degree	% and of milk lation	ity	degree	% and of milk lation	ity	Alc. % and degree of milk
period	!	Acidity	Experi- mented	Con- verted	Acidity	Experi- mented	Con- verted	Acidity	coagulation Converted
	5	0.165	70++	<b>69</b> +	0.155	66+	66+	0.160	67.5+
	6	0.165	66±	<b>67</b> +	0.155	66+	66+	0.160	66.5+
	7	0.160	66±	67+	0.150	66±	67+	0.155	67.0+
	8	0.160	66 ±	67+	0.150	66±	67+	0.155	67.0+
441	9	_		_	_		_		
4th	10	0.165	66±	67+	0.150	66+	<b>66</b> +	0.158	66.5+
	11	0.165	66±	67+	0.150	66±	67+	0.158	67.0+
	12	0.160	66±	67+	0.150	66±	67+	0.155	67.0+
	13	0.160	70++	69+	0.150	66±	67+	0.155	68.0+
	14	0.165	66±	67+	0.155	66±	67+	0.150	67 <b>.</b> 0+

Milk produced by the cows No. 154 and 146 was in each case coagulated with the same volume of 78% alcohol at the beginning of this experiment. In the 2nd period hay was replaced by rice straw, and 50% of the concentrates were replaced by rice bran. The cow's appetite decreased on account of the sudden change in ration from hay to the rice straw, so that it was necessary to increase the amount of concentrates somewhat but gradually the cows accustomed to the rice straw feeding. As soon as the feed was changed, the daily milk production of these cows decreased, yet the alcohol susceptibility of milk was not influenced.

In the 3rd period 150 g. "calcium" was added to the ration keeping the ration otherwise the same as in the 2nd period. The alcohol susceptibility and the acidity of the milk did not change at all throughout the 3rd period. Especially the acidity of milk of cow No. 146 did

not show any influence of the "calcium" dosage in spite of its original high acidity such as 0.22%.

In the 4th period the cows were not given the calcium material but the alcohol susceptibility and the acidity of the milk were almost constant.

On the other hand cows No. 115 and 110 secreted milk which were coagulated with the same volume of 66% alcohol at the beginning of the experiment but the health condition of these cows was very good and the basic ration were always the same throughout the experimental period. In the 3rd period each cow was given 150 g. of "calcium" daily, but the author did not find any change in the alcohol susceptibility and acidity in the milk comparing with the control. That is to say: these cows always secreted milk which was coagulated by the same volume of 66% alcohol even in the 3rd period in spite of the plentiful dosage of calcium materials.

It is recognized that the degree of alcohol stability of milk which is secreted by healthy cows can not be changed by addition of any calcium salts to a ration however strong was the alcohol susceptibility in the normal condition. But it is not difficult to imagine that the health of a cow may be disturbed by an unrational feeding for a long continued period. Under such a condition the milk production may be decreased gradually and the alcohol stability may become variable.

The author will show these experimental results in the graph as follows:

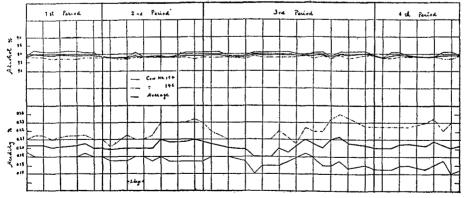


Fig. 15. Influence of Calcium dosage upon the alcohol susceptibility of milk.

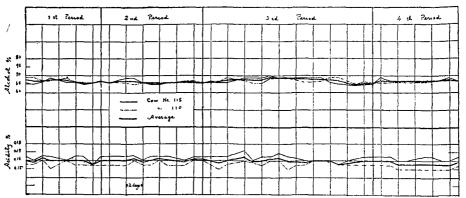


Fig. 16. Influence of Calcium dosage upon the alcohol susceptibility of milk.

# IX. Influence of Feeding a Ration Rich or Poor in Protein upon the Alcohol Susceptibility of Milk

There have been hitherto a good many experiments reported concerning the effect of a protein rich or poor ration for the cows upon the milk production. LINDSEY (43) obtained the experimental result that there was a 15% increase in the milk production by giving a double amount of protein in the feeding standard to milking cows.

It it well known fact that the balance of the protein in the ration is very important from various standpoint of feeding dairy cows. HART and HUMPHREY (25) reported that it is necessary to mix the various kind of protein feed whose origin is different for an increased milk production instead of using the same quality of protein whose origin is the same.

On the other hand August, Brahman and Kriss (2) reported ten years ago that the influence of the protein rich ration upon the milk production is not yet determinantly definite. According to the study of Kappeli and Schneider (38) the milk production and the body weight are beneficently influenced by the protein rich ration for the milking cow. Moreover Perkins and Monroe (60) concluded from the study of the influence of the protein rich ration upon the metabolism in the cow's body and the power to digest feed that a protein rich ration exerts a more favourable influence than a protein poor ration upon the utility of feed and water and also upon the balance of nitrogen, and calcium in the animal's body.

On the other hand HAYDEN and PERKINS (28) advocated that the protein rich ration for a milking cow does not exert a good influence upon the health of cow though it causes some increase in the milk production. HANSSON (24) likewise obtained the experimental result that the ration with protein rich feeds for a cow does not exert a good influence upon the health though the milk production increased about 10–20%, due to its temporary stimulation to the milk gland.

Recently SAVAGE (72) concluded that there is not any influence of excess protein ration upon the milk production, because the feeding with the protein comprising 20% in the total digestible nutrients rather than 16% or 24% is the most favourable condition for the milk production.

The author carried on experiments to learn whether the protein rich or poor ration has any influence upon the alcohol susceptibility of milk and also upon the content of P, Ca and Mg in milk.

### 1. Experimental methods

The following three Ayrshire cows were used in this experiment.

TABLE 93
Particulars as to the cows

Cow No.	97	111	114	
Date of birth	20/XI 1918	25/VIII 1921	29/III 1922	
Date of parturition	21/VII 1928	21/VIII 1928	23/VIII 1928	
Daily milk production kg.	8.0	7.0	8.0	

Each cow was milked twice, morning and evening, per day and the samples were taken in the evening.

The specific gravity, fat %, the alcohol susceptibility of milk and the ash constituents in milk were determined.

There were 5 periods (75 days) in this experiment, each of 15 days (6 days for preparation 9 days for the experiment itself). The

1st, 3rd and 5th were for control, the 2nd was the protein rich period and the 4th the period for feeding a protein poor ration.

The feeding standards used in this experiment were those of NILS HANSSON (23). Among the 3 experimental cows No. 97 was used for the control throughout the whole experimental period. The feeding stuffs used were hay, corn silage, soybean cake, wheat bran, malt sprouts and starch cake. Each of these feeds was analyzed before hand and the starch value and digestive protein were obtained by using the NILS HANSSON'S standard (23).

TABLE 94
Constituents of the feeding stuffs (%)

Feeds	Soybean cake	Wheat bran	Malt sprouts	Starch cake	Hay	Corn silage
Total solid	81.01	81.72	85.93	81.81	85.09	20.06
Crude protein	41.21	17.21	26,50	3.67	7.12	1.65
Crude fat	7.35	5.29	1.32	0.50	1.93	1.09
N-free extracts	26.85	55.74	41.86	59.35	45.80	9.50
Crude fiber	6.02	4.97	10.91	6.38	26.75	5.91
$\mathbf{A}\mathbf{s}\mathbf{h}$	5.59	4.52	5.34	11.90	5.49	1.88
Dig. protein	36.22	13.02	13.06	_	2.85	0.42
Starch value	76.40	52.67	38.11	48.25	35.75	9.30

Each cow was fed daily with 16 kg. of corn silage and 3 kg. of hay and the weight of feeding stuffs given daily in each period was as follows.

TABLE 95
Feeding table for each cow (kg.)

Experimental period	Feeding stuff	Amount by weight	Dig. protein	Starch value	Dig. protein	:	Starch value
Control	Wheat bran	2.12	0.28	1.12			
1st	Malt sprouts	1.32	0.17	0.50			
3rd and 5th	Starch cake	2.01	-	0.97			
	Total	5.45	0.45	3.59	1	:	5.75

Table 95 (Continued)

Experimental period	Feeding stuff	Amount by weight	Dig. protein	Starch value	Dig. protein	:	Starch value
Protein rich	Soybean cake	2.00	0.72	1.53	]		
2nd	Malt sprouts	1.50	0.20	0.57			
2nd	Wheat bran	1.00	0.13	0.53			
	Total	4.50	1.05	2.63	1	:	2.50
Protein poor	Wheat bran	1.40	0.13	0.38			
•	Malt sprouts	1.00	0.18	0.74			
4th	Starch cake	3.00	-	0.45			
	Total	5.40	0.31	2.57	1	:	8.29

# 2. Experimental results

The experimental results of this experiment are as follows.

TABLE 96

Influence of feeding a ration rich or poor in protein upon the alcohol susceptibility of milk

	Cow No.		97	1	111	]	14	Average of 111 and 114	
Experi- mental period	Date	degree	% and e of milk ulation	degree coag	% and e of milk culation	degree coag	% and e of milk ulation	Alc. % and degree of milk coagulation	
period	L	Experi- mented	Converted	Experi- mented	Converted	Experi- mented	Converted	_	
1st Control	15/X 16 17 18 19 20 21 22 23 24 24 25 26 27 28 29	82± 82± 82± 82± 86+ 86+ 86+ 86+ 86+ 86+ 86± 86±	83 + 83 + 83 + 83 + 86 + 85 + 86 + 85 + 86 + 85 + 86 + 87 + 87 + 87 + 87 + 87 + 87 + 88 +	86± 86+ 86+ 86+ 86+ 86+ 86+ 86+ 86++ 86+	87+86+86+86+86+86+86+85+85+85+85+86+86+86+86+86+86+86+86+86+86+86+86+86+	82±±+++ 86++ 86++±+ 86++++ 86++++ 86+++++ 86+++++	83+ 86+ 86+ 86+ 86+ 86+ 86+ 86+ 86+ 86+ 86	85.0 + 85.0 + 86.0 + 86.0 + 86.0 + 86.0 + 86.0 + 85.5 + 85.5 + 85.5 + 86.0 +	

TABLE 96 (Continued)

	Cow No.		97		111		114	Average of 111 and 114	
Experi-	Date	degree	% and e of milk culation	degree	% and e of milk ulation	degree	% and e of milk ulation	Alc. % and degree of milk	
2nd Protein rich		Experi- mented	Converted	Experi- mented		Experi- mented	Converted	coagulation Converted	
	30	86±	87+	86+	86+	86+	86+	86.0+	
	31	86+	86+	86+	86+	86 +	86+	86.0 +	
	1/XI	86+	86+	86+	86+	86±	87+	86.5 +	
	2	86+	86+	86+	86+	86 +	86+	86.0 +	
	3	86+	86+	$86\pm$	87+	+68	86+	86.5+	
	4	86+	86+	86++	85+	86±	87+	86.0+	
2nd	5	86+	86+	86+	86+	86±	87-+	86.5 +	
Duotoin	6	86±	87+	86+	86+	86±	87+	86.5 +	
	7	86+	86+	86±	87+	90++	89-+-	88.0 +	
	8	86±	87+	90++	89+	90++	89+	89.0 +	
	9	90++	89+	90++	89+	86±	87+	88.0 +	
	10	86±	87+	86±	87+	86	86+	86.5 +	
	11	90++	89+	86±	87+	86+	86+	86.5 +	
	12	86±	87+	86±	87+	86±	87+	87.0 +	
	13	90++	89+	86+	86+	86±	87+	86.5 +	
	14	86±	87+	86±	87+	86±	87+	87.0+	
	15	86±	87+	86±	87+	86+	86+	86.5+	
	16	86±	87+	86+	86+	86++	85+	85.5+	
	17	86±	87+	$86\pm$	87+	86+	86+	86.5+	
	18	86±	87+	86+	86+	86+	86+	86.0+	
	19	86±	87+	86+	86+	86±	87+	86.5 +	
0.1	20	86±	87+	86±	87+	86±	87+	87.0+	
31 <b>a</b>	21	90++	89+	$86\pm$	87+	90++	89-	88.0+	
Control	22	86±	87÷	86±	87+	90++	89-	88.0 +	
	23	86+	87+	86+	86+	86±	87+	86.5+	
	24	90++	89+	86±	87+	86±	87+	87.0+	
	25	86±	87+	86+	86+	86±	87+	83.5+	
	26	86+	87+	90++	89+	86+	87+	88.0+	
	27	90++	89+	86±	87+	86+	86+	86.5+	
	28	83±	87+	86+	87-+-	86±	87+	87.0+	

Table 96 (Continued)

	Cow No.		97		111		114	Average of 111 and 114
Experimental	Date	degree	% and e of milk ulation	degree	% and e of milk culation	degre	% and e of milk culation	Alc. % and degree of milk coagulation
mental period  4th  Protein poor  5th  Control		Experi- mented	Converted	Experi- mented		Experi- mented	Converted	Converted
	29	86±	87+	86±	87+	86±	87+	87 <b>.</b> 0+
•	30	86±	87+	86+	86+	90++	89+	87.5+
	1/XII	86±	87+	86+	86+	90++	89+	87 <b>.</b> 5+
	2	86±	87+	86±	87+	86±	87+	87.0+
	3	86÷	86+	86+	86+	86±	87+	86.5 +
į	4	86±	87+	86±	87+	86±	87+	87.0 +
4th	5	86±	87+	86+	86+	86±	87+	86.5 +
Protein	6	86-	86+	$86\pm$	87+	86+	86+	86.5+
	7	86+	86+	86±	87+	86+	86+	8 <b>6.</b> 5+
	8	86±	87+	86±	87+	86+	86+	86.5 +
	9	86±	87+	86+	86+	86+	86+	86.0 +
	10	86±	87+	86+	86+	86+	86+	86.0+
	11	86+	86+	86 <sup>.</sup> ±	87+	86± .	87+	87.0+
	12	86±	87+	$86\pm$	87+	87±	87+	<b>87.</b> 0+-
	13	86-+	86+	86±	8`+	86+	86+	$86\ 5+$
	14	86±	87+	86±	87+	86+	86+	86.5+
	15	90++	89+	86+	86+	86+	86+	86.0 +
	16	90++	89+	$86\pm$	87+	86+	86+	86.5 +
	17	86±	87+	$86\pm$	87+	86±	87+	87.0+
	18	86±	87+	$86\pm$	87+	86±	87+	87.0十
	19	86±	87+	$86\pm$	87+	86+	86+	86.5 +
5th	20	82±	87+	90++	89+	86+	86+	87.5 +
	21	90++	89+	$86\pm$	87+	$86\pm$	87+	87.0 +
Control	22	86±	87+	$86\pm$	87+	86+	86+	86.5 +
	23	86+	86+	$86\pm$	87+	86+	86+	86.5+
	24	86±	87 +	86+	86+	$86\pm$	87+	86.5 +
	25	86±	87+	$86\pm$	87+	$86\pm$	. 87+	87.0 +
	26	86±	87+	86+	86+	86+	86+	86.0+
	27	86±	87+	86±	87+	86+	86÷	86.5+
	28	90++	89+	86+	86+	$86\pm$	87+	86.5+

The quantitative determination of some physico-chemical properties of each milk which was made twice in each period is shown in the following table.

TABLE 97

Physico-chemical properties of milk of cow No. 97

Cow No.					9	7				
Experi- mental period	1st 2n		nd 3re		rd 4t		zh		5th	
Date of sample taken	21/X	29/X	5/XI	13/XI	20/XI	28/XI	5/XII	13/XII	20/XII	28/XII
Spec. gravity	1.0293	1.0305	1.0305	1.0305	1.0313	1.0305	1.0335	1.0337	1.0303	1.0300
Fat %	4.30	4.40	5.40	5.70	5.50	6.00	5.30	6.00	6.50	5.20
Total solid	13.675	13.376	13.998	14.527	15.023	14.940	15.560	14.910	15.684	15.180
Ash	0.706	0.716	0.701	0.722	0.743	0.755	0.759	0.754	0.754	0.785
CaO	21.264	21.488	21.577	20.110	21.785	20.883	22.193	<b>22.3</b> 50	21.684	21.549
Cl	15.192	15.887	15.372	14.856	14.554	14.554	14.463	14.554	14.857	14.554
MgO	2.144	2.150	2.252	2.628	2.801	2.652	2.425	2.504	2.259	2.277
$P_2O_5$	27.587	29.397	28.716	28.999	29.671	28.231	29.848	30.329	29.524	30.061

Table 98

Physico-chemical properties of milk of cow No. 111

Cow No.						11					
Experi- mental period	1:	st	<b>2</b> 1	nd	31	rd	41	th.	5th		
Date of sample taken	21/X	29/X	5/XI	13/XI	20/XI	28/XI	5/XII	13/XII	20/XII	28/XII	
Spec. gravity	1.0294	1.0295	1.0300	1.0310	1.0312	1.0324	1.0305	1.0310	1.0312	1.0308	
Fat %	3.70	3.90	4.25	4.20	3.80	4.60	5.20	5.30	5.10	5.80	
Total solid	13.345	13.142	13.220	13.540	13.140	13.875	14.755	14.862	14.889	14.512	
$\mathbf{A}\mathbf{s}\mathbf{h}$	0.750	0.738	0.725	0.731	0.759	0.781	0.781	0.786	0.797	0.776	
· CaO	20.590	21.757	21.174	20.491	20.547	2 <b>1.6</b> 00	21.068	20.866	20.872	20.383	
Cl	14.888	16.251	15.746	13.372	14.554	14.554	15.645	14.554	14.554	14.250	
MgO	2.155	2.132	2.180	2.596	2.703	2.548	2.407	2.483	2.332	2.348	
$P_2O_5$	28.868	29.308	29.531	29,133	31.487	30.406	29.310	29.800	31.001	30.25 <b>2</b>	

Table 99

Physico-chemical properties of milk of cow No. 114

Cow No.					1	14				
Experimental period	1:	st	2r	ıd	31	rd	41	t <b>h</b>	5th	
Date of sample taken	21/X	29/X	5/XI	13/XI	20/XI	28/XI	5/XII	13/XII	20/XII	28/XII
Spec. gravity	1.0288	1.0304	1.0300	1.0293	1.0315	1.0317	1.0300	1.0318	1.0304	1.0305
Fat %	5.25	4.70	4.20	4.90	4.40	5.00	5.30	5.60	5.20	5.70
otal solid	12.788	13.305	12.945	13.305	13.483	13.900	14.093	<b>15.14</b> 3	15.491	14.720
Ash	0.688	0.653	0.670	0.653	0.716	0.720	0.704	0.725	0.726	0.710
CaO	20.686	20.318	20.732	20.318	20.177	20.502	20.524	21.936	21.387	21.288
Cl	15.979	14.980	15.190	14.980	14.857	15.463	15.736	14.554	15.039	15.463
MgO	2.216	2.358	2.216	2.358	2.734	2.850	2.677	2.526	2.305	2.305
$P_2O_5$	28.242	29.993	30.052	29.993	30.688	39.395	28.347	30.278	30.385	30.189

Milk of cow No. 97 which was fed with the control ration throughout experimental period was almost constant in regard to its alcohol susceptibility. The average percentages of alcohol which caused the milk to coagulate in a degree of + in each period from 1st to 5th were as follows: 84.9, 86.7, 87.3, 86.6 and 86.7%.

