JOURNAL
OF
DAIRY SCIENCE

VOLUME XXV

JANUARY, 1942, TO DECEMBER, 1942

1942
AMERICAN DAIRY SCIENCE ASSOCIATION
THE OHIO STATE UNIVERSITY, COLUMBUS, OHIO

EDITORIAL BOARD

THE SCIENCE PRESS PRINTING COMPANY
LANCASTER, PENNSYLVANIA

CONTENTS

JANUARY, NO. 1

FEBRUARY, NO. 2

MARCH, NO. 3

APRIL, NO. 4

MAY, NO. 5

CONTENTS

JUNE, NO. 6

JULY, NO. 7

AUGUST, NO. 8

SEPTEMBER, NO. 9

OCTOBER, NO. 10

NOVEMBER, NO. 11

DECEMBER, NO. 12

JOURNAL OF DAIRY SCIENCE

Contents

Vol. XXV, No. 1, January, 1942

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION

WHAT IS Duraglas ?

TRADE-MARK REG. U.S. PAT. OFF.

YOU have seen our Duraglas advertisements in national magazines.* Now let us explain Duraglas more fully, point out its advantages to you in this industry.

Duraglas is not an advertising catchword casually adopted for our containers. The mere addition of a name to containers adds nothing to their quality.

NO—Duraglas is far more than that. Duraglas is the trade-mark that symbolizes the fruition of a glassmaking technique developed by scientific research. Only after our technique reached the perfection we set for ourselves, did we give it the name Duraglas.

A Step Forward for an Age-Old Industry

Duraglas is no overnight discovery. There is no "secret ingredient." But the Duraglas method —a milepost in the technological progress of an industry 5,000 years old—does represent epoch-making advances in every step of producing glass containers. Duraglas cannot be duplicated without painstaking research, special equipment and the experience which comes from making more glass containers, year after year, than any other manufacturer.

Duraglas Means a Better Container

To containers made by this technique, we give the name "Duraglas." They have greater strength and durability. In the case of returnable containers—milk, beverage and beer bottles—Duraglas results in longer life and lower trip costs. In one-trip containers, the strength of Duraglas makes possible lower weight, with resulting important economies. Where safe delivery of contents is paramount, Duraglas bottles offer greater confidence.

Duraglas brings better packaging not only to industries that normally use glass, but to hundreds of others whose products would serve and sell better in glass containers.

Duraglas is to Glass What Sterling is to Silver

We have established for Duraglas a standard to be zealously guarded.

Although most of the containers we now make are Duraglas, some few are not. Good containers though they are—the equal certainly of others in their fields—they are not yet called Duraglas. For each container group presents special problems; and month by month, as the Duraglas method is fitted to these containers, they will be awarded the trade-mark "Duraglas."

The Duraglas standard . . fixed, not by law as is sterling, but by our experience and integrity . . gives new meaning to our slogan—*"First in Glass."*

Owens-Illinois Glass Company, Toledo, Ohio.

**In The Saturday Evening Post, Collier's and Life*

OWENS-ILLINOIS GLASS

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION
Published at
NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

The Journal of Dairy Science is issued monthly. Subscription is by the volume and one volume is issued per year.

Manuscripts should be typewritten and carefully revised before submission to T. S. Sutton, The Ohio State University, Columbus, Ohio. Twenty-five reprints will be furnished gratis to authors. Cost of additional reprints and reprint order blank will be submitted with proof.

The use of material published in the Journal is encouraged and a liberal policy will be followed concerning reproduction of articles with proper notation as to source.

Subscriptions. Price; $6.00 per volume in United States and Canada; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, 19 Bridge Street, Sydney, Australia.

Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Correspondence regarding business policies of the Journal should be addressed to the Secretary-treasurer, R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Entered as second-class matter April 13, 1934 at the postoffice at Lancaster, Pa., under the act of March 3, 1879.

JOURNAL OF DAIRY SCIENCE

VOLUME XXV JANUARY, 1942 NUMBER 1

A COMPARISON OF HOT WATER, STEAM AND CHLORINE FOR SANITIZING ICE CREAM FREEZERS*

F. W. FABIAN, A. E. HOOK, AND G. L. NIELSEN

Michigan Agricultural Experiment Station, East Lansing, Michigan
Cooperating with G. J. TURNEY, *Chief Milk Inspector,*
Lansing Department of Health, Lansing, Michigan

The ice cream freezer is an important factor in the production of low bacterial count ice cream since the introduction of better and more sanitary methods for the preparation of ice cream mix containing relatively few bacteria. The present work was done in order to study the relative efficiency of the most commonly used physical and chemical methods.

METHOD

In the experiments reported herein, three ice cream plants were chosen, one was a small counterfreezer installation and two were commercial plants. The type of freezer in use in Plant A was a Mills 20-quart horizontal counter-freezer. Plant B used a 50-quart Creamery Package Mfg. Company batch freezer and Plant C a 40-quart Miller batch freezer.

The method of cleaning all freezers preparatory to sanitizing them was standardized. This was done so that the cleaning operations would be the same throughout and the bacterial reduction resulting from the cleaning of the freezers would be as nearly uniform as possible.

The same ice cream mix was used for testing the efficiency of each type of sanitization so that there would be approximately the same numbers and types of bacteria present to be killed. The ice cream mix used was a commercial one prepared in the usual way and allowed to stand for two days at room temperature until sufficient bacterial growth had taken place so as to insure the presence of sufficient numbers of bacteria in the freezer after it had been cleaned ready for sanitization.

Method of Cleaning Freezers.

1. Three, six and nine gallons respectively of cold water (60° F.) were placed in the freezer and the freezer run for two minutes.

2. Next, three, six and nine gallons respectively of warm water (140–

Received for publication August 1, 1941.

* Journal Article No. 543 (n.s.).

150° F.) to which had been added 0.5 per cent of Wyandotte dairy cleaner were placed in the freezer and the freezer run for two minutes.

3. Finally, three, six and nine gallons respectively of hot water (180° F.) were placed in the freezer and the freezer run for two minutes.

All the freezers were first cleaned in the above manner irrespective of the method which was to be used later in sanitizing them.

Hot Water Sanitization.

In the hot water method of sanitizing three, six and nine gallons, respectively, of water at approximately 200° F. were added to the freezer after it had been cleaned by the method previously outlined. The hot water was agitated in the freezer by running it for two minutes. After the last water had been drained from the freezer, one liter of sterile water was placed in the machine and agitated by running the freezer. (Tables 1, 4, 5 and 6.)

Samples of the mix and all the various rinse waters were taken for bacterial analysis. After the freezer was sanitized, it was disassembled and swabs made of the front and rear bearings. In one type of freezer, it was impossible to get a swab of the rear bearing due to the improper operation of the freezer over a long period. All samples were plated immediately on Standard agar for total plate count and on violet-red-bile agar for *Escherichia coli* counts. These data are given in table 4.

The temperature of the rinse waters was taken as they entered and left the freezer. In collecting these data, the freezer was operated according to the method in use in the factory where the ice cream was made. The results are given in tables 1, 4, 5, and 6.

Steam Sanitization.

After the freezer had been cleaned by the method just outlined, the steam hose was placed in the freezer and the steam turned on and allowed to run until the condensed steam dripping from the freezer had a temperature of 190° F.

At the end of this period the freezer was rinsed with one liter of sterile water and the water plated for bacterial counts. The head was removed and the bearings swabbed. The results are given in tables 2, 4, 5, and 6.

Chlorine Sanitization.

Chlorine or chemical sanitization was carried out the same as in the two types of physical sanitization except the freezers were rinsed with three gallons of cold water containing 94.5 to 132.65 p.p.m. of active chlorine after the freezers had been cleaned.

The freezers were then rinsed with one liter of sterile water which was collected in a flask containing sufficient sodium thiosulphate to neutralize the effect of the chlorine. Swabs were made of the front and rear bearings. The complete data are in tables 3, 4, 5 and 6.

Bacteriological Analysis.

Platings were made in duplicate from all rinse waters on Standard milk agar. They were incubated at 37° C. for 48 hours and counted. All procedures were according to Standard Methods for the Examination of Dairy Products (2).

The front and rear bearings where possible were swabbed with sterile cotton swabs kept in tubes containing 10 ml. of sterile saline. The recommended procedure (4) for swabbing dishes and utensils was followed.

TABLE 1

Temperatures of three gallons of water used to rinse freezers as it entered and as it left the freezers after a two minute rinse period during hot water sanitization

Plant	Cold water 60° F. E–L	Hot water 140° F. E–L	Hot water 180° F. E–L	Hot water 200° F. E–L
	°F.	°F.	°F.	°F.
A	68–52	140–108	180–153	198–166
B Run # 1	60–40	144–40	184–46	207–63
B Run # 2	60–49	141–66	182–58	202–77
B Run # 3	57–38	146–52	184–86	198–120
C Run # 1	61–31	143–52	184–102	202–132
C Run # 2	59–36	142–56	180–96	201–124

E = temperature of the rinse water as it entered the freezer.
L = temperature of the rinse water as it left the freezer after a two minute rinse period.

The coliform count was determined by using violet-red-bile agar according to Standard Methods for the Examination of Dairy Products (2).

Influence of Volume of Water Used to Rinse Freezers.

It early became apparent from the data collected in tables 1, 2 and 3 that there was considerable variation in the temperature of the water as it left the freezers. In the first series of tests only three gallons of water were used for the various rinse waters. A second series of tests were set up using one of the freezers, a 50-quart Creamery Package Mfg. Co. batch freezer in Plant B in which the amount of rinse water was increased from three (tables 1, 2 and 3) to six and finally nine gallons.

Proper Handling of Freezers.

Another factor influencing the temperature of the rinse water is the handling of the freezer during the rinsing operation. To study the influence of these factors, one experiment was conducted as follows:

TABLE 2

Temperature of three gallans of rinse water used to rinse freezers as it entered and as it left freezers after a two minute rinse period and temperature of steam drip after two minutes steaming

Plant	Cold water 60° F. E–L	Hot water 140° F. E–L	Hot water 180° F. E–L	Temp. of steam drip
	°F.	°F.	°F.	
A	68–52	140–108	180–153	198–166° F* 3 gallons 2 minutes
B Run # 1	59–51	150–68	181–74	Steam to 197° F. drip
B Run # 2	61–75	140–103	176–127	Steam to 190° F. drip
B Run # 3	54–76	142–102	181–126	Steam to 191° F. drip
C Run # 1	58–43	141–82	177–117	Steam to 194° F. drip
C Run # 2	60–38	140–82	181–123	Steam to 190° F. drip

E = temperature of rinse water as it entered the freezer.
L = temperature of rinse water as it left the freezer.
* = no steam available—hot water used.

TABLE 3

Temperature of three gallons of water used to rinse freezers two minutes and parts per million of chlorine used in rinsing freezers for chlorine sanitization

Plant	Cold water 60° F. E–L	Hot water 140° F. E–L	Hot water 180° F. E–L	Cold chlorine*
	°F.	°F.	°F.	
A	63–49	140–108	180–152	132.65–87.85 p.p.m. 2 minutes
B Run # 1	59–50	150–58	183–72	112.35–82.95 p.p.m. 2 minutes
B Run # 2	62–60	142–94	181–124	108.5–86.8 p.p.m. 2 minutes
B Run # 3	60–35	143–101	185–128	121.45–91.35 p.p.m. 2 minutes
C Run # 1	61–68	143–101	182–110	112.75–81.35 p.p.m. 2 minutes
C Run # 2	60–63	140–100	180–110	94.5–63.0 p.p.m. 2 minutes

E = temperature of the rinse water as it entered the freezer.
L = temperature of the rinse water as it left the freezer.
* The first figure in this column represents the parts per million of chlorine at the beginning and the second figure the parts per million of chlorine at the end of the two minute rinse period.

TABLE 4

Showing the standard bacterial plate counts, percentage of organisms removed and the Escherichia coli counts of ice cream mix and the influence of each subsequent operation on the bacterial content of the freezers using three gallons of water for each rinsing of the freezer

Material plated out	Plant A—3-gallon rinse			Plant B—3-gallon rinse			Plant B—3-gallon rinse		
	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.
Hot Water Sanitization									
1. Mix before starting	19,000,000		320,000	5,200,000		107	6,000,000		0
2. 60° rinse water	670,000	96.64	1,000	1,100,000	97.52	13	1,700,000	92.54	34
3. 140° rinse water	16,000	2.31	1,000	24,500	2.17	0	32,300	1.76	0
4. 180° rinse water	3,820	0.55	0	1,200	0.11	0	104,600	5.69	0
5. 200° rinse water	3,460	0.50	0	2,300	0.21	0	160	0.008	0
6. Sterile rinse water	2,640		0	97		0	65		0
7. Swab of front bearing	270*		10*	Not taken			250		0
8. Swab of rear bearing	510*		100*	Not taken			Not taken		
Steam Sanitization									
1. Mix before starting	17,900,000		290,000	7,000,000		122	100,000		0
2. 60° rinse water	117,000	98.73	0	655,000	98.92	38	70,000	98.26	2
3. 140° rinse water	1,200	1.01	0	6,350	0.96	0	950	1.33	0
4. 180° rinse water	130	0.11	0	765	0.12	1	230	0.32	0
5. Sterile rinse water	171	0.14	0	30	0.005	0	60	0.085	0
6. Swab of front bearing	70*		0	Not taken			2,200		0
7. Swab of rear bearing	60*		0	Not taken			Not taken		
Chlorine Sanitization									
1. Mix before starting	14,600,000		60,000	6,250,000		242	400,000		0
2. 60° rinse water	31,000	92.15	10,000	1,040,000	98.84	46	60,000	88.41	0
3. 140° rinse water	2,600	7.73	0	11,500	1.09	0	6,000	8.84	0
4. 180° rinse water	40	0.12	0	700	0.07	0	1,850	2.74	0
5. Chlorine rinse water	Not taken			13	0.003	0	12	0.002	0
6. Sterile rinse water	0		0	26		0	0		0
7. Swab of front bearing	20*		0	Not taken			0		0
8. Swab of rear bearing	30*		0	Not taken			Not taken		

* All bacterial counts from the swabs are total counts.

TABLE 4—(*Continued*)

Material plated out	Plant C—3-gallon rinse			Plant C—3-gallon rinse			Plant C—3-gallon rinse		
	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.
Hot Water Sanitization									
1. Mix before starting	760,000		1,630	131,500,000		10	456,000,000		0
2. 60° rinse water	200,000	25.284	106	29,300,000	99.43	37	18,410,000	97.06	3
3. 140° rinse water	135,000	17.07	59	123,000	0.42	0	460,000	2.42	5
4. 180° rinse water	381,000	48.17	4	29,000	0.10	1	51,900	0.27	7
5. 200° rinse water	75,000	9.48	0	14,500	0.05	0	46,000	0.24	0
6. Sterile rinse water	19,200		0	4,100		1	530		00
7. Swab of front bearing	8,910*		50	36,750		10	7,500		0
8. Swab of rear bearing	230,000		0	765,000		210	119,000		200
Steam Sanitization									
1. Mix before starting	450,000		460	30,000,000		0	353,000,000		0
2. 60° rinse water	130,000	59.04	145	1,705,000	97.54	8	1,890,000	80.32	1
3. 140° rinse water	86,000	39.05	2	43,000	2.46	0	339,000	14.41	0
4. 180° rinse water	3,000	1.36	2	5 pr.		0	124,000	5.27	1
5. Sterile rinse water	1,200	0.54	2	60	0.001	0	40	0.001	1
6. Swab of front bearing	10		20	1,800		0	150		0
7. Swab of rear bearing	28,500		80	27,900		50	34,900		0
Chlorine Sanitization									
1. Mix before starting	1,300,000		790	15,500,000		0	311,000,000		0
2. 60° rinse water	415,000	77.58	112	6,600,000	98.10	41	58,300,000	97.95	5
3. 140° rinse water	114,000	21.31	0	120,000	1.78	2	1,190,000	2.00	1
4. 180° rinse water	5,870	1.10	3	7,600	0.11	0	30,300	0.05	0
5. Chlorine rinse water	33	0.007	0	37	0.001	0	36	0.001	0
6. Sterile rinse water	16		1	spr.		00	61		0
7. Swab of front bearing	4,990*		0	57,000*		80*	320*		0
8. Swab of rear bearing	67,500*		10	396,700*		40*	470*		0

* All bacterial counts from the swabs are total counts.

TABLE 5

Showing the standard bacterial plate counts, percentage of organisms removed and the Escherichia coli counts of ice cream mix and the influence of each subsequent operation on the bacterial content of the freezers using six gallons of water for rinsing of the freezer

Material plated out	Plant B—6-gallon rinse			Plant B—6-gallon rinse			Plant B—6-gallon rinse		
	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.
Hot Water Sanitization									
1. Mix before starting	4,300,000		5,000	5,000,000		200,000	2,500,000		5,000
2. 60° rinse water	1,800,000	99.69	500	1,300,000	87.92	127,000	450,000	96.71	500
3. 140° rinse water	5,000	0.28	200	178,000	12.04	4,200	10,000	2.15	50
4. 180° rinse water	450	0.02	3	550	0.04	1	3,500	0.75	52
5. 200° rinse water	45	0.004	0	85	0.007	3	1,800	0.39	56
6. Sterile rinse water	12		0	22		0	983		28
7. Swab of front bearing	90*		0	1,090		20	15,000 (est.)		0
8. Swab of rear bearing	1,428*		0	1,750		280	15,000 (est.)		0
Steam Sanitization									
1. Mix before starting	3,000,000			445,000,000		0	1,500,000		10,000
2. 60° rinse water	700,000	98.28	1,000	63,000,000	99.90	209,500	950,000	99.73	200
3. 140° rinse water	12,000	1.68	0	60,000	0.09	8,100	1,500	0.16	55
4. 180° rinse water	250	0.03	0	3,000	0.005	89	1,020	0.11	11
5. Sterile rinse water	3	0.006	0	1		0	3	0.001	0
6. Swab of front bearing	10*		0	2,740		142	0		0
7. Swab of rear bearing	670*		0	150		0	10		0
Chlorine Sanitization									
1. Mix before starting	3,500,000		5,000	13,000,000		650,000	2,000,000		0
2. 60° rinse water	550,000	98.67	0	2,100,000	78.12	215,900	1,210,000	98.73	0
3. 140° rinse water	7,000	1.25	0	580,000	21.58	11,900	15,000	1.22	2
4. 180° rinse water	400	0.07	0	8,050	0.30	1	500	0.04	28
5. Chlorine rinse water	8	0.004	0	1		0			0
6. Sterile rinse water	5		0	0		0	10		0
7. Swab of front bearing	30*		0	80*		0	15,000*		10*
8. Swab of rear bearing	1,415*		0	1,120*		280*	15,000*		10*

* All bacterial counts from the swabs are total counts.

TABLE 6

Showing the standard bacterial plate counts, percentage of organisms removed and the Escherichia coli counts of ice cream mix and the influence of each subsequent operation on the bacterial content of the freezers using nine gallons of water to rinse the freezer

Material plated out	Plant B—9-gallon rinse			Plant B—9-gallon rinse			Plant B—9-gallon rinse		
	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.	Standard plate counts per ml.	Per cent organisms removed	*E. coli* counts per ml.
Hot Water Sanitization									
1. Mix before starting	52,000,000	.....	100,000	9,000,000	.....	2,500	14,000,000	.....	70,000
2. 60° rinse water	44,000,000	98.54	2,000	7,200,000	72.66	4,000	4,400,000	98.87	1,500
3. 140° rinse water	640,000	1.43	1,000	2,700,000	27.25	0	45,000	1.01	0
4. 180° rinse water	27,060	0.01	0	9,200	0.09	0	5,000	0.11	0
5. 200° rinse water	8,255	0.02	0	508	0.006	0	20	0.002	0
6. Sterile rinse water	74,300	.....	2	5,143	.....	4	317	.....	0
7. Swab of front bearing	11,000	.....	0	7,250	.....	20	1,890	.....	0
8. Swab of rear bearing	0	.....	0	2,400	.....	0	1,500	.....	0
Steam Sanitization									
1. Mix before starting	8,500,000	.....	0	7,300,000	.....	80,000	63,000,000	.....	60,000
2. 60° rinse water	6,200,000	98.10	0	5,800,000	96.43	8,500	7,100,000	95.14	9,500
3. 140° rinse water	110,000	1.74	0	208,000	3.46	0	360,000	4.82	0
4. 180° rinse water	5,000	0.08	0	6,600	0.11	0	2,400	0.03	0
5. Sterile rinse water	5,144	0.08	0	87	0.003	0	32	0.001	0
6. Swab of front bearing	9,530	.....	0	350	.....	0	1,480	.....	0
7. Swab of rear bearing	5,080	.....	0	700	.....	0	510	.....	0
Chlorine Sanitization									
1. Mix before starting	7,000,000	.....	450,000	8,600,000	.....	300,000	8,500,000	.....	70,000
2. 60° rinse water	4,600,000	99.36	7,000	6,500,000	77.37	10,000	8,000,000	97.28	11,000
3. 140° rinse water	24,000	0.54	0	1,900,000	22.61	100	220,000	2.67	0
4. 180° rinse water	800	0.001	0	1,600	0.02	0	32,000	0.040	0
5. Chlorine rinse water	5,000	0.11	0	3	0.001	0	1	.....	0
6. Sterile rinse water	1,715	.....	0	3	.....	0	1	.....	0
7. Swab of front bearing	6,350*	.....	0	6,350*	.....	0	205*	.....	0
8. Swab of rear bearing	10,000*	.....	0	5,080*	.....	0	2,500*	.....	10*

* All bacterial counts from the swabs are total counts.

The 50-quart freezer in Plant B, in a dry state, was allowed to come to −10° F. The valve to the expansion chamber was then closed, but the liquid ammonia line was left open. Three gallons of 60° F. rinse water was poured into the freezer, and the freezer run for two minutes. The rinse water was then run off, and the temperature taken. This procedure was repeated using 6 and 9 gallons of rinse water. Results are shown in table 7.

A second experiment was performed with both the valve to the expansion chamber and the valve to the liquor line closed, so that the freezer was entirely shut off. The amounts of rinse water and the initial temperature of the rinse waters were the same as for the first experiment. The results are shown in table 8.

TABLE 7

The effect of the amount of rinse water used to rinse an ice cream freezer on the temperature of the rinse water (expansion chamber closed; liquor line open)

Trial No.	Initial temp. of rinse water	Temperature of rinse water after running freezer for 2 minutes		
		3-gallon rinse	6-gallon rinse	9-gallon rinse
	°F.	°F.	°F.	°F.
	60	33	33	38
1	140	40	51	65
	180	50	65	81
	200	53	71	88
	60	34	33	35
2	140	44	56	67
	180	49	71	82
	200	54	71	87
	60	34	34	34
3	140	44	53	66
	180	50	66	82
	200	55	67	88
	60	33	33	34
4	140	42	51	67
	180	48	67	81
	200	53	68	88
	60	33.5	33	35
Average of four runs	140	42.5	53	66
	180	49	67	81.5
	200	53	69	88

It is obvious from tables 7 and 8 that the amount of water used to rinse the freezer as well as the method of handling the freezer during rinsing has an influence on the temperature of the rinse water. This in turn would influence the sanitizing value of any of the three methods used. For example when 200° F. water was used with the expansion chamber closed and the liquid ammonia line open, the average temperature of four runs with respective volumes of rinse water of three, six and nine gallons was 53, 69 and 80° F., respectively. Under the same conditions with the liquor line closed,

TABLE 8

The effect of the amount of rinse water used to rinse an ice cream freezer on the temperature of the rinse water (expansion chamber and liquid ammonia line closed)

Trial No.	Initial temp. of rinse water	Temperature of rinse water after running freezer for 2 minutes		
		3-gallon rinse	6-gallon rinse	9-gallon rinse
	°F.	°F.	°F.	°F.
	60	34	34	36
1	140	34	50	66
	180	44	67	80
	200	50	76	107
	60	34	32	35
2	140	35	50	65
	180	44	69	82
	200	51	80	110
	60	34	34	36
3	140	36	49	62
	180	47	69	82
	200	49	82	114
	60	34	33	37
4	140	35	50	68
	180	45	68	80
	200	50	78	112
	60	34	33	36
Average of four runs	140	35	50	65
	180	45	68	81
	200	50	79	111

the average temperature was 50, 79 and 111° F., respectively. These differences in temperatures are significant and show the value of plenty of rinse water and the proper handling of the freezers.

Method of Calculating Bacterial Reductions.

The method of calculating the percentage reduction in bacteria after each successive operation was as follows: The bacterial count of each rinse water was determined by plating in duplicate on Standard Milk agar. The average of the two counts per ml. for each rinse water was then totaled and the percentage bacterial reduction calculated by dividing the number per ml. found in each rinse water by this total. This gave the percentage of the total number removed by each operation in terms of bacteria per ml. The number per ml. found in the sanitized rinse water used to determine the number of bacteria left in the freezer after it had been sanitized was not used in the calculations.

GENERAL DISCUSSION

The data indicate that the preliminary treatment given to freezers is more important than the method used to sanitize them. When freezers are

properly and adequately rinsed, the bacterial load is reduced to a point where sanitization, by any of the three methods used in these experiments, is greatly facilitated. The advisability of using several rinse waters is also demonstrated since each successive rinse water greatly reduces the bacterial load. This is demonstrated in several instances in table 4 where the first rinse water removed as few as 73 per cent of the bacteria. Additional bacteria were removed by successive rinse waters until the number remaining was reduced in most cases to a satisfactory point by the sanitizing method used.

It should be noted in this connection that when the percentage reduction in the number of bacteria was small for the first rinse waters, the number remaining in the freezer after sanitizing was correspondingly large. This gives additional evidence for the necessity of adequate rinsing. Likewise, when the ice cream mix contained large numbers of bacteria, the number remaining after sanitizing was in many instances excessive. This was true despite the fact that the percentage reduction was large. The types of bacteria present would, of course, influence the percentage reduction which doubtless accounts for the exceptions found in some instances.

These data emphasize the necessity of an adequate and preferably an abundant supply of hot and cold water—a condition not possible in many retail ice cream manufacuring establishments such as soda bars, drug stores, restaurants and such places.

Sanitizing Front and Rear Bearings.

The results show that the rear bearing is harder to sanitize than the front bearing irrespective of the amount of water used or the method used to sanitize them. This does not bear out the assumption of Dalhberg and Marquardt (1) who state, "Although the rear bearings have not been considered in this study for obvious practical reasons, it is reasonable to assume that a procedure which sterilized the front bearings would also sterilize the rear bearings unless the front bearing is sterilized when the head of the freezer has been removed."

Steam was more effective than either hot water or chlorine in reducing the number of bacteria found in the bearings. This confirms the findings of the above authors (1) with respect to the front bearing. In the 8th edition (3) of Standard Methods for the Examination of Dairy Products, there is a new section entitled "Rinse Test as Applied to Ice Cream Freezers." The standard procedure recommended is 100-, 200- and 400-ml. portions of sterile rinse water for 10-, 20- and 40-quart freezers respectively. Freezers showing a total rinse count of 10,000, 20,000 and 40,000 for 10-, 20- and 40-quart freezers, respectively, are considered satisfactory. In these experiments, which were done before the standard procedure was formulated, one liter of sterile rinse water was used irrespective of the size of the freezer. However,

the bacterial content of the rinse water was determined on the ml. basis. The count per ml. was then multiplied by 1000 and this figure used as the basis of comparison with the bacterial counts considered satisfactory for ice cream freezers by the new standard methods.

On this basis, chlorine sanitization of freezers was best since 99.9 per cent of the rinse waters from chlorine sanitized freezers fell within the range considered satisfactory. Whereas, only 50 per cent of the rinse waters from steam sanitized freezers and 17 per cent of the rinse waters from hot water sanitized freezers fell within the satisfactory range.

However, when one considers the bacterial counts of the front and rear bearings, steam showed to better advantage than chlorine since the counts from the swabs were lower on the bearings of the freezers sanitized with steam than with chlorine. Hot-water sanitization was less effective than either steam or chlorine for both the freezer proper and for the front and rear bearings. The temperature data in table 8 show why hot water is the least effective since under ideal conditions the average temperature of 200° F. water as it entered the freezer was only 111° F. as it left it two minutes later.

It should be stated that the ice cream mixes used in these experiments had a bacterial count far in excess of what one would ordinarily find in commercial mixes. For this reason it is believed that the bacterial counts considered satisfactory in the 8th edition of Standard Methods for the Examination of Dairy Products (3) are reasonable and should be easily obtainable with any of the three methods used especially with chlorine and steam.

Significance of Escherichia coli Count.

From the data presented in tables 4, 5 and 6, it is obvious that the *Escherichia coli* were killed in practically all instances as judged by plating of the sterile rinse water. They were found more frequently in both the front and rear bearings than in the freezer proper. This would indicate the difficulty of sanitizing the bearings. Likewise, they were more numerous in the rear than in the front bearing indicating that the rear bearing is more difficult to sanitize than the front bearing of an ice cream freezer.

SUMMARY

1. The first rinse water caused the greatest reduction in the number of bacteria in the freezer. Each subsequent washing reduced the number of bacteria left in the freezer but to a lesser degree.

2. The amount of water used to rinse the freezer is important since there is a direct relationship between the volume of water used and the number of bacteria remaining in the freezer.

3. The rear bearing is harder to sanitize than the front bearing of an ice cream freezer.

4. For sanitizing both the front and rear bearings, of a freezer, the order of effectiveness is steam, chlorine and hot water.

5. The valves to the liquid ammonia line and expansion chamber should be closed during the rinsing and sanitizing of a freezer to prevent dissipation of heat.

6. For most effective cleaning and sanitizing, freezers should be completely filled with water or at least to 90 per cent of their capacity.

7. As judged by the bacterial content of the sterile rinse water, chlorine was best, steam the next best, and hot water the least efficient method of sanitizing freezers.

8. There are marked temperature changes in water as it enters and leaves the freezer if the freezer is washed immediately after use. Under the most favorable conditions, hot water entering the freezer at 200° F. had a temperature of only 111° F. after it had been agitated two minutes. This explains why hot water sanitization is the least effective of the three methods.

REFERENCES

(1) DAHLBERG, A. C., AND MARQUARDT, J. C. Sterilization of Ice Cream Freezers. New York State Agr. Expt. Sta. Bul. 628. April 1933.

(2) Amer. Pub. Health Assoc. Standard Methods for the Examination of Dairy Products. 7th Edition, 1939. New York.

(3) Amer. Pub. Health Assoc. Standard Methods for the Examination of Dairy Products. 8th Edition, Am. P. H. Assn. New York (Unpublished).

(4) Standard Technic Proposed by Sub-Committee on Standard Methods for the Examination of Dishwashing Devices of the Committee on Research and Standards. A. P. Hitchens, Chairman. 7th Annual Yr. Book 1936—37 pp. 45–58.

AGE, LIVE WEIGHT AND MILK-ENERGY YIELD—A CORRECTION

W. L. GAINES, C. S. RHODE AND J. G. CASH
University of Illinois, Urbana

This correction relates to a previous article (1) in which initial live weight (live weight measured within 31 days after calving) was estimated by the New York chest-girth live-weight tape. Since that time a new girth-weight scale has been developed (2) for the specific purpose of estimating initial live weight, where age and breed are known. The New York tape is designed without regard to age, breed, or stage of lactation and as compared with the new scale it leads to gross systematic errors in the weight estimates (2).

The old weight estimates may be changed onto the new scale readily since both are based on chest girth. In the present paper this change has been made for those records with age reported and the age-weight-yield relations are re-examined. The yield data used are the eight-months partial lactation milk-energy yields in terms of pounds of four per cent milk per day (FCM).

AGE, WEIGHT, YIELD

As before, the equation, $FCM = a + bW + dA$, is fitted to the observations at less than 7 years of age, with the results:

Holstein	Old scale, $FCM = 9.89 + .0128W + .96A$
	New scale, $FCM = 1.53 + .0217W + .54A$
Jersey	Old scale, $FCM = .48 + .0264W + .37A$
	New scale, $FCM = 3.80 + .0412W + .79A$

in which W = initial live weight in pounds and A = age in years.

The effect of removing the systematic errors in the New York tape has been to decrease the influence of age on FCM in the Holstein and increase it in the Jersey. In both breeds the effect of weight on FCM has been stepped up by about 60 per cent. The previous conclusion still holds that age independent of weight has only a negligible influence on yield. The influence of initial live weight, independent of age, is even more marked than before.

The mean weight on the new scale is practically the same as the mean weight on the old scale, in either breed but the range in weight is reduced on the new scale as compared with the old scale.

AGE AND FCM/W

In figure 1 milk-energy yield per unit live weight (FCM/W) is plotted against age. It is quite apparent that FCM/W is independent of age

Received for publication August 6, 1941.

through the whole age range. A statistical verification of this is afforded by an analysis of variance with respect to 1000 FCM/W between and within age groups:

	Holstein	*Jersey*
Variance between age groups	48.4	31.5
Variance within age groups	38.0	38.4
F	1.27	1.22
F, at 5% level	1.83	2.57

Clearly such differences as exist between age groups with respect to FCM/W are no more than might easily arise by reason of differences within age groups. The former conclusion remains: we may deal freely with FCM/W without regard to age of cow.

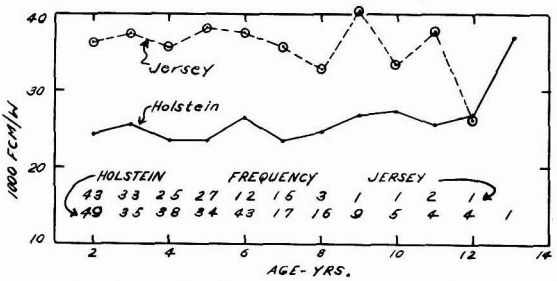


FIG. 1. Showing the change (or absence of change) in milk-energy yield per day per 1000 pounds live weight (1000 FCM/W) with age. There are no significant differences between the age groups with respect to FCM/W.

The independence of age (including all ages) and FCM/W is further tested by an analysis of covariance, taking account of a possible herd effect. This gives:

Correlation	*Holstein*	*Jersey*
Total	+.10	−.04
Between herds	−.03	+.10
Within herds	+.14	−.06

All of these correlations are below the five per cent level of significance, which together with their mixed nature in sign may be taken to mean that age and FCM/W are independent not only in the total population but also between and within herds. Age of cow may be ignored in FCM/W comparisons within a herd.

LIVE WEIGHT AND FCM/W

Analysis of covariance with reference to initial live weight (W) and milk-energy yield per unit initial live weight (FCM/W) gives the following:

Correlation	*Holstein*	*Jersey*
Total	+.06	+.04
Between herds	+.02	+.23
Within herds	+.08	−.01

All of these correlations are below the five per cent level of significance although in the Jersey breed the between-herds correlation is large enough to attract attention. It means, of course, that among the Jersey herds the herds with larger cows produce more milk-energy per unit live weight of cow than do the herds with smaller cows.

As compared to the old results a very important change is to be noted in the correlation between W and FCM/W within herds. *The present result shows that within herd and breed initial live weight and milk-energy yield per unit live weight are substantially independent.*

The correlation between W and FCM/W may be readily translated into the power equation, $FCM = aW^b$, if desired. In this power equation, as a close approximation,

$$b = 1 + r_{W(FCM/W)} V_{FCM/W} / V_W$$

where V indicates standard deviation divided by mean. Applying this formula it comes out that in the Holstein breed, and within herd, FCM is proportional to $W^{1.02}$; while in the Jersey breed, and within herd, FCM is proportional to $W^{.98}$. If it is accepted that the within herd correlations represent some degree of uniformity of environment, the present results give important support to the theory that milk-energy yield tends to be proportional to initial live weight. However, the disparity between the two breeds with respect to FCM/W remains as before (2).

The correlation between W and FCM may be readily derived from the correlation between W and FCM/W by the formula:

$$r_{WFCM} = \frac{V_W + r_{W(FCM/W)} V_{FCM/W}}{[V^2_W + V^2_{FCM/W} + 2r_{W(FCM/W)} V_W V_{FCM/W}]^{.5}}$$

Applying this formula the within herd correlation between W and FCM is + .44 in the Holstein records and + .55 in the Jersey records.

If the correlation between W and FCM/W is zero, the above formula reduces to terms of variability (V) in W and FCM/W, and if $V_W = V_{FCM/W}$ the expected correlation between W and FCM is + .71. Within a herd and breed variability in live weight (V_W) is not apt to exceed variability in energy yield per unit live weight ($V_{FCM/W}$) and hence the correlation between live weight and energy yield is not likely to exceed + .7.

DISCUSSION

The importance of having an estimate of live weight of the cow in connection with the estimate of her milk-energy yield is very evident. The estimate of yield under present systems may be regarded as satisfactory (although hardly precise). The estimate of live weight under present systems is not at all satisfactory. In the first place the live weight estimate is usually entirely absent—which may be better than badly erroneous estimates, or estimates made at indiscriminate stages of lactation.

An instance of the effect of systematic errors in the live weight estimates

has been noted above. Thus the estimate of live weight during the first month of lactation by the New York tape leads to the result that FCM is proportional to the $\frac{3}{4}$ power of live weight (1, footnote 3) while the estimate of live weight by the Nebraska-Illinois tape (2) for the same cows and FCM records leads to the result (above) that FCM is proportional to the first power of live weight. One result supports the $\frac{3}{4}$ power theory, the other supports the direct proportionality theory.

The basis of the difference in the two scales of estimating live weight from chest girth has been shown previously (2, figure 3). That it should lead to such a difference in the philosophical interpretation of the relation of live weight to yield emphasizes the necessity of *accuracy* in the estimate of live weight and the avoidance of systematic errors. Of no less importance is the *stage of lactation* at which live weight is measured (3). The confusion introduced by measuring live weight at indiscriminate stages of lactation may completely obscure the essential relation between size of cow and milk-energy yield.

SUMMARY

The correction of now known systematic errors in the estimate of live weight as previously used (1) shows the influence of weight on yield, independent of age, to be greater than previously found. The influence of age on yield, independent of weight, is negligible, as before.

Milk-energy yield per unit live weight is independent of age of cow, whether within herd, between herds or in total.

Within herd and breed (Holstein, Jersey) energy yield per unit live weight is independent of live weight. Or, if energy yield is expressed as a power function of live weight the within herd relation shows milk energy proportional to the 1.02 power of live weight in the Holstein records and proportional to the .98 power of live weight in the Jersey records.

Two essential points in the live weight measurements are stage of lactation and accuracy (avoidance of systematic errors). The present results are based on live weight measured within 31 days after calving.

REFERENCES

(1) GAINES, W. L., RHODE, C. S., AND CASH, J. G. Age, Live Weight and Milk-Energy Yield in Illinois Cows. JOUR. DAIRY SCI., 23: 1031–1043. 1940.

(2) GAINES, W. L., DAVIS, H. P., AND MORGAN, R. F. Estimation of Initial Live Weight at Each Lactation of Dairy Cows. JOUR. DAIRY SCI., 24: 983–992. 1941.

(3) GAINES, W. L. Live Weight of Cow at Various Stages of Lactation in Relation to Milk-Energy Yield. JOUR. DAIRY SCI., 24: 795–799. 1941.

A TEST FOR THE PROTEIN STABILITY OF MILK

ARNOLD B. STORRS

American Seal-Kap Corporation, Long Island City, N. Y.

The stability of the protein of milk is a matter of great importance in many phases of the dairy industry. Frequently an excessive loss of protein stability is the chief factor limiting the extent to which some treatments may be applied to milk or milk products. The problem is often of concern in the processing of such products as evaporated milk, ice cream, cream and some types of modified fluid milk.

It is not the purpose of this paper to enter into a lengthy review of protein stability since more adequate treatment of the subject may be found elsewhere (1, 2). The physical and chemical aspects of the problem will be mentioned only briefly. Fundamentally, the degree of stability of the milk proteins is dependent upon the hydration and the charge of the particles. These factors are influenced in turn by temperature, reaction, salts, previous heat treatment, or the action of other dehydrating or denaturing substances. The controlled application of one or more of these factors has been the basis for most methods of measuring protein stability.

One of the most widely used methods of studying protein stability has been that of subjecting the milk to high temperature under pressure in a manner simulating the sterilization of evaporated milk. The use of pilot sterilizers by condenseries is a routine operation for determining the degree of stability and the corrective measures needed. The technique of such tests is described by Hunziker (2). The same general methods have been applied by many investigators in studies of heat stability (3, 4, 5, 6, 7, 8). The use of a pilot sterilizer or similar equipment makes it possible to duplicate on a small scale many of the processing operations which affect stability, particularly with respect to the ability of the product in question to withstand sterilization. While the method is largely of value for the control and study of evaporated milk and related products, it is not well suited for use with many of the other dairy products.

Another method of measuring the coagulability of milk is the alcohol test (9). The test was originally devised as a measure of acidity but has been shown by Sommer and Binney (10) to be of little practical value due to the influence of the salts and other milk constituents. The test has been used for the detection of milk lacking stability during sterilization but there is some disagreement as to its accuracy for this purpose (2).

In 1935 Keith and Freeman (11), to determine the amount of HCl required to produce flocculation, employed an acid coagulation test which consisted of adding varying concentrations of N/40 HCl in distilled water to 5 ml. of ice cream mix.

Received for publication August 11, 1941.

Dahle and Rivers (12), in a study of ice cream, used a modified alcohol test essentially the same as the acid coagulation test of Keith and Freeman. The samples were observed for flocculation after the addition to the ice cream mix of varying concentrations of alcohol in distilled water instead of HCl.

Another method of estimating protein stability is a determination of the coagulating time with rennin. Mattick and Hallett (13) and others (6) have employed this technique in studies of heat stability.

In 1931 Ramsdell, Johnson and Evans (14) proposed a phosphate test for the detection of milk unstable to heat. After the addition of 0.5 M mono-basic potassium phosphate to 2 ml. of milk and mixing, the tubes were immersed in boiling water for five minutes, then cooled and examined for coagulation. A low "phosphate number" indicated low heat stability.

Various other methods of studying protein stability have been tried including such general tests as titratable acidity, pH measurements both with and without the use of coagulating agents, and simple boiling tests. These methods have been of little value except for some limited and specific applications, probably because results were too often influenced by factors having little or no relationship to protein stability.

The writer has been interested in the development of a process for modifying milk by the addition of a proteolytic pancreatic enzyme. One of the problems was a control system which would insure proper treatment of the milk and at the same time would be a simple and inexpensive routine for milk plants. Preliminary investigation indicated that the method best suited for the purpose was one involving a determination of protein stability and particularly with respect to the ability of the milk to withstand boiling without coagulation. A number of procedures were attempted before a test was developed which was both simple and accurate. A description of the test follows.

METHOD

This test for protein stability includes mixing increasing amounts of N/10 HCl with portions of the milk, boiling the mixtures for a specified length of time and then examining the samples for coagulation. While originally devised as a control test for the enzymatic treatment of milk, it need not be limited solely to that application.

Equipment and reagents for the test:

10-ml. volumetric pipette
1-ml. pipette, graduated in 0.1 or 0.05 ml.
Supply of test tubes, 16 × 150 mm. (Pyrex)
Test-tube rack
Water bath
N/10 hydrochloric acid

Procedure:

Arrange and number a series of test tubes as follows, adding N/10 HCl to each by means of the 1-ml. pipette in the amounts shown:

Tube no.	N/10 HCl (*ml.*)
0	0.00
5	0.05
10	0.10
15	0.15
20	0.20
etc., as needed	

(Note: HCl in the amounts 0.05, 0.15, 0.25 ml., etc., can be estimated satisfactorily with a pipette graduated in 0.1 ml.)

The tube numbers correspond to 100 × the ml. of N/10 HCl added to each tube. This eliminates the decimal point as well as any need for further interpolation of results.

Add to each tube by means of a volumetric pipette 10 ml. of the milk to be tested. All tubes are then placed in a water bath maintained at the boiling temperature. After 10 minutes the tubes are removed from the boiling water and examined for coagulation by tipping . The number of the first tube in the series which shows coagulation represents the end-point and is recorded as the stability number of the milk.

RESULTS

In applying this test to a variety of untreated fresh milks a range in stability of from 40 to 100 has been observed. A frequency table of these results is shown in table 1. Samples with extremely high or low stability

TABLE 1

Distribution of stability numbers of untreated fresh milk

Stability number	No. of times observed
40–49	2
50–59	15
60–69	32
70–79	41
80–89	11
90–99	3
100–109	1
Total observations	105
Ave. stability number	66.6

numbers have not been found to be common. The average stability number has been found to be about 60 to 70. Results have been consistent and

properly stored milk has maintained the same stability number for several days.

It was observed in some of the early work with this test that pasteurization seemed to increase the stability number of milk. Consequently, an experiment was run to determine more exactly what effect could be expected. The samples for this investigation were pasteurized in glass bottles in the laboratory. Preheating periods of 10, 20 and 30 minutes were used in conjunction with the usual holding process at 143–145° F. for 30 minutes. The data from this study are shown in table 2. Pasteurization increased the

TABLE 2

The effect of the preheating time upon the stability number of pasteurized milk

Raw milk	After pasteurization		
	Time of preheating		
	10 min.	20 min.	30 min.
50	60	55	60
70	80	80	85
65	75	75	75
70	85	85	90
60	70	70	70
60	75	80	80
50	60	60	55
70	80	80	80
65	75	75	75
65	75	75	75
Ave. 62.5	73.5	73.5	74.5

stability number of the milk slightly more than 10. In some cases the stability number increased as the length of the preheating period was increased. However, in the samples tested the average increase in the stability number as a result of longer preheating periods was negligible.

There was also an opportunity for a limited study of the effect of copper contamination upon protein stability. Known amounts of copper, in the form of copper sulfate, were added to the milk and the effect upon the stability number observed. As shown in table 3 copper contamination de-

TABLE 3

The effect of copper upon the stability number of milk

Copper added, in p.p.m.			
0.5	1.0	2.0	3.0
Decrease in stability number			
.....	10	15	20
.....	10	15	.....
5	10	.....	.....
Ave. 5	10	15	20

creased the stability of the milk, the loss of stability becoming greater as the amount of copper was increased.

In the application of this test to the enzymatic treatment of milk it is the general practice to control the amount of enzyme activity so that the stability of the milk is reduced to within the limits of from 20 to 40. This insures adequate treatment and at the same time provides a satisfactory margin against coagulation upon subsequent boiling.

With respect to simplicity the method is largely satisfactory. The apparatus and reagents are standard items which can be procured easily and inexpensively if not already on hand. A minimum of technical knowledge is necessary for proper performance of the test.

SUMMARY

A simple test for protein stability is described which includes mixing increasing amounts of N/10 HCl with portions of the milk, boiling the mixtures for a specified length of time and examining the samples for coagulation.

The stability number of untreated fresh milk has been found to average about 60 to 70 as indicated by the test.

Pasteurization tends to increase the stability of milk.

Copper contamination tends to lower the protein stability of milk.

ACKNOWLEDGMENT

The writer wishes to acknowledge the assistance and criticism of Professor E. O. Anderson of the Department of Dairy Industry, University of Connecticut, in the preparation of this manuscript.

REFERENCES

(1) SOMMER, H. H. Market Milk and Related Products. Published by the author. Madison, Wis. 1938.

(2) HUNZIKER, O. F. Condensed Milk and Milk Powder. Published by the author. La Grange, Ill. 1935.

(3) SOMMER, H. H., AND HART, E. B. Heat Coagulation of Milk. Jour. Biol. Chem., 40: 137. 1919.

(4) SOMMER, H. H., AND HART, E. B. Heat Coagulation of Milk. JOUR. DAIRY SCI., 5: 525. 1922.

(5) ROGERS, L. A., DEYSHER, E. F., AND EVANS, F. R. Relationship of Acidity to the Coagulating Temperature of Milk. JOUR. DAIRY SCI., 4: 294. 1921.

(6) HOLM, G. E., WEBB, B. H., AND DEYSHER, E. F. Heat Coagulation of Milk. I. JOUR. DAIRY SCI., 15: 331. 1932.

(7) WEBB, B. H., AND HOLM, G. E. Heat Coagulation of Milk. II. JOUR. DAIRY SCI., 15: 345. 1932.

(8) WEBB, B. H., AND BELL, R. W. Effect of Flash Forewarming upon the Heat Stability of Evaporated Milk. Paper presented at 36th Ann. Meeting Amer. Dairy Sci. Assoc. Abstract JOUR. DAIRY SCI., 24: 535. 1941.

(9) LABORATORY MANUAL. p. 236. Compiled and published by the International Association of Milk Dealers. Chicago, Ill. 1936.

(10) SOMMER, H. H., AND BINNEY, T. H. A Study of the Factors that Influence the Coagulation of Milk in the Alcohol Test. JOUR. DAIRY SCI., 6: 176. 1923.
(11) KEITH, J. I., AND FREEMAN, T. R. Ice Cream Rev., 17: 52. 1933.
(12) DAHLE, C. D., AND RIVERS, P. W. Protein Stability of Ice Cream Mixes and Its Effect on Certain Properties. Ice Cream Trade Jour. October, 1940.
(13) MATTICK, E. C. V., AND HALLETT, H. S. Jour. Agr. Sci., 19: 452. 1929.
(14) RAMSDELL, G. A., JOHNSON, WM. T., JR, AND EVANS, F. R. A Test for the Detection of Milk Unstable to Heat. JOUR. DAIRY SCI., 14: 93. 1931.

THE EFFECT OF NATURAL MILK ENZYMES, ACID, AND SALT UPON THE KEEPING QUALITY OF BUTTER STORED FOR SIX YEARS

B. J. SCHEIB, C. N. STARK, AND E. S. GUTHRIE
New York State Department of Agriculture and Markets, Albany, New York, and
New York State College of Agriculture, Cornell University, Ithaca, New York

In a previous publication, Guthrie, Scheib, and Stark (5) reported the results of their studies on the effect of certain factors upon the keeping quality of butter held for 36 days at 50° F. (10° C.). Other temperatures which would also permit the growth of microorganisms were tested. Quality was measured by scoring the butter on the basis of taste and odor. This measure of quality was used because it is recognized that the consumers' response to the flavor of butter largely determines its commercial value.

Pasteurization temperatures. It was found that pasteurization temperatures equivalent to 165° F. (73.9° C.) for 30 minutes were required to destroy the harmful natural enzymes of milk. It was suggested that a flash pasteurization of 200° F. (92.5° C.) or higher would probably be the equivalent of 165° F. for 30 minutes. Neither heating the cream to 145° F. (62.8° C.) nor 155° F. (68.3° C.) for 30 minutes was found to be sufficient to inactivate completely the harmful natural enzymes of milk. In our early preliminary studies on pasteurization temperatures, 180° F. (82° C.) for 30 minutes was tested. This temperature of pasteurization resulted in butter having noticeably cooked flavors with no improvement in keeping quality over butter made from cream pasteurized at 165° F. for 30 minutes. The reports by Kende (7) and Guthrie and Brueckner (4) of their studies on oxidized flavors in pasteurized milk, indicated the importance of these higher temperatures of pasteurization. In a personal communication, dated March 16, 1937, Mr. C. W. Fryhofer, Supervising Federal-State Grader of Minneapolis, Minnesota, mentions the results obtained by the Land O'Lakes Creameries as follows: "We found a decided improvement in the keeping quality of the butter when the pasteurization temperature was raised to 165 degrees and the cream held for thirty minutes at that temperature before cooling. When the cream was held for only fifteen minutes at a temperature of 165 the results were not satisfactory." Jensen (6), Wiley (11), Wilster (12), and Fabricius and Bird (2) have since reported that higher pasteurization temperatures than previously used definitely improved the keeping quality of butter.

Bacteria. It is not possible to evaluate correctly the part played by bacteria in the spoilage of butter made from raw cream since the action of

Received for publication August 18, 1941.

the harmful natural enzymes of milk partially or completely overshadow the effect of the bacteria present. It is possible to determine unmistakably the effects of certain harmful bacteria capable of growing in butter made from cream pasteurized at 165° F. for 30 minutes, since the natural enzymes of milk have been destroyed by this pasteurization process. Our preliminary studies, the object of which was to determine the part played by bacteria in the spoilage of butter, convinced us that a total count of the number of bacteria in butter meant little or nothing with regard to its keeping quality or the part played by bacteria in its deterioration. Stark and Scheib (10), in a study of bacteria growing in butter to numbers sufficiently large to be of significance in its keeping quality, if other important spoilage factors were absent, found that fat-splitting and casein-digesting, gram negative, non-spore-producing rods were the types usually predominant in butter of poor quality. Flake and Parfitt (3) have also reported that large numbers of rod-shaped organisms were generally associated with butter of poor keeping quality. Our tests showed that pasteurization of cream at 165° F. for 30 minutes destroyed these types of bacteria. However, in making experimental butter, difficulty was encountered in avoiding recontamination with these types of bacteria in cream pasteurized at such temperatures. It was suggested that the possible importance of the recontamination of commercial butter by these harmful types of bacteria should be recognized. Data presented on lots of sweet cream-unsalted butter made from cream pasteurized at 165° F. for 30 minutes and held for 36 days at 50° F. showed the uncontaminated butter to have a score of 92 at the end of this storage period, while lots of butter contaminated with these harmful types of bacteria had a score of 83.

Acid and salt. In a previous publication (Guthrie, Scheib and Stark, 5), it was stated: "In the presence of certain microorganisms and at a temperature at which they can grow, the *preserving* action of acid and salt is well known." . . . "The presence of either acid or salt in butter, not containing other spoilage factors considered in this study, resulted, after storage, in a *poorer quality* butter." "The combined *deteriorating* effect of both acid and salt was shown." Wiley (11) and Bendixen (1) have since confirmed the importance of salt and high acidity as deteriorating factors on the keeping quality of butter.

Rogers, *et al.* (9) made the following statement: "The most serious difficulty in experimental work on butter is in controlling the conditions under which butter is made. *So many apparently unimportant factors have an influence on the flavor that it is nearly impossible to make butter with a normal flavor and have only one varying factor.* The work is further complicated by the sequence of flavors that frequently occurs in butter held in storage. It is evident that the usual off flavors are in many cases a combination of flavors and that the flavors themselves are caused by a combina-

tion of circumstances and not by a single cause. It is probable also that identical flavors may be caused by different factors."

In all of our studies the aim has been to conduct experiments in such a manner that it would be possible to observe the quality of butter: first, in the absence of any of the deteriorating factors considered by us; second, in the presence of but one of each of these deteriorating factors; and third, in the presence of more than one of these deteriorating factors. Although commercial butter is not and cannot be made under such carefully controlled conditions, it is of extreme importance to know the combined significance of these factors and their relative importance as they affect butter quality.

All butters in each series of churnings were made from the same fresh cream of high quality. The extremely low bacterial content of the raw cream and the freshly made sweet cream-unsalted butter indicated the high quality of the cream used. A number of duplicate sets from five different series of butters were placed at different holding temperatures. This paper is a report on the influence of: pasteurization temperatures, milk enzymes, acid, and salt, in the absence of bacterial action, on the keeping quality of the duplicate sets of butter stored for 6 years at 0° F. (−17.8° C.) to −10° F. (−23.3° C.).

After 6 years of storage at 0° F. to −10° F., the duplicate samples of butter of each type for each series were scored for taste and odor, and total and differential bacterial counts were made. Other duplicate sets were held for one week at 50° F. for a further check on the action of enzymes, bacteria, salt, and acid, if these agents were present, on the keeping quality of such butter, when held at a temperature similar to that at which butter would normally be held under commercial conditions when removed from storage. The bacterial content of all the duplicate lots of butter, whether or not they were held for one week at 50° F., were all too low, from less than 1 to a few hundred per gram, to have been of any significance in the quality of the butter. These results are in accordance with what one would have anticipated, since it is well known that bacteria cannot grow in a frozen medium, and that the bacteria present in such an environment gradually die.

The results obtained from the storage of these lots of butter for six years at 0° F. to −10° F. are summarized in table 1. The judges in reporting their scores on the sweet cream-unsalted butters made from cream pasteurized at 165° F. for 30 minutes indicated these butters to have what is known as a slight storage flavor, indicating that these butters had lost only their original ideal freshness. This is the explanation offered for the decrease in score from 95 to 92.3 during this storage period. Using 92.3 as the score for butter which had present none of the deteriorating factors considered in this study, it is readily observed that the presence of salt in butter is harmful to its keeping quality but less harmful than is acid, or acid and

TABLE 1

The influence of pasteurization temperatures, milk enzymes, acid, and salt, in the absence of bacterial action, on the keeping quality of butter stored for six years at 0° F. to −10° F. (−17.8 to −23.3° C.)