Cows No. 111 and 114 which were used in the experiment secreted milk of almost the same quality in respect to the alcohol coagulation throughout the experimental period as shown in table 96; i.e., the percentages of alcohol which caused the milk of these cows to coagulate in each period were as follows, 85.9, 85.3% in the 1st, 86.7 and 86.9% in the 2nd, 86.4 and 86.9% in the 3rd, 86.6% and 86.7% in the 4th and 86.7 and 86.4% in the 5th period respectively.

The ash constituents of milk especially P, Ca and Mg in each period also were almost constant throughout the experimental period though there was some difference with individuals.

The results are shown as following.

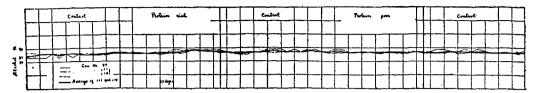


Fig. 17. Influence of feeding a ration rich or poor in protein upon the alcohol susceptibility of milk.

# X. Influence of a Sudden Change in Cow's Ration upon the Alcohol Susceptibility of milk

It is not favourable for a milking cow to change the kind of ration suddenly as it sometimes caused an interruption in the appetite or the hindrance of the digestive organs, so it is the general custom to change the ration slowly and to make substitutions through 7–10 days if necessary.

The author will report here the results of his experiments whether the sudden change of coarse or concentrated feed for a milking cow does influence the health and the alcohol coagulation of milk.

## A. Case of Sudden Change of Coarse Fodder

## 1. Experimental methods

Three Holstein cows were used in this experiment. They were fed a mixture of soybean cake, green peas, wheat bran, candy cake to which were added 1% of salt and bone meal as concentrates.

The whole experimental period comprised 40 days consisting of 4 periods. In the 1st, the cows were given 3.5 kg. of hay and 20 kg. of corn silage, but in the 2nd, 35 kg. of corn silage only, in the 3rd, 40 kg. of mangels only and in the 4th period the animals were returned to the ration of the 1st period. Of course the changing of the fodder was carried out suddenly on the morning of the first day of the each new period.

The author observed the health condition of cow and the alcohol susceptibility of the milk throughout the experimental period.

Some accounts on the cows are as follows.

Cow No.	237		243	3	31	2
Date of birth	12/IV	1923	28/VII	1923	10/IV	1930
Date of parturition	12/IV	1932	5/XI	1932	26/V	1932
Date of next parturition	1/IX	1933	25/XI	1933	7/XI	1933
Daily milk production kg.	13.		13.	0	10	.0

#### 2. Experimental results

The experimental results with each cow are as follows.

## 3. Discussion

The acidity of the milk of cow No. 237 was almost constant at 0.175% although the coarse fodder of hay and corn silage was changed to silage only, but when she was obliged to eat on mangels only in place of corn silage the acidity increased a little to 0.185%. In the 4th period when these mangels were replaced by hay and silage, the acidity of the milk decreased to 0.175%.

The cow No. 243 secreted normal milk in the 1st period whose acidity showed 0.160%. After 3 days in the 3rd period of mangel feeding her appetite decreased and she had a severe attack of diarrhoea. The milk production decreased suddenly. Therefore it was necessary to replace the mangels with the normal ration of hay and corn silage after 7 days of this period. Fortunately her health recovered generally and she was carried through the experiment without changing the coarse fodder. The acidity of her milk increased in the 3rd period showing 0.175% but in the 4th period it returned to the normal of 0.165%.

In the case of cow No. 312 the acidity of the milk increased a little in the 3rd period of the mangel feeding to 0.183%. This agrees well with the result obtained from cow No. 237.

Table 100

Influence of sudden change of coarse fodder upon the alcohol susceptibility of milk

	Cow No.		237			243			312		Averag	ge of 3 cows
Experi-	Date	Acidity	degree	% and of milk lation	Acidity	degree	% and of milk ulation	Acidity	degree	% and of milk	Acidity	Alc. % and degree of milk coagulation
period			Experi- mented	Converted		Experi- mented	Converted		Experi- mented	Converted		Converted
	24/II 25 26	0.170 0.175	74+ 74+	74+ 74+	0.170 0.170	82++ 82++	81+ 81+	0.185 0.185	78++ 74±	77+ 75+	$0.175 \\ 0.177$	77.3+ 77.0+
1st Hay and Corn silage	27	0.175 0.175 0.175 0.175 0.175 0.175	$74+74+70\pm 74+74+74+74+$	74+ 74+ 71+ 74+ 74+ 74+	0.170 0.165 0.165 0.140 0.145 0.150	$82++\ 82++\ 82+\ 82+\ 82+\ 82+\ -$	81+ 81+ 82+ 82+ 82+ 82+ 82+	0.185 0.175 0.180 0.175 0.175 0.175	$78++$ $78++$ $74\pm$ $74\pm$ $78\pm$ $82++$	77+ 77+ 75+ 75+ 79+ 81+	0.177 0.172 0.173 0.163 0.165 0.167	77.3+ 77.3+ 76.0+ 77.0+ 78.3+ 79.0+
2nd Corn silage	6 7 8 9 10 11 12 13 14 15	0.175 0.175 0.175 0.175 0.175 0.175 0.175 	74+ 74+ 74± 74+ 74+ 74+ 74+ 74+ 74+	74+ 74+ 75+ 75+ 75+ 74+ 74+ 75+ 74+	0.167 0.165 0.160 0.160 0.155 0.160 - 0.165 0.160 0.165	82 ± 82 ± 82 ± 82 ± 82 ± 82 ± 82 ± 82 ±	83+ 83+ 83+ 83+ 83+ 83+ 83+ 82+	0.183 0.175 0.175 0.175 0.175 0.180 	78± 78± 78± 78+ 78+ 78± 78± 78± 78±	79+ 79+ 79+ 78+ 78+ 79+ 79+ 79+ 79+	0.172 0.172 0.170 0.170 0.165 0.172 - 0.172 0.172 0.173	78.3+ 78.3+ 79.0+ 78.0+ 78.7+ 78.7+ 78.7+ 78.3+

	Cow No.		237			<b>2</b> 43			312		Averag	ge of 3 cows
Experi-	Date	Acidity	degree	% and of milk alation	Acidity	degree	% and of milk	Acidity	degree	% and of milk lation	Acidity	Alc. % and degree of milk coagulation
period			Experi- mented	Converted		Experi- mented	Converted		Experi- mented	Converted		Converted
3rd Mangels	16 17 18 19 20 21 22 23 24 25	0.175 0.185 0.18) - 0.185 0.185 0.190 0.190 0.180 0.185	74+ 74++ 74± - 74+ 74+ 74+ 74+ 74+ 74+	74+ 73+ 75+  75+ 74+ 74+ 75+ 73+ 75+	0.165 0.175 0.175 	$\begin{array}{c} 82 \pm \\ 82 \pm \\ 78 + \\ - \\ 66 \pm \\ 74 + \pm \\ 78 + \\ 78 \pm \\ 82 \pm \\ 82 \pm \end{array}$	83+ 83+ 78+  67+ 73+ 78+ 79+ 83+ 83+	0.180 0.180 0.180 	$78 \pm \\ 78 \pm \\ 78 \pm \\ - \\ 78 \pm \\ 82 + \\ 78 \pm \\ 82 + + \\ 78 \pm \\ 78 + + \\ 74 \pm $	79+ 79+ 79+ 79+ 79+ 81+ 79+ 75+	0.173 0.180 0.178 	78.3+ 78.7+ 77.3+ - 73.7+ 75.3+ 77.7+ 77.7+ 77.7+
4th Hay and Corn silage	26 27 28 29 30 31 1/IV 2 3 4	0.180 0.185 0.175 0.175 0.175 0.175 0.175 0.175	$ \begin{array}{c} -\\ 70 \pm\\ 70 \pm\\ 74 +\\ 74 \pm\\ 74 \pm\\ 74 \pm\\ 74 \pm\\ 74 \pm \end{array} $	71+ 71+ 74+ 75+ 75+ 74+ - 75+ 75+	0.175 0.170 0.165 0.165 0.165 0.165 0.165 0.165	86++ 86+ 86+ 82± 82+ 82± 82± 82± 82±	85+ 86+ 86+ 83+ 82+ 83+ - 83+ 83+	0.190 0.185 0.185 0.170 0.170 0.180 	$ \begin{array}{c} -74 \pm \\ 78 + \\ 78 + \\ 78 + \\ 78 + \\ 78 + \\ -78 + \\ 78 + \\ 78 \pm \end{array} $	75+ 78+ 78+ 79+ 78+ 78+ 78+ 79+	0.182 0.180 0.175 0.170 0.170 0.173 - 0.173 0.173	77.0+ 78.3+ 79.3+ 79.3+ 78.7+ 78.0+ - 78.7+ 79.7+

The alcohol susceptibility of milk secreted by cow No. 237 throughout the experimental period was almost constant. That is to say, the percentage of the alcohol which coagulated the milk to a degree of + averaged 74% in each period.

However, the alcohol susceptibility of milk of cow No. 243 increased suddenly as a result of her sickness at the beginning of the 3rd period, but it reduced gradually to the normal condition as soon as her health was recovered. Lastly it will be recognizes that the alcohol susceptibility of the milk of cow No. 312 was rather constant as in the milk of cow No. 237; the percentage of alcohol which coagulated the milk was 77–79%.

Therefore it may be stated that the alcohol susceptibility of milk did not change materially under a good healthy condition of the cow even though the kind of coarse fodder was changed suddenly from one to another.

The author obtained an experimental result that an exclusive mangel feeding as a roughage has a tendency to hind digestive actions especially under an unhealthy condition of cow, thereby increasing the acidity and the alcohol susceptibility of milk.

The experimental data will be graphed in the following figures.

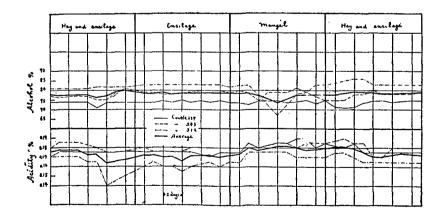


Fig. 18. Influence of a sudden change in coarse fodder upon the alcohol susceptibility of milk.

## B1. Case of Sudden Change of the Concentrates

# 1. EXPERIMENTAL METHODS

Three Holstein cows were used in this experiment. Each was fed with 4 kg. of hay and 25 kg. of corn silage daily as roughages throughout the experimental period.

The experimental period was divided into five sub-periods of 10 days each. In the 1st the cows were given wheat bran only as concentrates, in the 2nd period wheat bran was wholly replaced by soybean cake, in the 3rd period green peas were given instead of the soybean cake, in the 4th crushed oats only and in the 5th period yellow corn.

Determinations of the acidity and alcohol susceptibility of the milk were made daily throughout the experimental period. The milk samples were taken twice in each period for analysis of Cl, P, Ca and Mg.

Each of the salt and bone meal was added in amount of one percent in the concentrates daily to a cow.

Some accounts of the experimental cows are as follows.

Cow No.	2	277	28	34	308	
Date of birth	1/V	1927	23/IX	1927	3/XII	1929
Date of parturition	10/II	1932	13/I	1932	29/I	1932
Daily milk production kg.	1	0.0	8	.0	8	.0

#### 2. Experimental results

The obtained results were as follows.

Table 101

Influence of sudden change of the concentrates upon the alcohol susceptibility of milk

	Cow No.	[	277			281			308		Averag	ge of 3 cows
Experi- mental	Date	Acidity	degree	% and of milk lation	Acidity	degree	% and! of milk ulation	Acidity	degree	% and of milk lation	Acidity	Alc. % and degree of milk coagulation
period			Experi- mented	Converted		Experi- mented	Converted		Experi- mented	Converted	_	Converted
1st Wheat bran	10/X 11 12 13 14 15 16 17 18	0.145 0.150 0.145 0.145 0.150 0.175 0.160 0.165 0.155 0.160	86++ 82± 82± 82± 82± 82± 82± 82± 82± 82±	85+ 83+ 83+ 83+ 83+ 83+ 83+ 83+ 83+	0.160 0.165 0.160 0.160 0.175 0.175 0.165 0.165 0.160 0.175	86++ 90+ 90± 90++ 90+ 90+ 90+ 90++ 86+	87+ 90+ 91+ 89+ 91+ 90+ 89+ 89+ 86+	0.200 0.215 0.210 0.205 0.205 0.200 0.200 0.205 0.190 0.205	86++ 86++ 86± 86+ 86+ 86± 82± 82± 82± 82±	85+ 85+ 87+ 86+ 86+ 87+ 83+ 83+ 83+ 83+	0.168 0.177 0.172 0.170 0.177 0.183 0.175 0.178 0.168 0.180	86.0+ 86.0+ 87.0+ 85.7+ 86.7+ 87.0+ 85.0+ 85.3+ 85.3+ 84.0+
2nd Soybean cake	20 21 22 23 24 25 26 27 23 29	0.160 0.160 0.165 0.170 0.175 0.165 0.170 0.175 0.170 0.180	82 ± 82 ± 82 ± 82 ± 82 ± 82 ± 82 ± 82 ±	83+ 83+ 83+ 83+ 83+ 83+ 83+ 85+ 86+	0.160 0.170 0.175 0.170 0.170 0.170 0.165 0.175 0.165	90++ 86± 90++ 90++ 86± 90++ 90+ 90± 90±	89+ 87+ 90+ 89+ 87+ 89+ 90+ 91+ 91+ 89+	0.200 0.205 0.205 0.210 0.210 0.215 0.200 0.210 0.215 0.210	82± 86++ 86+ 86± 86± 82± 82± 82± 82±	83+ 85+ 86+ 86+ 87+ 83+ 83+ 83+ 83+	0.173 0.178 0.182 0.183 0.185 0.183 0.178 0.187 0.183	85.0+ 85.0+ 86.7+ 86.0+ 86.3+ 86.3+ 85.7+ 85.7+ 86.3+ 87.3+
3rd Green peas	30 31 1/XI 2 3	0.175 0.165 0.175 0.165 0.170	86+ 86+ 86+ 86++	86+ 86+ 86+ 85+ 85+	0.170 0.170 0.170 0.165 0.165	90+ 86± 86± 90++ 90+	90+ 87+ 87+ 89+ 90+	0.205 0.205 0.210 0.200 0.200	86± 86+ 86++ 82± 86++	87+ 86+ 85+ 83+ 85+	0.183 0.180 0.185 0.178 0.178	87.7+ 86.7+ 86.3+ 85.3+ 86.3+

Table 101 (Continued)

	Cow No.		277			284			308		Avera	ge of 3 cows
Experi- mental	Date	Acidity	degree	% and of milk alation	Acidity	degree	% and of milk lation	Acidity	degree	% and of milk	Acidity	Alc. % and degree of milk
period			Experi- mented	Converted		Experi- mented	Converted		Experi- mented	Converted	220,010,	coagulation Converted
3rd Green peas	4 5 6 7 8	0.170 0.165 0.170 0.170 0.170	$82+\ 82\pm\ 82++\ 82\pm\ 78\pm$	82+ 83+ 81+ 83+ 79+	0.165 0.160 0.155 0.160 0.165	$\begin{array}{ c c c }\hline 90++\\ 90++\\ 90++\\ 90+\\ 90+\\ \end{array}$	89+ 89+ 89+ 90+ 90+	0.210 0.205 0.200 0.205 0.205	86++ 86++ 82± 82± 82+	85+ 85+ 83+ 83+ 82+	0.182 0.177 0.175 0.178 0.180	85.7+ 85.7+ 84.3+ 85.3+ 83.7+
4th Oats	9 10 11 12 13 14 15 16 17 18	0.165 0.170 0.175 0.170 0.170 0.175 0.165 0.170 0.165 0.170	82++ 82+ 82+ 82± 86++ 86+ 82± 82+ 82+ 82+	81+ 82+ 82+ 83+ 85+ 86+ 83+ 82+ 82+ 82+	0.170 0.170 0.170 0.165 0.170 0.165 0.165 0.160 0.165 0.170	90++ 86± 90++ 90++ 90++ 90++ 86± 86± 86++ 86±	89+ 87+ 89+ 89+ 89+ 87+ 87+ 87+	0.200 0.205 0.210 0.200 0.210 0.205 0.200 0.205 0.200 0.210	82++ 78± 82± 82+ 82+ 86++ 86++ 82± 82± 82±	81+ 79+ 83+ 82+ 83+ 85+ 85+ 83+ 83+	0.178 0.182 0.185 0.178 0.183 0.182 0.177 0.178 0.177	83.7+ 83.0+ 84.7+ 84.7+ 85.3+ 86.7+ 85.0+ 84.0+ 83.3+ 84.0+
5th Yellow corn	19 20 21 22 23 24 25 26 27 28	0.175 0.170 0.165 0.175 0.175 0.170 0.165 0.175 0.165 0.175	$82 \pm \\ 82 + \\ 78 \pm \\ 78 + \\ 78 \pm \\ 78 \pm \\ 82 + \\ 78 + \\ 82 + \\ 78 \pm $	83+ 81+ 79+ 78+ 79+ 79+ 81+ 78+ 81+ 79+	0.175 0.175 0.170 0.170 0.165 0.160 0.150 0.165 0.175	86± 86++ 86++ 86++ 86+ 86± 86± 86++ 86++	87+ 85+ 86+ 85+ 86+ 87+ 87+ 85+	0,215 0,205 0,205 0,210 0,215 0,200 0,200 0,200 0,210 0,205	$\begin{array}{c} 82 \pm \\ 82 + \\ 82 + \\ 82 + \\ \end{array}$	83+ 83+ 83+ 82+ 83+ 83+ 83+ 83+ 81+	0.188 0.183 0.180 0.185 0.186 0.177 0.175 0.180 0.183 0.183	84.3+ 83.0+ 82.7+ 81.7+ 82.7+ 82.7+ 82.7+ 82.7+ 82.7+

In the next table the results of the quantitative determination of Cl, P, Ca and Mg in each milk in each period are given.