No. of series of churnings	No. of samples	Description of butter	Average score of fresh butter	Average of score after storage for 6 years at 0 to −10° F. (−17.8 to −23.3° C.)	Average score on 6-year-storage butter after holding 1 week at 50° F. (10° C.)	Decrease in average score of 6-year-storage butter after holding 1 week at 50° F. (10° C.)
Butter made from cream pasteurized at 165° F. (73.9° C.) for 30 minutes						
5	10	Sweet—unsalted	95	92.3	92.3	0.0
5	10	Sweet—salted	95	90.8	90.8	0.0
5	10	Sour —unsalted	95	87.5	87.0	0.5
5	10	Sour —salted	95	85.4	84.6	0.8
Butter made from cream pasteurized at 145° F. (62.8° C.) for 30 minutes						
5	10	Sweet—unsalted	95	90.4	88.6	1.8
5	10	Sweet—salted	95	88.4	87.2	1.2
5	10	Sour —unsalted	95	86.4	85.8	0.6
5	10	Sour —salted	95	84.8	84.2	0.6
Butter made from raw cream						
2	4	Sweet—unsalted	93.5	83.0	83.0	0.0
2	4	Sweet—salted	93.5	83.0	83.0	0.0
2	4	Sour —unsalted	93.5	85.0	84.0	1.0
2	4	Sour —salted	93.5	83.0	83.0	0.0

The flavor of the pasteurized cream butters, with the exception of the 165° F. sweet cream-unsalted butters were all oily or tallowy. The raw sweet cream butters were all rancid-bitter; the raw sour cream butters were all oily or tallowy. The butters were scored by E. S. Guthrie, W. E. Ayres, and B. J. Scheib. Many samples, probably, should have been scored below 83. This figure, however, was as far from the thresholds of smell and taste as the judges dared to venture.

salt combined. The scores 90.8, 87.5, and 85.4 indicate the proportional deteriorating effect of salt, acid, and salt and acid on the keeping quality of butter stored at 0° F. to −10° F. for 6 years when examined immediately after removal from storage. The scores obtained on duplicate samples of these butters after an additional one week of holding at 50° F. further indicate hastened deterioration of these butters by acid. In the absence of harmful natural milk enzymes or spoilage bacteria, the lowered score of the sweet cream-salted butter, which is 1.5 points less than the sweet cream-unsalted butter, and the lowered score of the sour cream-unsalted butter which is 4.8 points less than the sweet cream-unsalted butter, and the decrease in score of the sour cream-salted butter which is 6.9 points less than the sweet cream-unsalted butter, indicate roughly the relative value of salt and acid as spoilage factors in storage butter. The spoilage relationships, according to these results, appear to be approximately—salt is to acid as one is to three (1 : 3), or that acid causes about three times as much decrease in the butter score as is caused by salt. As a possible indication that the relationships hold and are also cumulative, it may be observed that acid and

salt together showed approximately four times as much decrease in score as did salt alone. These findings seem to be significant and tend to confirm the statement made by Rogers, *et al.* (8): ". . . the change in the pasteurized ripened-cream butter stored at 0° F. was four times as great as that in the pasteurized sweet-cream butter at the same temperature, . . ."

The difference between the scores of sweet cream-unsalted butter, 92.3, for butter made from cream pasteurized at 165° F. for 30 minutes, and 83 for the sweet cream-unsalted butters made from raw cream shows a difference in score of 9.3 points which may be attributed to the action of harmful natural milk enzymes. To continue further the analogy previously suggested, this indicates that the ratio of salt is to acid, is to natural milk spoilage enzymes, as one is to three, is to six (1:3:6). The scores obtained on sweet cream-unsalted butters made from cream pasteurized at 145° F. for 30 minutes indicate that this degree of pasteurization is roughly only 80 per cent effective. The continued and more rapid deterioration of the sweet cream butters made from cream pasteurized at 145° F. for 30 minutes when the butters were held an additional week at 50° F. also indicates the failure of this degree of pasteurization to inactivate effectively the natural spoilage enzymes of milk.

SUMMARY

The effect of harmful natural milk enzymes, acid, salt, and acid and salt upon the keeping quality of butter held at 0° F. (−17.8° C.) to −10° F. (−23.3° C.) for 6 years, has been studied. Five series of butters consisting of 192 samples made and held under known and carefully controlled conditions have been examined.

Pasteurization of cream at 165° F. (73.9° C.) for 30 minutes inactivates the harmful natural milk enzymes; whereas pasteurization of cream at 145° F. (62.8° C.) for 30 minutes is apparently only about 80 per cent effective.

The presence of either acid or salt in butter, not containing other spoilage factors considered in this study, resulted, after storage, in a poorer quality butter. The relationship appears to be salt is to acid as one is to three (1:3).

The combined deteriorating effect of acid and salt was shown to be approximately four times as great as the harmful effect of salt alone.

It appears that the relationship between the spoilage factors of salt, acid, both acid and salt, and the natural milk-spoilage enzymes, is approximately one is to three, is to four, is to six (1:3:4:6).

REFERENCES

(1) BENDIXEN, H. A. Acid Values and Acid Ratios as Related to the Keeping Quality of Salted Butter. JOUR. DAIRY SCI., 23: 275. 1940.

(2) FABRICIUS, N. E., AND BIRD, E. W. The Quality of Butter Made from Vacuum-pasteurized and Vat-pasteurized Lots of the Same Cream. Iowa Sta. Res. Bul., 284: 149. 1940.

(3) FLAKE, J. C., AND PARFITT, E. H. Some Causes for the Deterioration in 10 Days at 15.5° C. of Salted Butter Made from Sour Cream. JOUR. DAIRY SCI., **21**: 545. 1938.

(4) GUTHRIE, E. S., AND BRUECKNER, H. J. The Cow as a Source of "Oxidized" Flavors of Milk. Cornell University Agr. Expt. Sta. Bull. 606. 1933.

(5) GUTHRIE, E. S., SCHEIB, B. J., AND STARK, C. N. The Effect of Certain Factors upon the Keeping Quality of Butter. JOUR. DAIRY SCI., **19**: 267. 1936.

(6) JENSEN, C. Pasteurizing Cream for Buttermaking. North Dakota Sta. Bimo. Bul. 1, No. 5: 25, 26. 1939.

(7) KENDE, S. Untersuchungen über "öligtalgige," "schmirgelige" Veränderungen der Milch. Milchw. Forsch., **131**. 1932.

(8) ROGERS, L. A., THOMPSON, S. C., AND KEITHLEY, J. R. The Manufacture of Butter for Storage. U.S.D.A. Bur. Anim. Indus. Bul. 148. 1912.

(9) ROGERS, L. A., BERG, W. N., POTTEIGER, C. R., AND DAVIS, B. J. Factors Influencing the Change in Flavor in Storage Butter. U.S.D.A. Bur. Anim. Indus. Bul. 162. 1913.

(10) STARK, C. N., AND SCHEIB, B. J. A Study of Fat Splitting and Casein Digesting Bacteria Isolated from Butter. JOUR. DAIRY SCI., **19**: 191. 1936.

(11) WILEY, W. J. Oxidation of the Fat of Butter During Cold Storage. Jour. Dairy Res., **10**: 300. 1939.

(12) WILSTER, G. H. Vacuum Pasteurization of Cream for Butter. Ore. Agr. Expt. Sta. Bul. 368. 1940.

IDENTIFICATION OF THE WHITE PARTICLES FOUND ON RIPENED CHEDDAR CHEESE*

F. L. DORN AND A. C. DAHLBERG

New York Agricultural Experiment Station, Geneva, N. Y.

When canned natural cheddar cheese had ripened for 5 to 7 months, white particles about the size of a small pin head began to appear on the surface and throughout the body of the cheese, especially along the cracks between the curd particles. They also appeared quite heavily on both sides of the parchment paper in which the cheese was wrapped. Similar granules have been observed by other authors from time to time on various types of natural cheese but reports as to their identity have been quite varied and conflicting. These particles are very common in well ripened cheddar cheese and have undoubtedly been submitted to many examinations not reported in the literature.

In 1909, Van Slyke and Publow (11) mentioned the formation of white particles in and on cheddar cheese ripened at low temperatures, and, after partial analysis, concluded that they were probably calcium soaps; calcium combined with some of the higher fatty acids.

Dox (2) described the occurrence of these white particles on the surface and especially in the crevices between the curd particles of Roquefort cheese. Chemical tests and microscopical examination of the recrystalized material indicated that it was composed largely of tyrosine.

More recent investigations have shown the particles to be calcium lactate. X-ray analysis of the white specks isolated from well ripened cheddar cheese, according to Tuckey, Ruehe, and Clark (10), showed them to be calcium lactate. Then McDowall and McDowell (7) isolated 0.9 gram of the particles and subjected it to chemical analysis. They found it to be calcium lactate with 11.3 per cent of protein and 17.2 per cent of fat present as cheese adhering to the specks.

EXPERIMENTAL RESULTS

The description of the physical appearance of the material found by different authors seemed the same, but three different chemical entities are reported. Of course it is conceivable that the chemical composition may vary somewhat under different conditions. It would be rather surprising,

Received for publication September 2, 1941.

* Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 465, August, 1941.

This investigation was supported by Bankhead-Jones fund.

This article is based upon a portion of the Doctor's Thesis presented to the Graduate School of Cornell University by F. L. Dorn, June, 1941.

however, if a compound as soluble as calcium lactate should accumulate in sufficient quantities to be precipitated in a normal cheese.

Ordinarily the material is difficult to collect in any quantity without contaminating it with particles of cheese. However, in canned cheese collection of the material was fairly easy for much of it would collect on the surface of the parchment paper. The particles could be obtained from the paper free from any insoluble cheese particles by allowing the wrapper to dry and scraping the material off with a spatula, or by washing off with cold water any cheese particles adhering to the surface of the wrapper and extracting the material with boiling water for a few minutes. The material was recovered by evaporating until the solution was highly concentrated and finally dried over concentrated sulphuric acid. In the course of several months a composite sample from several batches of canned cheddar cheese was collected on which chemical analyses were made in an attempt to identify the material.

The material was quite insoluble in cold water but was slightly more soluble in boiling water, a fact which indicated that it contained little or no calcium lactate. A qualitative test for lactic acid, as described by Troy and Sharp (9), was made using a 0.1 gm. sample. Another sample of the material to which some calcium lactate had been added served as the control and was treated in the same way. The test for lactic acid on the control was strongly positive while the test on the white material alone was almost completely negative. Against a white background there was a slight indication of a pink color which developed after 15 to 20 minutes, standing. According to Troy and Sharp, this test will detect 0.002 per cent lactic acid, beginning with 125 cc. of milk of which about 65 gm. were finally used as filtrate, or about 1.3 mg. of lactic acid. Assuming the same sensitivity, 1.3 mg. would be equal to 1.3 per cent lactic acid when 0.1 gm. sample was used. The weakness of the test, however, showed that if any lactic acid were present it was present only in minute traces as an incidental contaminant. The test for calcium with ammonium oxalate was positive though not very strong. These tests eliminated the possibility of calcium lactate as an important constituent.

Certain other qualitative tests were also made on the solution of the material, among them the Biuret test for proteins which was negative. The Xanthoproteic test was strongly positive as well as the reaction with Millon's reagent. These results pointed strongly in favor of tyrosine.

A sample of the material was found to contain 1.57 per cent ash which was far too low for calcium lactate. The ash was further analyzed for calcium and phosphorus. The calcium content amounted to 40.19 per cent, expressed as CaO while the phosphorus made up 38.94 per cent, expressed as P_2O_5. These results would indicate that calcium phosphate salts were present as an impurity or as a minor constituent of the compound.

A portion of the material was also analyzed for nitrogen by the Kjeldahl method and duplicate determinations showed that 7.63 and 7.57 per cent nitrogen was present. Correcting this value to allow for the ash the nitrogen content of the combustible material was 7.72 per cent. The theoretical value for tyrosine is 7.73 per cent nitrogen.

The next step was to purify some of the material and identify it in pure form. This was accomplished by dissolving the material in hot water and recrystallizing three times. Microscopic examination of the crystals showed them to be long white needle-like crystals in the characteristic sheave-like arrangement of tyrosine. The melting point of these crystals was 293° C. but after two more recrystallizations from water it was 310° C. No further purification seemed necessary although the melting point of tyrosine is around 315° C. as reported by Cole (3). Cole states that tyrosine is laevorotatory in aqueous solutions.

As a final identification of the material the dibenzoyl derivative was made by adding benzoyl chloride to an aqueous solution containing sodium bicarbonate and some of the purified material from the cheese wrapper. The purpose of the sodium bicarbonate was to keep the reaction mixture weakly alkaline during benzoylation.

The procedure was as follows: 0.1 gm. of l-tyrosine or purified cheese material was placed in 75 ml. of distilled water with 5 gm. of sodium bicarbonate and the mixture warmed until complete solution was obtained. It was then cooled to 20° C. or below and 2.5 ml. of benzoyl chloride was added slowly with shaking. The mixture was placed in the cold room (4–7° C.) for at least an hour after which it was acidified with hydrochloric acid, allowed to stand for half an hour and then filtered. The precipitate was washed with water, air dried, and the benzoic acid removed by extraction with warm petroleum ether.

Several solvents were tried on the precipitate remaining after the petroleum ether extraction. Dibenzoyl tyrosine did not crystallize well from glacial acetic acid. Chloroform dissolved the dibenzoyl tyrosine readily and it could be completely precipitated by adding about an equal volume of petroleum ether, but the material seemed to precipitate in an amorphous form rather than yielding good crystals. Absolute ethyl alcohol gave the best results. The dibenzoyl tyrosine was dissolved in hot absolute alcohol and when completely in solution, water was added dropwise until a slight opalescence developed. Needle-like crystals formed on cooling. After several recrystallizations from this solvent the dibenzoyl tyrosine was obtained pure. The melting point of dibenzoyl tyrosine according to Abderhalden and Brockman is 216–217° C. (1). Dibenzoyl tyrosine prepared from chemically pure tyrosine and benzoyl chloride by the above method had a melting point of 216.8° C. (corrected). The melting point of the product made with the white material from cheese and benzoyl chloride was

also 216.8° C. (corrected). The melting point of a mixture (50–50) of pure dibenzoyl tyrosine and the unknown was 217.0° C. (corrected). Since the mixed melting point remained unchanged, the unknown compound prepared from the purified white cheese particles must have been dibenzoyl tyrosine and the white particles obtained from the cheese wrapper must have been largely tyrosine.

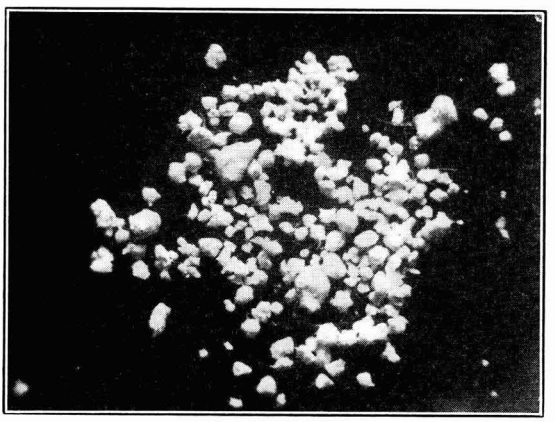


FIG. 1. The white particles as removed from the parchment wrapper on the canned cheddar cheese. ×30.

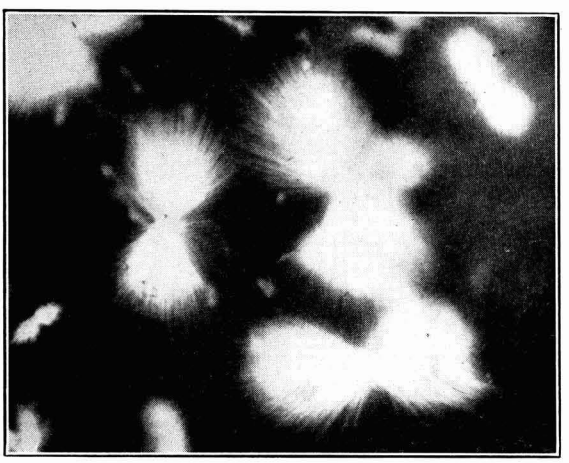


FIG. 2. The sheath-like crystals typical of tyrosine prepared by recrystallization of the white particles from aqueous solution. ×45.

Photomicrographs (figs. 1 and 2) were taken of some of the granules showing them as they appeared respectively after they were scraped from

the parchment wrapper with a spatula and after they had been purified by recrystallization from water.

DISCUSSION

The white particles so often observed in well ripened cheese have been reported as calcium soap, tyrosine, and calcium lactate. It seems doubtful that such different results could be obtained on the same material so there is the possibility of several types of white particles. However, a discussion of the various results may aid in interpreting previous findings.

Van Slyke and Publow (11) found calcium and no phosphorus. The particles were not especially gritty and smeared readily between the figures in a greasy manner. They then concluded the particles were calcium soap. Dox (2) found the particles burned on platinum wire without melting and with no ash. He secured a strong Millon reaction and Piria's test. Recrystallization gave typical tyrosine crystals so he concluded the particles were tyrosine. The observed facts in these two investigations are not contradictory so far as they duplicate each other. The observations in the present study agree with them except that small amounts of phosphorus were found and Piria's test was not made.

It is difficult, however, to reconcile these findings with those showing the particles to be calcium lactate. Since the particles are crystallized from a solution containing calcium lactate there must be traces of calcium lactate as impurities on the particles. This may explain the X-ray pattern of calcium lactate secured by Tuckey, Ruehe, and Clark (10). Such impurities cannot explain the data of McDowall and McDowell (7) who found 35.1 per cent lactic acid in the particles. Either two different types of particles are involved or gross contamination or errors occurred.

Certain facts tend to indicate against the particles being calcium lactate. According to Suzuki, Hastings, and Hart (8) the lactic acid content of cheese was high within 3 days and increased for 3 to 5 months. It was lowest in 10-month-old cheese. This means that calcium lactate particles should appear promptly and gradually disappear after 5 months. Actually they begin to appear in 5 to 7 months and increase with increased age of cheese.

Then there is the question of solubility of calcium lactate. The highest concentration of lactic acid reported by Suzuki, Hastings, and Hart (8) amounted to 3.1 per cent calcium lactate in the water present in cheese. The solubility of calcium lactate with 5 molecules of water of crystallization varies from 3.0 per cent at 0° C. to 7.3 per cent at 30° C., Hodgman (4). These solubilities must mean that particles picked from cheese at room temperature should be dissolving into the cheese as the cheese is not saturated with calcium lactate at room temperature. Actually this solution does not occur for the particles are insoluble even when rinsed with water.

Tyrosine is a normal constituent of well ripened cheese, and was first isolated and recognized by Liebig in 1846–47 (5, 6). Since it is practically

insoluble in cold water (1 part in 2500 of water at room temperature) its precipitation from cheese ought to be expected. In the present investigation all results indicated the particles were tyrosine.

CONCLUSIONS

Chemical analyses were made to determine the identity of the white particles appearing in ripened cheddar cheese. The insolubility of the material in water, its low ash content, and a negative test for the lactate radical eliminated the possibility that the material was calcium lactate. The large amount of calcium and phosphorus present in the ash suggested a calcium phosphate salt as an impurity or a minor constituent of the white material.

The chemical reactions of the material were all characteristic for tyrosine. It was very insoluble in cold water but fairly soluble in boiling water. Tests for the hydroxyphenyl group were positive and the nitrogen content corresponded with the calculated value for tyrosine. The crystal formation was characteristic for tyrosine and the melting point was only slightly below the value reported for this amino acid. Confirmatory proof was obtained by the melting point of the dibenzoyl derivative.

The results of these tests seemed sufficient proof that the white material was principally tyrosine.

REFERENCES

(1) ABDERHALDEN, E., AND BROCKMAN, H. Vergleichende Studien über die Abspaltbarkeit von Benzoyl-bzw. Halogenbenzoylgruppen, die in Verschiedener Bindung mit aromatischen und aliphatischen Verbindungen (Tyrosin, tyrosinhaltigen Polypeptiden, Colamin, Serin, Gylcinanhydrid) verknüpft sind. Biochem. Ztschr., 225: 426–440. 1930.

(2) DOX, A. W. The Occurrence of Tyrosine Crystals in Roquefort Cheese. Jour. Amer. Chem. Soc., 33: 423–425. 1911.

(3) COLE, S. W. Practical Physiological Chemistry. Williams and Wilkins Company, Baltimore, Md., 142. 1928.

(4) HODGMAN, CHARLES D. Handbook of Chemistry and Physics, 19th Edition, 1934. Chemical Rubber Publishing Co., Cleveland, Ohio.

(5) LIEBIG, JUSTUS. Baldriansäure und ein Neuer Körper Aus Kasestoff. Ann. der Chemie und Pharmacie, 57: 127–129. 1846.

(6) LIEBIG, JUSTUS. Über die Bestandtheile der Flussigkeiten des Fleisches. Ann. der Chemie und Pharmacie, 62: 257–369. 1847.

(7) MCDOWALL, F. H., AND MCDOWELL, A. K. R. The White Particles in Mature Cheddar Cheese. Jour. Dairy Res., 10: 1, 118–119. 1939.

(8) SUZUKI, S. K., HASTINGS, E. G., AND HART, E. B. The Production of Volatile Fatty Acids and Esters in Cheddar Cheese and Their Relation to the Development of Flavor. Wis. Agr. Expt. Sta. Res. Bul., 11, 1910.

(9) TROY, H. C., AND SHARP, P. F. Detection of Lactic Acid in Milk and Cream. JOUR. DAIRY SCI., 17: 12, 759–762. 1934.

(10) TUCKEY, S. L., RUEHE, H. A., AND CLARK, G. L. X-ray Diffraction Analysis of White Specks in Cheddar Cheese. JOUR. DAIRY SCI., 21: 161. 1938.

(11) VAN SLYKE, LUCIUS L., AND PUBLOW, CHARLES A. The Science and Practice of Cheese-Making. Orange Judd Pub. Co., Inc., New York, 332–333. 1909.

SUPERHEATED SOFT CURD MILK*

J. L. DIZIKES AND F. J. DOAN

The Pennsylvania Agricultural Experiment Station,
State College, Pa.

A careful review of the literature dealing with the use of modified cow's milk as a substitute for breast milk in the feeding of infants (particularly the new born) leads inescapably to the conclusion that the most satisfactory types of milk for the purpose are those which have undergone an evident degree of precoagulation in the modifying process. Examples of such milks are: acidified milk, evaporated milk, boiled milk, dried milk and some of the proprietary powdered preparations. The first two types mentioned have undergone perceptible coagulation and almost invariably exhibit curd tension values of zero. Boiled milk and dried milk while not perceptibly coagulated in most cases, have suffered some degree of protein denaturation and loss of "soluble nitrogen" which may be looked upon as the first stages of such a coagulation. Such milks are not always reduced to zero curd tension values but rarely give readings in excess of five or six grams.

The dairy industry has produced for years a product which undergoes a very evident protein coagulation, induced by heat, in the process of manufacture. This product is known in dairy circles as superheated plain condensed milk. On a reconstituted fluid basis, it should have a zero curd tension value; it should produce a very fine curded structure under peptic digestion conditions; and, judging from results secured with acidified milk and evaporated milk, it should prove equally as suitable for the feeding of infants.

In view of the fact that so many radically treated forms of milk have been advanced as more digestible soft curd types, presumably useful in infant feeding, it was felt that reconstituted superheated milk, a form which results from a process long established in the industry should be studied and described. The investigation presented here was accordingly undertaken.

METHODS

Fresh fluid whole milk was analyzed for solids, preheated at 145° F. for 30 minutes, drawn into a small vacuum pan and concentrated by removing water until a condensation ratio of approximately three to one was obtained as indicated by a Baume hydrometer. The product was drawn from the pan and analyzed for solids. Then, together with a portion of the original fluid milk, the concentrated milk was superheated in a hot water bath or by

Received for publication September 4, 1941.

* Authorized for publication on August 25, 1941, as paper No. 1046 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

means of a heating coil at temperatures varying from 194° F. to 205° F. (in different trials) for periods of time necessary to obtain the desired thickening or the desired degree of protein coagulation. The time required varied from about 10 minutes to about 30 minutes. The fluid and concentrated portions of the milk were then set in cold water and cooled to avoid further thickening or coagulation. Portions of the fluid preheated milk, the fluid milk heated to the superheating temperature, the unsuperheated concentrated milk, the concentrated superheated milk, the unsuperheated concentrated reconstituted milk and the superheated concentrated reconstituted milk were homogenized at a pressure of 3000 lbs. and cooled to 40° F. Samples of the same lots of milk were also taken and held for examination in the unhomogenized state. Reconstitution was accomplished by diluting the concentrated product with water to the original solids content.

Curd tension determinations were made using the American Curd-O-Meter and in a few cases the Submarine Signal Co. Curd Tension Meter (corrected) employing the procedure recommended by the American Dairy Science Association Committee on Methods of Determining the Curd Tension of Milk, in 1938.

In order to determine the degree of coagulation obtained in the superheating process more accurately than by visual means, reconstituted samples were centrifuged at 2500 r.p.m. for five minutes and the per cent of sedimentation measured. This made it possible to evaluate the effect of the degree of coagulation on reduction of curd tension and on digestibility.

Peptic digestibility was measured using the procedure employed by Doan and Flora[1] with some modifications, the results being based on the percentage of total nitrogen which passed a 10-mesh screen after three hours of digestion employing a progressive lowering of the pH to approximately pH 3.5 at two and one-half hours.

EXPERIMENTAL RESULTS

Reduction of Curd Tension

The average reduction in the curd tension value of superheated condensed, reconstituted and homogenized milk, based on four different trials, is shown in table 1, as are also similar data for samples of milk taken at different stages of the manufacturing process and used as controls for the sake of furnishing comparative evidence of the effects of the various treatments employed.

The results indicate that superheated condensed and reconstituted milk can be produced with a curd tension of zero (sample 8) without difficulty. However, homogenization of the superheated product either before or after reconstitution to the fluid state is a necessary practice since in most cases the unhomogenized product contained fine flakes of protein. After homogeniz-

[1] Doan, F. J., and Flora, C. C. Comparative Digestibility of Some Soft Curd Milks in Vitro. Bul. 380, The Pa. Agr. Expt. Sta. 1939.

ing the texture is absolutely smooth and indistinguishable from ordinary homogenized fluid milk. As indicated by samples 8, 9 and 10 in table 1,

TABLE 1

The effects of condensing, superheating, reconstituting and homogenizing on the curd tension value of fluid whole milk

(Average data obtained in four trials where the superheating temperature varied from 194° F. to 205° F. and the time varied from 10 to 30 minutes)

Treatment of milk	Curd tension value
	grams
1. Preheated at 145° F. for 30 minutes (fluid)	37.6
2. Preheated and homogenized (fluid)	14.2
3. Preheated and superheated (fluid)	2.8
4. Preheated, superheated and homogenized (fluid)	4.2
5. Preheated, condensed, and reconstituted	28.2
6. Preheated, condensed, reconstituted and homogenized	17.0
7. Preheated, condensed, homogenized and reconstituted	20.2
8. Preheated, condensed, superheated and reconstituted	0.0
9. Preheated, condensed, superheated, reconstituted and homogenized	0.25
10. Preheated, condensed, superheated, homogenized and reconstituted	0.25

homogenization has a tendency to increase the curd tension of the superheated product and the same effect is noticeable for the fluid milk treated at superheating temperatures (samples 3 and 4). In only one trial of the four, however, did samples 9 and 10 give other than zero curd tension readings. In this trial the superheating process was not carried to a point resulting in definite protein flakes. Subsequent studies indicated that definite flaking is required to obtain zero curd tension values of the finished homogenized milk.

Table 1 indicates quite conclusively that the superheating treatment alone, or in conjunction with homogenization, will not reduce the curd tension value of fluid milk to zero (samples 3 and 4). The condensing process alone is considerably less effective in producing a milk of extreme soft curd character (sample 5). Condensing coupled with homogenization results in considerable reduction in curd tension (samples 6 and 7) but not to the extent that superheating alone reduces the value in the case of fluid milk (sample 3). From these data it is apparent that a partial coagulation of the milk is required to lower the curd tension to a zero value. For fluid milk this would undoubtedly require heating under pressure or boiling for some period of time but for concentrated milk it can be had in the more convenient temperature range of 180° F. to 205° F.