TABLE 102
Effects upon the milk of Cow No. 277 (mg/dl)

	Cow No.				277		
Experimental period	Date of sample taken	Cl	P	Ca	Mg	Acidity	Alc. % and degree of milk coagulation Converted
1st	12/X	222.30	50.00	133.00	15.31	0.145	83+
	18/X	229.91	55.56	137.00	16.54	0.155	83+
2nd	22/X	222.30	51.28	139.00	15.31	0.165	83+
	28/X	224.06	55.56	128.00	13.30	0.175	85+
3rd	1/XI	233.42	52.63	135.00	13.30	0.175	86+
	7/XI	234.00	54.05	139.00	15.31	0.170	83+
4th	11/X (	243.36	52.63	140.00	13.64	0.175	82+
	17/XI	242.19	51.28	134.00	16.54	0.165	82+
5 <b>t</b> h	21/XI	252.72	50.00	134.00	16.02	0.165	79+
	25/XI	243.95	52.63	134.00	16.02	0.165	81+

TABLE 103
Effect upon the milk of Cow No. 284 (mg/dl)

41.00	Cow No.				284		
Experimental period	Date of sample taken	Cl	P	Ca	Mg	Acidity	Alc. % and degree of milk coagulation Converted
1st	12/X	185.45	55.56	114.00	14.71	0.160	91+
	18/X	211.77	55.56	120.00	16.24	0.160	89+
2nd	22/X	190 65	52.63	115.00	15.31	0.175	90+
	28/X	191.88	54.06	121.00	14.50	0.165	91+
3rd	1/XI	201.83	52.63	111.00	15.63	0.170	87+
	7/XI	212.94	52.63	116.00	14.42	0.160	90+
4th	11/XI	197.73	52.63	115.00	14.90	0.170	89+
	17/XI	187.20	51.28	113.00	15.50	0.165	85+
5th	21/XI	203.58	48.78	108.00	16.44	0.170	86+
	25/XI	199.49	52.63	120.00	16.24	0.160	87+

TABLE 104
Effects upon the milk of Cow No. 308 (mg/dl)

	Cow No.				308		
Experimental period	Date of sample taken	Cl	P	Ca	Мg	Acidity	Alc. % and degree of milk coagulation Converted
1st	12/X	159.12	66.67	110.00	16.59	0.210	87+
	18/X	183.69	58.89	117.00	15.67	0.190	83+
2nd	22/X	175.50	66.67	115.00	17.50	0.205	85+
	28/X	175.03	62.63	110.00	15.24	0.215	83+
3rd	1/XI	175.50	64.50	109.00	15.67	0.210	85+
	7/XI	168.48	62.50	10 <b>1.</b> 00	15.98	0.205	83+
4th	11/XI	159.12	64.50	110.00	14.71	0.210	83+
	17/XI	204.17	64.50	102.00	17.36	0.200	83+
5th	21/XI	184.24	61.87	118.00	18.87	0.205	83+
	25/XI	175.50	68.79	113.00	17.36	0.220	83+

In the following figure the above experimental results are graphed to show at a glance whether or not the sudden change of the concentrates has any influence upon the acidity and the alcohol susceptibility of milk.

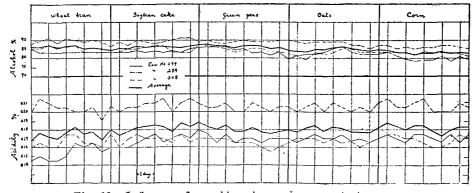


Fig. 19. Influence of a sudden change in concentrates upon the alcohol susceptibility of milk.

This experiment was divided into five periods of 10 days each. The various kinds of concentrates were suddenly changed from one to another from the morning immediately after each period. Wheat bran was fed in the 1st period, soybean cake in the 2nd, green peas in the 3rd, crushed oats in the 4th and yellow corn in the 5th period respectively.

The cows passed the whole experimental period without any alteration in their health in spite of sudden changings of the concentrates.

The average acidities in the milk of cow No. 277 were respectively 0.155, 0.170, 0.170, 0.170 and 0.172% in order of the periods. It was also nearly constant in the milk of cow No. 284 being 0.166, 0.170, 0.165, 0.167 and 0.171%. The acidity was quite uniform also in cow No. 308 though the degree was higher than the others being 0.204, 0.210, 0.205 and 0.211%.

On the other hand there was not any noticeable influence on the alcohol susceptibility of milk by sudden change of the concentrates for cow. The average percentages of alcohol which coagulated milk in each period to a degree of + were as follows: 83.0, 83.5, 83.0, 82.0 and 80.0% for cow No. 277, 89.0, 89.0, 89.0, 88.0 and 86.0% for cow No. 284 and 84.0, 85.0, 84.0, 82.0 and 83.0% for cow No. 308.

The average acidities of these cow's milk in each period were 0.175, 0.183, 0.180, 0.180 and 0.180% and it seems to increase a little in the 2nd period, that of soybean cake feeding. The average alcohol percentages which coagulated these milk were 85.0, 86.0, 86.0, 84.0 and 83.0% showing gradual increase though slight as the period being progressed, but the average graph concerning the alcohol susceptibility throughout the experimental period showed almost a straight line as seen in figure 19.

It may be said that there is a tendency to increase the acidity of milk somewhat by sudden change of concentrates for cow. However, there was not any influence recognizable upon the inorganic matter especially Cl, P, Ca and Mg contents in milk, as results of sudden change of the concentrates as shown in the following table.

TABLE 105

The average experimental results concerning the inorganic matters in milk of each period (mg/dl)

Experimental period		18	at					
Constituents	Cl	P	Ca	Mg				
277 284 308	229.91 211.77 183.69	55.56 55.56 58.89	137.00 120.00 117.00	16.54 16.24 15.67				
Average of 3 cows	208.46	56.67	124.66	16.15				
Experimental period		2n	ıd	·				
Cow No.	Cl	Р	Ca	Mg				
277 284 308	224.06 191.88 175.03	55.56 54.06 62.63	128.00 121.00 110.00	13.30 14.50 15.24				
Average of 3 cows	196.66	57.42	119.66	14.35				
Experimental period	3rd							
Cow No.	Cl	Р	. Ca	Mg				
277 284 308	234.00 212.94 168.48	54.05 52.63 62.50	139.00 116.00 101.00	15.31 14.42 15.98				
Average of 3 cows	205.14	56.38	118.66	15.24				
Experimental period		4t	h					
Cow No.	Cl	P	Ca	Mg				
277 284 308	242.19 187.20 204.17	51.28 51.28 64.50	137.00 113.00 102.00	16.54 15.50 17.36				
Average of 3 cows	211.19	55.69	117.33	16.47				
Experimental period		5t	h					
Cow No.	Cl	P	Ca	Mg				
277 284 308	243.95 199.49 175.50	52.63 52.63 68.79	134.00 120.00 113.00	16.04 16.24 17.36				
Average of 3 cows	206.31	58.02	122.33	16.55				

Nevertheless the author obtained the experimental result, as related in the following section, that the alcohol susceptibility of milk was influenced by a sudden change of concentrates due indirectly to the disturbance of the intestine.

# B2. Case of Sudden Change of Concentrates

## 1. Experimental methods

Three Holstein cows were used in this experiment for 20 days. In the 1st period of 5 days the cows were fed a normal ration consisting 10 kg. of candy cake and 5 kg. of a mixture of wheat bran, oats and cotton seed cake beside the normal coarse fodder including 1% of salt and bone meal. However, from the 6th day of the experiment the author substituted for the mixture 5 kg. of soybean cake only which was soaked in hot water over night. This feeding was continued for 10 days, but on the 16th day the soybean cake ration was displaced by the normal concentrates as in the 1st period.

The author determined the acidity and the alcohol susceptibility of milk daily throughout the experimental period.

Some accounts of the cow are as follows.

Cow No.	249		252		243	
Date of birth	7/111	1924	6/VII	1924	28/VII	1923
Date of parturition	30/IX	1928	28/VIII	1928	. 10/IX	1928
Daily milk production kg.	20.0		18.0		2).0	

## 2. Experimental results

The experimental results which were obtained for each cow are as follows.

• Table 106
Influence of sudden change of concentrates upon the alcohol susceptibility of milk

	Cow No.		249		252			243			Average of 3 cows	
Experimental period	Date	Acidity	Alc. % and degree of milk coagulation		Acidity	Alc. % and degree of milk coagulation		Acidity	Alc. % and degree of milk coagulation		Acidity	Alc. % and degree of milk
			Experi- mented	Converted		Experi- mented	Converted		Experi- mented	Converted		coagulation Converted
1st	31/III 1/IV 2 3 4	0.143 0.153 0.154 0.152 0.151	$94\pm 94\pm 94\pm 94\pm 94\pm 94\pm$	95+ 95+ 95+ 95+ 95+	0.134 0.132 0.135 0.133 0.133	90± 90+ 90+ 90+ 90+	91+ 90+ 90+ 90+ 90+ 90+	0.158 0.158 0.158 0.158 0.158 0.158	90++ 90++ 90++ 90++ 90++	89+ 89+ 89+ 89+ 89+	0.145 0.148 0.149 0.148 0.148	91.7+ 91.3+ 91.3+ 91.3+ 91.3+
2nd	5 6 7 8 9 10 11 12 13 14	0.150 0.165 0.170 0.168 0.163 0.158 0.158 0.158 0.158 0.155	94 ± 90 ± 90 + + 90 + 90 ± 94 + 94 + 94 ± 94 ±	95+ 91+ 89+ 90+ 91+ 94+ 94+ 95+ 95+ 95+	0.132 0.141 0.139 0.143 0.143 0.134 0.136 0.136 0.136	86 ± 86 + 86 + 82 ± 86 + 86 ± 86 ± 90 + 90 +	87+ 86+ 86+ 83+ 85+ 87+ 87+ 89+ 99+	0.156 0.172 0.170 0.164 0.161 0.158 0.158 0.158 0.158 0.158	86 ± 82 ± 86 + + 82 + 86 + + 86 ± 86 ± 90 + + 90 + + 90 ±	87+ 83+ 85+ 85+ 87+ 87+ 89+ 91+	0.148 0.154 0.160 0.158 0.156 0.150 0.151 0.151 0.154 0.147	89.7+ 86.7+ 86.7+ 85.0+ 87.0+ 89.3+ 89.3+ 91.0+ 91.0+ 92.0+
3rd	15 16 17 18 19	0.150 0.154 0.150 0.150 0.150	98++ 94± 98++ 98++ 94±	97+ 95+ 97+ 97+ 95+	0.135 0.136 0.139 0.136 0.134	90++ 86± 86± 90++ 90+	89+ 87+ 87+ 89+ 90+	0.157 0.158 0.156 0.154 0.154	90++ 86+ 86+ 86± 86+	89+ 86+ 86+ 87+ 86+	0.147 0.149 0.148 0.147 0.146	91.7+ 89.3+ 90.0+ 91.0+ 90.3+

#### 3. DISCUSSION

It was recognizable that the health of each cow was disturbed on the 2nd day after the normal concentrates were replaced by the fermented viscid soybean cake without exception. They had each a weak attack of diarrhoea. At the same time the acidity and also the alcohol susceptibility of the milk increased: e.g., in cow No. 249 to 0.165-0.172% from 0.150-0.152% of acidity, and to 91-89% from 95% of alcohol. But as the cows became accustomed to the ration they gradually recovered and the acidity and also the alcohol susceptibility of milk were also recovered to normal as shown in the above table.

Therefore it is clearly ascertained from this experiment that the acidity and also the alcohol susceptibility of milk will temporarily at least be influenced by a sudden change of the concentrates from one to another which was fermented, such as viscid fermented soybean cake which directly caused a disturbance of the health of the cows.

A graph of the experimental results will serve to show this point:

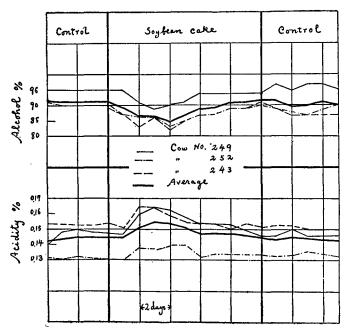


Fig. 20. Influence of sudden change in concentrates upon the alcohol susceptibility of milk

# XI. Influence of Rich or Poor Supply of Balanced Ration for the Cow upon the Alcohol Susceptibility of Milk

It is impossible for a cow to display her milk producing ability without rational feeding and careful management. It is natural that the health and the milk productivity would decline when the cow is fed with a deficient supply of ration for a long time.

The author determined the influence of the quantitative supply of feed upon the alcohol susceptibility of milk by feeding cows with a plentiful or deficient amount of ration.

#### 1. EXPERIMENTAL METHODS

Three Ayrshire cows were used in this experiment for 36 days which were divided into 3 periods. The 1st period was for control, the 2nd was the period of feeding abundant amounts of balanced ration and the 3rd was that of poor allotment of feed. The feeding table for each cow in each period is as follows.

TABLE 107
Feeding table for experiment (kg.)

Feeds Experimental period	Corn silage	Нау	W heat bran	Soybean cake
1st	19.0	1.2	1.2	0.4
2nd	28.0	3.5	2.2	0.8
3rd	14.0	1.5	0.8	0.4

1% of salt and bone meal were added in the ration throughout the experimental period. Some accounts of the cows are as follows.

Cow No.	114	99	94	
Date of birth	29/III 1922	20/XI 1919	23/IV 1918	
Dats of parturition	19/IX 1929	19/IX 1929	30/VII 1929	
Daily milk production kg.	4.0	4.0	4.0	

#### 2. EXPERIMENTAL RESULTS

The experimental results which were obtained in this experiment are as follows.

#### 3. Discussion

Viewing the above experimental results the author will discuss here results whether or not the abundant or scant amount of balanced ration for cows does influence the acidity and the alcohol susceptibility of milk.

The average acidity of the milk of cow No. 114 was about 0.146% in the 1st period, 0.150% in the 2nd, and 0.147% in the 3rd. And the average acidity of the others was almost constant in each period. On the other hand there was no influence on the alcohol susceptibility of milk resulting from the giving of a high or low amount of ration to milking cows for about 12 days. That is to say that the average percentage of alcohol which coagulated the milk of cow No. 114 was about 80.0, 81.5 and 81.0% in each period. Nearly the same results were obtained with cows 97 and 94.

From these results it may be concluded at least that there is not any noticeable influence on the acidity nor on the alcohol susceptibility of milk as a result of feeding cows with a high or low amount of ration for a period of 12 days only.

These experimental results will be shown with graph.