Digestibility

Digestion analyses were made on all of the samples collected in the four trials mentioned previously. The results are presented in table 2, the values given being the per cent of total nitrogen passing through a 10-mesh screen after 3 hours of digestion. There are a number of interesting deductions

TABLE 2

The effects of condensing, superheating, reconstituting and homogenizing on the in-vitro digestibility of fluid whole milk

Treatment of milk	Digestibility—% total N passing 10 mesh screen after 3 hrs. of digestion			
	Trial 1 C.T.–34.2*	Trial 2 C.T.–30.2*	Trial 3 C.T.–37.0*	Trial 4 C.T.–48.8*
1. Preheated at 145° F. for 30 minutes (fluid)	59.8	65.8	59.9	47.34
2. Preheated and homogenized (fluid)	65.7	78.6	68.9	47.13
3. Preheated and superheated (fluid)	.....	79.0	83.4	62.39
4. Preheated, superheated and homogenized (fluid)	.....	87.3	86.7	82.53
5. Preheated, condensed and reconstituted	61.3	59.3	58.9	49.7
6. Preheated, condensed, reconstituted and homogenized	65.8	64.6	64.1	57.48
7. Preheated, condensed, homogenized and reconstituted	61.5	62.6	62.9	52.15
8. Preheated, condensed, superheated and reconstituted	68.7	97.3	93.6	96.3
9. Preheated, condensed, superheated, reconstituted and homogenized	69.4	94.1	92.2	90.0
10. Preheated, condensed, superheated, homogenized and reconstituted	75.2	94.6	87.0	90.9

* Curd tension value of the fluid milk after preheating:
Trial 1—Superheated at 194° F. to a heavy body but not protein flakes
Trial 2— " " 205° F. to a visible coagulation
Trial 3— " " 201° F. " " " "
Trial 4— " " 205° F. " " " "

which can be made from the data shown. Of prime importance in this study, however, are the high figures for digestibility of the reconstituted superheated condensed milk in trials 2, 3 and 4 (samples 8, 9 and 10). With one exception (sample 10, trial 3) these are all in excess of 90 per cent which indicates a high degree of digestibility comparable to that of reconstituted evaporated milk, eight samples of which had digestibility values ranging from 92.3 to 98.9 with an average of 95.3 per cent; and superior to ordinary boiled milk, eight samples of which had values ranging from 70.3 to 84.4 with an average of 76.8 per cent; but inferior to properly acidified milk and breast milk both of which exhibited values of practically 100 per cent.

It can therefore be assumed that properly made supreheated soft curd milk with a curd tension value of zero would be as suitable for infant feeding purposes as is evaporated milk.

The data in table 2 indicate that the superheated samples of trial 1 had inferior digestibility characteristics to those of trials 2, 3, and 4. The explanation is that the milk in trial 1 was not superheated far enough to produce a definite flaking of the protein and the degree of precoagulation, therefore,

was insufficient to prevent considerable pepsin coagulation in the digestion determination. A definite precoagulation in superheating is indicated as necessary by the digestion data just as in the case of the curd tension data of table 1.

If the results presented in table 2 are compared with those shown in table 1 certain anomalies are to be noted in the relationship between curd tension and digestibility. Homogenizing fluid milk that has been heated to superheating temperatures increases curd tension values to some extent but definitely improves digestibility. On the other hand, homogenizing reconstituted supreheated condensed milk has little or no effect on curd tension values but the digestibility, in these cases, is detrimentally affected. An exception to the latter statement is to be noted in the case of trial 1 which was not superheated enough to produce a product of high digestibility. Here homogenization, particularly when applied to the concentrated rather than the reconstituted milk, very appreciably improved the digestion characteristics but raised the curd tension from zero to 1 gram. This was the sample responsible for the average tension of 0.25 grams in samples 9 and 10 of table 1. The other three had zero values.

Sedimentation

It was noted that superheated condensed milk, after reconstitution, tended to separate, depending on the degree of superheating. In some cases a very noticeable sediment collected in the bottom of the container. Since a definite degree of flaking was found necessary to produce a highly digestible type of milk with a curd tension value of zero, it was believed that the sedimentation tendency might be made use of in controlling proper superheating. By centrifuging reconstituted samples various degrees of coagulation, produced by the superheating process, could be easily noted. Table 3 shows some preliminary data obtained in a study to determine the degree of coagulation required to insure a zero curd tension value after the homogenization process was applied.

It will be noted that, while all of the condensed, superheated and reconstituted samples exhibited zero curd tension values, only those coagulated to a point where 30 or 32 per cent sediment appeared on centrifuging showed zero values after homogenizing. It is believed that a superheating treatment sufficient to produce a 32 per cent sedimentation is the minimum heat treatment necessary to produce highly digestible milk. The percentages of sedimentation for sample 8 of the four trials included in table 2 were 20.0, 37.0, 32.0 and 35.0 respectively. Trial 1 showed poorest digestibility being no better than average boiled milk, even after homogenization which in the case of this trial improved the digestibility.

Notwithstanding the fact that homogenization somewhat lowers the digestibility of superheated milk it is a process necessary to smooth texture

TABLE 3

The effects of superheating to various degrees on the curd tension values and on the sedimentation of reconstituted, superheated milk before and after homogenization

Treatment of milk	Curd tension (grams)						Sedimentation on centrifuging (%)					
	Trial						Trial					
	1	2	3	4	5	6	1	2	3	4	5	6
Condensed, superheated, and reconstituted	0.0	0.0	0.0	0.0	0.0	0.0	17.0	18.0	24.0	28.0	30.0	32.0
Condensed, superheated, reconstituted and homogenized	2.0	1.0	2.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
Condensed, superheated, homogenized and reconstituted	2.0	1.5	2.2	1.0	1.0	0.0	0.0	0.5	0.5	0.5	1.0	1.0

and must be applied. There seems to be little choice in the case of properly coagulated milk whether this is accomplished while the milk is in concentrated form or after reconstitution as far as the digestibility results are concerned but, as can be noted in table 3, the sedimentation tendency is not overcome to quite as great a degree when the homogenization is applied to the concentrated milk as when it is accomplished after reconstitution. It seems, therefore, to be better practice to reconstitute the product first and then follow with homogenization.

Process

In the preparation of a highly digestible superheated soft curd milk, the following process would appear to give consistently satisfactory results, from evidence collected in this study:

Select a high grade of milk of average composition. Preheat or forewarm this to such a temperature and hold for such a period of time as will result in satisfactory superheating conditions; or if pasteurization is required, satisfy the legal requirement for temperature and time. Condense the milk in a vacuum pan to a concentration of three to one. Superheat the product in the pan, or after withdrawing, at a temperature between 180° F. and 205° F. and hold until a definite flaking is evident. A sedimentation test of the reconstituted milk, such as is described under methods should give a reading of 32 to 35 per cent at this point. Immediately after the proper degree of coagulation is obtained the milk should be cooled under the coagulation point, to about 160° F. Water previously heated to 160° F. is then added in exact amount, on a weight basis, to bring the composition to the same level as in the original fluid milk. The product is then homogenized at 3000 pounds pressure followed by filling into the final container. A sedimentation test of the finished product should not give a reading of more than one per cent and the product should be absolutely smooth in texture with a noticeable cooked but not caramelized, flavor.

SUMMARY AND CONCLUSIONS

A method of producing soft curd milk by heat treatment similar to that used in manufacturing superheated plain condensed milk is described. This type of soft curd milk has been designated as superheated soft curd milk.

Superheated soft curd milk properly processed, has a curd tension value of zero, is superior in digestion characteristics to boiled milk and comparable to evaporated milk, both of which have been long and successfully used as substitutes for breast milk. It is not, however, as digestible as acidified milk properly acidified to the isoelectric point.

It is believed that superheated soft curd milk has an advantage over acidified milk in that the flavor is not sour. Therefore, less difficulty would be experienced in substituting it in the case of infants started on breast milk.

The product, being equally as digestible as evaporated milk, could be used in place of the canned milk by those preferring a fresh milk product. Furthermore, superheated soft curd milk would provide the fluid milk dealer with a highly satisfactory type of soft curd milk (requiring no boiling in the home if carefully prepared) capable of competing with evaporated milk.

Since superheated soft curd milk contains no added foreign material, has had nothing removed and has not been treated in any way unusual to long established dairy plant practice, there should be less objection among health officials and pediatricians to this method of preparing a soft curd milk suitable for infant use than to some of the modifications previously advocated.

REPEATABILITY OF TYPE RATINGS IN DAIRY CATTLE*

LESLIE E. JOHNSON AND JAY L. LUSH
Iowa State College, Ames, Iowa

INTRODUCTION

The usefulness of type for predicting production in dairy cattle has been investigated often. Although the correlations found between type and production have usually been small, type has persisted as a criterion in the selection of breeding animals. It appears that differences in type will continue to affect the price which buyers will pay for animals, or at least the ease with which different animals can be sold. Hence it is desirable to know something about the permanence, accuracy and other characteristics of estimates or ratings of type. The present study was undertaken to ascertain the amount and kind of variation occurring in type ratings when such ratings were made by different judges at intervals throughout an animal's life. The specific questions investigated were (1) the comparative accuracy of ratings made at different ages, (2) the repeatability of ratings separated by varying intervals of time, (3) the degree of agreement between judges in ratings given the same cow, (4) the specific causes of large changes in ratings, and (5) the extent to which future ratings can be predicted from one or more past ratings.

SOURCE AND NATURE OF DATA

The data for this investigation were taken from the Holstein-Friesian herd at the Iowa State College during the period 1930 to 1940. Only the females were used in the present analyses, since few of the males were classified more than once. In all, 229 females were included, most of which were born in the college herd. The herd was fed and managed at a level sufficient to maintain good production. None of the animals was shown at fairs or expositions.

Two sets of independent ratings were made. One set was made primarily by the second author. A graduate assistant and the herdsman were usually present when these ratings were made and each of the three reached his own opinion before any of the others announced theirs. Then the junior author decided what the rating would be, sometimes shifting his original opinion as much as two points (two-thirds of an official grade) if the ratings of both of the other men were distinctly above or below his own. Thus, these ratings have something of the nature of an average. Scrupulous care was taken never to look at the record of any previous rating until the current one had

Received for publication September 6, 1941.

* Journal paper No. J-929 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 31.

been recorded. Each animal was rated during the weeks when it reached the ages of six months, 1, 2, 3, 4, 5, and 7 years. The terms and rating standards of the Holstein-Friesian Official Herd Classification were used as the standard except that the grades (excellent, very good, good plus, good, fair, and poor) were each subdivided into a high, medium and low. In this article this set of ratings is called "station ratings."

The other set of ratings, called "judges ratings" here, was made by nationally known dairy judges all but three of whom, at the time they made the ratings, were on the Official Herd Classification Committee of the Holstein-Friesian Association of America. The entire herd was classified at one time each year, usually during October or November. The 1930, 1932 and 1936 ratings were made by the same judge. A different man was used for each of the other years. The judges were asked to follow the principles and standards of Official Herd Classification except that they were to extend the classification to heifers as young as any on which they would venture an opinion. This usually included animals down to the ages of five or six months.

Considering only the cows that had freshened at least once, the official judges classified 2.2 per cent very good, 9.2 per cent good plus, 54.6 per cent good, 25.7 per cent fair, and 8.3 per cent poor. By contrast, the cows officially classified in the Holstein-Friesian voluntary plan up to Dec. 31, 1939, were distributed[1] as follows: 4.9 per cent excellent, 22.7 per cent very good, 24.0 per cent good plus, 41.2 per cent good, 6.6 per cent fair, and .6 per cent poor. These figures indicate that this herd was poorer in type than most of those classified voluntarily, but the real difference is doubtless less than the figures imply. In the voluntary system it seems inevitable that many of the owners would select a time at which their herds would show to best advantage, would give some special care and preparation to the animals prior to classification, and would eliminate some of the poorer individuals in type if such animals were not greatly needed in the breeding herd. In the herd which was the subject of this investigation there was little opportunity to cull for type during the period studied. The recorded causes and numbers of cows and heifers leaving the herd after the age of six months were: Bang reactors, 31; other breeding troubles, 24; tuberculosis reactors, 18; death, 13; mastitis, 12; low production, 10; dairy purposes, 3; poor type, 1; and miscellaneous, 13. However, the decision to remove cows for mastitis, low production, and some miscellaneous causes may have been influenced in part by their type.

For computation, the six grades of the judges' ratings were coded: poor, 1; fair, 4; good, 7; good plus, 10; very good, 13; and excellent, 16. The same code was used for the station ratings with the intervening numbers being used for the subdivisions of the classes.

[1] Norton, H. W. Extracts from A. R. Reports. Holstein-Friesian World 37: 618. 1940.

RESULTS

Variation in the Rating Levels of the Different Judges

Table 1 shows the annual averages of the station and judges' ratings. The station ratings tended to increase over the period studied. There ap-

TABLE 1

Annual averages and standard deviations of the ratings

Year	Station ratings		Judges' ratings	
	Mean	Standard deviation	Mean	Standard deviation
1930	.....	.....	5.9*	2.94
1931	6.8	1.82	.....	.....
1932	6.9	2.56	6.0*	2.41
1933	7.2	2.49	7.6*	2.00
1934	7.5	2.81	.....	.....
1935	8.3	3.26	7.8	3.84
1936	8.9	2.70	6.8*	3.21
1937	8.8	3.00	5.7*	2.58
1938	8.6	3.16	9.5	3.40
1939	9.0	2.62	6.8*	1.87
1940	9.0	2.46	6.7	2.91

* These ratings were made by judges on the official herd classification committee.

peared, however, to be no objective method of determining whether this increase was due to improvement in the herd, a change in the ideals of those classifying the cows, or both. In the judges' ratings no regular trend existed. There was, however, considerable variation between the annual averages, there being approximately 4 points or $1\frac{1}{3}$ official grades between the high and low averages. If only those who were on the official classification committee are included, the range between their averages was 1.9 points or about two-thirds of an official grade. Again there was no objective method for analyzing the causes of this variation. The lack of any consistent trend here, however, would seem to indicate that much of the variation was due to differences in the rating levels which the various judges used. That those who were on the official committee differed less in their averages than the others seems to indicate, as might be expected, that the meetings held to unify and standardize the official terms had some influence in the desired direction. However, the difference between consecutive judges' ratings, when omitting men not on the official classification committee, was statistically significant.[2] This, of course, could mean either that the judges levels genuinely differed, or that the average type of the herd had really changed, or both. Naturally about a fifth (or a little more) of the animals

[2] In this article the word "significant" has been used to indicate that such a value would occur by chance in not more than five per cent or less than one per cent of the trials when no such difference existed in the population from which these data are considered a random sample. "Highly significant" has been used to indicate that a value that large or larger would occur by chance in less than one per cent of the trials.

classified one year would be gone from the herd before the next classification and would be replaced by nearly an equal number of heifers.

To eliminate the variation in the rating levels of different judges, all ratings were adjusted to a common level in those analyses subsequent to table 1 wherever judge-to-judge differences in general rating levels could have affected the findings.

Comparative Accuracy of Ratings at Different Ages

Table 2 shows for both sets of ratings the correlation coefficients between ratings at each pair of ages. The coefficients varied considerably

TABLE 2

Correlations of all age ratings

Station ratings			Judges' ratings		
Ages correlated	D/f	r	Ages correlated	D/f	r
½ yr. with 1 yr.	133	.43++	1st with 2nd	58	.21
1 " " 2 "	111	.49++	2nd " 3rd	76	.27+
2 " " 3 "	93	.49++	3rd " 4th	56	.30+
3 " " 4 "	64	.55++	4th " 5th	56	.48++
4 " " 5 "	42	.83++	5th " 6th	27	.40+
5 " " 7 "	13	.61+	6th " 7th	10	.37
			7th " 8th	3	−.28
All consecutive ratings		.52	All consecutive ratings		.32
½ yr. with 2 yrs.	103	.16	1st with 3rd	40	−.03
1 " " 3 "	79	.44++	2nd " 4th	67	.22
2 " " 4 "	61	.48++	3rd " 5th	21	.16
3 " " 5 "	37	.66++	4th " 6th	27	.49++
4 " " 7 "	10	.36	5th " 7th	10	.43
			6th " 8th	3	−.23
All with two-rating interval		.37	All with two-year interval		.20
½ yr. with 3 yrs.	71	.34++	1st with 4th	16	.00
1 " " 4 "	51	.33+	2nd " 5th	31	.34+
2 " " 5 "	32	.56++	3rd " 6th	5	.56
3 " " 7 "	8	.22	4th " 7th	10	.36
			5th " 8th	3	−.22
All with three-rating interval		.37	All with three-year interval		.26
½ yr. with 4 yrs.	45	.21	1st with 5th	4	.59
1 " " 5 "	25	.44+	2nd " 6th	11	.65+
2 " " 7 "	4	−.45	4th " 8th	3	−.46
			2nd " 7th	6	.62
			2nd " 8th	2	−.88
All with four-rating interval		.24	All with four-year interval		.46
½ yr. with 5 yrs.	21	.01			
1 " " 7 "	2	.30			
All with five-rating interval		.01			

+ indicates a significant correlation.
++ indicates a highly significant correlation.

but much of this is presumably due to the small number of animals in several of these comparisons.

The coefficients between consecutive age ratings increased slightly and rather steadily with age up to four or five years and then decreased. Although none of the consecutive differences were large or statistically significant in these data, they suggest that type ratings are slightly more repeatable near maturity than at very young or very old ages. Contrary to our expectation there was no noticeable increase in repeatability following a heifer's first freshening.

In table 3 the correlation coefficients have been averaged to show the relation of each age rating with all others. The first ratings were distinctly the

TABLE 3

Correlations of each age rating with all other ratings

Station ratings			Judges' ratings		
Ages correlated	Pairs compared*	r	Ages correlated	Pairs compared	r
½ yr. with all others	385	.28	1st with all others	126	.11
1 " " " "	413	.44	2nd " " "	265	.26
2 " " " "	416	.40	3rd " " "	208	.27
3 " " " "	364	.47	4th " " "	249	.31
4 " " " "	285	.48	5th " " "	166	.38
5 " " " "	182	.57	6th " " "	95	.42
7 " " " "	49	.33	7th " " "	49	.36

* Each age rating was used several times in comparing it with all others, thus the number of degrees of freedom is somewhat less than the pairs compared. Since it is not readily apparent how many degrees of freedom should be deducted because of this repetition, the number of pairs of ratings compared has been given in place of degrees of freedom.

least like the other ratings. For all ratings after the first the coefficients were of approximately the same magnitude but there was some slight (statistically non-significant) increase up to four and five years of age. Apparently type can be judged on heifers as young as one year almost as accurately as on mature cows. Both the station and judges' ratings showed this trend.

TABLE 4

Correlation of each age rating with all others when omitting six-month station ratings and judges' first ratings

Station ratings			Judges' ratings		
Ages correlated	Pairs compared	r	Ages correlated	Pairs compared	r
1 yr. with all others	278	.44	2nd with all others	205	.28
2 " " " "	311	.48	3rd " " "	166	.29
3 " " " "	291	.50	4th " " "	231	.33
4 " " " "	238	.53	5th " " "	160	.37
5 " " " "	159	.65	6th " " "	95	.42
7 " " " "	47	.35	7th " " "	49	.36

If the lower correlation coefficients involving the oldest ages need an explanation, perhaps it can be found in the fact that some cows are then beginning to show broken udders and other age effects that affect type ratings seriously.

Table 4 shows the correlation of each age rating with all others when the first ratings were omitted. This increased the size of the coefficients but in no way changed the relationships just discussed.

Station Ratings Compared with Judges' Ratings

It is noticeable in this study that the station ratings were more repeatable than the judges' ratings. Although the differences between the two classifications did not reach the level of statistical significance at most ages, they were consistently in favor of the station ratings. The reasons for this are not certain. Unquestionably the judges had far more experience in judging and had studied type more intensively than the station men. Factors that might have operated to make the station ratings more repeatable than the judges ratings are: (1) the station ratings were somewhat of an average of three independent opinions, (2) the judges were different men most years and may thus have differed more in their ideals than the station men, some of whom were the same throughout the study, (3) the station men may have been influenced by past knowledge of the animals although every precaution was taken to avoid looking at or remembering previous ratings, and (4) the classes in the station ratings were subdivided into high, medium, and low which would permit greater accuracy of classification. The fourth explanation seems not to have been important when tested by Sheppherd's correction. Of the remaining three we incline to think that the first cause is the most important with the second coming next and the third of practically no consequence, but we can find no objective way of verifying this belief.

Effect of Length of Interval

Table 2 includes separately the averages of the correlations between ratings separated by one, two, three, four and five rating intervals. In the station ratings the size of the correlations decreased with an increase in the time interval. In the judges' ratings no such general trend was observed. Since the ratings made at the youngest age were somewhat less repeatable than the others the averages of the correlations were computed after omittting all of the first ratings. The correlation coefficients for the station ratings under these conditions were .55++, .51++, .40++, and .38++ when separated by intervals of one, two, three, and four years, respectively. The corresponding averages for the judges' ratings were .34++, .26++, .34++ and .45, respectively. Thus, consecutive ratings when made at or above one year of age, were little if any more repeatable than non-consecutive ones. Apparently most of the things which cause changes in the type ratings of an animal operate over a relatively short period, so that their incidence from one year to the next is

almost random. Ratings of the same animal made only one year apart differ almost as much as ratings separated by two or more years.

Agreement between Different Judges

Correlation coefficients were calculated for each pair of judges between the ratings they made on the same cows in different years. This was done to test whether certain judges agreed with each other more than others or whether the variation was no more than might reasonably occur when sampling from one population. The first age ratings were included in these comparisons as otherwise the numbers of animals involved would have been very small. Table 5 is a nearly typical one of these correlation tables. The coefficients for all of them are shown in table 6. The herd was not classified by judges in 1931 or in 1934. The same judge made the 1930, 1932, and 1936 ratings.

TABLE 5

Sample correlation table between ratings which two official judges made on the same cows

1936 judge	1937 judge							
	Poor		Fair		Good		Good plus	
Very good				(1)		(3)		
Good plus		(1)	2	(2)	1	(3)		(1)
Good	1			(2)	4	(5)	1	(1)
Fair	4	(2)	2	(3)	1	(1)		
Poor	1	(1)		(2)		(2)		

The numbers in parentheses were individuals that had not yet freshened when the 1936 rating was made. A few of these had still not freshened in 1937. Numbers not in parentheses were cows which had already freshened in 1936 and for which both judges could therefore see how the udder looked after freshening. $r = +.37$ for the whole group, which is a shade higher than the average of the judges' ratings.

The averages involving different judges (correlations weighted and averaged by the z-method of Fisher) were:

1930, '32, and '36 judge with himself	.56
1930, '32, and '36 judge with others	.31
1933 judge with others	.27
1935 judge with others	.30
1937 judge with others	.26
1938 judge with others	.25
1939 judge with others	.28
1940 judge with others	.21

With the exception of the one judge with himself, the correlation coefficients varied no more than if all of them had been random samples from one population. Attempts to pick groups of two or more judges who agreed among themselves but not with the others failed or were inconclusive. Thus, the 1935 and 1936 judges agreed more than average with each other and with the 1932 judge but the three of them were not consistent in disagreeing with

TABLE 6

Correlations between ratings by different judges*

Ratings correlated	D/f	r
1932–1933	19	.38
1935–1936	46	.46++
1936–1937	45	.37++
1937–1938	74	.24++
1938–1939	78	.39++
1939–1940	88	.24++
All consecutive ratings		.32
1930–1932	18	.57++
1933–1935	42	.16
1935–1937	22	.14
1936–1938	35	.12
1937–1939	58	.21
1938–1940	63	.18
All with 2 year intervals		.18
1930–1933	15	.36
1932–1935	10	.61+
1933–1936	19	.23
1935–1938	15	.13
1936–1939	25	.23
1937–1940	45	.28
All with 3 year intervals		.26
1932–1936	2	.46
1933–1937	4	.63
1935–1939	12	.27
1936–1940	19	−.04
All with 4 year intervals		.12

* The 1935, 1938, and 1940 judges were not on the Holstein-Friesian Official Classification Committee.

the others. The 1933 judge agreed well with both the 1930 and the 1932 ratings but less than the average amount with the 1936 ratings although all three of these were by the same man. These comparisons seem to indicate that the judges could not be separated into groups within each of which the ideals were similar but distinctly different from those held in other groups. If the judges did differ genuinely in ideals, each apparently had his own peculiarities. The judges not on the official classification committee agreed with each other and with those on the official committee about as closely as the latter agreed with each other. As previously noted in table 1, however, these judges varied more in rating levels than did the members of the committee. This suggests that conferences and practice, such as are held in official classification work, help to unify judges in the general level of their rating standards. These conferences probably do little to promote a higher degree of agreement about the relative merits of different individual cows. At least this was true in these data where all of the judges had considerable judging experience.

It would be interesting to know how far the imperfectness of agreement concerning individual animals was due; (1) to genuine and permanent differences in ideals from judge to judge, (2) to the animal really changing in

appearance from one year to another, (3) to temporary fluctuation in the judge's ideal, and (4) to the unsuitability or clumsiness of this classification system as a means of recording exactly what the judge thought of the animal. The experimental design did not permit separating these different causes of disagreement. We believe that (2) was the most important and (1) the least important of these causes but this opinion is based on considerations not absolutely conclusive in themselves. These include such things as experience in estimating rate of gain and final values in beef steers,[3] some unpublished analyses of scoring technique as applied to swine, and some general considerations such as personal knowledge of individual cases where cows changed widely in general appearance within the space of one or two years.

Causes of Major Shifts in Type Ratings

In the station classification brief descriptions of the animals were recorded each time a rating was made. In making these descriptions care was taken not to look at the previous records until after the current description had been written. A summary of the 132 animals that had three or more ratings showed that 42 changed one official grade or less, 64 changed two or less official grades but more than one, and 26 changed more than two grades. The causes of the larger changes were sought by examining the verbal descriptions. Among the cows that changed more than two official grades during the test period, it appeared that 50 per cent of these large shifts were caused mainly by udder changes, 26 per cent by obvious changes in general health, 12 per cent by inferior body conformation which later improved, and 12 per cent by miscellaneous causes. The larger shifts occurred frequently between consecutive ratings and apparently as often in the older animals as in the younger ones.

Value of Knowing More Than One Rating

The correlation coefficient for consecutive ratings was compared with multiple correlation coefficients in which the third rating was dependent on the first and second ratings, and the fourth rating was dependent on the first, second, and third ratings. The first age ratings in both classifications (those made at less than ten months of age) were omitted in these correlations. In the station classification 61 animals had four or more ratings. The correlation coefficient of the third and fourth ratings was .62; of the fourth as dependent on the second and third ratings, .65 −; and of the fourth as dependent on the first, second, and third ratings, .65 +. In the judges' classification 33 cows had four or more ratings. The correlation coefficient of the third and fourth ratings was .10; of the fourth as dependent on the second

[3] Partly published in 1931 in Jour. Agr. Res. 42: 853–881, and partly unpublished studies at the Iowa Station.

and third ratings, .50; and of the fourth as dependent on the first, second, and third ratings, .57. Thus, little was gained in the station ratings by using the second preceding rating while much was gained in the judges' ratings. That the advantage was small in the former classification was probably due to the fact that the correlation coefficient between the third and fourth ratings was unusually high. When the first age ratings were omitted the correlation coefficients for all consecutive ratings was .55 in the station classification and .34 in the judges' classification. In data with such correlations one would generally expect to get larger gains by the use of two or more ratings than was found in the station classification and smaller gains than was found in the judges' classification. If the correlations between the three ratings all equalled .55, one would expect to make about 1.14[4] times as much improvement in the third rating by selecting on the average of the first two ratings as by selecting on either one alone. If all the correlations equalled .34, the corresponding figure would be 1.22. Although there were not enough animals in either comparison to determine accurately the value of using more than one rating, the use of two ratings appears desirable whenever possible. The use of three or more ratings, however, would further increase the gains only a little even if all the correlations were equal, and perhaps not enough to be worth attention if correlations between consecutive ratings really are a little higher than those between ratings separated by longer intervals of time.

DISCUSSION

The small correlation of the first ratings (those made at 10 months of age or less) with all future ratings shows that little confidence can be placed in ratings made under one year of age. At or above one year of age the repeatability of the ratings was somewhat higher and of approximately the same magnitude at all ages. Thus, estimates of type at any age above one year are about equally valid for predicting a cow's future type.

The repeatability of type, .55 in the station ratings and .34 in the judges' ratings, is of the same order as the repeatability of intra-herd production records. Intra-herd correlation coefficients between yearly or lactation fat records made by the same cow have been in most studies about .4, rarely being below .3 or above .5. The repeatability of milk records appears to be about as high, although milk records have not been studied for this as much as fat records. Thus, one type rating made at or above one year of age is about as indicative of a cow's future type as one production record is of her future production records. In view of this it appears that breeders should be conservative in culling or selecting for type on the basis of a single inspection. The animal may appear much better or worse next year.

[4] This equals $\sqrt{\frac{2}{1+r}}$.