Table 108

Influence of rich or poor amount of balanced ration upon the alcohol susceptibility of milk

	Cow No.		114			97			94		Averag	ge of 3 cows		
Experi- mental	Date	Acidity	degree	% and of milk	Acidity	Acidity Alc. % and degree of milk coagulation		Acidity	Alc. % and degree of milk coagulation		degree of a		Acidity	Alc. % and degree of milk
period			Experi- mented	Converted		Experi- mented	Converted		Experi- mented	Converted	11014103	coagulation Converted		
1st normal	2J/XI 21 22 23 24 25 26 27 28 29 30 1/XII	0.150 0.148 0.150 0.148 0.142 0.140 0.144 0.145 0.144 0.150 0.146	$\begin{array}{c} 82 + + \\ 78 \pm \\ 78 \pm \\ 78 \pm \\ 82 + + \\ 82 + + \\ 82 + + \\ 82 + + \\ 78 \pm \\ 82 + + \\ 82 + + \\ 78 \pm \\ 78 \pm \end{array}$	81+ 79+ 79+ 79+ 81+ 81+ 81+ 81+ 79+ 81+ 79+ 79+	0.140 0.135 0.138 0.140 0.142 0.188 0.143 0.144 0.150 0.148 0.148	90++ 90++ 86± 86± 86± 90++ 90++ 90++ 90++	89 + 87 + 87 + 87 + 87 + 87 + 89 + 89 + 89 + 89 + 89 +	0.150 0.145 0.145 0.145 0.145 0.150 0.145 0.148 0.148 0.142 0.143	$78 \pm \\82++\\78+\\78\pm \\78\pm \\78\pm \\82++\\82++\\78\pm \\78\pm \\78\pm \\78\pm \\78\pm \\$	79+ 81+ 79+ 79+ 79+ 81+ 81+ 79+ 79+ 79+ 79+	0.147 0.143 0.144 0.144 0.145 0.145 0.145 0.147 0.146	83.0+ 83.0+ 81.3+ 81.7+ 82.3+ 82.3+ 83.0+ 83.0+ 82.3+ 82.3+ 82.3+		
2nd rich	2 3 4 5 6 7	0.149 0.150 0.150 0.150 0.150 0.150	82++ 82++ 82++ 82++ 82+ 82+	81+ 81+ 81+ 81+ 82+ 82+	0.148 0.145 0.148 0.150 0.150 0.148	86 ± 90++ 90++ 90++ 86 ± 86 ±	87+ 89+ 89+ 89+ 87+ 87+	0.143 0.148 0.150 0.150 0.145 0.150	82++ 82++ 78± 82++ 82++ 82++	81+ 81+ 79+ 81+ 81+ 81+	0.147 0.148 0.149 0.150 0.148 0.149	83.0+ 83.7+ 83.0+ 83.7+ 83.3+		

Table 108 (Continued)

	Cow No.		114			97			94		Averag	ge of 3 cows
Experi-	Date	Acidity	Alc. % and degree of milk coagulation		Acidity	degree	% and of milk	Acidity	degree	% and of milk ulation	Acidity	Alc. % and degree of milk
period			Experi- mented	Converted		Experi- mented	Converted		Experi- mented	Converted		coagulation Converted
2nd rich	8 9 10 11 12 13	0.150 0.148 0.150 0.150 0.150 0.150	82++ 82++ 82+ 82+ 82++ 82+	81+ 81+ 82+ 82+ 81+ 82+	0.148 0.140 0.145 0.150 0.148 0.148	86± 86± 90++ 86± 86± 86±	87 + 87 + 83 + 87 + 87 + 87 +	0.150 0.150 0.150 0.150 0.150 0.147	78± 78± 78± 82++ 82++ 78±	79+ 79+ 79+ 81+ 81+ 79+	0.149 0.146 0.148 0.150 0.149 0.148	82.3+ 82.3+ 82.7+ 83.3+ 83.0+ 82.7+
3rd poor	14 15 16 17 18 19 20 21 22 22 23 24 25	0.150 0.150 0.150 0.145 0.143 0.145 0.145 0.145 0.150 0.150	82+ 82+ 82++ 82++ 82++ 82+ 82+ 82+ 82+ 8	82 + 82 + 81 + 81 + 81 + 82 + 82 + 82 + 82 + 82 + 82 + 82 +	0.148 0.148 0.146 0.144 0.145 0.145 0.145 0.145 0.145 0.145	90++ 90++ 86± 86+ 86++ 86++ 86± 90++ 86± 86± 86± 86±	89+ 89+ 87+ 87+ 86+ 85+ 87+ 89+ 87+ 87+ 87+	0.145 0.145 0.145 0.145 0.150 0.150 0.145 0.145 0.145 0.145 0.150	$78 + \\ 78 + \\ 78 + \\ 78 \pm \\ $	78+ 78+ 79+ 79+ 79+ 79+ 79+ 79+ 79+ 79+ 79+	0.148 0.148 0.147 0.145 0,146 0.147 0.145 0.145 0.145 0.147 0.148	83.0+ 83.0+ 82.3+ 82.3+ 82.0+ 82.3+ 82.7+ 83.3+ 82.7+ 82.7+ 82.7+ 82.7+

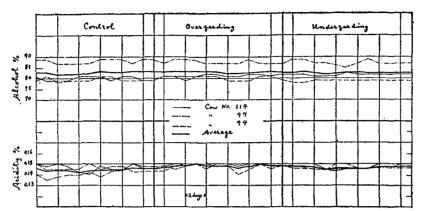


Fig. 21. Influence of over or underfeeding upon the alcohol susceptibility of milk.

# XII. Relation of Fat Content upon the Alcohol Susceptibility of Milk

It is quite distinct that there is a close relation between the alcohol susceptibility and the salt balance in milk as has already been ascertained in the former experiments.

On the other hand SENO (78) found that the casein and albumin content in milk does not exert any influence upon the coagulation of milk by alcohol. But the author can not find any detailed literature concerning the relation of fat content to the alcohol coagulation of milk, so he made a determination of this problem as related in this chapter.

#### 1. Experimental methods

Six Holstein cows were used in this experiment for 58 days. The fat percentage, acidity and the alcohol stability were determined daily.

The feeding and management of these cows were uniform and they all passed through the whole experimental period in healthy condition.

#### 2. Experimental results

The experimental results throughout the experimental period are given in the following tables.

Table 109

Influence of fat content upon the coagulation of milk by alcohol

Cow No.		2	60			. 2	92	
Date	Acidity	degree	% and of milk ulation	Fat %	Acidity	degree	% and of milk	Fat %
		Experi- mented	Converted			Experi- mented	Converted	- 40 /0
12/XI	0.185	82+	82+	3.60	0.160	82+	82+	3.21
13	0.175	$82\pm$	83+	3.19	0.155	$82\pm$	83+	3.31
14	1.170	82++	81+	3.10	0.155	82+	82+	3.41
16	0.155	82 +	82+	3.29	0.150	86++	85+	3.10
17	0.150	82++	81+	3.00	0.155	86++	85+	3.20
18	0.155	82+	82+	2.89	0.160	82+	82+	3.35
19	0.160	$82\pm$	83+	3.50	0.150	86++	85+	3.25
20	0.150	82++	81+	3.20	0.150	86++	85+	3.10
21	0.160	$82\pm$	83+	3.00	0.150	86++	85+	3.50
23	0.165	$82\pm$	83+	3.10	0.155	$82\pm$	83+	3.30
24	0.160	$82\pm$	83±	2.91	0.150	86++	85+	3.30
25	0.155	$82\pm$	83+	3.25	0.150	86++	85+	3.40
26	0.155	82+	82+	3.30	0.155	86++	85+	3.40
27	0.158	$82\pm$	83+	3.10	0.170	86++	85+	3.50
28	0.160	$82\pm$	83+	3.20	0.170	86++	85+	3.50
<b>3</b> 0	0.155	82++	81+	3.70	0.155	86+	86+	3.31
1/XII	0.160	82++	81+	3.41	0.157	86+	86+	3.41
2	0.160	82++	81+	3.65	0.165	86 +	86+	3.65
4	0.163	82++	81+	3.70	0.165	86+	86+	3.70
5	0.158	82+	82+	3.59	0.160	86+	86+	3.59
7	0.165	$82\pm$	83+	3.75	0.168	86±	87+	3.75
8	0.163	82+	82+	3.40	0.170	86++	85+	3.40
9	0.165	82+	82+	3.40	0.175	86+	86+	3.60
10	0.175	82++	81+	3.50	0.165	$82\pm$	83+	3.40
11	0.170	$78\pm$	79+	3.30	0.175	82++	81+	3.25
12	0.168	74+	74+	3.60	0.170	82++	81+	3.50
14	0.150	82++	81+	3.45	0.165	$82\pm$	83+	3.30
15	0.160	8 <b>2</b> +	82+	3.60	0.165	82±	83+	3.45
16	0,170	82++	81+	3.30	0.170	82++	81+	3.50

Table 109 (Continued)

Cow No.		2	60			2	92	
Date	Acidity	degree	% and of milk plation	Fat %	Acidity	degree	% and of milk lation	Fat %
		Experi- mented	Converted			Experi- mented	Converted	
17	0.175	82+	82+	3.10	0.170	82++	81+	<b>3</b> .35
18	0.170	82+	82+	3.60	0.170	$82\pm$	83+	3.30
19	0.180	82++	81+	3.30	0.175	82+	82+	3.20
22	0.175	$78\pm$	79+	3.35	0.175	82+	82+	3.25
23	0.170	82++	81+	3.20	0.175	$82\pm$	83+	3.30
24	0.160	82++	81+	3.30	0.170	86++	85+	3.45
26	0.165	82++	81+	3,45	0.160	82±	83+	3.40
28	0.160	78±	79+	3,30	0.170	82+	82+	3.35
29	0.175	78±	79+	3.45	0.160	$82\pm$	83+	3.30
30	0.180	78+	78+	3,40	0.170	86++	£5+	3.30
31	0.185	74±	75+	3.30	0.175	82++	81+	3.60
1/I	0.165	74+	74+	3.45	0.175	82++	81+	3.40
2	0.165	78±	79+	3.00	0.155	82+	82+	3.50
4	0.170	82++	81+	2.60	0.160	$82\pm$	83+	2.90
5	0.170	82++	81+	2.60	0.170	82++	81+	3.30
6	0.170	82++	81+	3.40	0.175	82++	81+	3.45
7	0.165	82++	81+	3.10	0.170	82+	82+	3.20
8	0.169	78±	79+	3.35	0.170	82++	81+	3.30
9	0.160	82++	81+	3.30	0.165	$82\pm$	83+	3.35
11	0.170	82++	81+	3.50	0.170	82+	82+	3.40
12	0.170	$78\pm$	79+	3.30	0.168	$82\pm$	83+	3.15
13	0.160	82+	82+	3.40	0.165	$82\pm$	82+	3.40
14	0.155	82++	81+	3.10	0.170	$82\pm$	83+	3.35
15	0.160	82++	81+	3.00	0.170	82+	82+	3.35
16	0.165	82++	81+	3.50	0.170	82 +	82+	3.50
18	0.170	78±	79+	3.15	0.160	82±	83+	3.25
19	0.165	78±	79+	3.90	0.165	82+	82+	3.30
20	0.160	82++	81+	3.30	0.170	82+	82+	3,55
21	0.160	78±	79+	3.20	0.170	82+	82+	3.40

## KENTARO MITAMURA

Table 109 (Continued)

Cow No.		3	00			2	50	
Date	Acidity	degree	% and of milk plation	Fat %	Acidity	degree	% and of milk lation	Fat %
		Experi- mented	Converted			Experi- mented	Converted	
12/XI	0.145	78±	79+	3.01	0.160	90++	89+	3.29
13	0.140	82±	83+	2.90	0.165	90 +	90+	3.35
14	0.140	$82\pm$	83+	2.10	0.160	90+	90+	3.25
16	0.135	86++	85+	2.70	0.165	<b>9</b> 0+	90+	3.00
17	0.145	$82\pm$	83+	2.90	0.165	90++	89+	3.30
18	0.150	$82\pm$	83+	2.70	0.165	90+	90+	3.25
19	0.140	86++	85+	2.83	0.160	90++	89+	3.45
20	0.140	86++	85+	2.85	0.150	<b>9</b> 0+	90+	3.40
21	0.140	86++	85+	2.80	0.165	$86\pm$	87+	3.85
23	0.135	86++	85+	2.92	0.155	$86\pm$	87+	3.10
24	0.135	86++	85+	2.70	0.150	<b>9</b> 0+	90+	3.30
25	0.135	86++	85+	2.80	0.150	90+	90+	3.55
26	0.140	86++	85+	2.65	0.140	86±	87+	2.95
27	0.150	86+	86+	3.05	0.160	$86\pm$	87+	3.15
28	0.150	86+	86+	3.00	0.163	$86\pm$	87+	3.15
30	0.140	86+	86+	3.00	0.155	90++	89+	3.10
1/XII	0.145	86+	86+	2.90	0.160	90±	91+	3.31
2	0.143	86+	86+	2.90	0.160	$90\pm$	91+	3.40
4	0.145	86+	86+	2.90	0.153	$90 + \dot{+}$	89+	2.90
5	0.140	86 +	86+	2.98	0.152	<b>9</b> 0+	90+	3.00
7	0.153	86 +	86∔	3.10	0.175	$90 \pm$	91+	3.05
8	0.148	86++	85+	3.22	0.170	<b>9</b> 0+	90+	<b>3.3</b> 0
9	0.140	86+	86+	3.59	0.170	$90\pm$	91+	2.87
10	0.145	86++	85+	3.10	0.170	90 ±	91+	3.35
11	0.158	86+	86+	2.95	0.173	90++	89+	3.25
12	0.140	86++	85+	3.10	0.160	90+	90+	4.60
14	0.140	86++	85+	2.90	0.160	90±	91+	3.00
15	0.150	86+	86+	3.00	0.160	90 ±	91+	3.40
16	0.145	86±	87+	3.00	0.155	90±	91+	3.35

Table 109 (Continued)

Cow No.		3	00			2	50	
Date	Acidity	degree	% and of milk ilation	Fat %	Acidity	degree	% and of milk lation	Fat %
		Experi- mented	Converted			Experi- mented	Converted	
17	0.145	86±	87+	2.90	0.165	90±	91+	3.20
18	0.145	86±	87+	2.90	0.160	90+	90+	3.00
19	0.140	$86\pm$	87+	2.70	0.180	90++	89+	3.55
22	0.140	86±	87+	3.60	0.175	$90\pm$	91+	3.15
23	0.140	86+	86+	2.90	0.168	$90\pm$	91+	<b>3.3</b> 5
24	0.150	86±	87+	3.00	0.170	94++	93+	2.80
26	0.160	86±	87+	2.90	0.170	90±	91+	3.40
28	0.140	86±	87+	3.00	0.170	$90\pm$	91+	3.10
29	0.160	86±	87+	3.00	0.180	90±	91+	3.20
30	0.155	86++	85+	3.00	0.175	90+	90+	3.20
31	0.150	86++	85+	2.80	0.175	90++	89+	3.70
1/ <b>I</b>	0.160	82+	82+	3.00	0.180	90+	90+	3.30
2	0.140	86+	86+	2.90	0.170	90±	91+	3.60
4	0.145	86++	85+	2.60	0.173	94++	93+	3.00
5	0.145	86+	86+	2.70	0.165	90±	91+	2.75
6	0.145	86+	86+	2.70	0.160	90±	91+	3.10
7	0.150	86++	85+	2.80	0.175	90++	89+	3.10
8	0.150	86+	86+	2.60	0.170	86+	86+	3.25
9	0.150	86±	87+	2.80	0.165	90++	89+	3.70
11	0.150	86+	86+	3.00	0.170	$90 \pm$	91+	3.50
12	0.150	86+	86+	2.75	0.170	94++	93+	2.90
13	0.145	86±	87+	2.90	0.170	90±	91+	3.00
14	0.150	86±	87+	2.90	0.170	94++	93+	3.30
15	0.145	86±	87+	2.90	0.170	90±	91+	3.10
16	0.145	86++	85+	2.90	0.170	90 ±	91+	3.10
18	0.145	86+	86+	2.80	0.170	90+	90+	3.20
19	0.145	86±	87+	3.00	0.165	90±	91+	3.40
20	0.145	86+	86+	3.20	0.160	90±	91+	3.40
21	0.145	86+	86+	2.85	0.160	90±	91+	3.25

Table 109 (Continued)

Cow No.		2	93			2	94	
Date	Acidity	degree	% and of milk plation	Fat %	Acidity	degree	% and of milk alation	Fat %
		Experi- mented	Converted			Experi- mented	Converted	
12/XI	0.145	82+	82+	3.72	0.150	70 ±	71+	3.15
13	0.150	86++	85+	3.60	0.155	$74\pm$	75+	3.50
14	0.150	82±	83+	3.30	0.150	74++	73+	3.10
16	0.140	82+	82+	3.45	0.150	74++	73+	3.60
17	0.135	82±	83+	3.69	0.150	74+	74+	3.20
18	0.135	82+	82+	3.40	0.150	$74\pm$	75+	3.22
19	0.145	82±	83+	3.50	0.150	74+	74+	3.60
20	0.135	86++	85+	3.65	0.150	74+	74+	3.41
21	0.145	82±	82+	3.80	0.150	$74\pm$	75+	3.30
23	0.145	82±	83+	3.70	0.159	74+	74+	3.15
24	0.135	82+	82+	3.25	0.145	74+	74+	3.49
25	0.140	82±	83+	3.50	0.150	74+	74+	3.20
26	0.135	86++	85+	3.90	0.150	74+	74+	3.65
27	0.135	86++	85+	3.80	0.150	74+	74+	3.45
28	0.140	82+	82+	3.60	0.155	74+	74+	3.45
30	0.143	82±	83+	3.75	0.152	74±	75+	3.90
1/XII	0.150	82+	82+	3.75	0.155	74++	73+	3.70
2	0.150	82+	82+	3.70	0.155	74++	73+	3.75
4	0.150	$82\pm$	83+	3.70	0.160	$70\pm$	71+	3.60
5	0.140	82±	83+	3.70	0.155	74+	74+	3.29
7	0.155	82+	82+	3.83	0.173	74+	74+	3.70
8	0.153	82±	83+	3.90	0.160	$74\pm$	75+	3.60
9	0.158	82++	81+	3.80	0.158	$74\pm$	75+	3.55
10	0.150	82++	81+	3.65	0.155	74++	73+	3.80
11	0.158	82++	81+	<b>3.</b> 70	0.162	74+	74+	3.80
12	0.150	82±	83+	3.70	0.158	$74\pm$	75+	3.50
14	0.155	82+	82+	3.65	0.158	74+	74+	3.70
15	0.150	78+	78+	3.70	0.160	$74\pm$	75+	3.70
16	0.150	82+	82+	3.60	0.165	74++	73+	3.50

Table 109 (Continued)

Cow No.		2	93			2	94	
Date	Acidity	degree	% and of milk clation	Fat %	Acidity	degree	% and of milk plation	Fat %
_		Experi- mented	Converted			Experi- mented	Converted	
17	0.150	78±	79+	<b>3</b> .60	0.160	74+	74+	3.40
18	0.153	78+	78+	3.45	0.163	$74\pm$	75+	3.60
19	0.150	78±	79+	3.40	0.172	74±	75+	3.60
22	0.145	78±	79+	3.50	0.160	74±	75+	3.45
23	0.150	$78\pm$	79±	3,65	0.162	74±	75+	3.50
24	0.150	78±	79+	3.45	0.160	74±	75+	3.50
26	0.150	78±	79+	3.45	0.155	78++	77+	3.50
28	0.155	78±	79+	3.50	0.160	78+	78+	3.50
29	0.160	78+	78+	3.40	0.155	78+	78+	3.45
30	0.155	78++	77+	3.20	0.160	74+	74+	3.40
31 .	0.150	78++	77+	3.40	0.165	74±	75+	3.60
1/I	0.160	78++	77+	3.35	0.165	74+	74+	3.50
2	0.150	78+	78+	3.35	0.160	$74\pm$	75+	2.90
4	0.155	78++	77+	3.00	0.162	78++	77+	3.35
5	0.150	78++	77+	2.80	0.160	78++	77+	3.20
6	0.148	78++	77+	3.50	0.160	78++	77+	3.35
7	0.155	$74\pm$	74+	3.20	0.155	74+	74+	3.30
8	0.155	74++	73+	3.30	0.155	74++	73+	3.55
9	0.150	78++	77+	3.45	0.160	74+	74+	3.35
11	0.150	78++	77+	3.40	0.155	74±	75+	3.40
12	0.155	74±	75+	3.30	0.160	74±	75+	3.25
13	0.155	78+	78+	3.50	0.165	78++	77+	3.35
14	0.150	78+	78+	3.50	0.160	78±	79+	3.20
15	0.155	78±	79+	3.25	0.160	78++	77+	3.20
16	0.160	78±	79+	3.30	0.160	78+	78+	3.40
18	0.150	78±	79+	3.25	0.155	78±	79+	3.20
19	0.150	78+	78+	3.45	0.155	78++	77+	3.65
20	0.150	78++	77+	3,20	0.160	74±	75+	3.40
21	0.150	74±	75+	3.40	0.155	74±	75+	3.40

#### 3. DISCUSSION

By observation of tables it will be recognized that the fat percentage, acidity and the alcohol coagulation of milk in each individual are almost constant throughout the experimental period. The averages of the various values for each cow are given in the following table.

Table 110

Average experimental results for each cow

Cow No.	260	292	300	250	293	234
Acidity %	0.165	0.165	0.145	0.165	0.149	0.157
Alc. % to coagulate milk	80.75	83.24	85.56	90.10	80.02	74.83
Fat %	3.31	3.37	2.91	3.30	3.51	3.45

The average results for cow No. 260 were 0.165% for acidity, 3.31% for fat content and 80.75% alcohol for coagulation of milk to a degree of +. The average values of these properties for cow No. 292 were 0.165% for acidity, 3.37% for fat content and 83.24% alcohol for coagulation.

Let us observe how these properties correlate with each other.

It may be said that the milk having a high acidity is not necessarily sensitive to the alcohol; the milk of cow No. 260 was coagulated with 80.75% alcohol while that of cow No. 250 was coagulated only with 90.1% though the acidity of the both was just the same at 0.165%. Moreover there is a case that the milk of cow No. 249 having a low acidity at 0.149% was coagulated with almost the same percentage of alcohol as that of cow No. 260 whose acidity was 0.165%.

On the other hand the author can not recognize any special relation between the coagulation by alcohol and the fat percentage of milk. In this experiment it was found that the coagulation of the milk of cow Nos. 250 and 260 took place with different strength of alcohol notwithstanding the fact that the fat percentages were almost the same.

Furthermore the milk of cow No. 300 having a low fat percentage such as 2.91 was coagulated with 85.56% alcohol while the milk of cow No. 250 having a higher fat percentage of 3.30 was coagulated with 90.1% alcohol. For another example the milk of cow No. 294 which was coagulated with as low a percentage of alcohol as 74.83 had the relatively higher fat percentage of 3.45.

The acidity, fat percentage and the alcohol coagulation differed with individuals, but these properties were almost constant in each individual throughout the experimental period. From these facts it may be concluded that there is not any correlation among these.

# XIII. Relation between the Inorganic Salts in Blood and the Alcohol Susceptibility of Milk

There have been many investigations reported concerning the inorganic salts in bovine blood. According to the study of McCay (49) the haemoglobin and phosphorus contents in bovine blood are almost constant no matter what the breed of cattle, the amount of the milk production, on the difference in the lactation. Palmer, Cunningham and Echles (59) determined the phosphorus content in blood of milking cow as 4-24.4 mg/dl.

AWDEJEWA, GERVSIMOWITSCH, IWANOWA and other co-workers (5) found that the contents of Fe, Ca, and Cl in bovine blood are respectively 40.7 mg, 10.6 mg and 545.85 mg/dl on averages MAREK, WELLMANN and ULBANEK (46) reported that the contents of calcium and phosphorus in horse and swine blood serum are each influenced by the dosage of a definite amount of calcium and phosphorus contrary to the case of matured cattle. Recently AWDEJEWA, BORISSENKO and other co-workers (6) showed by experiment that there is not any close correlation between the content of the inorganic salts in cow's blood and milk.

FLEISCHMANN and WEIGMANN (16) also declared that the inorganic salt in milk is not produced directly from the blood.

The author performed this experiment with the purpose of finding whether or not the alcohol susceptibility of milk would be influence indirectly by the inorganic constituents of the blood.

#### 1. Experimental methods

Twenty cows including Ayrshire, Holstein and Guernsey were used in this experiment. Determinations were made of the content of Cl, Ca, Na, K, inorganic P, Fe and Mg in bovine blood by the following methods when the animal's lactations were almost normal.

#### a. Estimation of Cl

 $0.15\,\mathrm{cc}$  of blood was taken in Ehrenmeyer flask of 50 cc (1.0 cc in the case of milk or milk serum) added 2.0 cc each of N/100 AgNO<sub>3</sub>, and HNO<sub>3</sub> (50%), and boiled. Next the content was sufficiently oxidised by pouring in 15–20 drops of saturated KMnO<sub>4</sub> and if necessary added some powdered pure grape sugar. Added further 2–3 drops of saturated Fe(NH<sub>4</sub>) (SO<sub>4</sub>)<sub>2</sub>, and then titrated the content by using N/100 (NH<sub>4</sub>)CNS solution from the burette.

The calculation was as follows.

s, ...... cc of N/100 AgNO<sub>3</sub> 1.0 cc of this solution equals to 0.585 mg of NaCl.
r, ..... cc of N/100 (NH<sub>4</sub>) CNS.
b, ..... cc of blood sample taken.
x, ..... Cl (NaCl) mg/dl in blood.

 $x = \frac{(s-r) \times 0.585 \times 100}{b} mg/dl.$ 

#### b. Estimation of inorganic P

1. Removal of protein in blood:— Put  $5.0\,\mathrm{cc}$  of blood sample into  $25\,\mathrm{cc}$  measuring flask containing  $13\,\mathrm{cc}$  of distilled water and added  $5\,\mathrm{cc}$  of trichloracetic acid (CCl<sub>3</sub>COOH) ( $20\,\%$ ) with shaking, filled up to  $25\,\mathrm{cc}$  by pouring in water.

After 10 minutes filtered the precipitate by using the centrifuger, named the filtrate "f".

2. Poured 10 cc of "f" into 25 cc measuring flask and then added 1 cc of ammonium molybdate solution; mixture of 10 g (NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub> and 200 cc N/1 H<sub>2</sub>SO<sub>4</sub> and added 2 cc of hydrochinon solution; mixture of 4 g C<sub>6</sub>H<sub>4</sub>(OH)<sub>2</sub>, 2 g Na<sub>2</sub>SO<sub>3</sub>, 200 cc H<sub>2</sub>O and 2 cc H<sub>2</sub>SO<sub>4</sub> (15%), after standing 5 minutes added sodium sulphite and sodium carbonate solution (fill up to 300 cc by adding H<sub>2</sub>O to the mixture of 40 g of Na<sub>2</sub>CO<sub>3</sub>, 8 g of Na<sub>2</sub>SO<sub>3</sub>, and filtrate, keep in brown bottle),

filled up to 25 cc by pouring in  $\rm H_2O$  and finally determined colourimetrically in the DUBOSCQ colorimeter by comparing with the standard phosphorus solution.

The standard phosphorus solution: Dissolve  $0.8772 \,\mathrm{g}$  of  $\mathrm{KH_2PO_4}$  to 200 cc of  $\mathrm{H_2O}$  and then dilute  $0.5 \,\mathrm{cc}$  of this solution into 100 cc of  $\mathrm{H_2O}$ , 1 cc of this solution is equal to  $0.005 \,\mathrm{mg}$  P.

3. Took 10 cc of the standard phosphorus solution into 25 cc measuring flask and treated as in the case of the unknown sample.

The calculation was as follows.

d<sub>0</sub>, ...... Height of the standard solution in the Duboscq colorimeter (mm).

d, ...... Height of the unknown sample in the colorimeter.

c, ...... Concentration of P in the unknown sample.

c<sub>0</sub>, ...... Concentration of P in the standard solution.

$$c=c\frac{d_0}{d}mg/dl$$

## c. Estimation of Na K, Ca and Mg

Removal of protein:— Weighed the 50 cc measuring flask containing about 25 cc of  $H_2O$  and let the weight be taken as "a" and poured about 8 cc of fresh blood into it, shook the contents some seconds and then weighed, this total weight known as "b". Then b—a equals the weight of the blood.

Poured 1–2 drops of kaprylalcohol with shaking, mixed 15 cc of trichloracetic acid (10%) in it, after standing 10 minutes filled up to 50 cc by adding  $\rm H_2O$ , and then filtered the precipitate sufficiently without loss by using the centrifuger for 10 minutes and assumed the filtrate as 35 cc, placed it in the evaporating porcelain dish and evaporated, dried the contents on the water bath. Then took it into a 10 cc measuring flask by agency of N/10 HCl. This light yellow solution to be known as "Bf" will be used for the determination of each element.

#### a. Estimation of Na

Poured 4 cc of "Bf" into a small type evaporating porcelain dish, evaporated half of the content on the water bath and added 1 drop of phenol-red, colour will be changed yellow; next made it alkaline by adding some drops of KOH (10%) solution: the colour changed

to red, then mixed 10 cc of  $K_2H_2Sb_2O_7$  solution, and 3 cc of pure alcohol, after allowing to stand about 30 minutes covered it with a watch glass, the precipitate was removed completely into weighed filter which was washed 3 times with 30% alcohol and dried at  $100^{\circ}\text{C}$  for 30 minutes, cooled and weighed; the increase in the weight of filter was called "Nd".

The calculation was as follows.

$$\frac{\text{Nd}}{11.08} = \text{Na mg in 4 cc of "Bf"}$$

But the weight of blood corresponding to 4 cc of Bf is

$$\frac{(b-a)\times 35\times 4}{50\times 10}g$$

Therefore Na content in 100 g of blood is x.

$$x = \frac{Nd(100 \times 50 \times 10)}{11.08(b-a) \times 35 \times 4} mg/100 g.$$

## $\beta$ . Estimation of K

Poured 0.5 cc of "Bf" into the settling tube and then mixed each 0.5 cc of H<sub>2</sub>O and the sodium nitrite solution (dilute 50 g of NaNO<sub>2</sub> into 100 cc of H<sub>2</sub>O), after standing 5 minutes filled up to 4 cc with H<sub>2</sub>O and then mixed 2 cc of the treated solution which was obtained in the following manner (dissolve 3 g of Co(NO<sub>3</sub>)<sub>2</sub>6H<sub>2</sub>O in 250 cc of H<sub>2</sub>O and mix 6.3 cc of glacial acetic acid, and then put it into the air passing bottle which contains sodium nitrite solution (mixture of 60 g of NaNO<sub>2</sub> and 90 cc of H<sub>2</sub>O); next removed NO<sub>2</sub> gas by continuing to the water pump wholly, kept in the ice room. Separated the filtrate by using the centrifuger for 30 minutes and then removed the filtrate wholly, washed the precipitate with 5 cc of water 3 times, added N/50 KMnO<sub>4</sub> and 1 cc of 4N/1 H<sub>2</sub>SO<sub>4</sub> stirred well with glass rod and dissolved the material on the water bath; the resultant solution must be quite clear light pink colour. Caused the colour to fade by mixing  $2\,cc$  of  $N/100~Na_2C_2O_4$ , then titrated it with N/50 KMnO<sub>4</sub> solution from the burrete.

The calculation was as follows.

 $v_1$ ......cc of N/50 KMnO<sub>4</sub> added in the precipitate of  $K_2$ Na  $Co(NO_2)_6$ .

 $v_2$ .....cc of N/50 KMnO<sub>4</sub> required in the titration.

 $v_3.\dots..cc$  of  $N/100\ Na_2C_2O_4$  consumed for the fading of excess  $KMnO_4$  .

1 cc of N/100 KMnO<sub>4</sub> corresponds to 0.071 mg K.

Therefore K content in "Bf" =  $x = (v_1 + v_2) \times 2 - v_3 \times 0.071$ .

K content in 100 g blood is equal to

$$\frac{x \times 10}{36} \times \frac{50}{35} \frac{(100)}{(b-a)} = mg/100 g blood$$

### y. Estimation of Ca

Poured 4 cc of "Bf" into the settling tube and mixed 1 cc of saturated ammonium oxalate solution and 2 cc of sodium acetate (25%) solution, let calcium oxalate settle well after standing at least 1 hour and then added 1 cc of  $\rm H_2O$ , stirred well and then separated the precipitate in the centrifuger, took out the filtrate carefully, without loss and measured. This was saved for use in the determination of Mg content. After the precipitate of calcium oxalate was washed well 4 times by admixing 10 cc of  $\rm H_2O$  and using the centrifuger, 5 cc of  $\rm N/1~H_2SO_4$  were added, stirred dissolved the mixture, heated to  $\rm 80^{\circ}C$  and titrated with  $\rm N/100~KMnO_4$  solution from the burette.

The calculation was as follows.

1 cc of N/100 KMnO<sub>4</sub> equal to 0.20 mg Ca.

Bf ..... 4 cc.

n ....... cc of N/100 KMnO<sub>4</sub> consumed for the titration

x ....... Ca content in Bf =  $n \times 0.2 \times 4$  mg

Ca content in 100 g of blood =  $x \times \frac{10}{Bf} \times \frac{50}{35} \times \frac{100}{(b-a)}$ 

#### $\delta$ . Estimation of Mg

Transferred the whole amount of the filtrate of calcium oxalate to the settling tube and added 1 cc of ammonium phosphate solution; mixture of 5 g of  $(NH_4)_2HPO_4$ , 1.5 cc of  $NH_4OH$  (10%) and 100 cc of  $H_2O$ , then 2 cc of  $NH_4OH$  (25%) were added in it. After standing 5–6 hours separated the precipitate by using the centrifugal

machine, removed the filtrate, washed with the mixture of ammonia and alcohol. Put the preparate in the electric oven to dry and then dissolved the contents in 0.5 cc of N/10 HCl, filled this up in the measuring flask of 25 cc with  $\rm H_2O$ . Then, finally, determined the P content in this precipitate by the Bell Doisy colorimetric method and obtained the Mg content indirectly.

#### d. Estimation of Fe

Took 0.1 cc of blood accurately into the micro KJELDAHL flask and then poured in 0.5 cc of concentrated  $\rm H_2SO_4$ , heated on the micro Bunsen burner about 2 minutes, after the production of white smoke, cooled, then added 0.5 cc of  $\rm H_2O_2$  drop by drop and repeated this treatment until the mixture become colourless; after that removed the preparate wholly into a measuring flask of 25 cc by using 4–5 cc of  $\rm H_2O$ , again and again, and then 0.4 cc of concentrated HCl, and 10 cc of KCNS (15 g/dl); next filled up to the measure by adding  $\rm H_2O$ , and compared it with the standard Fe solution.

The standard Fe solution:— Took 1 cc of Fe  $(NH_4)$   $(SO_4)_2$   $12H_2O$  solution  $(8.62 \,\mathrm{g/dl})$  in the 50 cc measuring flask and diluted this to the measure with N/1 HCl. And further diluted 5 cc of this solution to 50 cc by mixing N/1 HCl. One cc of this solution contains  $0.02 \,\mathrm{mg}$  Fe..... $2 \,\mathrm{mg/dl}$ .

Put 5 cc of this standard solution into the measuring flask of 25 cc and treated as in the case of unknown sample as described above.

The calculation was as follows.

 $c_0 \dots 0.4 \text{ mg/dl}$ .

 $d_0$ .....Height of the standard Fe solution in the colorimeter (mm).

d..... Height of the unknown sample in the colorimeter.

c.....Content of Fe in the sample.

But 0.1 cc of the sample was diluted to 25 cc therefore  $c \times 250 = Fe \text{ mg/dl blood.}$ 

The author will next tabulate some accounts concerning the experimental cows from which the blood samples were taken, practically at the same time, keeping the cows in normal condition.

TABLE 111
Particulars as to the cows

Breed of cow	Cow No.	Date of	birth	Date partur	of ition	Date sample	
	144	24/VII	1927	24/IV	1931	13/X	1931
	127	6/V	1925	21/I <b>V</b>	1931	13/X	1931
	97	20/XI	1918	15/XI	1930	21/X	1931
	97	20/XI	<b>1</b> 918	15/XI	1930	19/XI	1931
4 15	94	23/IV	1918	25/IX	1930	21/X	1931
Ayrshire	110	28/VI	1921	28/VII	1930	27/X	1931
	115	28/IX	1921	10/VI	1931	27/X	1931
	111	29/111	1922	22/III	1931	9/XII	1931
	114	29/111	1924	15/XI	1931	11/XII	1931
	142	27/IV	1927	19/I	1931	19/XI	1931
	270	4/IV	1926	20/X1I	1930	5/XI	1931
	277	11/V	1927	28/XII	1930	5/XI	1931
	255	6/VIII	1924	15/XI	1930	13/XI	<b>1931</b>
Holstein	243	28/VI1	1922	10/I <b>I</b>	1931	2/XII	1931
	254	9/VIII	1924	6/XI	1931	2/XII	1931
	239	23/VI	1922	24/X	1931	15/XII	1931
	299	8/111	1929	13/X	1931	15/XII	1931
	217	3/VIII	1927	23/IX	1931	13/XI	1931
Guernsey	227	29/XII	1928	18/VII	1931	26/XI	
	214	23/VIII	1926	14/IV	1931	26/XI	1931

# 2. EXPERIMENTAL RESULTS

Next are shown the experimental results concerning the inorganic constituents in the blood and the alcohol susceptibility of milk when the blood samples were taken. The author divided these data into two parts for convenience.