If animals do genuinely change in type from time to time as much as we think, a person wishing to measure type or select for it will gain considerably by having each animal rated more than once during its life. More is to be gained by that than by striving to get some one particular judge to do the rating. If, as these data indicate, about 30 per cent of the variance in single type ratings made by one judge is due to things which are permanent for those individual cows and will be agreed to by subsequent judges, then in comparable populations, in which each cow had been rated in two different years, about 46 per cent $(=\frac{2r}{1+r})$ of the variance in the averages for individual cows would be due to genuine and permanent differences between the cows. If each cow had three ratings, the corresponding figure would be 56 per cent. If the average repeatability is higher than 30 per cent, the figures corresponding to these just given would be higher but would not increase so rapidly with additional ratings. One desiring seriously to breed for type and to use the services of judges not biased by personal knowledge of the production or preceding histories of the animals might well make it a regular practice to have his herd classified once every two years. If in addition the classification were extended to heifers as young as one year, and the present data indicate that the accuracy would not be lowered much thereby, then nearly all cows which freshen at all would be classified at least once, many of them twice, and some three or more times. The expense, the owner's opinion of how much he needs such help, and the advertising value of such a classification would need to be considered before adopting such a plan.

Proponents of type may wish to reinvestigate the old type-production problems to determine what changes in production might be accomplished by selecting for type when several type ratings and several production records are considered. If the lack of repeatability in type ratings is in large part due to random errors of classification, two or more ratings should show more relation to average future production than one.[5]

If type classification becomes more general, many more judges than are now being used to classify herds will be needed. This raises the questions: (1) Who is qualified to classify herds? (2) Are ratings made by different

[5] This was sketchily investigated in this herd with the following results:

	D/f	*r*
Station ratings with six-month ratings omitted:		
1 type rating with 1 production record	129	.25
2 type ratings with 2 production records	83	.45
3 type ratings with 3 production records	47	.26
Judges' ratings with 1st ratings omitted:		
1 type rating with 1 production record	143	.19
2 type ratings with 2 production records	89	.38
3 type ratings with 3 production records	49	.18

judges comparable? Although this experiment was not designed to answer these questions specifically it has thrown some light upon them. The agreement between judges in ratings given the same cows was of practically the same magnitude for both official and non-official judges, indicating little if any difference in the ideals of those rating the herd. (However, the non-official judges were men of wide experience in judging major shows.) It seems likely that special conferences can do little to unify experienced men in their relative rankings of the individual animals. In the average rating levels or standards there was considerable variation between judges. The conferences of the official judges may well have helped some in this respect. Within the official group, however, there was evidence of genuine differences in levels. How much of this could have been eliminated by more conferences among the judges is uncertain. Certainly if it becomes necessary for many men to classify herds, and if customers come to attach much importance to such ratings the judges should participate in such conferences and be given special schooling in rating standards, so far as that contributes much to making their rating levels alike. But it does not appear that any amount of such training will lead to near-unanimity of their ratings of individual animals, at least if those ratings are separated by a year or more in time.

SUMMARY

Type as shown by consecutive ratings was only a moderately permanent attribute of a dairy cow, being roughly similar in repeatability to her intra-herd production records. The repeatability of type when omitting ratings made at 10 months or less of age was .34 in the judges' ratings and .55 in the station ratings.

Ratings made under one year of age were somewhat less repeatable than those made at older ages. The increase in permanency of ratings after one year of age was small.

Consecutive ratings were only slightly if any more alike than ratings separated by two, three, and four years.

Some judges agreed with each other more closely than they did with other judges but these differences did not reach the level of significance here and seemed no more than would be expected from the fluctuations of random sampling.

Changes in the udder and in the health of the cow appeared to be the chief causes of large shifts in type ratings. Two ratings had an advantage over one for predicting future type but little if any more seemed to be gained from using more than two.

SIZE OF THE RABBIT MAMMARY GLAND WITH SUCCESSIVE LACTATIONS*†

A. A. LEWIS AND C. W. TURNER

Department of Dairy Husbandry, University of Missouri

Milk and butterfat production of dairy cows have been shown to increase with advancing age up to about eight years. Based on official records this amounts to an increase of between 35 and 50 per cent of mature records over the two-year-old lactations (see Turner (6) for review). Under D. H. I. A. conditions the increase has been found to be 20 to 29 per cent in milk production (3, 1).

It is interesting to speculate as to the causes of this increase of production with age. The most obvious changes in dairy cattle with advancing age are an increase in body size and the recurrence of pregnancy and lactation. It would appear from studies which have been made of this subject (2, 5, 7) that from 40 to 80 per cent of the increase in production with age is due to recurring pregnancy and lactation and the development of organs and glands accompanying these conditions. Since size and quality of the udder have been shown to have a higher correlation with production than other body measurements (4) it appeared likely that much of this increase in production might be due to increasing development of the mammary glands with succeeding pregnancies. Measurement of the volume of the mammary gland in the living cow is impractical. The comparatively simple mammary glands of laboratory animals are spread over the ventral body wall in a fairly thin layer. This should render possible the measurement of changes in volume of the gland with successive lactations.

This study is a report of an attempt to demonstrate whether there is a lateral extension of the mammary glands with successive pregnancies in a laboratory animal, the rabbit. It was thought possible that lateral increase would give a good approximation of increase in volume of the mammary gland in the rabbit.

Virgin, sexually mature does of the New Zealand White breed were selected and bred. Soon after the first parturition, when the mammary glands were filled with milk, a line was tattooed on the lateral body wall from the anterior to the posterior leg through the shaved skin outlining the lateral extent of the mammary glands. A hand electric tattooing needle was used with India ink. The rabbits were immobilized on an operating board for the tattooing and later examinations.

Received for publication September 15, 1941.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 773.

† Aided in part by a grant from the International Cancer Research Foundation.

After weaning of the litters the does were rebred. Following the second and successive parturitions, the extent of the mammary glands was examined in relation to the tattoo line. Three does were carried through two, one through three and two through four parturitions. On ten examinations of the mammary glands in relation to the tattoo line there was no lateral extension apparent. In one case, after the second parturition, the mammary glands on the left side of the body extended uniformly one-quarter inch beyond the tattoo line.

These almost uniformly negative results do not preclude the possibility that the thickness and density of the mammary glands may have increased. That the anterior-posterior extent of the glands could have increased is doubtful, except for the first and last glands on a side, for the glands on the same side of the body are adjacent even at the first lactation. The glands also practically meet at the mid-ventral line.

SUMMARY

It was thought that the rabbit might illustrate the influence of mammary gland development in dairy cows as a cause of the increase in milk production with succeeding pregnancies. This increased production is greater than can be accounted for by the increase in body weight. The lateral extension of the mammary glands in succeeding lactations was compared in rabbits with lines tattooed in the skin at the lateral extent of the mammary glands early in the first lactation. In eleven succeeding lactations only one case was found in which the mammary glands on one side extended past the tattoo line. The lateral extent of the glands did not increase in the other cases.

REFERENCES

(1) ANONYMOUS. New Age Conversion Factors. D.H.I.A. Letter. Vol. 9, No. 9. Bur. Dairy Indus., U.S.D.A. 1933.

(2) GAINES, W. L. Age and Size of Cow Affects Milk Yields About Equally. Ill. Agr. Expt. Sta. Rept. p. 144. 1935.

(3) RAGSDALE, A. C., AND GIFFORD, W. Selecting the Dairy Sire. Mo. Agr. Expt. Sta. Bul. 274. 1929.

(4) SWETT, W. W., MILLER, F. W., GRAVES, R. R., AND CREECH, G. T. Quality, Size Capacity, Gross Anatomy, and Histology of Cow Udders in Relation to Milk Production. Jour. Agr. Res., 45: 577. 1932.

(5) TURNER, C. W. The Relation between Weights and Fat Production of Guernsey Cattle. JOUR. DAIRY SCI., 12: 60. 1929.

(6) TURNER, C. W. The Physiology and Biochemistry of Milk Secretion. Univ. Missouri. Mimeograph, pp. 158–205. 1932.

(7) TURNER, C. W., RAGSDALE, A. C., AND BRODY, S. The Relation between Age, Weight and Fat Production in Dairy Cows. Mo. Agr. Expt. Sta. Res. Bul., 221. 1924.

FURTHER STUDIES ON THE USE OF SALT FOR IMPROVING THE QUALITY OF CREAM FOR BUTTERMAKING[1]

F. E. NELSON, W. J. CAULFIELD AND W. H. MARTIN

Kansas Agricultural Experiment Station

Improvement of the quality of cream for buttermaking purposes by the addition of salt has received considerable attention during the past several years, although the method has not been accepted by either creamerymen or regulatory officials. The literature on the subject was reviewed in an earlier publication from this Station (1). In this earlier publication the addition to cream of 10 to 13 per cent salt, calculated on a fat-free serum basis, was shown to improve considerably the quality of the cream and the butter made therefrom under laboratory conditions simulating those on Kansas farms during the summer months (1). These studies did not answer a number of important questions, especially questions relative to the general applicability of this method to the handling of cream on the farm.

The studies herein reported were designed for the following purposes:

1. To study under controlled laboratory conditions the feasibility of adding at the beginning all of the salt necessary for the final volume of cream collected in daily increments.

2. To obtain information as to the applicability of the method to practical farm conditions, especially where all of the salt for a given quantity of cream was added to the container at the time collection was begun.

3. To follow the quantitative and qualitative changes in microflora under different holding conditions.

4. To obtain further data on the comparative scores of butters made from salted and unsalted creams.

5. To determine the effect of salt added to the cream on the containers used for collection and delivery.

METHODS

In each of four series of laboratory trials, commercial salt was added in amounts, calculated on a fat-free serum basis, to give 10 or 13 per cent concentration in 2 liters of cream. In these series cream standardized to 30 per cent butterfat was used. Sterile gallon-size glass jugs were used in series I, II and III and single-service gallon tinned ice cream cans were used in series IV. In series I and II all of the salt and cream were mixed together on the first day and the resulting mixture held without addition for 10 days. In series III and IV the salt for the entire period was added to 200 ml. of cream

Received for publication September 12, 1941.

[1] Contribution No. 207 from the Department of Bacteriology and No. 137 from the Department of Dairy Husbandry.

the first day and 200 ml. of fresh cream was added with thorough stirring on each of the following nine days.

Four additional series of trials were conducted under farm conditions. In each case a portion of the cream from each separation was added to a control can, and another portion of the same cream was added to a can in which had been placed at the beginning of the collection period enough salt to give a final 13 per cent concentration in the fat-free serum of all cream added during the collection period. The cans of cream were agitated after each addition of cream, but the salt in cream VIIB was not completely incorporated due to lack of sufficient agitation during collection. In three of the four series of farm studies the cream (30–36 per cent butterfat) was cooled by immersion of the cans in a tank through which well water flowed. In the fourth series the cans of cream were allowed to stand at atmospheric temperature which averaged about 85° F. After the cans of cream reached the laboratory, samples for bacteriological and chemical analysis were taken and portions were then neutralized, when necessary, pasteurized at 149° F. for 30 minutes, cooled and churned in gallon glass churns. In each series butter from unsalted cream was salted to give as closely as possible the same salt concentration as was found in butter made from salted cream. No additional salt was added to the butter churned from the salted cream.

All grading of cream was done organoleptically by two or more judges working independently on samples designated by code. Cream grades as defined in the Kansas dairy law (2) were used as a basis for grading. Saltiness was not considered an undesirable flavor defect in samples to which salt had been added. Butter samples were graded in a similar manner, using generally accepted commercial grades as a basis.

Direct microscopic counts were made by the methods outlined in Standard Methods for the Examination of Dairy Products (3), except that dilutions of one part of cream in nine parts of water were prepared from those samples in which so many organisms were present that enumeration in an undiluted sample was difficult. Molds and yeasts were determined on acidified potato-dextrose agar by the methods outlined for butter in Standard Methods. Total plate count and acid formers were determined on lactose beef infusion agar to which 0.1 ml. of 0.16 per cent aqueous brom-cresol-purple per plate had been added. The plates were incubated at 20° C. and colonies of acid-formers were counted after 2 and 4 days. Total counts were obtained after 4 days. Counts of proteolytic and lipolytic bacteria were determined by the use of beef infusion agar to which 0.5 ml. of 3 per cent cottonseed oil emulsified in 0.5 per cent agar and 0.5 ml. sterile skim milk per plate were added. The proteolytic colonies were recognized by the surrounding cleared areas in the somewhat opaque medium and lipolytic colonies by the blue color of the fat globules around the colonies after the plates had been flooded for several minutes with 0.1 per cent aqueous nile

blue sulfate. In many instances counts of proteolytic and lipolytic organisms undoubtedly were inaccurate due to overgrowth by the many other organisms present.

Titratable acidity and formol titration were determined by the usual laboratory procedures.

The effect of salt upon metals was determined by immersion to a depth of 2 inches of polished strips of dairy metal, tinned copper and two kinds of stainless steel in cream containing 13 per cent salt (serum basis). Temperatures of 90° F. for 10 days and 150° F. for 18 hours were used, the temperature in the latter series being maintained for 1, 8, 6, and 3 hours, respectively, on each of four successive days. Corrosion was measured by visual observation and loss of weight by the metal strips as a result of immersion.

EXPERIMENTAL RESULTS

Laboratory studies on the effect on cream and butter quality of the addition of salt to cream. Two series (I and II) of studies were made to show the effect of adding all the salt to the full volume of cream at one time, the mixture being held for ten days at 70 and 82° F. Two additional series (III and IV), which were otherwise identical except that different lots of cream were used, were conducted to show the effect of adding all of the salt at the beginning and adding the cream in daily increments. The grade and chemical data on these four series are shown in table 1. The data indicate that the addition of salt markedly improves the quality of cream and the butter made therefrom, either when the salt and cream are all mixed at the beginning and held, or when all of the salt is added at first and the cream is added in daily increments. All of the cream to which salt was added remained sweet for four days and none of it had dropped to second grade after ten days. None of the butter made from salted cream held for ten days at 70 or 82° F. before churning graded below 90 when churned, and much of it graded 91 or 92. These results are in marked contrast to those obtained on the control lots. Much of the control cream was third grade (illegal) after ten days and the butter made from this cream graded from 86 to 89 immediately after churning. The data on acidity and formol titration emphasize the degree to which the salt reduces chemical changes caused by microorganisms while the cream is being accumulated on the farm. Increases in acidity and formol titration in the salted cream were small, especially in the two series in which all the salt was added at first and cream accumulated from day to day. This undoubtedly was due to the very high salt concentration during the first few days. This greater preservative action of the initially higher concentration of salt is not reflected in significantly better grades of cream and higher scores of butter. At the same salt concentration the differences in grade are smaller at 70° F. than at 82° F. Individual lots of cream seem to differ in the degree of improvement resulting from salt

TABLE 1

Laboratory studies on the effect of salt on the quality of cream and the butter made therefrom

Series No.	Storage temp. (°F.)	Cream data														Butter scores					
		Acidity (per cent)				Formol titration				Cream grades						Fresh			After 60 days at 0° F.		
		0 days	10 days			0 days	10 days			After 4 days			After 10 days								
% salt		0	0	10	13	0	0	10	13	0	10	13	0	10	13	0	10	13	0	10	13
All cream added at the start																					
I	70	0.11	1.35	0.21	0.155	2.25	4.62	2.65	2.60	1	Sw*	Sw	3	1	1–	89	91	90	85	89	90
II	70	0.11	0.77	0.125	0.12	1.80	3.05	1.75	1.70	1	Sw	Sw	2–	Sw	Sw	87	91	92	88	91.5	92
I	82	0.11	1.96	0.29	0.21	2.30	6.05	2.75	2.70	2	Sw	Sw	3	1–	1–	89	91	90	85	90	89
II	82	0.11	1.60	0.19	0.12	1.80	4.30	2.10	1.80	2–	Sw	Sw	3	Sw	Sw	86	92	92	87	92	91.5
All salt added at start, cream accumulated daily (VI in glass container, VII in tinned container)																					
III	70	.....	0.69	0.12	0.125	.....	2.25	1.70	1.60	1	Sw	Sw	2–	1	1	88	91	91	87	92	92
IV	70	.....	0.68	0.105	0.11	.....	2.40	1.80	1.60	1	Sw	Sw	2–	Sw	Sw	87	92	92	84	91.5	91
III	82	.....	1.60	0.145	0.135	.....	3.10	1.85	1.70	1–	Sw	Sw	3	1–	1	86	90	90	86	91	92
IV	82	.....	1.63	0.115	0.12	.....	3.70	1.85	1.65	1–	Sw	Sw	3	Sw	Sw	86	91.5	91.5	84	91	91.5

* Sweet.

addition, better creams and butters being usual in series II than in series I and series IV being somewhat superior to series III in this respect. The extremely low scores after storage for the butters from unsalted creams of series IV were due to a pronounced cheesy and putrid flavor apparently the result of bacterial action rather than to any change which might be attributed to the use of metal containers.

The data relative to total plate count and counts of acid-formers are presented in table 2. Total bacterial counts on series I and II show that when salt in the concentrations used was added to the cream, the count usually decreased somewhat on the first day and then increased to the end of the ten-day period. The highest counts reached by the salted creams were appreciably below the maximum levels reached in the unsalted creams. The superior quality of the original cream and the lower maximum counts of the salted creams explain why salted creams and the butters made therefrom were better in series II than in series I. In series III and IV, where the cream was accumulated over ten-day periods, both the magnitude and duration of the decrease in count resulting from the addition of salt were greater than in series I and II when all the cream and salt was added at the beginning, and the levels reached after 10 days tended to be slightly lower although of the same general magnitude.

In series I and II the acid forming organisms constituted almost 100 per cent of the total viable populations of the unsalted control creams until the total counts had declined considerably below the maxima, after which the count of acid-formers dropped more rapidly than did the total count. The daily addition of fresh unsalted cream to series III and IV controls tended to maintain a predominantly acid-forming bacterial flora at 70° F., but at 82° F. the proportion of acid-forming bacteria decreased significantly toward the end of the ten-day accumulation period. In salted creams the count of acid-forming bacteria usually dropped below the total count almost at once and acid-forming bacteria made up only a small proportion of the total count at the end of the ten-day period. This suppression of acid-forming bacteria explains why cream to which salt has been added seldom develops a high degree of acidity.

The direct microscopic counts given in table 3 also indicate the marked inhibitory action of salt upon bacterial growth but emphasize the fact that bacteria do grow extensively at the salt concentrations used. The counts at 10 days are not the maximum counts obtained in all instances, even on the salted samples, but in no series did a pronounced drop in microscopic count parallel that observed for the plate counts. In the unsalted creams the organisms observed were usually predominantly large paired and clumped cocci, probably micrococci, and occasional sarcina types were encountered. These are organisms which usually cause comparatively little change in the substratum in which they develop.

TABLE 2

Changes in plate counts of total and acid-forming organisms under laboratory conditions

Series	Age days	Total count (thousands) on sample:						Acid-formers (thousands) on sample:					
		70° Control	70°–10%	70°–13%	82° Control	82°–10%	82°–13%	70° Control	70°–10%	70°–13%	82° Control	82°–10%	82°–13%
I	0	45,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	1	150,000	37,000	34,000	470,000	47,000	37,000	.....	.....	.....	.....	.....	.....
	2	960,000	54,000	45,000	410,000	64,000	50,000	.....	.....	.....	340,000	.....	.....
	4	540,000	83,000	49,000	19,000	120,000	87,000	540,000	72,000	48,000	12,000	110,000	77,000
	6	91,000	190,000	60,000	4,900	220,000	100,000	90,000	21,000	49,000	7,000	40,000	21,000
	8	29,000	290,000	81,000	30	220,000	150,000	16,000	6,000	1,000	Molds	4,000	2,000
	10	9,900	230,000	101,000	750	180,000	170,000	Molds	6,500	<1,000	Molds	<1,000	<1,000
II	0	16	.....	.....	.....	.....	.....	16	.....	.....	.....	.....	.....
	1	550,000	6	8	1,100,000	230	9	.....	2	.....	1,100,000	.....	7
	2	1,300,000	120	11	1,100,000	6,500	58	.....	9	.....	.....	26,600	6
	4	1,300,000	1,000	700	1,100,000	28,000	4,600	1,300,000	<10	<10	1,100,000	27,000	<100
	6	920,000	26,000	2,800	9,200	93,000	9,600	920,000	200	<100	9,100	12,000	100
	8	850,000	53,000	6,000	300	120,000	16,000	840,000	<1,000	<100	Molds	<1,000	<100
	10	370,000	81,000	18,000	110	200,000	36,000	80,000	<1,000	<100	Molds	<1,000	<100
III	0	3,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	1,500,000	21	18	1,200,000	17	15	1,500,000	16	10	1,100,000	14	8
	4	820,000	33	56	500,000	43	18	820,000	3	3	480,000	<1	3
	7	580,000	3,600	310	330,000	6,000	4,500	570,000	>100	>100	330,000	>100	>100
	10	340,000	54,000	18,000	650	61,000	14,000	340,000	200	200	<100	50	150
IV	0	73	.....	.....	.....	.....	.....	49	.....	.....	.....	.....	.....
	2	1,100,000	1,300	1,700	1,000,000	1,100	1,500	1,100,000	1,300	1,700	1,000,000	1,100	1,500
	4	830,000	690	500	610,000	340	220	830,000	690	470	600,000	280	220
	7	340,000	2,700	1,000	13,000	20,000	1,500	220,000	400	150	3,500	500	<100
	10	470,000	53,000	26,000	1,300	90,000	53,000	450,000	1,000	1,200	<100	3,000	<1,000

TABLE 3

Microbiological data on laboratory studies of the effect of salt on the quality of cream (samples incubated 10 days)

Series	Salt %	Temp.	Plate count per ml. of cream				Total plate count per ml. after past. 149°–30 min.	Direct microscopic count per ml.
			Proteolytic	Lipolytic	Yeasts	Molds		
I‡	0	70	*	*	12,000,000	>1,000,000	2,500	948,000,000
	10	70	29,000,000	10,000,000	20	110	34,000	260,000,000
	13	70	15,000,000	2,000,000	140	150	28,000	140,000,000
	0	82	*	*	4,200,000	>100,000	1,100	936,000,000
	10	82	20,000,000	10,000,000	80	<10	34,000	264,000,000
	13	82	19,000,000	5,000,000	250	<10	46,000	372,000,000
II‡	0	70	200,000	<1,000,000	<100,000	700,000	.....	1,240,000,000
	10	70	3,500,000	200,000	1	2	.....	205,000,000
	13	70	150,000	200,000	0	3	.....	72,000,000
	0	82	*	*	1,000,000	11,000,000	.....	1,385,000,000
	10	82	9,000,000	6,500,000	0	0	.....	420,000,000
	13	82	1,000,000	700,000	0	3	.....	141,000,000
III†	0	70	180,000,000	<1,000,000	1,300,000	<100,000	11,600	606,000,000
	10	70	<100,000	300,000	<10	150	9,700	88,000,000
	13	70	100,000	100,000	<10	150	10,000	36,000,000
	0	82	<100,000	.....	450,000	<100,000	150	1,400,000,000
	10	82	4,500,000	<100,000	<10	100	8,800	42,000,000
	13	82	50,000	100,000	.....	140	10,400	47,000,000
IV†	0	70	<1,000,000	<1,000,000	2,900,000	450,000	55,000	1,060,000,000
	10	70	200,000	<100,000	<10	20	56,000	86,000,000
	13	70	<100,000	<100,000	10	60	68,000	82,000,000
	0	82	<10,000	<100,000	1,400,000	280,000	650	624,000,000
	10	82	100,000	300,000	<10	20	71,000	92,000,000
	13	82	150,000	100,000	<10	20	68,000	108,000,000

* Molds made proper plates uncountable.
† All salt added at once, cream added over 10-day period.
‡ All salt and cream added at once and held 10 days.

Data on the numbers of microorganisms of certain types developing in the different lots of cream are given in table 2, only values at the end of the 10-day period being recorded. Salt does not entirely prevent the development of proteolytic and lipolytic organisms in cream, but it is effective in preventing the development of molds and yeasts. Plate counts after pasteurization indicate that when cream was held at 70° F., the pasteurization efficiency was the same on salted cream as on unsalted cream. When cream was held at 82° F. the pasteurization efficiency on the unsalted cream was much higher than on the same cream held at 70° F., but no similar increase in efficiency occurred in the case of the creams to which salt was added.

The data definitely indicate that the addition of salt to cream is effective because of both quantitative and qualitative changes in the microflora which result.

Farm studies. Four series of studies of the effect of salt on the quality of cream and butter made therefrom were conducted on farms through the cooperation of two local dairy farmers. The results of these series are presented in tables 4 and 5. In all instances the cream to which salt had been added or the butter made from such cream was superior to the cream and butter from the same source without added salt. The effect of salt was more apparent when higher cream storage temperatures were used. The salted creams had lower acidities, lower formol titration values, lower total plate counts, lower proportions of acid-forming bacteria, much lower plate counts of yeasts and molds, lower direct microscopic counts and were higher in grade than were the unsalted control creams. The butters made from the salted creams scored one to five points higher than butters from the same lots of cream unsalted and also frequently maintained their grade better in storage. The studies made under farm conditions thus corroborate the results obtained under laboratory conditions and indicate that the addition of salt to cream as a means of delivering higher quality cream might be applicable to practical farm conditions.

Corrosive effect of salt in cream on metals used in dairy equipment. A product known as dairy metal, a tinned copper and two types of stainless steel were used in these studies. The results of the study are presented in table 6. Both types of stainless steel seemed very resistant to corrosion by salt in cream, either at 90° or 150° F. The recorded losses in weight probably were within the limits of experimental error and no change in appearance occurred. Dairy metal showed definite susceptibility to corrosion under the test conditions, the strips decreasing an appreciable amount in weight and showing slight visible corrosion at the point of contact between the metal and the surface of the cream. Tinned copper was corroded quite badly by the salted cream, all strips losing appreciably in weight and showing considerable corrosion at the point of contact between metal and cream surface. The tinned cans used in one series showed no visible corrosion attributable

TABLE 4

Farm studies of the effect of salt on the quality of cream and butter made therefrom

Series	Patron	Temp. range (°F)	Salted or unsalted	Cream data (7 d.)			Butter data									
				Grade	Acidity %	Formol titration	Moisture content %	Salt content %	Scores							
									Fresh		7 days at 70°F.		30 days at 40°F.		60 days at 0°F.	
									Score	Difference	Score	Difference	Score	Difference	Score	Difference
V	A	58–62	Un	1–	0.52	2.40	15.5	1.3	88	3.0	86	4.5	87	3.5	87	5.0
			S	Sw	0.11	1.75	15.4	0.7	91		90.5		90.5		92	
	B	60–68	Un	1	0.59	3.00	15.6	1.3	90	1.5	89	1.0	88	3.0	89.5	2.5
			S	Sw	0.13	2.00	15.7	1.3	91.5		90		91		92	
VI	A	58–62	Un	1–	0.51	2.32	15.6	0.8	89	2.0	87	3.0	89	3.0	88	4.0
			S	Sw	0.20	2.00	14.6	0.7	91		90		92		92	
	B	62–66	Un	1–	0.55	2.82	13.2	1.0	90	1.0	89	1.5	88	3.5	88	4.0
			S	Sw	0.26	2.05	14.8	0.7	91		90.5		91.5		92	
VII	A	80–92 (air)	Un	3	1.10	2.85	16.8	1.0	87	3.0	87	3.0	87	3.0	87	2.0
			S	Sw–	0.18	1.65	16.4	0.7	90		90		90		89	
	B	80–92 (air)	Un	3	1.19	3.10	15.4	0.7	89	2.0	89	1.0	87	4.0	88	3.0
			S	Sw–	0.15	1.65	14.4	1.0	91		90		91		91	
VIII	A	59–62	Un	2	0.61	2.90	16.0	1.4	86	5.0	86	4.5	86	5.0	86	4.5
			S	Sw–	0.11	2.00	17.0	0.9	91		90.5		91		90.5	

TABLE 5

Microbiological data on farm studies of the effect of salt on the quality of cream (7-day accumulation period)

Series	Patron	Temp. (°F.)	Salted or unsalted	Plate counts per ml.						Total direct microscopic
				Total	Acid-formers	Proteolytics	Lipolytics	Yeasts	Molds	
V	A	58–62	Un	440,000,000	> 400,000,000	< 10,000	80,000	11,000	8,000	680,000,000
			S	4,300,000	1,700,000	110,000	30,000	60	30	11,000,000
	B	60–68	Un	700,000,000	700,000,000	600,000	200,000	2,000	350,000	670,000,000
			S	15,000,000	2,500,000	260,000	160,000	< 10	70	53,000,000
VI	A	58–62	Un	440,000,000	> 400,000,000	100,000±	< 100,000	40,000	450,000	740,000,000
			S	10,800,000	5,400,000	130,000	10,000±	70	< 10	60,000,000
	B	62–66	Un	660,000,000	650,000,000	2,000,000	< 1,000,000	< 10,000	200,000	780,000,000
			S*	55,000,000	14,000,000	2,700,000	< 100,000	< 10	370	380,000,000
VII	A	80–92 (air)	Un	47,600,000	46,000,000	10,000	.....	150,000	580,000	1,220,000,000
			S	18,000,000	2,700,000	40,000	20,000	> 10,000	< 10	?
	B	80–92 (air)	Un	1,300,000	800,000	35,000	10,000	< 1,000	71,000	1,075,000,000
			S	11,400,000	600,000	400,000	350,000	> 500	> 50	38,500,000
VIII	A	59–62	Un	306,000,000	300,000,000	200,000	< 100,000	3,200,000	500,000	1,455,000,000
			S	27,200,000	750,000	900,000	< 100,000	160	10	120,000,000

* Salt poorly incorporated.