Table 112

Influence of inorganic salts in blood upon the alcohol susceptibility of milk

Breed of	Cow	Alc.	_		in	blood			
cow	No.	coag. milk	Cl mg/dl	Ca mg/100 g	Na "	K ,,	inorg. P mg/dl	Fe	Mg ,,
Ayrshire	97	66++	567.8	10.42	245.6	64.00	6.33	43.0	2.54
,,	94	66++	554.5	9.13	238,8	48.50	5.81	44.3	2.95
Holstein	270	66++	602.6	5.05	335.7	53.28	9.90	58.7	2.35
,,	277	66+	628.8	5.08	310.9	52.52	5.80	50.0	2.15
Ayrshire	97	66±	593.8	9.20	318.0	62,62	5.54	57.6	3.55
Holstein	254	70±	504.9	5.80	290.1	39.34	5.88	48.7	2.04
Ayrshire	115	74±	468.6	7.13	281.7	53.60	7.14	41.8	2.41
,,	142	74++	564.5	7.73	306.5	64.66	5.00	58.7	2.75
Guernsey	214	74+	427.1	8.38	302.8	53.33	4.35	37.5	1.66
Average		70++	545.8	7.55	308.3	54.65	5.75	47.8	2.49
Ayrshire	144	78±	555.7	8.40	305.5	48.01	6.40	40.0	2.28
,,	110	78++	503.6	6.50	318.1	61.40	7.14	44.3	2.48
Guernsey	227	78±	480.2	5.90	338.9	52.05	5.00	46.0	1.55
Holstein	243	78±	608.4	5.61	305.2	45.57	5.56	62.5	2.36
Guernsey	217	<b>78</b> +	564.5	11.80	306.3	64.26	4.36	44.8	2.57
Ayrshire	111	78+	573.3	8.75	3038	43.35	4.76	41.7	2.59
,,	114	78±	564.5	7.36	309.1	51.03	5.88	45.5	3.35
Holstein	239	78+	526.5	6.16	292.1	51.22	5.88	51.7	1.95
,,	299	78±	506.9	6.69	279.2	57.81	6.25	55.5	2.34
Ayrshire	127	82±	552.8	8.40	305.5	48.01	6.40	40.0	2.28
Holstein	255	82 ±	579.2	11.53	336.1	60.38	5.88	46.7	2.39
Average		78±	546.9	7.92	309.1	53.01	5.77	47.1	2.39

## 3. Discussion

The above experimental results have been arranged into two parts: in the first are gathered the blood samples of cows whose milk were coagulated with 66-74% alcohol, and in the other one those whose milk were coagulated with 78-82% alcohol. The author will

discuss here whether or not these inorganic constituents in blood exert any influence upon the alcohol susceptibility of milk.

There were 9 cows whose milk was coagulated with 66–74% alcohol, on an average 70%, to a degree of ++. The average inorganic constituents in the blood of these cows were as follows: 545.8 mg/dl of Cl, 7.55 mg/100 g of Ca, 308.3 mg/100 g of Na, 54.65 mg/100 g of K, 5.75 mg/dl of inorg. P, 47.8 mg/dl of Fe and 2.49 mg/dl of Mg. On the other hand the average values obtained from 11 cows whose milk was coagulated with 78–82%, average 78%, alcohol to a degree of ±, were as follows: 546.9 mg/dl of Cl. 7.92 mg/100 g of Ca, 309.1 mg/100 g of Na, 53.01 mg/100 g of K, 5.77 mg/dl of inorg. P, 47.1 mg/dl of Fe and 2.39 mg/dl of Mg.

One can recognize that the corresponding inorganic constituents in both groups were almost the same. Therefore it may be concluded that the inorganic constituents in the blood of the healthy cow has not any relation to the coagulation of the milk with alcohol.

# XIV. Influence of Inorganic Matters in Whole and Dialyzed Milk Serum upon the Alcohol Susceptibility of Milk

GRIMMER (20) proposed that the milk consists for the most part of water as medium, fat as micron, casein as submicron, albumin as amicron, lactose as molecule and inorganic matters as ultra-micron, amicron, molecule or ion itself.

The present writer has already ascertained that there is a close relation between the inorganic constituents and the alcohol coagulation of milk. Furthermore the author has determined, as reported in this chapter, the contents of Ca, inorganic P, Mg and Cl in milk microquantitatively, and the accounts of these matters the soluble form which is dialyzible through the collodion membrane. Thus there arise an interesting problem whether these water soluble inorganic matters especially Ca, P, Mg and Cl in milk have any important meaning in respect to the alcohol coagulation of milk.

Rona and Michaelis (66) found by experiment that 40-50% of Ca in milk is soluble in form. According to the study of György (22) there is 40-50% of inorganic P in milk soluble by the ultrafiltration. Moreover Söldner (79) determined that the most part

of K, Na and Cl in milk are soluble. Söldner and Daclaux (80) reported that the water soluble Mg in milk is about 65% and also Winkler (97) found that 50% of Ca and 40% of inorganic P are each in soluble form in the normal milk.

## 1. EXPERIMENTAL METHODS

The author applied Sudo's methods (88) which are very convenient and accurate for the micro-quantitative determination of the various elements of inorganic matters in blood and urine in the determination of these materials in milk.

Took 5 cc of milk in the 50 cc measuring flask containing 10 cc of  $H_2O$ , added 5 cc of trichloracetic acid to produce the precipitate of casein and filled up to the measure with  $H_2O$ , filtered after standing 5 minutes; the filtrate was later used for the determination of the inorganic elements except Cl content.

On the other hand the author obtained the milk serum by the following method. Prepared some collodion membran having 3 centimeter diameter and holding 100 cc of the content, poured 10 cc of whole milk in it and tied the upper part with a gum string to prevent the inroad of water and then hung carefully in a beaker of 300 cc containing about 200 cc of  $H_2O$ . Changed the content in beaker 5 times every day during 5 days and gathered all the water solution containing the soluble matters which had dialyzed through the collodion membrane. Then evaporated and concentrated in the electric oven keeping at  $80^{\circ}C$ , after that remove the preparate to a 25 cc measuring flask, filled up to the measure with  $H_2O$  keeping at  $15^{\circ}C$ .

The method of analysis will be described in the following.

#### a. Estimation of inorganic P

Took 2 cc of sample into a 25 cc measuring flask and at the same time took 2 cc of standard phosphorus solution containing 0.1 mg P into another measuring flask of 25 cc, and then added 5 cc of  $\rm H_2O$ , 1 cc of ammonium molybdate solution, and 1 cc of hydrochinon solution; after standing 5 minutes poured in 2 cc of sodium sulphite sodium carbonate solution then filled up to 25 cc with  $\rm H_2O$ ; compared both in the Duboscq colorimeter after 10 minutes.

The calculation follows.

 $d_0$ .....Height of standard phosphorus solution in colorimeter (mm).

d..... Height of unknown sample solution (mm).

c<sub>0</sub>.....Concentration of the standard solution 5 mg/dl.

c.....Concentration of unknown sample

$$c = c_0 \frac{d_0}{d} mg/dl$$
.

But as milk was diluted 10 times and also the milk serum was diluted in 2.5 times multiply by the value of c 10 or 2.5 respectively.

#### b. Estimation of Ca

Took 2 cc of sample into the settling tube, admixed 1 cc of saturated ammonium oxalate solution, stirred well and after standing at least 2 hours separated the precipitate of  $CaC_2O_4$  by adding 5 cc of  $H_2O$  to it. The filtrate had to be kept without loss for the determination of Mg. After removing the filtrate 10 cc of  $H_2O$  were added into the tube to wash the precipitate which treatment was repeated 3 times. Then mixed 5 cc of N/1  $H_2SO_4$ , to the precipitate of  $CaC_2O_4$ , stirred well and warmed at  $80^{\circ}C$ , titrated with N/100 KMnO<sub>4</sub> from the burette.

The calculation was as follows.

1 cc of N/100 KMnO<sub>4</sub> is equal to 0.20 mg of Ca.

h.....cc of sample taken.

n.....cc of N/100 KMnO<sub>4</sub> required for titration.

x.....Ca mg/dl of sample.

$$x = n \times 0.2 \frac{100}{h} \text{ mg/dl.}$$

But as the sample was diluted 10 times and also the milk serum 2.5 times so must each of the values must be multiplied by 10 or 2.5 respectively.

#### c. Estimation of Mg

Took the filtrate which was obtained from the separation of CaC<sub>2</sub>O<sub>4</sub> into the settling tube containing 1 cc of ammonium-phosphate

solution (10%) and mixed 2 cc of  $NH_4OH$  (25%), stirred well, after standing 20 hours got the precipitate of  $MgNH_4PO_46H_2O$ , after taking out the filtrate washed the content with 10% of  $NH_4OH$  two times and then washed further with ammonium alcohol mixture, then dried up in the electric oven, then dissolved it by pouring in 1 cc of N/10 HCl, and filled up to the 25 cc mark of the measuring flask with  $H_2O$ .

Took 10 cc of this solution into a 25 cc measuring flask and on the other hand took 10 cc of standard magnesium solution (0.1 mg. Mg) in another flask. Then mixed 1 cc of ammonium molybdate solution and 1 cc of hydrochinon solution, after standing 5–8 minutes added a further 1 cc of sodium sulphite and sodium carbonate solution, filled up to the measure with  $H_2O$ , and then determined the Mg content by comparing the two flasks in the Duboscq colorimeter.

The calculation was as follows.

c<sub>0</sub>.....Concentration of Mg in the standard magnesium solution 0.4 mg/dl.

 $d_0$ ..... Height of standard solution in the colorimeter (mm).

d.....Height of the unknown sample in the colorimeter (mm).

c.....Concentration of Mg in the unknown sample mg/dl.

$$c = c_0 \frac{d_0}{d}.$$

The volume of the original sample was 2 cc but the samples used in this determination were taken in 1/2.5 parts of the original and further diluted 25 cc. Therefore the values obtained by the above formula must be multiplied by 31.25, that is  $c = 0.4 \frac{d_0}{d} \times 31.25$ .

Further as the original milk sample was diluted 10 times, and milk serum 2.5 times, so again the values must be multiplied by 10 or 2.5 respectively.

Some details regarding the experimental cows appear in the following.

TABLE 113
Particulars as to the cows

Breed of cow	Cow No.	Date of	birth	Date parturi		Date sample	
	250	2/V	1923	27/V	1931	25/IV	1932
	275	19/I	1927	13/V	1931	25/IV	1932
	294	25/IX	1927	1/V	1931	25/IV	1932
	309	7/XII	1929	19/I	1932	25/IV	1932
	260	18/VI	1924	29/VI	1931	19/V	1932
	272	20/VII	1925	9/XII	1931	19/V	1932
	298	11/II	1929	24/IX	1931	19/V	1932
	301	22/IV	1929	20/VII	1931	19/V	1932
	243	28/VII	1922	11/V	1932	10/VI	1932
	261	11/VI1	1924	20/V	1932	10/VI	1932
	280	20/VII	1927	12/V	1932	10/VI	1932
	283	2/VIII	1927	9/I	1932	10/VI	1932
	237	12/IV	1922	12/IV	1932	2/VII	1932
	279	13/VI	1927	28/V	1932	2/VII	1932
	309	7/XII	1929	19/I	1932	2/VII	1932
Holstein	312	10/IV	1930	26/V	1932	2/VII	1932
	239	26/VI	1922	24/X	1931	19/ <b>V</b> II	1932
	252	6/VII	1925	18/I	1932	19/VII	1932
	272	20/VII	1925	9/XII	1931	19/VII	1932
	284	23/IX	1927	13/I	1932	19/VII	1932
	280	20/VII	1927	12/V	1932	3/VI1I	1932
	283	2/VIII	1927	9/I	1932	3/VIII	1932
	301	22/IV	1929	20/VII	1932	3/VIII	1932
	309	7/XII	1929	19/I	1932	3/VIII	1932
	277	1/V	1927	10/II	1932	2/IX	1932
	279	13/IV	1927	28/V	1932	2/IX	1932
	293	10/IX	1928	3/VI	1932	2/IX	1932
	230	3/VII	1921	22/II	1932	16/IX	1932
	239	26/VI	1922	24/X	1931	16/IX	1932
	304	24/VIII	1929	19/VIII	1932	16/IX	1932
	308	2/XII	1929	24/I	1932	16/IX	1932

## 2. EXPERIMENTAL RESULTS

The author will show the experimental results in the following table.

TABLE 114

Influence of inorganic matters upon the alcohol susceptibility of milk (mg/dl)

ri- tai		Alc. % and degree of	iı	n whole	milk		i	n milk	serum	
Experi- mental No.	Cow No.	milk coagulation	Cl	Inorg. P	Ca	Mg	Cl	Inorg. P	Ca	Mg
12	383	66+	173.16	47.61	161.00	18.45	162.69	38.46	87.50	14.77
3	294	70±	172.58	56.18	134.00	18.22	170.13	48.58	71.60	13.02
9	243	70++	172.58	55.56	145.00	18.24	152,85	41.66	72.00	14.44
	Average	70+	172.58	55.87	139.50	18.23	161.49	45.32	71.80	13.73
2	275	74±	167.89	57.14	125.00	17.36	160.88	51.78	67.00	12.50
8	301	74++	179.99	51.26	138.00	17.05	176.12	39.47	67.25	11.19
22	283	74++	190.82	55.56	125.00	19.25	180.75	51.50	70.00	14.25
25	277	$74\pm$	187.90	52.63	124.00	18.52	190.98	50.00	80.50	12.20
	Average	74+	181.65	54.15	128.00	18.05	177.18	48.19	71.19	12.54
	Average	(66-47)+	177.58	53.71	136.00	18.16	173.49	45.98	73.69	13.20
6	272	78+	172.26	54.07	107.00	14.71	163.82	51.72	50.50	11.19
7	298	$68\pm$	170.24	60.00	108.00	14.88	157.95	58.18	62.50	10.93
10	261	78±	145.08	57.14	105.00	12.02	141.15	50.00	46.50	11.81
11	280	78+	149.18	62.50	107.00	14.53	140.04	55.45	45.50	13.26
14	279	78±	168.48	52.63	118.00	16.45	170.15	40.30	59.00	12.20
18	252	78±	177.32	52.55	125.00	15.59	170.59	44.89	68.00	12.85
19	272	78++	179.68	₹2.63	126.00	15.89	172.22	43.98	68.00	12.85
31	308	78±	170.82	62.50	113.00	14.88	155.03	45.46	62.00	12.83
	Average	78+	166.63	56.76	113.63	14.89	158.86	48.75	57.75	12.24
5	260	82++	173.16	51.72	105.00	15.00	170.65	43.57	49.85	10.68
13	237	82++	156.20	54.05	103.00	14.53	151.35	43.33	47.50	11.10
16	312	82++	153.86	57.14	104.00	14.88	151.35	44.48	50.50	11.10
17	239	82++	165.50	57.62	122.00	15.30	<b>163.2</b> 0	41.66	62.00	9.65
20	284	82++	173.85	54.05	120.00	15.30	170.59	50.00	65.00	11.00

Experi- mental No.	Cow No.	Alc. % and degree of		in whol	le milk		i	n milk	serum	
EXE Design	COW NO.	milk coagulation	Cl	Inorg. P	Ca	Mg	Cl	Inorg. P	Ca	Mg
26	279	82++	164.39	59 22	115.00	17.61	160.88	55.56	75.00	11.36
27	293	82+	165.56	62.50	120.00	17.22	160.88	52.63	77.50	10.64
28	230	82±	169.07	64.52	119.00	15.43	152.10	55.56	80.00	10.41
29	239	82 ±	171.41	52.63	128.00	13.50	163.80	45.46	67.00	11.63
30	304	82++	170.24	52.63	126.00	14.60	159.71	50.00	85.00	10.86
	Average	82++	166.32	57.60	116.20	15.34	160.45	48.23	61.94	10.84
	Average	(78-82)+	166.46	57.22	115.06	15.13	159.75	48.46	60.08	11.30
4	309	86±	137.00	66.67	119.00	14.76	138.03	58.50	57.50	10.96
21	280	86++	163.80	71.43	110.00	17.64	140.40	62.50	62.50	13.48
23	301	86++	167.90	68.18	106.00	13.42	159.65	56.81	63.50	12.77
	Average	86+	156.23	68.76	111.67	15.27	146.03	59.27	57.83	12.40
24	309	90+	142.16	75.00	93.00	15.25	131.70	57.95	50.00	12.12
1	250	94++	150.93	62.50	108.00	15.58	145.97	57.69	56.50	12.02
15	309	94++	136.31	66.61	96.00	13.03	122.10	55.45	50.50	11.35
	Average	94++	143.67	64.56	102.00	14.31	134.04	56.57	53.50	11.69
	Average	(86-94)+	149.68	68.40	105.33	14.95	139.64	58.15	50.83	12.12

TABLE 114 (Continued)

#### 3. Discussion

There were 31 samples of milk coagulated with 66 to 94% alcohol to a degree of + in this experiment. These samples may be arranged in 8 groups which were coagulated with similar concentrations of alcohol: viz., 66, 70, 74, 78, 82, 86, 90 and 94% alcohol.

Again these groups will be classified into 3 groups: viz., 66–74, 78–82, 86–94% alcohol coagulation. Then a consideration may be made on the content of Ca, P, Mg and Cl in the whole milk or milk serum as to their relation to the alcohol coagulation of milk.

The average experimental results concerning the inorganic matters in 31 milk samples were as follows.