TABLE 6

Corrosive effect of salt in cream on certain dairy metals

Metal used	Initial weight	Final weight	Loss in weight	Visual change
	Metal strips immersed to a depth of two inches in cream containing 13 per cent salt serum basis for 10 days at 90°F.			
	grams	*grams*	*grams*	
Stainless steel 2B	12.4064	12.4064	0.0000	No visible change
Stainless steel 2B	12.3879	12.3878	0.0001	No visible change
Stainless steel 4B	10.7157	10.7157	0.0000	No visible change
Stainless steel 4B	11.0255	11.0254	0.0001	No visible change
Dairy metal	14.2577	14.2560	0.0017	Slight corrosive effect at surface of cream
Dairy metal	14.1633	14.1620	0.0013	Slight corrosive effect at surface of cream
Tinned copper	19.0582	19.0522	0.0060	Visible corrosion near surface of cream
Tinned copper	18.9674	18.9620	0.0054	Visible corrosion near surface of cream
	Metal strips immersed in salted cream as described above and cream held at 150°F. for total of 18 hours			
Stainless steel 2B	12.3175	12.3170	0.0005	No visible change
Stainless steel 4B	10.9085	10.9085	0.0000	No visible change
Dairy metal	13.2388	13.2376	0.0012	Slight corrosive effect at surface of cream
Tinned copper	19.1886	19.1851	0.0035	Slight corrosive effect at surface of cream

to the use of salt, since some corrosion was apparent in cans which had contained both salted and unsalted creams. Had the cans been used repeatedly or had the immersion tests with the metal strips been repeated a number of times on the same strips, greater susceptibility to corrosion might have been apparent. The results indicate that if salt were added to cream on the farm, containers of more resistant types than some of those commonly employed probably would be necessary. Use of plant equipment made of the more resistant metals also might be necessary in processing salted cream.

CONCLUSIONS

The data herewith presented confirm previously reported studies demonstrating that the addition of 10 to 13 per cent salt to cream markedly increases the quality of the cream and of the butter made therefrom.

Placing all of the salt in the container at the beginning of the accumulation period and adding the cream in daily increments with thorough stirring each time was found satisfactory in both laboratory and farm studies. Such a procedure would be applicable to practical farm conditions.

The added salt not only markedly reduces the numbers of microorganisms which develop in the cream but also affects the types. The number of acid-forming bacteria in the cream is reduced markedly, usually being less

at the end of the ten-day holding period than in the original cream. Salt in the concentrations used apparently inhibits completely the development of yeasts and molds. The data on proteolytic and lipolytic bacteria, while not conclusive, indicate that salt is not consistently more inhibitory to such organisms than is the acidity which normally develops in unsalted cream.

Microscopic examination of the cream revealed that the flora of salted cream is predominantly large paired and clumped cocci and a few sarcina types, instead of the small cocci in pairs and short chains, frequently followed by lactobacilli, which predominate in unsalted control lots of cream.

Salted cream apparently is non-corrosive to stainless steel of the types tested, but dairy metal and tinned copper are subject to noticeable corrosion and probably would not be suitable materials for equipment used in collecting and processing salted cream.

The addition of salt to cream as it is produced on the farm offers an apparently feasible method for considerable improvement of the quality of butter manufactured over a considerable section of the country. Acceptance by regulatory officials and by creamerymen must be obtained before the method can be placed in the hands of the cream producer.

REFERENCES

(1) CAULFIELD, W. J., NELSON, F. E., AND MARTIN, W. H. Effect of Salt on the Keeping Quality of Cream. JOUR. DAIRY SCI., 23: 1215–1227. 1940.

(2) KANSAS STATE BOARD OF AGRICULTURE. The Permit System of Cream Buying. Report of the Kansas State Board of Agriculture, Dairy Division, for the Quarter ending December, 1938, p. 9. 1938.

(3) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. Seventh edition. Amer. Pub. Health Assoc., New York, N. Y. 1939.

NEW DEVELOPMENTS IN THE PHYSIOLOGY AND BIOCHEMISTRY OF LACTATION; A REVIEW[1]

W. E. PETERSEN

University of Minnesota, St. Paul, Minnesota

The physiological and biochemical aspects of lactation may be divided broadly into three phases.

I. Development of the mammary glands.

II. Lactation.

III. Ejection of milk from the smaller gland recesses.

A comprehensive review of the literature pertaining to all of these would require far more space than is available. It is, therefore, proposed to deal only superficially with the endocrine aspects of gland development and the lactogenic hormones. The biochemical aspects will be dealt with more extensively but not exhaustively. It shall rather be the purpose to review the more recent pertinent literature with a view of establishing the present status of research in this field.

I. DEVELOPMENT OF THE MAMMARY GLANDS

The literature pertaining to the growth of the mammary glands has recently been reviewed by Nelson (167), Turner (253), Folley (45), and Riddle (215, 216). For anatomical features of mammary development, see Turner (254, 255) and Espe (38). No attempt is made here to cover all of the areas reviewed in these papers, but an attempt will be made to incorporate the recent work with a view of establishing the status of the problem at the present time.

It is well established that mammary development in the normal animal is stimulated by ovarian hormones falling into two categories—estrogens and progesterins. The classical example is where the estrogen causes duct development and the progesterin causes alveolar development. While estrogen is needed for duct development in all species progesterin is not essential for the alveolar development in some species.

In the rat and the rabbit some alveolar development is observed, while for the guinea pig, Nelson (167), the goat, de Fremery (61) and Folley *et al.* (50), the monkey, Gardner and Von Wagenen (67), and the cow, Walker (262) and Turner (253), complete alveolar development is obtained from estrogen administration. Walker and Stanley (262) obtained complete mammary development as well as initiation of lactation from diethylstilbestrol administration to an ovariectomized heifer, while Turner (253)

[1] Scientific Journal Series Paper No. 1948, Minnesota Agricultural Experiment Station. Prepared with the assistance of Work Projects Administration, Official Project No. 65-1-71-140. Sponsor: University of Minnesota.

obtained good udder development and considerable secretion from a spayed heifer with ovarian transplant.

The generally observed failure of mammary development in estrogen-treated hypophysectomized animals led to the conclusion that the hypophysis is involved. The main question is as to whether the estrogens and progesterins stimulate the hypophysis to form new hormones which are capable of causing mammary development or whether such formed hormones act synergistically with the sex hormones or whether normally present hormones of the hypophysis act with the sex hormones.

While Corner (31) suggested the pituitary might produce mammogenic hormones, evidence for mammogenic hormone formation by the pituitary through stimulation of the sex hormones comes chiefly from workers at Missouri. Gomez *et al.* (75,78) obtained mammary development in hypophysectomized guinea pigs by implantation of pituitaries from rats treated with estrogen and negative results from pituitaries of nontreated rats. Gomez and Turner (77) again reported that extracts from anterior pituitaries of pregnant cattle stimulated mammary growth in hypophysectomized rabbits and rats while the extracts from pituitaries of nonpregnant cattle were ineffective. Lewis and Turner (141) reported that the mammogenic factor in the pituitary is soluble in fat solvents and Lewis *et al.* (142, 144) reported on methods for biological assay also further confirming the effect of pregnancy on the pituitary content of the hormone. Reece and Leonard (206) found that implants of pituitaries from estrogen-treated and nontreated rats stimulated mammary development in hypophysectomized rats although they supported the mammogen hypothesis. In more recent work two mammogenic hormones are reported—mammogen I (Lewis (140)) for the development of ducts and Mammogen II (Mixner (162)) for the alveolar lobular development—and Mixner and Turner (163) have reported on a method for assaying the lobular alveolar growth promoting activity of the anterior pituitary.

In opposition to the "mammogen" hypothesis are the reports of failure to confirm the basic experiments leading to the development of the theory, the fact that estrogens have a local effect when applied topically, the possibility that the failure of mammary development in the hypophysectomized animal may be due to inanition and some evidence that the growth hormone is essential.

The results of Corner (31) and Nelson and Tobin (175), where crude pituitary extract together with estrogen caused mammary development in hypophysectomized rabbits and rats, respectively, might be explained by the supposition that the pituitaries they used contained the mammogenic hormone, and the negative results of Selye and Collip (222) could be explained by postulating the absence of the mammogenic hormone in the pituitaries they used. Nelson (168, 170) observed no difference in the development

of mammary glands in hypophysectomized rats from implants of pituitaries from estrogen-treated and nontreated rats. Similar results were obtained by Reece and Leonard (207). More recently Greep and Stavely (95) extracted bovine pituitaries according to the method of Lewis and Turner (141) and found the extract to be inactive while implants of the powdered pituitaries and extracted residue were active although the potency of the latter was somewhat reduced.

Topical application of estrogen to the nipple area of individual glands has been observed to cause development of the treated gland only with little or no effects upon the untreated one for: the rat by Lyon and Sako (151); woman by MacBryde (152); the goat by Folley *et al.* (51); the monkey by Speert (240); the guinea-pig by Nelson (171), and the bovine by Petersen *et al.* (186). These experiments may be interpreted as showing that the estrogen acts directly upon the mammary gland in a synergistic manner with other pituitary hormone or hormones.

Astwood *et al.* (4) suggested that the inanition state of the hypophysectomized animal may be a factor in the failure of mammary development, for intact rats restricted to a food intake comparable to that of the hypophysectomized ones resulted in failure of mammary gland response to estrogen. The report of Nathanson *et al.* (164) supports the view that under-nutrition is a factor in the failure of mammary growth stimulation to estrogen in hypophysectomy. Samuels *et al.* (220) force fed hypophysectomized rats by stomach tube so as to gain in weight but noted no effect on the mammary development. It should be noted, however, that while forced feeding caused increases in weight such might be due to adipose tissue increments and not to real growth.

That the growth hormone in conjunction with estrogen is needed for mammary growth is indicated by the work of Nathanson *et al.* (164), who noted that in eight of 24 hypophysectomized rats treated simultaneously with estrogen and a growth complex there were weight increases and marked mammary development. In six animals there were no weight increases and moderate gland development, and in ten animals with weight loss gland development varied from moderate to no effect. Greep and Stavely (95) observed body growth from pituitary powder implants although the amount of body growth and the extent of mammary development were not always correlated. More recently, Reece and Leonard (207) report on positive evidence of growth hormone effect with estrogen on mammary growth. Samuels *et al.* (220a), using a more highly purified growth hormone, corroborate the findings of Reece and Leonard, but contend other anterior pituitary hormones are also needed.

The observation of Gardner (65) that large doses of estrogens have an inhibitory effect on the mammary gland development is of great importance to workers in the field. Other important considerations are the effects of

other endocrines of which merely mention is made. Some of the androgens (see Folley (45) and Bottomley and Folley (15)) have mammogenic properties. The adrenal hormone, desoxycorticosterone, has been shown by Van Heuverswyn *et al.* (259) to be growth-promoting for the mammary gland of male mice. It must also be mentioned that Beall and Richstein (10) isolated progesterone from the adrenals, which may in part explain alveolar proliferation from estrogen administration alone. Another interesting observation was made by Butcher (25) in that the mammary glands of underfed adrenalectomized rats developed more rapidly than in the intact animals. Ovariectomy together with adrenalectomy produced similar results, indicating that the effect is not one in which the ovary is involved. The author has noted that the mammary gland of the thyroidectomized bovine develops much more slowly than in the normal. Because of the specific effects of the hypophysis upon other endocrine glands and in turn their probable effect upon the development of the mammary gland, complete development of the mammary gland in hypophysectomized animals no doubt will be found to require more than one fraction of the anterior pituitary.

II. LACTATION

The complicated phenomenon of lactation may be divided, for ease of discussion into the following five parts: A. Endocrine; B. When milk is secreted; C. Equilibria between milk and blood; D. Relation of pressure to milk secretion; E. Synthesis of milk.

A. *Endocrine Factors.* The initiation and maintenance of lactation is dependent upon endocrines. From the literature it is apparent that the anterior pituitary, the thyroid and the adrenals are involved in complete lactation. Endocrines also play a part in inhibiting lactation. For reviews see Nelson (167), Turner (253), Folley (45, 46), and Riddle (215, 216).

1. Anterior Pituitary. Since Stricker and Greuter (245) in 1928 first discovered that injection of an aqueous extract of the anterior pituitary would initiate lactation in ovariectomized pseudo-pregnant rabbits a large number of experiments dealing with anterior pituitary lactogenic hormones have been reported. The literature is so large that only a small portion can be cited here. For further literature citations the reader is referred to the reviews (39, 43, 167, 216, 222, 254).

The main pituitary lactogenic hormone studied is known as prolactin (also as galactin by Turner and mammatropin by Lyons). Prolactin is universally accepted as a lactogenic hormone, but Riddle (216) rightly argues against calling it the lactogenic hormone, for, as will be shown, there are probably other lactogenic hormones. Although other methods of assay have been proposed (66, 166), the international unit is based upon the growth produced in the crop-gland of the pigeon. Therefore, prolactin refers specifically to crop-gland-stimulating activity.

The standard method of isolation of prolactin is that of Riddle and Bates (217, 218). While the chemistry is not known, in its purest form prolactin reacts like a protein (Riddle and Bates (217, 218), Bates *et al.* (8), Young (268), and McShan and French (156)).

While the anterior lobe of the pituitary is the chief source of prolactin, this hormone is also found elsewhere. Lyons and Page (150), Ehrhardt and Voller (37), and Turner and Meites (257) detected prolactin in the urine of lactating women; Lyons (148), in the urine of babies secreting witches milk and in the urine of normal human males. Leblond (136) has demonstrated prolactin in the blood of pregnant and lactating mares. Lessman (139), Ehrhardt (36), and Turner and Meites (257) have found the lactogenic hormone in placentas. A prolactin-like substance was found by Rabald and Voss (203) in normal beef and hog livers, but not in the liver of the horse.

That the anterior pituitary varies in its prolactin content as to species, physiologic stage, and age was first reported by Bates *et al.* (9). Chance *et al.* (29) report progressive increases in prolactin for the following species: horse (only 4% of the ox), swine, man, ox, and sheep. Reece and Turner (209) report a higher prolactin content in dairy than in beef catle pituitaries. They also report a lesser concentration of the hormone in calves than in adults. In another report, these workers (210) found estrogen administration to increase the lactogen content of rat pituitaries. In guinea pigs, according to Reece (205), the hormone is greatest during lactation, next greatest in late pregnancy, followed by decreasing amounts in estrum, early pregnancy, and diestrum. Holst and Turner (110) found no increase of prolactin in early pregnancy, little in late pregnancy, but large increases following parturition of rabbits and guinea pigs. Ehrhardt and Voller (37) report two peaks of prolactin in the urine of post-partum women, one at menstruation and the other at mid-cycle corresponding to ovulation.

The question of the mechanism responsible for the secretion of prolactin to time with parturition has long been and still is a matter of speculation. In general, the theories advanced fall into five groups—1, the corpus luteum inhibits lactation; 2, the placenta inhibits lactation; 3, the lactation is inhibited by mechanical distension of the uterus; 4, estrogen secretion of pregnancy inhibits lactation, and 5, some unknown factor during pregnancy inhibits the production of prolactin.

The theory of the inhibitory action of the corpus luteum is supported by the work of Drummond-Robinson (34) and Asdell (2), who reported that removal of the corpora lutea from pregnant goats with well-developed mammary glands resulted in lactation. Selye *et al.* (224) observed that ablation of the corpora lutea in rats with well-developed mammary glands and treated with the luteinizing hormone also resulted in lactation. Hammond (101) as early as 1917 advanced the theory that the corpus luteum inhibited lactation.

The reports of Anselmino and Hoffmann (1) and Folley and Kon (48), who injected progesterin into lactating animals and failed to inhibit lactation, must be cited as evidence against the theory, although Anselmino and Hoffman suggested that some other corpus-luteum factor than progesterin is responsible for the inhibition. Lactation in the human, cow, goat, and other species during pregnancy would also indicate that the corpus luteum is not the chief inhibitory factor.

In support of the placental inhibition theory are the reports of Frankl (59), Nelson (165), and Smith and Smith (238). These workers observed inhibition of lactation when placentas were retained, and Frankl also obtained inhibition of lactation by placental implantation. Litt (146), however, was unable to observe any inhibitory effect on lactation by the implantation of placentas in rabbits as was also Selye *et al.* (224) with mice. Other objections to the theory come from the lactations during pregnancy and the often observed fact the retained placentas in cattle, at least, do not completely inhibit lactation in that species.

That the mechanical distension of the uterus may inhibit lactation finds support in the report of Selye *et al.* (224), as when the young in rats were removed by Caesarian section and the uteri filled with paraffin, lactation failed, although Bradbury (17), using the same technique, could not confirm the work of Selye and others. Freud and Wijsenbeck (62) found that transferring rat fetuses from the uterus to the abdomen inhibited lactation until removed therefrom.

That estrogens inhibit lactation is supported by too many reports to be reviewed here. Among the many papers suppression of lactation by estrogen administration has been reported for the mouse (122, 219), the rat (48, 209), the cow (42, 46), the guinea pig (84, 123, 135), and woman (58, 128, 137, 204, 267). It must be noted that heavy doses of estrogen are required for inhibitory effects on lactation; in fact, larger amounts than would be present in pregnancy. Nelson (167) advances the theory that estrogen during pregnancy acted through two channels: (a) by suppressing the secretion of the lactogenic hormone by the hypophysis, and (b) by direct action on the mammary gland. Turner and Meites (257) point out that this cannot account for lactation in pregnancy in the several species where this phenomenon is observed nor does it explain the initiation of lactation when diethylstilbestrol is used for mammary development such as has been observed by Folley *et al.* (50) and Lewis and Turner (143) for the goat and by Walker (262) for the cow. The observations of Folley and Watson (49), Folley *et al.* (52), and Spielman *et al.* (241), that injection of large doses of diethylstilbestrol into lactating cows did not inhibit milk production but merely altered the composition of the milk, suggest some species differences in response to estrogen as diethylstilbestrol has been shown to inhibit lactation in other species. It must also be stated that this theory of Nelson does not agree with the obser-

vations of Reece and Turner (209), who reported increases in the prolactin content of the pituitaries following estrogen administration.

Turner and Meites (257), after criticizing the theories advanced relative to the initiation of lactation and presenting evidence to show that pregnancy does not lower the lactogenic hormone of the pituitary, propose that copious lactation can be initiated only when (a) there is a well-developed mammary gland present and (b) when there is a high lactogen content of the anterior pituitary.

Since it is well established that lactation can take place during pregnancy, and accepting the suggestion of Turner and Meites (257) that high lactogen content of the pituitary is essential for copious lactation, it appears to the reviewer that initiation of lactation at the time of parturition is not due to the removal of some inhibitory factor but rather is due to the introduction of some stimulating factor. This stimulating factor may well come from the posterior pituitary as the oxytocic principle of that gland is secreted at the onset of labor. The observations by Reece and Turner (208) of the effect of suckling upon the prolactin content of the pituitary support such a hypothesis as does also the theory of Ely and Petersen that milk is ejected from the alveoli through the action of oxytocin in turn liberated by the milking stimulus. The recent report by Hooker and Williams (111) that injection of the lactogenic hormone retarded involution of the mammary gland emphasizes the dual role of this hormone—maintaining the mammany gland as well as stimulating lactation.

That prolactin is not the only anterior pituitary hormone needed for the initiation and maintenance of lactation is indicated by a number of experiments (Folley and Young (53, 54, 56)). The earlier work with success of initiating and maintaining lactation in the hypophysectomized animal can be explained by assuming that the extracts used were relatively crude. This seems to be the explanation for the success of Riddle *et al.* (218) with rats; Stricker and Grueter (245) with rabbits; Lyons *et al.* (149) and Housay (112, 113) with the dog; and McPhail (155) with the cat. Failures of Nelson (167) and Selye *et al.* (223) with the use of crude anterior pituitary extracts are difficult to explain. Later, however, Nelson and Gaunt (172) found that a purified preparation failed, while a crude extract of the anterior pituitary succeeded, in initiating lactation in hypophysectomized guinea pigs. Gomez and Turner (71), also working with hypophysectomized guinea pigs, found their purified preparation of prolactin failed to initiate lactation.

Thyroxin has been ruled out as the missing hormone by Gomez and Turner (75, 76), as the simultaneous administration of thyroxin and purified prolactin failed to initiate lactation. Leonard and Reece (138), however, report that thyroidectomy of rats resulted in a thickening of the ducts and an increase in the number of end and lateral buds to indicate an effect

of the thyroid upon the growth of the gland. Atrophy of the adrenals accompanies hypophysectomy, and it is not surprising that simultaneous administration of the adrenocortical hormone and prolactin is effective in initiating lactation as has been reported by Gomez and Turner (71, 73, 74) and by Nelson and Gaunt (172, 173, 174). Climenko and McChesney (30) observed that injection of epinephrin with prolactin and adrenal cortex hormone augmented milk flow.

In addition to laboratory animals, injections of anterior pituitary extracts have been reported for the cow, goat, sheep and the human. Gruter and Stricker (98), Evans (39, 40), Asimov and Krouze (3), and Folley and Young (53, 54, 55) injected rather crude extracts into lactating cows and observed significant rises in milk production, Evans (40) reporting as much as 25 to 50 per cent increases. In the main, the effects were temporary and most pronounced in cows during the downward trend of the lactation curve, and some cows did not respond at all. Asimov and Krouze reported the injections to be more effective in the first half of the lactation. Stockklausner and Daum (244) found that injections of anterior pituitary extracts caused a decline in milk production of cows. Experiments by Folley and Young (54, 55) showed that while "purified" preparations of prolactin caused increases in milk production, preparations containing the glycotropic factor were more effective even though such extracts contained less of the pigeon-crop-gland stimulating factor.

For goats, anterior pituitary extracts have been shown to stimulate milk production similarly to the cow by Grueter (97), Evans (39), and Asdell *et al.* (2). For sheep, Kabak and Kisilstein (125) have reported stimulation of lactation following administration of anterior pituitary extracts.

Prolactin has been used with conflicting results in attempts to increase lactation in women. Kenny and King (131) report 74 per cent of 43 women responded with satisfactory lactation following injections of prolactin. Kurzrok *et al.* (133) also report favorable results. On the other hand Stewart and Pratt (243) reported negative results with 14 women and Werner (265) describes some severe reactions from prolactin injections.

2. Thyroid. Since the thyroid regulates body metabolism, this gland would be expected to exert a marked influence over lactation. Jones (121) points out that the thyroid hormone may influence milk secretion in three ways: 1, by affecting the level of the blood precursors of milk; 2, by influencing the rate of blood flow through the gland, and 3, by a direct effect on the gland secretory cells. Studies on the thyroid-milk-secretion relationships have been made by two general methods—thyroidectomy and administration of the thyroid hormone to intact animals.

The reports on the effects of thyroidectomy are conflicting for reasons that are obscure. For lactating cows, Graham (84) reported only a slight decrease in milk production following thyroidectomy, while Spielman and

Petersen (242) have observed not only a slowing of the development of the mammary gland but a complete cessation of lactation in 180 days following thyroidectomy. Trautmann (252) reported thyroidectomy of goats resulted in a significant decrease in milk yield, while Hibbs *et al.* (105) obtained lactation for more than a year in the thyroidectomized goat. Nelson and Tobin (176) and Nelson (169) have reported observing no effect of thyroidectomy in the rat on lactation while Folley (44) reports marked diminution of milk secretion following the operation on this species. Dragstedt *et al.* (33) report apparent normal milk secretion in the thyroidectomized bitch provided that tetany was prevented.

In the administration of thyroid and thyroxine to intact animals there is better agreement as to its effect in increasing milk secretion. Graham (84, 85), Jack and Bechdel (118), Folley and White (57), Herman *et al.* (103), and Hurst *et al.* (115) have reported on increased milk production in the cow following either thyroid feeding or injection of thyroxine. De Fremery (60) reported opposite results in goats with thyroxine, which Folley (45) explains as probably being due to too large doses. All observing increased milk production in the cow, with the exception of Jack and Bechdel (118), also observed increased fat percentage, pointing to the thyroid being especially involved in milk-fat synthesis. Folley and White (57) observed that thyroxine injections raised milk production to a peak, and with continued injection milk production declined at a normal rate but remained at the higher level. Attention is again called to the reports of Folley and Young (55, 56), where anterior pituitary extracts containing the thyrotropic hormone are more effective in increasing lactation than preparations without this principle. However, Grumbrecht and Von Düsterlo (99) reported the thyrotropic hormone decreased lactation in the guinea pig. Di-iodotyrosin has been reported as increasing milk production in the guinea pig by Grumbrecht and Von Düsterlo (99) and in women by Küstner (134). Turner (256) has reported oral administration of iodized skim milk to increase milk production in goats comparable to thyroid, the active principle being termed thyrolactin (256). Heathman and Turner (104) have reported an assay method for thyrolactin.

Graf *et al.* (83) noted no effect upon amount of milk by administration of small doses of dinitrophenol but marked changes in composition. On large toxic doses there was a diminution in milk with more marked changes in composition. Brower and Martin (19) observed marked declines in milk flow as well as changed composition when this drug was administered to goats.

3. Adrenals. There is an increasing literature that the adrenal cortex hormone is needed for normal lactation, as ablation of the adrenals has been reported by Carr (27), Swingle and Pfiffner (249), Gaunt (69), Britton and Kline (18), Nelson and Gaunt (173), Gaunt and Tobin (70),

Brownell *et al.* (23), and others to prevent normal lactation. The need for the adrenal cortex hormone in conjunction with prolactin to initiate lactation in hypophysectomized animals has previously been cited. The mode of action of the adrenal cortical hormone is speculative. Brownell *et al.* (23) suggested a special lactation hormone in the adrenals but Folley (42) and Nelson and Gaunt (173) suggest that the action of the adrenals is indirect. They propose that the general upset in metabolism in the adrenalized animal is responsible for the adverse effects on lactation. The work of Climenko and McChesney (30) showing that epinephrin administration augmented the effect of the cortical hormone and prolactin in hypophysectomized animals is of interest and adds emphasis to the fact that complete lactation is dependent upon the interaction of a number of endocrine secretions.

B. *When Milk Is Secreted.* The older idea that milk secretion takes place in two phases, one in the interim between milking and the other at milking time due to the milking stimulus, was supported, among others, by the reports of Isaachsen (117), who stated that cows producing 5 to 6 kilos per day secreted 2 to $2\frac{1}{2}$ kilos during milking, and of Maxwell and Rothera (157), who concluded at least 40 per cent of the milk produced by rats was secreted during the nursing act. The capacity of udders for containing all the milk produced at a milking has been reported by Gaines (63) for goats and by Zwart (270) and Swett (247) for cows by injecting solutions into the udder after withdrawal of the milk. Zwart (270), Gaines and Sanmann (64), Gowen and Tobin (79), Petersen *et al.* (190), and Swett *et al.* (248), slaughtered cows before milking at the regular milking time and estimated the amount of milk in the udder by either post-mortem milking or by chemical analysis of the udders, and were able to account for all or nearly all the milk predicted on the basis of pre-slaughter records. On the basis of the evidence at hand, it must be concluded that the milk is secreted in the interval between milkings and that there is no evidence for increased rate of secretion during the milking act. The reports of Shaw and Petersen (233) would indeed indicate that during the milking act there is complete cessation of secretion, for the mammary venous blood at this time contains more fat, calcium, and phosphorus than the arterial blood.

C. *Equilibria between Milk and Blood.* While milk is isotonic with blood, each having an osmotic pressure of 6.6 atmospheres, the two substances are not in equilibrium. According to Simms (225), milk contains, on a molar basis, 20 times the fat, 40 times the sugar, 7 times the potassium, 14 times the calcium, 4 times the magnesium, and 7 times the PO_4 content of blood. On the other hand, blood contains 2 times the protein, 8 times the sodium, and 4 times the chlorine content of milk. Simms dialyzed milk against blood serum and found a shift toward the same concentration for the salts, but magnesium and calcium were still 2.1 and 4.4 times as concentrated, respectively, in the milk as in the serum at the end of the dialysis.

For the above-mentioned constituents the mammary gland acts in a selective manner, preferentially absorbing some and repelling others. For other substances the mammary gland behaves as a permeable membrane in which the levels are the same in both the blood and milk. Peskett (183) has shown that urea concentration of blood and milk are identical. The same is probably true for other normal blood constituents such as uric acid, creatine and creatinine. Many substances administered orally or inhaled will likewise pass from the blood into the milk.

Any disturbance within the udder, such as mastitis, introduction of foreign substances, and continued pressure within the gland, will interfere with the normal behavior of the gland and cause it to behave more like a simple membrane. Petersen and Rigor (194) studied the effect of leaving milk in the udder for 24, 36 and 120 hours and found the total solids, protein, pH, and ash values to increase while the lactose decreased. Garrison and Turner (68) noted that suspending milking for 24 hours tended in the same direction and also observed the catalase and chloride content of the milk to increase. Porcher and Muffet (202) observed that the casein content of retained milk declined and the globulin increased.

Udder irrigations have shown the mammary gland to behave contrary to that expected on a basis of known physical laws. Filling the udder with distilled water immediately after milking decreased production but slightly according to Petersen and Rigor (195) and Garrison and Turner (68) and lowered the lactose and increased the total solids, protein, pH, and ash contents slightly. Hucker and Lee (114) found a 0.12 per cent sodium chloride solution to have little effect. Increasing the concentration of salts, sugar, and a mixture of salts intensified the disturbance, which lasted for several days after the injections. When hypertonic solutions were injected (195) it was observed that practically all of the injections were resorbed in 12 hours where it was expected that water would pass from the blood into the more concentrated solution to increase the volume. Still harder to explain is the effect of reinjecting the milk withdrawn from the udder, which has been reported by Jackson and Rothera (120), Davidson (32), and Garrison and Turner (68) to have the same effects as injecting salt solutions of about isotonic concentration. Petersen and Turner (201) have found that injection of blood serum and a 6 per cent gum acacia concentration in Ringer's solution had the severe effects of a hypertonic salt solution.

Oral or subcutaneous administration of toxic doses of dinitrophenol (19, 83) have been shown to increase the permeability of the mammary gland to the sodium bicarbonate of the blood, which phenomenon is responsible for an increase in the pH of the milk.

It should be noted that with all injections, or leaving the milk in the udder, there is a great increase in the cell count of the milk; or, in other words, the characteristics of milk retained in the udder or following injection of any kind into the udder are similar to those of mastitic milk.

As a result of the studies on maximum pressures developed and equilibria phenomena established in the udder and ultimate resorption of the milk by refraining from milking, Wayne *et al.* (263) reported that no harmful effects could be observed from drying off cows by suddenly stopping milking.

D. *Relation of Pressure to Rate of Milk Secretion.* Not only is all milk secreted in the interim between milkings but due to the intra-alveolar pressure developed by the accumulating milk the rate of secretion diminishes with time and in high producing cows may be completely stopped before milking. Neusch (177), Isaachsen (117), and Tgetgel (251) reported measurements of the milk pressure within the gland cistern at milking and Petersen and Rigor (193) measured the maximum pressure developed by not milking cows. While in general these measurements showed that pressure increased with the accumulation of milk, and that with not milking a maximum pressure is developed, followed by a decline, the method does not measure the intra-alveolar pressure and is also affected by the "letting down" of milk phenomenon. Petersen and Rigor (193) maintained constant air pressures in the gland during the interim between milkings and found a progressive decrease in the rate of milk secretion as the air pressure was raised from 10 mm. to 25 mm. of mercury, when secretion was stopped. Garrison and Turner (68) used oxygen pressures at 10 and 40 mm. mercury levels and reported small secretion at the higher level, which may be due to residual milk in the gland at the time the pressure was applied, for the milk had a high fat content.

The inhibitory effect of pressure upon the rate of secretion is of great practical importance and is the chief explanation for the increase in milk production obtained by more frequent milking. The report of Ludwick *et al.* (147), where milking one side of the udder 3 times daily increased milk production as much as 16 per cent without affecting the production on the other side milked but twice daily, would indicate that the effect of more frequent milking is due to the lowering of the pressure within the gland and not to the more frequent stimulation of milking.

E. *The Synthesis of Milk.* The synthesis of the various ingredients of milk has been studied by determining the uptake of probable blood precursors by the mammary gland, by altering the level of the probable precursor in the blood, by analysis of the mammary glands, and by perfusing the surviving mammary gland.

Kaufmann and Magne (126) first used the technique of determining the difference between jugular blood and mammary vein blood which has since been used by a number of investigators. The results are erroneous, as pointed out by Blackwood and Stirling (13), for first it is assumed that the maintenance requirement of the head, drained by the jugular, is the same as that of the udder. Secondly, the composition of the jugular blood is

greatly influenced by the secretion of saliva. Blackwood and Stirling introduced the technique of using simultaneously taken arterial blood and mammary veinous blood for determination of blood precursors of milk. They used radial artery blood. Since then arterial blood has been taken from the left ventricle of goats by Lintzel (145), the internal iliac artery, through the rectal wall, by Graham *et al.* (90), the internal pudic artery, through the vaginal wall, by Maynard *et al.* (159), and by exteriorlizing the carotid artery in goats by Graham *et al.* (92). The arterio-venous difference technique, while superior to the Kaufmann-Magne method, is still subject to a number of criticisms (188). The samples must be taken simultaneously and without disturbance to the animal, for Shaw and Petersen (232) have shown as much as 14 per cent concentration of blood passing through the mammary gland during excitement. The returning lymph from the mammary gland undoubtedly alters the composition of the blood and is unaccounted for by this method, and there is no means of knowing what are the maintenance requirements of the gland itself. To obviate the effect of excitement, Reineke *et al.* (212) have suggested anesthetizing the animal. However, such procedure is found to have other effects upon the general body metabolism. Perfusion experiments described by Petersen *et al.* (200), while also subject to criticism, obviate some of the criticisms of the in-vivo experiments. Among the advantages given by Petersen *et al.* (199) for perfusion in studying milk secretion are: elimination of the metabolic factors of the body, effect of depletion of some precursor upon milk secretion, and the addition of certain substances to the perfusion that cannot be done in the intact animal. A combination of in-vivo and perfusion studies is, therefore, desirable. Study of the analysis of the mammary gland, which has not been resorted to, to any great extent, offers possibilities of contributing much to the knowledge of the metabolism within the gland.

Before proceeding to a discussion of the literature pertaining to the synthesis of the various milk ingredients there are a few problems of general importance in milk secretion to consider. First is the ratio of blood flow through the gland to the amount of milk. Assuming glucose to be quantitatively converted into lactose and on the basis of the glucose uptake by the mammary gland of cows, Graham *et al.,* (89) calculated 500 volumes of blood passes through the gland for each volume of milk produced. On the same basis Lintzel (145) calculated 256 volumes of blood per volume of milk for goats. Shaw and Petersen (231) found the ratio of blood to milk (in cows) to be 387 to 1 on the basis of calcium uptake; and 391 to 1 on the basis of combined glucose and lactic acid uptake assuming the latter two are quantitatively used for lactose synthesis. Direct measurements of blood flow by Graham (86) by means of a thermostromuhr gave only about one half the values. Jung (124) found using stromuhr essentially the same. The disagreement among the reports of ratio of blood flow to milk is difficult

to explain. It is believed that determination of the use of calcium by the mammary gland is the most accurate means of measurement and that, therefore, in the cow, the ratio is about 400 volumes blood per volume of milk.

Only one paper has appeared upon the difficult task of calculating the energy used by the mammary gland—that of Graham *et al.* (87), who estimated about 10 per cent of the total energy uptake of the gland from the blood was used by the gland itself.

1. Fat Metabolism in the Mammary Gland. Theoretically it is possible for the milk fat to come from blood fat, protein, or carbohydrate. Of the blood fats, neutral fat, phospholipids, or sterol esters must be considered as the probable precursors of milk fat. The evidence now points to neutral fat as the chief, if not exclusive, blood precursor of milk fat. Foa (41) and Petersen *et al.* (192) perfused mammary glands with oil emulsions, the former finding the oil in the milk while the latter observed none of the stained fat in the milk but reported 3 per cent of the dye was found in the gland fat. In each case, therefore, the gland took up neutral fat. Since then neutral fat of the blood has been shown to be taken up by the mammary gland by Blackwood (12), Lintzel (145), Graham *et al.* (89), Maynard *et al.* (159), Shaw and Petersen (233), and Voris *et al.* (261), all comparing arterial blood with mammary vein blood. These workers, together with Aten and Hevesey (5), have shown that the mammary gland does not take up phospholipids as was advanced by Meigs *et al.* (160), using the Kaufmann-Magne technique. Aylward *et al.* (6) reported iodized tryglycerides fed to produce lipemia in cows caused but little iodine to be detected in the phospholipid fraction of the blood and large increases in the iodized fat of the milk.

Shaw and Petersen (233) have shown that the mammary gland takes up more than enough neutral fat from the blood to account for the milk fat and support the suggestion of Hilditch and Thompson (107) and Hilditch and Paul (106) that the short chain fatty acids come from a breakdown of oleic tryglycerides. Shaw and Knodt (227) have shown that the mammary gland uses β-hydroxybutyric acid, but for what purpose is still speculative, although these authors are inclined to believe it is used for energy purposes. Shaw and Petersen (233) have reported that the uptake of fats from the blood is practically nil during milking and increases for several hours after milking.

In further support of the breakdown of higher fatty acids for the formation of the lower fatty acids of milk are the reports of Petersen *et al.* (191) and Gowen and Tobey (80) that the fat in an active mammary gland is intermediate between body fat and milk fat. This would indicate that the fat stored in the gland is gradually changed to milk fat. The location of free fatty acids in the basal part of the secretory epithelium by Kelly and Petersen (130) and the detection of lipase in the active mammary gland also tend to support the breakdown of higher acid theory.

The finding of a respiratory quotient of the mammary gland above unity by Graham *et al.* (87) and Reineke *et al.* (212) led them to postulate the formation of fat from carbohydrate, although their data of uptake of carbohydrate from the blood were not equal to the requirements for the production of lactose. Shaw (226) reported on somewhat lower respiratory quotients on perfused udders. It is generally agreed that the respiratory quotient by itself is not reliable in deducing the type of metabolism that takes place in an organ. (See Soskin (239).)

The synthesis of milk fat seems to be more or less independent of the other ingredients of the milk, as the fat content may be increased or decreased without affecting the amount of milk. The literature on the effects of the diet upon milk fat is entirely too large to review here, but it may be said that the character of the fat is greatly altered by the type of fat fed (158) and by inanition (237). Fat content of milk may be increased by increasing certain fats in the diet and decreased by cod liver oil feeding (184). Administration of thyroid, dinitrophenol, and diethylstilbestrol causes increases in fat content of the milk without necessarily affecting the quantity of the milk. Daily secretion of milk fat is more persistent than that of milk, with the decline of milk with the advance of lactation, there is an increase in fat percentage.

2. Lactose Synthesis. That blood glucose is taken up by the mammary gland has been shown by arterio-venous differences observed by Blackwood and Stirling (14), Lintzel (145), Graham *et al.* (87, 89, 90), Shaw *et al.* (227), and others, all of whom postulated blood sugar as being the precursor of milk sugar. Hypoglucemia has been produced by insulin administration by Petersen *et al.* (189), Brown *et al.* (20), Gowen and Tobey (82), Guisti and Rietti (100), Nitzecu and Nicolean (179), Macchiarno (154), and Bucciardi (24), all noting decrease in lactose content of the milk following insulin use. Similar results have been reported following phloridzin administration by Paton and Cathcart (182) and Gowen and Tobey (82). Lowered lactose contents of milk following inanition with hypoglucemia has been reported by Overman and Wright (181) and Gowen and Tobey (81).

Hyperglucemia has been produced by intravenous injections of glucose by Brown *et al.* (21), Nitzescu (178), and others, by introduction of large quantities of glucose into the stomach by Whitnah *et al.* (266), by resorption of sugars introduced into the mammary gland by Brown *et al.* (22), by thyroxine injection by Jones (121), and by implantation of adrenalin tablets by Bottomley *et al.* (16). Petersen and Boyd (187) infused glucose through the intact mammary gland through the external pudic artery to greatly increase the glucose content of the blood going through the mammary gland. In these experiments increased lactose content of the milk was reported with observed hyperglucemia except by Brown *et al.* (21) and Petersen and Boyd. Brown *et al.* consider the intravenous injection of

sugar as of doubtful value in studying lactose synthesis because of other effects of the injections. In the case of failure, of lactose increase due to infusion of large quantities of glucose in the external pudic artery, Petersen (185) has suggested that increased amounts of lactose may be formed in the gland as the result of more available glucose, but, because of increased osmotic pressure, the excess (above normal) lactose diffuses back into the blood. This explains why it is possible to reduce lactose markedly by hypoglucemia, while it can be increased but slightly by hyperglucemia.

That glucose is the only precursor of lactose is to be doubted as there is good evidence that lactic acid is involved in lactose synthesis. Graham (86) and Shaw *et al.* (227) reported that the mammary gland takes up lactic acid from the blood and the latter calculated the glucose plus lactic acid uptake by the mammary gland, would be about enough to account for the lactose while the glucose uptake alone is not adequate. The possibility of glycoproteins being a source of sugar to the mammary gland complicates matters still further, as Reineke *et al.* (213) have reported lactating glands to take up glucose equivalent to more than 2 mgm. per 100 ml. of plasma from this source.

Several have attempted to establish the lactose precursors by the method of synthesis. Of these, only Grant (93, 94) and Petersen and Shaw (196) have definitely identified lactose as the end product. Grant demonstrated lactose formation from glucose added to fresh slices and observed no increase from the addition of hexose monophosphates or phosphoglycerate. Petersen and Shaw, however, could not demonstrate lactose formation from glucose and macerated mammary gland tissue, but did so when lactic acid was added. Since Petersen and Shaw (197) demonstrated that the active mammary gland contains 0.20 per cent glycogen, the question is raised as to the possibility that the breakdown of glycogen is essential for the formation of lactose and that Grant's mammary slices contained considerable glycogen. Weinbach's (264) postulation that the mammary gland contains a non-reducing precursor for lactose fits in with such a conjecture.

3. Nitrogen Metabolism in the Udder. Less is known about the synthesis of milk proteins than any of the other ingredients of milk. The only safe statement at the present time is that the precursors of milk proteins must be some of the blood nitrogen compounds, with some good evidence to indicate that blood globulin is involved. Cary (28), using the Kaufmann Magne technique, was the first to claim that blood amino acids were the precursors of milk protein, which was supported by Blackwood (11), using radial artery and mammary veinous bloods for analysis. Graham *et al.* (87, 91), Shaw and Petersen (229, 231), and Reineke *et al.* (211), however, showed that the uptake of amino acids by the mammary gland was inadequate to account for the milk proteins, and, further, Graham *et al.* (88) and Shaw and Petersen (229) reported that the mammary gland produced

urea in quantities to account for the amino acid nitrogen taken up by the gland. Shaw and Petersen (229) reported that the mammary gland did not take up uric acid, creatine, and creatinine. Graham *et al.* (91) and Reineke *et al.* (211) claim that the mammary gland takes up considerable quantities of globulin from the blood, and, recently, Reineke *et al.* (213) have reported that glycoproteins, which are globulins, are taken up in appreciable quantities. The latter, together with the report of Jackson and Gortner (119) that in lactating glands globulin predominates and in nonlactating glands albumin predominates, indicates that probably globulin of the blood is a precursor of the milk proteins. The fact that in the blood there may be reversible shifts from albumin to globulin must be recognized as a possible explanation for an apparent uptake of globulin from the blood.

The report of finding arginase in the mammary gland by Shaw and Petersen (230) does not simplify the picture. Does the arginase merely split off urea from incoming arginine? Or, as a result of deamination of amino acids, is urea formed from CO_2 and ammonia? Much more work is required before these questions and other fundamental questions relating to milk protein formation can be definitely answered.

While arterio-venous blood analysis has contributed much to a better understanding of milk synthesis, merely ascertaining the uptake of a blood constituent by the mammary gland does not necessarily prove such is a definite precursor for a definite milk constituent. Before any blood constituent can be definitely established as a percursor for any milk constituent, its metabolism in the udder must be determined. To date, very little work has been done in this direction. The synthesis of lactose by the mammary gland from glucose and lactic acid is a small beginning; even that does not go far enough, for it is possible and even probable that both must go through glycogen.

The reports by Kelly (129) and Virtanen (260) of finding lipase in the mammary glands suggest that studies be made on the mammary gland, involving the use of this enzyme. Phosphatase, reported to be present in mammary glands by Kay (127) and found to be similar to the kidney phosphatase and to be present in large quantities in the mammary gland by Folley and Kay (47), suggests the great importance of phosphorus metabolism in milk synthesis. Folley and White (57) suggest the mammary gland synthesizes large quantities of phosphatase, part of which is excreted in the milk.

Very little work has been reported on the proteolytic enzymes in the mammary gland. Tateyama (250) reported finding a peptidase in human breasts, and Shaw and Petersen (230) have reported finding arginase in bovine glands.

Of carbohydrate enzymes, Grimmer (96) and Tateyama (250) have reported finding amylase, and Kleiner and Tauber (132) have reported finding maltase.

III. THE EJECTION OR "LET DOWN" OF MILK

Many postulations have been advanced to account for the commonly observed response to the milking or nursing act, in which the gland becomes turgid. The oldest postulate is that most of the milk was secreted during the milking act as the result of a nervous stimulus. The reports of Swett *et al.* (248), Petersen *et al.* (190), Gaines and Sanmann (64), and others, where all or nearly all the milk expected was obtained from excised udders, rather definitely established the fact that all the milk drawn at a milking is present at the beginning of a milking.

Hammond (102) advanced the hypothesis that the milk was forced out of the alveoli by erection. The milking stimulus reflexly caused the teat and gland to become engorged with blood, according to this hypothesis. The regression in the size of the udder with the progress of milking must be taken as evidence against the validity of the theory.

In another theory, supported by the arguments of Zeitzschmann (269), a contractile mechanism in the udder and teats is postulated as being responsible for the retaining of the milk. The failure for "let down" of the milk is, therefore, a positive act according to this postulate.

That the nervous system is involved in milking is supported by a number of experiments, but how the nerves are involved has only recently been clarified. Sympathectomy, by Ingelbrecht (116), Selye (221), Seyle *et al.* (224), Bacq (7), Cannon and Bright (26), and Simeone and Ross (234), was observed in most cases to inhibit lactation to a greater or less extent, but, in some cases, not until after the next gestation following the operation. In the case of Ingelbrecht, resection of the spinal cord was made so the front breasts of rats were inervated and the posterior denervated. He observed that the young nursing the posterior breasts died of starvation unless the front breasts were nursed at the same time. Ribbert (214) observed that transplanted mammary tissue would function; such transplant being free of nervous connections. Ely and Petersen (35) resected the inguinal nerve trunk to the one side of the udder of cows and noted no effect upon either the growth of the udder or the amount of milk let down. Petersen and Shaw (198) observed that cows under anesthesia failed to let down their milk even though the udders were filled with milk, as proven by the let down of milk to intravenous injections of the oxytocic hormone. With the exception of the last, all of these experiments indicate the involvement of the nervous system, which can best be explained after considering the hormonal relationship to milk "ejection."

The posterior pituitary extract has been studied in its effect upon milk secretion and reported to be either a galactogogue or to cause more complete evacuation of the milk in the gland, by Ott and Scott (180), Gaines (63), Simpson and Hill (235, 236), Hill and Simpson (108, 109), Hammond (101), Turner and Slaughter (258), McCandlish (153), and others, in

various species. For these experiments the extract contained both the pitressor and oxytocic principles. Since Kamm succeeded in separating the two fractions, Ely and Petersen (35), on the basis of experimental work, concluded that the oxytocic fraction causes a contraction of the musculature shown by Swanson and Turner (246) to be found around the alveoli. Ely and Petersen suggest that the sensory endings on the teat and afferent pathways are the only nerves needed for milking response. A stimulation of the nerve endings on the teat and udder, carried upward through the central nervous system, caused the oxytocic principle to be ejected from the posterior pituitary into the blood, whence it is carried to the udder. This reflex may become conditioned to many other stimuli, such as feeding, rattling of milking utensils, washing of the udder, etc. Gomez (72) has reported upon the need for posterior pituitary extract for lactation in the hypophysectomized rat in support of the theory.

Ely and Petersen (35) noted that fright inhibited a response to the milking stimulus. Miller and Petersen (161) have reported that stimulating cows to let down their milk by washing the udder 20 minutes before milking resulted in incomplete milking and gradual "drying off." Taking too long to milk (more than 8 minutes) had the same effect. Almost any factor which attracts the attention of the cow during milking will detract from a complete response to the milking stimulus.

From the foregoing, it is apparent that research work during the past decade has contributed much to a better understanding of the fundamentals underlying the phenomenon of lactation. Crucial evidence for all the pituitary factors involved in mammary gland development is still wanting. Much more work needs to be done on the blood precursors and on the mode of synthesis of the various milk constituents in the mammary gland before the complete picture of milk secretion is at hand. The blood precursors of milk protein and particularly their synthesis in the mammary gland is obscure as is also the forces in the gland secretory tissue that select certain constituents from the blood and hold back others. The phenomenon of ejection or "let down" of milk, although not completely solved, is now fairly well established, and this knowledge promises to contribute much to better milking practices.

Lastly comes the important problem of practical application of the newly found knowledge, an attack of which has scarcely begun. Will it be possible to increase mammary growth and subsequent lactation through application of the knowledge now at hand? Will it be possible to increase and prolong lactation at a higher level due to the administration of lactogenic substances? These and other questions can only be answered after much more research has been done.

REFERENCES

(1) ANSELMINO, K. J., AND HOFFMANN, F. Zentbl. Gynak, **60**: 501. 1936.

(2) ASDELL, S. A., BROOKS, H. J., SALISBURY, G. W., AND SEIDENSTEIN, H. R. Cornell Agr. Expt. Sta. Mem. 198. 1936.
(3) ASIMOV, G. G., AND KROUZE, N. K. JOUR. DAIRY SCI., 20: 289. 1937.
(4) ASTWOOD, E. B., GESCHICTER, C. F., AND RAUSCH, E. O. Anat. Rec., 61: 373. 1937.
(5) ATEN, A. H. W., AND HEVESY, G. Nature, Lond., 142: 111. 1938.
(6) AYLWARD, F. X., BLACKWOOD, J. H., AND SMITH, J. A. B. Biochem. Jour., 31: 130. 1937.
(7) BACQ, Z. M. Amer. Jour. Physiol., 99: 444. 1932.
(8) BATES, R. W., RIDDLE, O., AND LAHR, E. L. Soc. Expt. Biol. and Med. Proc., 31: 1223. 1934.
(9) BATES, R. W., RIDDLE, O., AND LAHR, E. L. Amer. Jour. Physiol., 113: 259. 1935.
(10) BEALL, D., AND RICHSTEIN, T. Nature, Lond., 142: 479. 1938.
(11) BLACKWOOD, J. H. Biochem. Jour., 26: 357. 1932.
(12) BLACKWOOD, J. H. Biochem. Jour., 28: 1346. 1934.
(13) BLACKWOOD, J. H., AND STIRLING, J. D. Biochem. Jour., 26: 357. 1932.
(14) BLACKWOOD, J. H., AND STIRLING, J. D. Biochem. Jour., 26: 362. 1932.
(15) BOTTOMLEY, A. C., AND FOLLEY, J. S. Roy. Soc. Proc. Ser. B. 126: 224. 1938.
(16) BOTTOMLEY, A. C., FOLLEY, S. J., WALKER, F. H. A., AND SCOTT WATSON, H. M. Jour. Endocrinology, 1: 284. 1939.
(17) BRADBURY, J. T. Endocrinology, 29: 393. 1941.
(18) BRITTON, S. W., AND KLINE, R. F. Amer. Jour. Physiol., 115: 627. 1936.
(19) BROWER, E., AND MARTIN, J. Le Lait, 18: 337. 1938.
(20) BROWN, W. R., PETERSEN, W. E., AND GORTNER, R. A. JOUR. DAIRY SCI., 19: 147. 1936.
(21) BROWN, W. R., PETERSEN, W. E., AND GORTNER, R. A. JOUR. DAIRY SCI., 19: 177. 1936.
(22) BROWN, W. R., PETERSEN, W. E., AND GORTNER, R. A. JOUR. DAIRY SCI., 19: 243. 1936.
(23) BROWNELL, K. A., LOCKWOOD, J. E., AND HARTMAN, F. A. Soc. Expt. Biol. and Med. Proc., 30: 783. 1933.
(24) BUCCIARDI, C. Boll. Soc. Biol. Sper. 3: 527. 1928.
(25) BUTCHER, E. O. Soc. Expt. Biol. and Med. Proc., 42: 571. 1939.
(26) CANNON, W. B., AND BRIGHT, E. M. Amer. Jour. Physiol., 97: 319. 1931.
(27) CARR, J. L. Soc. Expt. Biol. and Med. Proc., 29: 131. 1931.
(28) CARY, C. A. Jour. Biol. Chem., 43: 477. 1920.
(29) CHANCE, M. R. A., ROWLANDS, I. W., AND YOUNG, F. G. Jour. Endocrinology, 1: 239. 1939.
(30) CLIMENKO, D. R., AND MCCHESNEY, E. W. Endocrinology, 28: 710. 1941.
(31) CORNER, G. W. Amer. Jour. Physiol., 95: 43. 1930.
(32) DAVIDSON, F. A. Jour. Agr. Res., 33: 873. 1926.
(33) DRAGSTEDT, S. R., SUDAN, A. C., AND PHILLIPS, K. Amer. Jour. Physiol., 59: 477. 1924.
(34) DRUMMOND-ROBINSON, G., AND ASDELL, S. A. Jour. Physiol., 61: 608. 1926.
(35) ELY, F., AND PETERSEN, W. E. JOUR. DAIRY SCI., 24: 211. 1941.
(36) EHRHARDT, K. München. Med. Wchnschr., 29: 1163. 1939.
(37) EHRHARDT, K., AND VOLLER, H. F. Endokrinologie, 22: 19. 1939.
(38) ESPE, D. Secretion of milk. Collegiate Press Inc., Ames, Iowa. 1938.
(39) EVANS, E. I. Soc. Expt. Biol. and Med. Proc., 30: 1372. 1933.
(40) EVANS, E. I. Amer. Jour. Physiol., 116: 45. 1936.
(41) FOA, CARLO. Arch. di Fisiol., 10: 402. 1912.