			TABLE I	115			
Influence	$\mathbf{of}$	soluble	inorganic	matters	upon	the	alcohol
		coagula	ation of m	ilk (mg	/dl)		

Number	Alc. %		in who	ole milk	-		in milk	serum	
of samples	coag. milk	Cl	P	Ca	Mg	Cl	P	Ca	Mg
1	66+	173.16	47.61	161.00	18.45	162.69	38.46	87.50	14.77
2	70+	172.58	55.86	139.50	18.23	161.49	45.32	71.80	13.73
4	74+	181.65	54.15	128.00	18.03	177.18	48.19	71.19	12.54
8	78+	166.63	56.76	113.63	14.86	158.85	48.75	57.75	12.24
10	82++	166.32	57.60	116.20	15.34	160.45	48.23	61.94	10.84
3	86+	156.23	68.76	111.67	15.27	146.03	59.27	57.83	12.40
1	90+	142.16	75.00	93.00	15.25	131.70	57.95	50.00	12.12
2	94++	143.62	65.56	102.00	14.31	134.04	56.57	53.50	11.69
7	(66-74)+	177.85	53.71	136.00	18.16	173.49	45.98	73.69	13.20
18	(78-82)+	166.46	57.22	115.06	15.13	159.75	48.46	60.08	11.80
6	(86-94)+	149.68	68.40	105.33	14.95	139.64	58.15	50.83	12.12

By observing the above table it may be clearly recognized that the milk which was coagulated with a low percent alcohol contains a larger amount of Cl, Ca and Mg but a less amount of P and that the same phenomena also occur in the milk serum of the corresponding milk samples.

In other words, these inorganic salts especially Ca, P and Mg in milk which exist in the colloidal form or in combination of casein are always constant no matter whether the alcohol susceptibility is strong or not, and only water soluble inorganic salts mainly Ca, P and Mg take part in the coagulation of milk with alcohol.

The author wants to emphasise these facts more on the basis of the experimental results which have been obtained, by placing all these samples into 3 large groups, those coagulated by 66-74, 78-82, and 86-94% alcohol respectively.

The average P and Ca contents in the whole milk samples which were coagulated by 66--74% alcohol are each 53.71~mg/dl and 136.00~mg/dl; these contents in the samples which were coagulated by the more strong alcohol such as 78--82% are each 57.22~mg/dl and 115.06~mg/dl.

The inorganic matters in milk which was coagulated by the highest alcohol percent such as 86–94 especially P increased to 68.4 mg/dl but Ca decreased, on the contrary, to 105.33 mg/dl. The same

tendency may be noted in milk serum as in the whole milk. Namely average P content in the milk serum which was obtained from the dialysis of milk whose alcohol susceptibility was by 66–74% alcohol was 45.98 mg/dl. In serum of milk coagulated with 78–82% alcohol the values was 48.46 mg/dl, and further it increased to 58.15 mg/dl on an average in the serum of milk coagulated with 86–94% alcohol.

On the contrary the more the increase in Ca content in the milk serum the more the increase in the alcohol susceptibility of milk; Ca content in milk serum which was obtained from the dialysis of milk whose alcohol susceptibility was with 86-94% alcohol was 50.83 mg/dl and next 60.08 mg/dl in the case of 78-82% alcohol and 73.69 mg/dl in the case of 66-74% alcohol.

# XV. Influence of Di-potassium Phosphate upon the Alcohol Susceptibility of milk

It was made clear in the preceding chapter that the alcohol susceptibility of milk mainly depends on the water soluble inorganic matters especially the balance of calcium and phosphorus, i.e., the more the calcium content in milk, the greater the alcohol susceptibility of milk, but the more the phosphorus content the less is the alcohol susceptibility. This property of milk may therefore be adjusted by balancing these two elements either by lessening calcium from or by adding phosphorus to milk. It is practically impossible to draw out the calcium from milk, but it is possible to add some phosphorus to it.

Concerning the heat stability and salt balance Hunziker (33) stated that the addition of sodium citrate or di-sodium phosphate did remedy the heat coagulation difficulties. In general the addition of from 4 to 10 ounces of crystalline di-sodium phosphate per 1,000 pounds of evaporated milk changed an unsafe sterilizing process to one that is satisfactory, and the remedy, the use of di-sodium phosphate to prevent troublesome heat coagulation, is already in extensive use in the industry.

The present author has ascertained already about the alcohol susceptibility of milk from the stand point of salt balance especially of the souble forms of calcium and phosphorus. Therefore the author experimented further as detailed in this chapter to see whether the alcohol susceptibility can be adjusted by adding a certain amount of phosphorus calculated from the results of analysis.

#### 1. EXPERIMENTAL METHODS

The summary of experimental results given in Table 115 in the preceding chapter may be reproduced in the following.

• Table 116

Average content of certain inorganic matters in milk (mg/dl)

CI)	Alc. % coag.		in who	le milk	
Class	milk	Cl	P	Ca	Mg
1st	(66-74)+	177.85	53.71	136.00	18.16
2nd	(78~82)+	166.46	57.22	115.06	15.13
3rd	(86-94)+	149.68	68.40	105.33	14.95

The calcium content in milk of 1st and 3rd classes was adjusted to that in milk of 2nd class to equalize the amounts of calcium in all classes, and the adjustment was also made on other elements to keep the same ratio with the original calcium as follows:

TABLE 117

Average content of certain inorganic matters in milk, converted values (mg/dl)

C1	Alc. % coag.		in who	le milk	
Class	milk	Cl	P	Ca	Mg
1st	(66-74)+	150.47	45.44	115.06	15.36
2nd	(78-82)+	166.46	57.22	115.06	15.13
3rd	(86-94)+	163.51	74.72	115.06	16.33

By observing the above table it may be recognized that the higher the alcohol susceptibility of milk the less the phosphorus content in it despite the fact that the content of calcium, chlorine and magnesium are in almost constant relation.

Therefore the balance between the calcium and phosphorus in milk is the most important problem to solve the question of the alcohol susceptibility of milk. Then the question arises how large an amount of phosphorus must be added to the milk coagulated by 66-74% alcohol in order to bring it up to the class of milk which is sensitive to 78-82% alcohol. With this in mind the ratio of P and Ca in this milk may be adjusted to the ratio of these elements in milk of the 2nd class as follows.

$$57.22:115.06 = x:136.00$$
  
 $x = 67.63 \text{ (mg/dl)}$ 

The alcohol susceptibility of thus adjusted milk may be the same as that of the milk of the 2nd class. As the milk contains 53.71 mg in 100 cc, theoretically the amount of phosphorus to be added would be the difference of these:  $67.63-53.71=13.92 \, (\text{mg/dl})$ . In other words it will be necessary to add  $13.92 \, \text{mg}$  of phosphorus to  $100 \, \text{cc}$  of this milk which was originally coagulated with  $70 \, \%$  alcohol in order to adjust its alcohol susceptibility to that coagulated with  $80 \, \%$  alcohol.

The author selected di-potassium phosphate as the source of phosphorus in order to control the balance of phosphorus content in this milk. The molecular weight of  $K_2HPO_4$  is 174.25 g and the amount of phosphorus in a molecular is 31.04 g.

100 cc of M/2  $K_2HPO_4$  solution contain 8.7125 g with while 1.552 g. P. Therefore 1 cc of this solution contains 15.52 mg of P or 0.896 cc of this solution will contain 13.92 mg of P.

In order to adjust the milk which was coagulated with 70% alcohol to that of 80%, will be necessary to add 0.896 cc of M/2  $K_2HPO_4$  to 100 cc or 0.18 cc of the solution to 20 cc of this milk.

To see if it works out in practical application the following experiment was carried out.

Milk	$\mathrm{H}_2\mathrm{O}$	M/2 K <sub>2</sub> HPO <sub>4</sub>
20.0 ce	0.6 cc	0.0 сс
,,	0.5 ,,	0.1 ,,
,,	0.4 ,,	0.2 ,,
,,	0.3 ,,	0.3 ,,
,,	0.2 ,,	0.4 ,,
,,	0.1 ,,	0.5 ,,
. 22	0.0 ,,	0.6 ,,

As usual 2 cc of the mixture of these solutions are taken for the determination of its alcohol susceptibility.

## 2. EXPERIMENTAL RESULTS

The author has obtained the following experimental results.

Table 118 Influence of  $K_2HPO_4$  upon the alcohol susceptibility of milk Example 1.

Milk	M/2 K <sub>2</sub> HPO <sub>4</sub>	H <sub>2</sub> O	Alc. % and de coagula	Acidity	
cc	cc	cc	Experimented	Converted	
20.0	0.0	0.6	82±	83+	0.151
,,	0.1	0.5	86±	87+	0.167
,,	0.2	0.4	90+	90+	0.189
,,	0.3	0.3	90±	91 +	0.201
,,	0.4	0.2	94++	93 +	0.210
,,	0.5	0.1	94±	95+	0.219
,,,	0.6	0.0	98±	99 +	0.229

Example 2.

Milk cc	M/2 K <sub>2</sub> HPO <sub>4</sub>	$_{12}$ O	Alc. % and de coagul	Acidity	
	ec	cc	Experimented	Converted	
20.0	0.0	0.6	78±	79+	0.167
,,	0.1	0.5	82+	82+	0.176
,,	0.2	0.4	86±	87+	0.194
,,	0.3	0.3	90±	91+	0.211
,,	0.4	0.2	94+	94+	0.222
,,	0.5	0.1	94±	<b>95</b> +	0.229
,,	0.6	0.0	98±	99+	0.238
	<u> </u>	J			J

Example 3.

Acidity	Alc. % and degree of milk coagulation		$\mathrm{H}_2\mathrm{O}$	M/2 K <sub>2</sub> HPO <sub>4</sub>	Milk
	Converted	Experimented	cc	cc	c <b>c</b>
0.167	78+	78+	0.6	0.0	20.0
0.176	83+	82±	0.5	0.1	,,
0.185	85+	86++	0.4	0.2	,,
0.202	89+	90++	0.3	0.3	,,
0.211	93+	94++	0.2	0.4	,,
0.229	97+	98++	0.1	0.5	,,
0.239	99+	98±	0.0	0.6	,,

# Example 4.

Milk ec	M/2 K <sub>2</sub> HPO <sub>4</sub>	H <sub>2</sub> O cc	Alc. % and degree of milk coagulation		Acidity
			Experimented	Converted	
20.0	0.0	0.6	78±	79+	0.158
,,	0.1	0.5	82±	83 +	0.176
,,	0.2	0.4	90++	89+	0.196
,,	0.3	0.3	90 ±	91 +	0.211
,,	0.4	0.2	94-+	94+	0 222
,,	0.5	0.1	94±	95+	0.229
,,	0.6	0.0	98±	99+	0.238

# Example 5.

Milk	M/2 K <sub>2</sub> HPO <sub>4</sub>	H <sub>2</sub> O cc	Alc. % and degree of milk coagulation		Acidity
сс			Experimented	Converted	
20.0	0.0	0.6	82++	81+	0.158
,,	0.1	0.5	82±	83 +	0.176
,,	0.2	0.4	90++	89+	0.186
,,	0.3	0.3	90±	91+	0.202
,,	0.4	0.2	94+	94+	0.211
,,	0.5	0.1	98++	97+	0.229
,,	0.6	0.0	98±	99±	0.238

#### 3. Discussion

The author will show the average experimental results in the following.

Table 119

Influence of  $K_2HPO_4$  upon the alcohol sensibility of milk (average)

Milk ec	M/2 K <sub>2</sub> HPO <sub>4</sub> cc	H <sub>2</sub> O cc	Alc. % and degree of milk coagulation Converted	Acidity
20.0	0.0	0.6	80+	0.160
,,	0.1	0.5	84+	0.174
,,	0.2	0.4	88+	0.189
,,	0.3	0.3	91+	0.205
,,	0.4	0.2	94+	0.215
,,	0.5	0.1	96+	0.227
,,	0.6	0.0	99+	0.236

As has already been explained above, the alcohol susceptibility of milk was determined of the mixture of 20 cc of milk, 0.0–0.6 cc of M/2  $\rm K_2HPO_4$  and 0.6–0.0 cc of  $\rm H_2O$ . By observing above table it will be recognized that the greater the increase in the amount of M/2  $\rm K_2HPO_4$  in milk the more the decrease in the alcohol susceptibility, but the greater the acidity of the mixture. That is to say that the acidity of the milk mixture containing only 0.6 cc of  $\rm H_2O$  in 20 cc of milk was 0.160%. When 0.1 cc of M/2  $\rm K_2HPO_4$  and 0.5 cc  $\rm H_2O$  were added into 20 cc of milk the acidity was increased to 0.174% and it was further increased to 0.236% in the milk to which 0.6 cc of M/2  $\rm K_2HPO_4$  was added. On the other hand the alcohol susceptibility of these milk mixtures decreased from 80% alcohol to 84% and finally to 99% as the concentration of M/2  $\rm K_2HPO_4$  in the milk became greater.

Thus in this experiment the author has proved that the alcohol susceptibility of milk can be controlled by the addition of a definite amount of M/2  $K_2HPO_4$  as the source of phosphorus, and also that this finding just coincides with the experimental results obtained in the preceding chapter.

However, there is still some room for further consideration concerning the added amount of  $K_2HPO_4$  and the consequent acidity of milk. Sommer and Hart (83) have already shown by experiment that there is some relation between the heat stability and the acidity of milk. Benton and Albery (8) reported that the heat stability of milk does not necessarily exert influence upon the alcohol stability, and that it is impossible to increase the heat stability of milk even by the addition of citrate salts or phosphate salts as buffer materials unless the salt balance does not hold well.

They suggested that the most suitable concentration of pH value in milk is 6.58-6.65.

Therefore the author may propose in this connection that the addition of  $0.1-0.2\,cc$  of  $M/2\,K_2HPO_4$  in  $20\,cc$  of milk which was coagulated by  $70\,\%$  alcohol has the most suitable effect upon the salt balance and also the acidity of milk.

It has been customary until the present di-sodium phosphate or sodium citrate was added to milk simply as a buffer material in manufacture of evaporated milk, but the author has determined the suitable amount of di-potassium phosphate to added to milk not only on the basis of the experiment but also on the theoretical basis.

# XVI. Relation between the Iso-electric Point of Milk Casein and the Alcohol Susceptibility of Milk

There have not been very many experiments reported concerning the iso-electric point of milk casein up to the present. NAKA-YAMA (57), YASUI (99) or DEMUTH (10) obtained results on the human or cow milk, but recently SATO and MURATA (70, 71) ascertained the iso-electric point not only of cow milk casein but also that of various animal milk and of dairy products. They pointed out that the iso-electric point of milk casein lies between 3.8 and 4.0.

MICHAELS (54) reported that the optimum coagulating point of the mixture of casein and buffer solution coincides well with the isoelectric point of its casein; therefore the author planned the experiment as described in this chapter to ascertain if there is any relation between the iso-electric point of milk casein and the alcohol susceptibility of milk.

### 1. Experimental methods

Determinations were made of the iso-electric point of milk caseins whose alcohol susceptibility is different by SATO and MURATA's method (70) as follows: Take milk sample into a collodion sack and dialyze for 7 days in cold running water if necessary with ice, then dialyze again in cold distilled water for one day, take away the floating fat on the surface carefully, then mix the half volume of this dialyzed milk into a definite amount of WALPOLE's buffer solution. After standing for 6 hours the author determined the iso-electric point of these milk caseins.

Walpole's buffer solution is as follows.

TABLE 120
WALPOLE'S buffer solution

2N/10 CH <sub>3</sub> COOH cc	2N/10 CH <sub>3</sub> COONa cc	pН	2N/10 CH₃COOH cc	2N/10 CH <sub>3</sub> COONa cc	pН
19.5	0.5	3.4	14.7	5.3	4.2
19.0	1.0	3.5	13.6	6.4	4.3
18.5	1.5	3.6	12.6	7.4	4.4
18.0	2.0	3.7	11.4	8.6	4.5
17.6	2.4	3.8	10.2	9.8	4.6
16.9	3.1	3.9	9.1	10.9	4.7
16.4	3.6	4.0	8.0	12.0	4.8
15.5	4.5	4:1			

### 2. Experimental results

The author obtained the following results.

### 3. Discussion

By observing the above table it may be recognized that the isoelectric point of milk casein is in each case almost constant no matter whether the alcohol susceptibility of milk is weak or strong, and it lies between 3.8 and 4.3. That is to say, there is not any relation between the iso-electric point of milk casein and the alcohol coagulation of milk.

 ${\bf TABLE~121}$  Influence of the iso-electric point of milk case in upon the alcohol susceptibility of milk

Milk	Alc. % and degree	ree										·				
sample No.	of milk coagula- tion	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8
279	78±	±	±	+	+-+	+++	+++	+++	+++	++	++	++	+	+	_	_
283	70±	-•	_	±	+	++	+++	+++	+++	+++	++	++	++	+	±	_
309	86±	_	_	±	土	++	++	+++	+++	+++	++	++	++	+	±	_
310	82++	_	_	士	+	++	++	+++	+++	+++	++	++	+	+	土	
279	78+	土	士	+	++	+++	+++	+++	++	++	++	+	+	_	_ :	_
283	70 <u>.</u> ±	-	_	±	+	++	++	+++	+++	+++	++	++	++	+	_	_
309	86±	-	土	士	++	++	+++	+++	+++	+++	+++	++	+	+	_	_
304	78±	1	<del>-</del>	±	+	++	++	++	+++	+++	+++	++	++	+	±	
312	82 ±	-	_	+	+	++	++	+++	+++	+++	+++	++	++	+	+	_
322	86±	_	± ,	+	++	+++	+++	+++	+++	+++	+++	++	+	+	±	_

It may be stated here that the soluble inorganic salts are almost entirely drawn out from the milk by the dialysis through the collodion membrane, and only the colloidal milk casein remains in the collodion sack.

## XVII. Influence of Freezing upon the Alcohol Susceptibility of Milk

It is a well known fact that the physico-chemical properties of milk are influenced by freezing: for example, the rising of fat globules, the change of the colloidal condition of casein, or the case of putrefaction after melting. MAI (45) reported that the acidity has been increased by freezing, and FULD and WHOLGEMUTH (19) proved that the casein has become easily coagulable by the acid or rennet enzyme. Moreover WINKLER (98) has cited BERGEN's experiment in his book that the reductase or catalase content had increased in the frozen milk. However, the author could not find any report concerning the influenced freezing on the alcohol susceptibility of milk. For this reason the following experiment was carried out.

### 1. EXPERIMENTAL METHODS

Five samples of normal Holstein milk whose alcohol susceptibilities were different were taken separately into 200 cc glass bottles and carried to the laboratory as soon as possible and each sample was filled into two beakers of 50 cc. One from each sample was frozen completely in a refrigerator at a temperature of  $-25^{\circ}$ C. for one and half hours, and then they were taken out for melting. The other beakers were left in the laboratory keeping the temperature at  $5-10^{\circ}$ C. Then the author performed a comparative experiment with these samples keeping the temperature at  $15-20^{\circ}$ C testing the alcohol susceptibility and acidity of milk.

## 2. Experimental results

The experimental results are shown in the following table.

TABLE 122

Influence of freezing upon the alcohol susceptibility of milk

Sample No.	·	1 hour after milking	Frozen milk	Control milk
	Acidity %	0.170	0.180	0.170
1	Alc. % {Experimented coag. milk {Converted	78± 79+	78+ 78+	78+ 78+
	Acidity %	0.190	0.205	0.190
2	Alc. % Experimented coag. milk Converted	86+++ 84+	82± 83+	86++ 85+
	Acidity %	0.185	0.190	0.185
3	Alc. % {Experimented coag. milk {Converted	78+++ 76+	74++ 73+	78++ 77+
	Acidity %	0.175	0.180	0.170
4	Alc. % {Experimented coag. milk {Converted	82 ± 83 +	82± 83+	82± 83+
	Acidity %	0.175	0.180	0.170
5	Alc. % {Experimented coag. milk {Converted	$94++\ 93+$	90 ± 91+	94++ 93+
	Acidity %	0.180	0.185	0.180
6	Alc. % {Experimented coag. milk {Converted	70± 71+	70++ 69+	70± 71+
	Acidity %	0.175	0.175	0.175
7	Alc. % Experimented coag. milk Converted	74+ 74+	70+ 70+	74++ 73+
-	Acidity %	0.185	0.190	0.185
8	Alc. % {Experimented coag. milk {Converted	74+ 74+	70++ 69+	74+ 74+
	Acidity %	0.190	0.195	0.190
9	Alc. % {Experimented coag. milk (Converted	78+ 78+	74++ 73+	78+ 78+
	Acidity %	0.180	0.180	0.180
10	Alc. % (Experimented coag. milk (Converted	78+ 78+	78++ 77+	78+ 78+
	Acidity %	0.181	0.186	0.180
Average	Alc. % Converted	79.0+	76.6+	79.0+

### 3. DISCUSSION

The alcohol susceptibility and the acidity of the frozen milk have a tendency to increase a little in comparison with the control; i.e., the frozen milk was coagulated by 76.6% alcohol but the control milk was coagulated by 79% alcohol on an average 10 examples as described above, and the acidity of the frozen milk tested 0.186% against 0.180% of the control. This is perhaps due to the change of casein properties making them easily coagulable by the alcohol.

# XVIII. Influence of Original Catalase Content in Milk upon the Alcohol Susceptibility of Milk

There are many enzymes such as hydrolase, oxydase, catalase, zymase, diastase, amylase, peroxydase, or solorase in milk. But FLEISCHMANN (17) and GRIMMER (21) reported that the content of the original enzymes with the exception of catalase in milk is not very great. These enzymes are almost all produced after milking by the development of bacteria. From the study of LENSEN (42) one learns that the content of amylase in the fresh normal milk is almost constant and it has a close relation to the pathological condition of the cow's udder.

FLEISCHMANN (17) states in his book that the diastase will be increased in the mastitis milk mainly appearing in the milk fat.

Now the catalase is in fresh milk in comparatively a large amount and it has a close relation to the physiological condition of the cow. It is the well known fact that this enzyme has a power to decompose H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O, and according to the studies of ROEDER and WASSERMANN (64) and FLEISCHMANN (17) it exists in colostrum, abnormal milk, and milk at the end stage of lactation more than in normal milk. Recently the physiological meaning of this enzyme has become clear; viz., it takes a part in the resolving the peroxide materials in the living cell. TADOKORO (89) states in his book that the catalase content in blood of malnourished animal is increased about 22% in comparison with normal. Further he suggested that the important function which separates oxygen from the oxyhaemoglobin and causes it to nourish the living cell is due to the catalase. This will explain the increase of this enzyme at the stage of gestation.

The author experimented on this subject for the reason that as already found the stage of lactation, sickness, or oestrum has an influence not only upon the alcohol susceptibility of milk but also upon the catalase content.

#### 1. Experimental methods

The author applied TSUCHIBASHI's method (92) for the determining of the catalase content in milk as described hereafter.

Take 1 cc each of  $H_2O_2$  (1%), the mixture of  $Na_2HPO_4$  and  $KH_2PO_4$  (same volume of M/15  $Na_2HPO_4$  and M/15  $KH_2PO_4$ ) (pH = 7.0),  $H_2SO_4$  (20%) and milk sample in the EHRENMEYER flask of 50 cc, and make up to 4 cc. On the other hand as control take 1 cc of the mixture of  $Na_2HPO_4$  and  $KH_2PO_4$  without milk and titrate it parallel to the milk preparate with N/10  $KMnO_4$  from the burette at the room temperature. Thus the amount of catalase of milk is shown by the difference of these two readings. Let the minimum amount of N/10  $KMnO_4$  required for the titration of the tested milk be 100, and obtain the comparative number in each example. Thus the author tried to compare easily the vitality of the catalase in milk. The chemical reaction in the titration is as follows:

$$2KMnO_4 + 5H_2O_2 + 4H_2SO_4 = 2KHSO_4 + 8H_2O + 5O_2$$

### 2. Experimental results

The experimental results concerning the influence of catalase upon the alcohol susceptibility of milk are as follows.

Table 123
Influence of catalase content upon the alcohol susceptibility of colostrum milk

Milk No.	Cow No.	Breed of	Date of parturition		Date of next	Date of sample taken	Alc. % and degree of milk coagu- lation	N/10 KMnO <sub>4</sub> cc.	Comparison of the catalase vitality
1	332	Holstein	17/III	1934	_	19/III 1934	58+	3.9	260
	317	,,	6/III	1934	_	19/III 1934	78+	2.8	187
	250	,,	25/III	1934		28/III 1934	62++	2.5	167
. 4	<b>2</b> 75	,,	25/III	1934		28/III 1934	62++	2.8	187
A	verage		:		· · · · · · · · · · · · · · · · · · ·			3.0	200

TABLE 124

Influence of catalase content upon the alcohol susceptibility of normal milk

Milk No.	Cow No.	Breed of	Date partur	-	Date of next parturition		sample degree		N/10 KMnO <sub>4</sub> cc.	Comparison of the catalase vitality
1	322	Holstein	30/X	1933			19/III 1934	86++	2.0	133
2	304	,,	9/XII	1933	_		21/III 1934	86±	1.5	100
3	279	,,	20/VII	1933	13/XII 1934		22/III 1934	82++	2.1	140
4	287	,,	<b>3</b> 0/ <b>V</b>	1933	3/IX 1934		22/III 1934	78+	1.9	127
5	311	,,	22/X	1933	12/IX 1934		22/III 1934	82++	2.6	173
6	333	,,	4/I	1934	-		22/III 1934	86±	1.6	107
7	292	. ,,	27/I	1934	-		22/III 1934	78±	1.7	113
8	329	,,	27/I	1934	-		23/III 1934	86+	1.8	120
9	230	,,,	<b>3</b> 0/ <b>X</b> I	1933			23/III 1934	74+	1.7	113
10	330	,,	18/XI	1933	_		23/III 1934	86±	2.0	133
11	312	,,	18/XII	1933	23/XII 1934		23/III 1934	86++	1.9	127
Average								1.9	127	

Table 125
Influence of catalase content upon the alcohol susceptibility of milk at the end stage of lactation

Milk No.	Cow No.	Breed of		Date of parturition		next tion	Date of sample taken	Alc. % and degree of milk coagu- lation	N/10 KMnO <sub>4</sub> cc.	Comparison of the catalase vitality
1	238	Guernsey	19/IV	1933	9/X	1934	19/III 1934	78±	3.0	200
2	244	Holstein	29/IX	1933			21/III 1934	86+	2.1	140
3	277	,,	24/V	1933	29/VI	1934	21/III 1934	70+	3.3	220
4	283	,,	6/IV	1933	26/VIII	1934	21/III 1934	78++	3.2	213
5	294	,,	6/III	1933	16/X	1934	21/III 1934	82++	2.2	147
6	316	,,	3/III	1933	12/IX	1934	22/III 1934	86++	2.3	153
A	Average									179

### 3. Discussion

By observing the above tables it is recognized that the content of original catalase is largest in the colostrum, and also that the milk at the end stage of lactation has a larger content than that of normal milk. However, in the normal milk there is not any definite correlation between the alcohol susceptibility and the catalase content of milk: e.g., it may be seen that the catalase content of milk of cow No. 230 whose alcohol susceptibility is strong is almost the same as that of cow No. 333 whose alcohol susceptibility is weak; on the other hand the catalase contents of the milk of cows No. 330 and 304 are different in spite of the fact that the susceptibility to alcohol is almost the same.

## Summary and Conclusion

In this paper the results of experiments made on various lines of the alcohol coagulation of normal milk secreted from healthy cows have been reported from the standpoints of chemistry and feeding.

In the following paragraphs a summary of the findings will be presented and in conclusion the writer proposes to state a few opinions.

- 1. Generally speaking the alcohol susceptibility of normal fresh milk is almost constant regardless of the breed of cow, although there are cases where the milk was coagulated with the alcohol ranging 66–94% by volume in the degree of +, the most milk samples were coagulated with 75–82% alcohol. The average concentration of alcohol which caused coagulation is  $78.82 \pm 0.10\%$  for 1, 195 samples of Holstein milk,  $81.17 \pm 0.10\%$  for 927 Ayrshire milk samples, and  $79.26 \pm 0.15\%$  for 582 samples of Guernsey milk. The average alcohol percentage which coagulated the same volume of milk is  $79.72 \pm 0.07\%$  for the whole number of these samples.
- 2. The alcohol susceptibility of the milk is specific to individuals, and it is almost constant in the same individual throughout the normal lactation period.

Determinations were made of the alcohol stability of Ayrshire milk for 2 years and 3 months by using 15 heads of cows throughout the lactation period.

The coagulation of colostrum milk was very marked, but it diminished gradually after 7-28 days, an average of 12.4 days for 27 samples.

In some cases even the normal milk secreted from the healthy cow gave a positive reaction to the alcohol test. This phenomenon is characteristic of certain individual animals.

There were 10 cases out of 25 samples of milk at the end stage of lactation in which milk showed entirely negative reaction to the alcohol test. Two cows always showed positive reaction to the test throughout the lactation period from the beginning to the end, continuing for over two lactations. These cows are closely related to each other in linage. It may be considered, therefore that this property is inheritive. Eleven milk samples reacted positively to the test, for 10 days at the shortest, 42 days at the longest, and 29.6 days on an average before drying of the milk.

Taking every thing in consideration, it may be generally recognized that the alcohol susceptibility of the milk increases at the advanced stage of lactation.

3. The hydrogen ion concentration of the milk has no direct relation to the alcohol susceptibility of milk.

The alcohol susceptibility of whole milk is decreased by dialysis, due to the dialyzing of the soluble salt in milk through the collodion membrane.

The susceptibility of milk to alcohol is increased by addition of the neutral salt containing cations of various valencies. The degree of coagulation is proportional to the valencies of cations of neutral salts, and it has a close relation to the concentration of these salts added to the milk, e.g., the greatest susceptibility to alcohol was found in case where 3N/10-7N/10 solution of divalent cation of neutral salt was added to milk in ratio of 1:4.

The alcohol susceptibility of milk containing a definite amount of mono-, di-, or trivalent cation of neutral salt is proportional to the concentration of added salt when a di- or trivalent cation of salt is further added to the milk mixture. But the alcohol susceptibility of milk containing a definite amount of di- or trivalent cation of neutral salts is inversely proportional to the concentration when monovalent cation of neutral salt is added to the milk mixture.

From these experimental results it is clear that the alcohol susceptibility of milk is distinctly governed by the salt balance in the milk.

4. The constituents of the inorganic salt in milk vary according to the stage of lactation; the total ash content of the colostrum and that of the milk at the advanced stage of lactation is much greater than that of the normal milk. The chlorine content is less in the colostrum, but it has a tendency to increase gradually as the lactation advances. The contents of calcium and magnesium are greater in the colostrum and also in the milk at the advanced stage of lactation. The sodium and phosphorus contents are generally constant throughout the lactation period, but the potassium content is greatest in the normal milk.

The total ash and the contents of calcium, magnesium and chlorine in milk which is positive to alcohol test are higher than those in milk which is always negative to alcohol test. The potassium content, on the other hand, is less in the former. There are no remarkable differences in the contents of phosphorus, sodium and iron in these two sorts of milk.

After all the ash constituents in milk which shows a positive reaction to alcohol test resemble the corresponding values of colostrum or the milk at the advanced stage of lactation.

- 5. The testing temperature distinctly influences the alcohol susceptibility of milk; both high and low temperatures cause the susceptibility to decrease and the milk is most sensitive to alcohol at a temperature between 15–20°C.
- 6. When the milk samples having different alcohol susceptibilities were kept at a definite temperature as at 30°C for certain hours, the milk which was originally more sensitive to alcohol increased its sensitiveness more, and at the same time the stability for heat decreased more.
- 7. Some physico-chemical properties of milk are affected by the oestrum; the fat content, total solid and total ash decrease a little, and the alcohol susceptibility of milk is materially increased. The contents of chlorine and calcium in the milk increased in reverse to the phosphorus.
- 8. The alcohol susceptibility and acidity of milk from the cows which received 150-300 g of bone meal in addition to the regular daily ration for 12-15 days and produced at least 4-14 kg. per day, was not changed in comparison with the control.

The acidity and the alcohol susceptibility of milk are not affected by the continual dosage of 40 g each of calcium lactate, di-potassium phosphate, sodium citrate or sodium carbonate in the ration for 9–24 days separately to cows giving of 3–4 kg. milk per day. Furthermore, the alcohol susceptibility and the acidity, also the contents of chlorine, phosphorus, calcium or magnesium are not influenced by the continual feeding of 80 g. of di-sodium phosphate, 120 g. of calcium carbonate or 50 g. of magnesium carbonate separately to the cows producing of 8–9 kg. milk per day. Moreover these characteristics of milk are almost invariable even though 140–150 g. of either "calcium" or "phoscalpin" in addition to the daily ration are given for 20–30 days to the cows producing of 15 kg. milk per day.

- 9. The alcohol susceptibility, the acidity and also the contents of chlorine, calcium, magnesium and phosphorus in milk are not affected by either the protein rich or protein poor ration for a short time, e.g., for 15 days, in comparison with the control.
- 10. The susceptibility to alcohol and the acidity of milk are not influenced by the sudden change of roughages, such as hay, corn silage, or mangels each separately after continual feeding of 10 days. In course of the experiment, however, a cow became unhealthy and had a attack of diarrhoea and these chemical properties of milk were disturbed at once. The same can be said of the effect of the sudden change of the concentrates, such as wheat bran, soybean cake, green peas, oats, or corn each separately after continual feeding for 10 days each.
- 11. The alcohol susceptibility of milk is not affected at all by either over or under feeding of the milking cow for a short time, at any rate for 12 days.
- 12. The fat content in milk has no relation whatsoever to the alcohol coagulation of milk.
- 13. Each of the inorganic matters in the blood of a milking cow is almost constant and it is not recognized that these salt constituents in blood have a direct relation to alcohol susceptibility of milk.
- 14. The inorganic salts in normal milk, especially the contents of chlorine, calcium, phosphorus and magnesium, have a close relation to the alcohol coagulation. That is to say, the greater the alcohol susceptibility of milk the greater the content of chlorine, calcium and magnesium, but on the contrary the less the susceptibility to alcohol the more the phosphorus in milk. The author recognized just

the same phenomena in the water soluble inorganic salts in milk, in other words, these inorganic salts which exist in colloidal form or as the casein compounds are almost constant in normal milk regardless of the alcohol susceptibility. Therefore the author can conclude that the alcohol susceptibility of milk is almost exclusively influenced by the content of these soluble inorganic salts, the greater the susceptibility of milk to alcohol the greater is found to be the contents of soluble calcium, chlorine and magnesium, but on the contrary the less the alcohol susceptibility the greater the soluble inorganic phosphorus in milk.

- 15. It is possible to adjust the alcohol susceptibility of milk by addition of a definite amount of phosphorus calculated on basis of the salt balance in the milk from the data on soluble inorganic salts obtained by analysis. Generally one can obtain a satisfactory result in adjustment of alcohol susceptibility of milk which coagulates with 60-70% alcohol by adding  $13.92 \, \mathrm{mg}$  of phosphorus to  $100 \, \mathrm{cc}$  of the milk, or by mixing  $0.896 \, \mathrm{cc}$  of  $M/2 \, K_2 HPO_4$  solution to  $100 \, \mathrm{cc}$  with a result that the milk coagulates only by addition of 78-82% alcohol.
- 16. The iso-electric point of the milk casein has no relation to its alcohol susceptibility.
- 17. The alcohol susceptibility of milk is increased slightly when it is frozen and melted.
- 18. The original catalase content is almost constant in fresh normal milk regardless of the alcohol susceptibility, but its contents are greater in the colostrum and the milk at the end stage of lactation. Therefore it may be considered that some indirect relation may exist between the catalase content and the alcohol sensitivity of the colostrum and of the milk at the end stage of lactation as these milk are usually very sensitive to alcohol test.

It may be concluded from the above summaries of experiments that the alcohol susceptibility of normal milk is almost constant, that it is a characteristic of the individual cow, and that it has a close relation to the content of the soluble inorganic salts in milk especially to the balance of calcium and phosphorus.

It is impossible to adjust these properties by feeding so long as the milking cow is kept in a healthy condition, but it is not difficult to imagine that the health of a cow may be disturbed when she is fed with unbalanced ration or under poor management for a long time bringing finally the change in constituents of the milk.

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