(42) FOLLEY, S. J. Biochem. Jour., 30: 2262. 1936.
(43) FOLLEY, S. J. Lancet, 235: 389. 1938.
(44) FOLLEY, S. J. Jour. Physiol., 93: 401. 1938.
(45) FOLLEY, S. J. Biol. Rev., 15: 421. 1940.
(46) FOLLEY, S. J. Lancet, Jan., p. 40. 1941.
(47) FOLLEY, S. J., AND KAY, H. D. Biochem. Jour., 29: 1837. 1935.
(48) FOLLEY, S. J., AND KON, S. K. Roy. Soc. Proc. Ser. B., 124: 476. 1937.
(49) FOLLEY, S. J., AND WATSON, H. M. S. Lancet, 2: 423. 1938.
(50) FOLLEY, S. J., SCOTT WATSON, H. M., AND BOTTOMLEY, A. C. Jour. Physiol., 98: 15. 1938.
(51) FOLLEY, S. J., SCOTT WATSON, H. M., AND BOTTOMLEY, A. C. Jour. Physiol., 98: 15. 1940.
(52) FOLLEY, S. J., WATSON, H. M. S., AND BOTTOMELEY, A. C. Jour. Dairy Res., 12: 1. 1941.
(53) FOLLEY, S. J., AND YOUNG, F. G. Chem. Indus. Lond., 56: 96. 1937.
(54) FOLLEY, S. J., AND YOUNG, F. G. Proc. Roy. Soc. B., 126: 45. 1938.
(55) FOLLEY, S. J., AND YOUNG, F. G. Biochem. Jour., 33: 192. 1939.
(56) FOLLEY, S. J., AND YOUNG, F. G. Lancet, March, p. 380. 1941.
(57) FOLLEY, S. J., AND WHITE, P. Roy. Soc. Proc. Ser. B., 120: 346. 1936.
(58) FOSS, G. L., AND PHILLIPS, P. Brit. Med. Jour., ii: 887. 1938.
(59) FRANKL, O. Amer. Jour. Obst. and Gynec., 6: 399. 1923.
(60) DE FREMERY, P. Jour. Physiol., 87: 50. 1936.
(61) DE FREMERY, P. Arch. Néerland. de Zool., 3: 48. 1938.
(62) FREUD, J., AND WIJSENBECK, I. A. Acta brev. Néerland. Physiol., 8: 11. 1938.
(63) GAINES, W. R. Dissertation University of Chicago, Ogden Graduate School. 1915.
(64) GAINES, W. L., AND SANMANN, F. P. Amer. Jour. Physiol., 80: 691. 1927.
(65) GARDNER, W. U. Endocrinology, 28: 53. 1940.
(66) GARDNER, W. U., AND TURNER, C. W. Mo. Agr. Expt. Sta. Res. Bul. 196. 1933.
(67) GARDNER, W. U., AND VAN WAGENEN, G. Endocrinology, 22: 164. 1938.
(68) GARRISON, E. R., AND TURNER, C. W. Mo. Agr. Expt. Sta. Res. Bul. 234. 1936.
(69) GAUNT, R. Amer. Jour. Physiol., 103: 494. 1933.
(70) GAUNT, R., AND TOBIN, C. E. Amer. Jour. Physiol., 115: 588. 1936.
(71) GOMEZ, E. T., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., 34: 404. 1936.
(72) GOMEZ, E. T. JOUR. DAIRY SCI., 23: 537. 1940.
(73) GOMEZ, E. T., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., 35: 365. 1936.
(74) GOMEZ, E. T., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., 36: 78. 1937.
(75) GOMEZ, E. T., AND TURNER, C. W. Mo. Agr. Expt. Sta. Res. Bul. 259. 1937.
(76) GOMEZ, E. T., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., 36: 80. 1937.
(77) GOMEZ, E. T., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., 37: 607. 1938.
(78) GOMEZ, E. T., TURNER, C. W., AND REECE, R. P. Soc. Expt. Biol. and Med. Proc., 36: 286. 1937.
(79) GOWEN, J. W., AND TOBEY, E. W. Jour. Gen. Physiol., 10: 949. 1927.
(80) GOWEN, J. W., AND TOBEY, E. R. Jour. Gen. Physiol., 12: 123. 1928.
(81) GOWEN, J. W., AND TOBEY, E. R. Jour. Gen. Physiol., 15: 45. 1931.
(82) GOWEN, J. W., AND TOBEY, E. R. Jour. Gen. Physiol., 15: 66. 1931.
(83) GRAF, G. C., LUDWICK, T. M., AND PETERSEN, W. E. JOUR. DAIRY SCI., 23: 539. 1940.

(84) GRAHAM, W. R., JR. Jour. Nutr., 7: 407. 1934.
(85) GRAHAM, W. R., JR. Biochem. Jour., 28: 1368. 1934.
(86) GRAHAM, W. R. Jour. Biol. Chem., 122: 1. 1937.
(87) GRAHAM, W. R., HOUCHIN, O. B., PETERSEN, V. E., AND TURNER, C. W. Amer. Jour. Physiol., 122: 150. 1938.
(88) GRAHAM, W. R., HOUCHIN, O. B., AND TURNER, C. W. Jour. Biol. Chem., 120: 29. 1937.
(89) GRAHAM, W. R., JR., JONES, T. S. G., AND KAY, H. D. Roy. Soc. London Proc., Ser. B., 120: 330. 1936.
(90) GRAHAM, W. R., JR., KAY, H. D., AND MCINTOSH, R. A. Roy. Soc. Proc., Ser. B., 120: 319. 1936.
(91) GRAHAM, W. R., PETERSON, V. E., HOUCHIN, O. B., AND TURNER, C. W. Jour. Biol. Chem., 122: 275. 1938.
(92) GRAHAM, W. R., TURNER, C. W., AND GOMEZ, E. T. Mo. Agr. Expt. Sta. Res. Bul. 260. 1937.
(93) GRANT, G. A. Biochem. Jour., 30: 1905. 1935.
(94) GRANT, G. A. Biochem. Jour., 30: 2027. 1936.
(95) GREEP, R. O., AND STAVELY, H. E. Endocrinology, 29: 18. 1941.
(96) GRIMMER, W. Chemie und Physiologie der Milch. 2nd ed. Paul Parey, Berlin. 1926.
(97) GRUETER, F. 2nd Internatl. Cong. Sex Res. Proc., p. 443. 1930.
(98) GRÜTER, F., AND STRICKER, P. Klin. Wchnschr., 8: 2322. 1929.
(99) GRUMBRECHT, P., AND VON DÜSTERLO, G. Klin. Wchnschr., 16: 513. 1937.
(100) GUISTI, L., AND RIETTI, C. T. Rev. de la Assoc. Med. Agr. Soc. d Biol., 36: 279. 1923.
(101) HAMMOND, J. Roy. Soc. Proc., Ser. B., 89: 534. 1917.
(102) HAMMOND, J. Vet. Rec., 16: 519. 1936.
(103) HERMAN, H. A., GRAHAM, W. R., JR., AND TURNER, C. W. Mo. Agr. Expt. Sta. Res. Bul. 275. 1938.
(104) HEATHMAN, W. W., AND TURNER, C. W. JOUR. DAIRY SCI., 23: 534. 1940.
(105) HIBBS, J. W., SUTTON, T. S., AND KRAUSS, W. E. JOUR. DAIRY SCI., 24: 498. 1941.
(106) HILDITCH, T. P., AND PAUL, H. Biochem. Jour., 30: 1905. 1936.
(107) HILDITCH, T. P., AND THOMPSON, H. M. Biochem. Jour., 30: 677. 1936.
(108) HILL, R. L., AND SIMPSON, S. Soc. Expt. Biol. and Med. Proc., 11: 82. 1914.
(109) HILL, R. L., AND SIMPSON, S. Quart. Jour. Expt. Physiol., 8: 103. 1914.
(110) HOLST, S., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., 42: 479. 1939.
(111) HOOKER, C. W., AND WILLIAMS, W. R. Endocrinology, 28: 42. 1941.
(112) HOUSAY, B. A. Soc. Biol. Paris, Compt. Rend., 120: 496. 1935.
(113) HOUSAY, B. A. Soc. Biol. Paris, Compt. Rend., 120: 496. 1935.
(114) HUCKER, G. G., AND LEE, D. New York Agr. Expt. Sta. Bul. 205. 1932.
(115) HURST, V., REECE, R. P., AND BARTLETT, J. W. JOUR. DAIRY SCI., 23: 635. 1940.
(116) INGELBRECHT, P. Soc. de Biol., Compt. Rend., 120: 1369. 1935.
(117) ISAACHSEN, N. World's Dairy Congress Proc., 2: 1021. 1923.
(118) JACK, E. L., AND BECHDEL, S. I. JOUR. DAIRY SCI., 18: 195. 1935.
(119) JACKSON, S. M., AND GORTNER, R. A. Jour. Biol. Chem., 123: 719. 1938.
(120) JACKSON, L. C., AND ROTHERA, A. C. Biochem. Jour., 8: 1. 1914.
(121) JONES, T. S. G. Jour. Soc. Chem. Indus. Lond., 54: 928. 1935.
(122) DEJONGH, S. E. Acta. Brev. Néerl. Physiol., 3: 52. 1933.
(123) DEJONGH, S. E., AND DINGEMANSE, E. Arch. Néerl. and Physiol., 16: 288. 1931.
(124) JUNG, L. Le Lait, 13: 307. 1933.

(125) KABAK, J., AND KISILSTEIN, M. Ztschr. f. Zucht. B., 29: 301. 1933.
(126) KAUFMANN, M., AND MAGNE, H. Acad. Sci. Paris, Comp. Rend., 143: 799. 1906.
(127) KAY, H. D. Biochem. Jour., 19: 433. 1925.
(128) KELLAR, R. J., AND SUTHERLAND, J. K. Amer. Jour. Obst. and Gynec., 46: 1. 1939.
(129) KELLY, P. L. JOUR. DAIRY SCI., 21: 122. 1938.
(130) KELLY, P. L., AND PETERSEN, W. E. JOUR. DAIRY SCI., 22: 7. 1939.
(131) KENNY, M., AND KING, E. J. Lancet, 237: 828. 1939.
(132) KLEINER, I. S., AND TAUBER, H. Jour. Biol. Chem., 99: 241. 1932.
(133) KURZROK, R., BATES, R. W., RIDDLE, O., AND MILLER, E. G. Endocrinology, 18: 18. 1934.
(134) KÜSTNER, H. München. Med. Wchnschr., 81: 1261. 1934.
(135) LACQUER, E., BORCHARDT, E., DINGEMANSE, E., AND DE JONGH, S. E. Dtsch. Med. Wchnschr., 54: 465. 1928.
(136) LEBLOND, C. P. Soc. Biol. Paris, Compt. Rend., 124: 1062. 1937.
(137) LEHMANN, J. München Med. Wchnschr., 85: 1781. 1938.
(138) LEONARD, S. L., AND REECE, R. P. Endocrinology, 28: 65. 1941.
(139) LESSMANN, F. Ztsch. f. geburts u gynäk., 119: 271. 1939.
(140) LEWIS, A. A. JOUR. DAIRY SCI., 23: 540. 1940.
(141) LEWIS, A. A., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., 39: 435. 1938.
(142) LEWIS, A. A., AND TURNER, C. W. Mo. Agr. Expt. Sta. Res. Bul. 310. 1939.
(143) LEWIS, A. A., AND TURNER, C. W. Amer. Soc. Anim. Prod. Proc., 33: 63. 1940.
(144) LEWIS, A. A., TURNER, C. W., AND GOMEZ, E. T. Endocrinology, 24: 157. 1939.
(145) LINTZEL, W. Ztschr. f. Zücht. B, 29: 219. 1934.
(146) LITT, S. Amer. Jour. Obst. and Gynec., 26: 37. 1933.
(147) LUDWICK, T. M., SPIELMAN, A., AND PETERSEN, W. E. JOUR. DAIRY SCI., 24: 505. 1941.
(148) LYONS, W. R. Soc. Expt. Biol. and Med. Proc., 37: 207. 1937.
(149) LYONS, W. R., CHAIKOFF, I. L., AND REICHERT, F. L. Soc. Expt. Biol. and Med. Proc., 31: 303. 1933.
(150) LYONS, W. R., AND PAGE, E. Soc. Expt. Biol. and Med. Proc., 32: 1049. 1936.
(151) LYONS, W. R., AND SAKO, Y. Soc. Expt. Biol. and Med. Proc., 44: 398. 1940.
(152) MACBRYDE, C. M. Jour. Amer. Med. Assoc., 112: 1045. 1939.
(153) MCCANDLISH, A. C. JOUR. DAIRY SCI., 1: 6. 1918.
(154) MACCHIARNLO, O. Bol. Soc. Ital. Biol. Sper., 3: 973. 1928.
(155) MCPHAIL, M. K. Roy. Soc. Proc., Ser. B., 117: 34. 1935.
(156) MCSHAN, W. H., AND FRENCH, H. E. Jour. Biol. Chem., 117: 111. 1937.
(157) MAXWELL, A. L. J., AND ROTHERA, A. C. H. Jour. Physiol., 49: 483. 1915.
(158) MAYNARD, L. A., MCCAY, C. M., AND MADSEN, L. L. JOUR. DAIRY SCI., 19: 49. 1936.
(159) MAYNARD, L. A., MCCAY, C. M., ELLIS, G. H., HODSON, A. Z., AND DAVIS, G. K. N. Y. (Cornell) Agr. Expt. Sta. Mem., 211. 1938.
(160) MEIGS, E. B., BLATHERWICK, N. R., AND CARY, C. A. Jour. Biol. Chem., 37: 1. 1919.
(161) MILLER, K., AND PETERSEN, W. E. JOUR. DAIRY SCI., 24: 225. 1941.
(162) MIXNER, J. P. JOUR. DAIRY SCI., 23: 542. 1940.
(163) MIXNER, J. P., AND TURNER, C. W. Endocrinology, 29: 324. 1941.
(164) NATHANSON, I. T., SHAW, D. T., AND FRANSEEN, C. C. Soc. Biol. and Med. Proc., 42: 652. 1939.
(165) NELSON, W. O. Endocrinology, 18: 33. 1934.

(166) NELSON, W. O. Anat. Rec., **60**: 69. 1934.
(167) NELSON, W. O. Physiol. Rev., **16**: 488. 1936.
(168) NELSON, W. O. Anat. Rec. Suppl., **72**: 117. 1938.
(169) NELSON, W. O. Amer. Jour. Physiol., **126**: 592. 1939.
(170) NELSON, W. O. Anat. Rec. Suppl., **73**: 39. 1939.
(171) NELSON, W. O. Am. Physiol. Soc. Proc., 53rd Ann. Mtg., 209. 1941.
(172) NELSON, W. O., AND GAUNT, R. Soc. Expt. Biol. and Med. Proc., **34**: 671. 1936.
(173) NELSON, W. O., AND GAUNT, R. Cold Spr. Harb. Quart. Biol., **5**: 398. 1937.
(174) NELSON, W. O., AND GAUNT, R. Soc. Expt. Biol. and Med. Proc., **36**: 136. 1937.
(175) NELSON, W. O., AND TOBIN, C. E. Anat. Rec. Suppl., **67**: 111. 1936–7.
(176) NELSON, W. O., AND TOBIN, C. E. Endocrinology, **21**: 670. 1937.
(177) NEUSCH, J. Über das soganante Aufziehen der Milch. Paul Parey, Berlin. 1910.
(178) NITZESCU, I. I. Soc. de Biol. Compt. Rend., **93**: 1319. 1925.
(179) NITZESCU, I. I., AND NICOLEAN, O. Soc. de Biol. Compt. Rend., **91**: 1462. 1924.
(180) OTT, I., AND SCOTT, J. C. Soc. Expt. Biol. Med. Proc., **8**: 48. 1910.
(181) OVERMAN, O. R., AND WRIGHT, K. E. Jour. Agr. Res., **35**: 637. 1927.
(182) PATON, D. N., AND CATHCART, E. P. Jour. Physiol., **42**: 179. 1911.
(183) PESKETT, G. R. Biochem. Jour., **28**: 1657. 1934.
(184) PETERSEN, W. E. JOUR. DAIRY SCI., **15**: 283. 1932.
(185) PETERSEN, W. E. Amer. Soc. Anim. Prod. Proc., **28**: 59. 1935.
(186) PETERSEN, W. E., LUDWICK, T. M., AND SPIELMAN, A. Unpublished data.
(187) PETERSEN, W. E., AND BOYD, W. L. Soc. Expt. Biol. and Med. Proc., **37**: 539. 1937.
(188) PETERSEN, W. E., AND BOYD, W. L. JOUR. DAIRY SCI., **22**: 437. 1939.
(189) PETERSEN, W. E., HEWITT, E. A., BOYD, W. L., AND BROWN, W. R. Jour. Amer. Vet. Med. Assoc., **79**: 217. 1931.
(190) PETERSEN, W. E., PALMER, L. S., AND ECKLES, C. H. Amer. Jour. Physiol., **90**: 573. 1929.
(191) PETERSEN, W. E., PALMER, L. S., AND ECKLES, C. H. Amer. Jour. Physiol., **90**: 582. 1929.
(192) PETERSEN, W. E., PALMER, L. S., AND ECKLES, C. H. Amer. Jour. Physiol., **90**: 592. 1929.
(193) PETERSEN, W. E., AND RIGOR, T. V. Soc. Expt. Biol. and Med. Proc., **30**: 254. 1932.
(194) PETERSEN, W. E., AND RIGOR, T. V. Soc. Expt. Biol. and Med. Proc., **30**: 257. 1932.
(195) PETERSEN, W. E., AND RIGOR, T. V. Soc. Expt. Biol. and Med. Proc., **30**: 259. 1932.
(196) PETERSEN, W. E., AND SHAW, J. C. Science, **86**: 398. 1937.
(197) PETERSEN, W. E., AND SHAW, J. C. JOUR. DAIRY SCI., **21**: 168. 1938.
(198) PETERSEN, W. E., AND SHAW, J. C. Unpublished data.
(199) PETERSEN, W. E., SHAW, J. C., AND VISSCHER, M. B. JOUR. DAIRY SCI., **22**: 439. 1939.
(200) PETERSEN, W. E., SHAW, J. C., AND VISSCHER, M. B. JOUR. DAIRY SCI., **24**: 139. 1941.
(201) PETERSEN, W. E., AND TURNER, R. Unpublished data.
(202) PORCHER, C., AND MUFFET, E. Le Lait, **10**: 394 and 528. 1930.
(203) RABALD, E., AND VOSS, H. E. Ztschr. f. Physiol. Chem., **261**: 71. 1939.
(204) RAMOS, A. P., AND COLOMBO, E. Dtsch. Med. Wchnschr., **64**: 782. 1938.
(205) REECE, R. P. Soc. Expt. Biol. and Med. Proc., **42**: 54. 1939.
(206) REECE, R. P., AND LEONARD, S. L. Soc. Expt. Biol. and Med. Proc., **42**: 200. 1939.
(207) REECE, R. P., AND LEONARD, S. L. Endocrinology **29**: 297. 1941.
(208) REECE, R. P., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., **35**: 621. 1937.
(209) REECE, R. P., AND TURNER, C. W. Mo. Agr. Expt. Sta. Res. Bul. 266. 1937.
(210) REECE, R. P., AND TURNER, C. W. Soc. Expt. Biol. and Med. Proc., **36**: 283. 1937.

(211) REINEKE, E. P., PETERSON, V. E., HOUCHIN, O. B., AND TURNER, C. W. Mo. Agr. Expt. Sta. Res. Bul. 296. 1939.
(212) REINEKE, E. P., STONECIPER, W. P., AND TURNER, C. W. Amer. Jour. Physiol., 132: 535. 1941.
(213) REINEKE, E. P., WILLIAMSON, M. B., AND TURNER, C. W. Jour. Biol. Chem., 138: 83. 1941.
(214) RIBBERT, HUGO. Arch. f. Entwickl. Mech. der Organ., 7: 688. 1898.
(215) RIDDLE, O. Jour. Amer. Med. Assoc., 115: 2276. 1940.
(216) RIDDLE, O. Ann. Rev. Physiol., Vol. III: 573–616. 1941.
(217) RIDDLE, O., AND BATES, R. W. Sex and Internal Secretions, Williams and Wilkins. Baltimore, 2nd ed. 1088.
(218) RIDDLE, O., BATES, R. W., AND DYKSHORN, S. W. Amer. Jour. Physiol., 105: 191. 1933.
(219) ROBSON, J. M. Quart. Jour. Expt. Physiol., 24: 337. 1935.
(220) SAMUELS, L. T., REINECKE, R. M., AND PETERSEN, W. E. Soc. Expt. Biol. and Med. Proc., 46: 379. 1941.
(220a) SAMUELS, L. T., REINECKE, R. M., AND PETERSEN, W. E. Unpublished data.
(221) SELYE, H. Amer. Jour. Physiol., 107: 535. 1934.
(222) SELYE, H., AND COLLIP, J. B. Endocrinology, 20: 667. 1936.
(223) SELYE, H., COLLIP, J. B., AND THOMSON, D. L. Soc. Expt. Biol. and Med. Proc., 30: 588. 1932.
(224) SELYE, H., COLLIP, J. B., AND THOMSON, D. L. Endocrinology, 18: 237. 1934.
(225) SIMMS, H. S. Internatl. Dairy Cong. Proc., 2nd Sec., 50. 1931.
(226) SHAW, J. C. JOUR. DAIRY SCI., 22: 438. 1939.
(227) SHAW, J. C., BOYD, W. L., AND PETERSEN, W. E. Soc. Expt. Biol. and Med. Proc., 38: 579. 1938.
(228) SHAW, J. C., AND KNODT, C. B. Jour. Biol. Chem., 138: 287. 1941.
(229) SHAW, J. C., AND PETERSEN, W. E. Soc. Expt. Biol. and Med. Proc., 38: 632. 1938.
(230) SHAW, J. C., AND PETERSEN, W. E. Soc. Expt. Biol. and Med. Proc., 38: 631. 1938.
(231) SHAW, J. C., AND PETERSEN, W. E. Amer. Jour. Physiol., 123: 183. 1938.
(232) SHAW, J. C., AND PETERSEN, W. E. Soc. Expt. Biol. and Med. Proc., 42: 520. 1939.
(233) SHAW, J. C., AND PETERSEN, W. E. JOUR. DAIRY SCI., 23: 1045. 1940.
(234) SIMEONE, F. A., AND ROSS, J. F. Amer. Jour. Physiol., 122: 659. 1938.
(235) SIMPSON, S., AND HILL, R. L. Amer. Jour. Physiol., 36: 347. 1915.
(236) SIMPSON, S., AND HILL, R. L. Quart. Jour. Expt. Physiol., 8: 377. 1915.
(237) SMITH, J. A. B., AND DASTUR, N. N. Biochem. Jour. 32: 1868. 1938.
(238) SMITH, G. VAN S., AND SMITH, W. V. Amer. Jour. Physiol., 103: 356. 1933.
(239) SOSKINS, S. Physiol. Rev., 21: 140. 1941.
(240) SPEERT, H. Science, 92: 461. 1940.
(241) SPEILMAN, A., LUDWICK, T. M., AND PETERSEN, W. E. JOUR. DAIRY SCI., 24: 499. 1941.
(242) SPEILMAN, A., AND PETERSEN, W. E. Unpublished data.
(243) STEWART, A. L., AND PRATT, J. P. Endocrinology, 25: 347. 1939.
(244) STOCKKLAUSNER, F., AND DAUM, F. Milchw. Forsch., 13: 448. 1932.
(245) STRICKER, P., AND GRUETER, F. Soc. Biol. Paris, Compt. Rend., 99: 1978. 1928.
(246) SWANSON, E. W., AND TURNER, C. W. JOUR. DAIRY SCI., 24: 635. 1941.
(247) SWETT, W. W. JOUR. DAIRY SCI., 10: 1. 1927.
(248) SWETT, W. W., MILLER, F. W., AND GRAVES, R. R. Jour. Agr. Res., 45: 401. 1932.
(249) SWINGLE, W. W., AND PFIFFNER, J. J. Medicine, Baltimore, 11: 371. 1932.
(250) TATEYAMA, R. Biochem. Ztschr., 163: 297. 1925.
(251) TGETGEL, B. Schweiz. Arch. f. Tierheilk., 68: 335. 1926.

(252) TRAUTMANN, A. Pflügers Arch. f. die Gesam. Physiol., **177**: 239. 1919.
(253) TURNER, C. W. Mo. Agr. Expt. Sta. Res. Bul. 156. 1931.
(254) TURNER, C. W. The Comparative Anatomy of the Mammary Glands. Univ. of Mo. Cooperative Store, Columbia, Mo.
(255) TURNER, C. W. Sex and Internal Secretions, 2nd ed. Williams and Wilkins, Baltimore. Allen, Doisy and Danforth. 740–803. 1939.
(256) TURNER, C. W. JOUR. DAIRY SCI., **23**: 535. 1940.
(257) TURNER, C. W., AND MEITES, J. Endocrinology, **29**: 165. 1941.
(258) TURNER, C. W., AND SLAUGHTER, I. S. JOUR. DAIRY SCI., **13**: 8. 1930.
(259) VAN HEWERSWYN, J., FOLLEY, S. J., AND GARDNER, W. U. Soc. Expt. Biol. and Med. Proc., **41**: 389. 1938.
(260) VIRTANEN, A. I. Hoppe-Seylers Ztschr. f. Physiol. Chem., **137**: 1. 1924.
(261) VORIS, L., ELLIS, G., AND MAYNARD, L. A. Jour. Biol. Chem., **133**: 491. 1940.
(262) WALKER, S. M., AND STANLEY, A. J. Soc. Expt. Biol. and Med. Proc., **48**: 50. 1941.
(263) WAYNE, R., ECKLES, C. H., AND PETERSEN, W. E. JOUR. DAIRY SCI., **16**: 69. 1933.
(264) WEINBACH, A. P. Jour. Gen. Physiol., **19**: 829. 1936.
(265) WERNER, A. A. Endocrinology, **24**: 119. 1939.
(266) WHITNAH, C. H., RIDDELL, W. H., AND HODGSON, R. E. JOUR. DAIRY SCI., **16**: 347. 1933.
(267) WINTERTON, W. R., AND MACGREGOR, T. N. Brit. Med. Jour., **1**: 10. 1939.
(268) YOUNG, F. G. Biochem. Jour. **32**: 656. 1934.
(269) ZEITZSCHMANN, O. Le Lait, 2: 229. 1922.
(270) ZWART, S. G. Ztschr. f. Fleisch u. Milchyg., **26**: 291. 1916.

American Dairy Science Association Announcements

MICHIGAN INVITES YOU

To the Officers and Members of the
American Dairy Science Association:

Michigan State College is looking forward to the meeting of the American Dairy Science Association here on its campus next June.

This, the oldest of the agricultural colleges, has grown into a great university but continues to regard as its most important function service to the agricultural interests of the state and nation. There have been four heads of the dairy department at Michigan State College since the creation of that department, and each of them has at one time served as President of your Association: A. C. Anderson in 1918, O. E. Reed in 1924, Dean E. L. Anthony in 1931, and Earl Weaver in 1938.

This college has unusual facilities for entertaining your group and is setting aside the use of the auditorium and certain dormitories and such other facilities as are required. It will be a good time to bring the members of your families to enjoy the early summer beauty of Michigan.

We are looking forward to your coming and extend to you a most cordial welcome. All the facilities of Michigan State College will be made available for your use and enjoyment.

(Signed) JOHN A. HANNAH
President, Michigan State College

THIRTY-SEVENTH ANNUAL MEETING, MICHIGAN STATE COLLEGE, EAST LANSING, MICHIGAN, JUNE 22–25, 1942

FIRST CALL FOR TITLES

Titles of papers to be presented should be in the hands of the program committee not later than April 1, 1942. Program chairmen are as follows:

Extension section—J. F. Kendrick, Bureau of Dairy Industry, U.S.D.A.

Manufacturing section—E. H. Parfitt, Evaporated Milk Ass'n., 307 N. Michigan Blvd., Chicago, Illinois.

Production section—H. A. Herman, University of Missouri.

General chairman—H. W. Cave, Oklahoma A. and M. College.

Titles should be sent to the section chairman concerned.

AMERICAN DAIRY SCIENCE ASSOCIATION SECTIONAL OFFICERS AND COMMITTEES

OFFICERS OF THE EXTENSION SECTION

G. W. VERGERONT, Wisconsin, *Chairman*
J. F. KENDRICK, Bureau Dairy Industry, Washington, D. C., *Vice-Chairman*
E. C. SCHEIDENHELM, Michigan, *Secretary*

COMMITTEES OF THE EXTENSION SECTION

Program Committee

J. F. KENDRICK, Washington, D. C., *Chairman*
E. C. SCHEIDENHELM, Michigan
A. C. BALTZER, Michigan

Exhibit Committee

C. A. HUTTON, Tennessee, *Chairman*
R. G. CONNELLY, Virginia
C. J. FAWCETT, Massachusetts
R. T. HARRIS, Wisconsin
FLOYD JOHNSTON, Iowa

Sires Committee

E. J. PERRY, New Jersey, *Chairman*
J. F. KENDRICK, Washington, D. C.
S. J. BROWNELL, New York
O. J. HILL, Washington
G. E. GORDON, California

Type Rating Committee

J. W. LINN, Kansas, *Chairman*
F. J. ARNOLD, Iowa
E. C. SCHEIDENHELM, Michigan
R. W. STUMBO, Wisconsin
J. G. CASH, Illinois

Testing Committee

R. W. DICKSON, Virginia, *Chairman*
JOSEPH B. PARKER, Washington, D. C.
A. J. CRAMER, Wisconsin
E. H. LOVELAND, Vermont
C. R. GERHART, Pennsylvania

Feeding Committee

C. L. BLACKMAN, Ohio, *Chairman*
RICHARD E. WATERS, Mississippi
C. G. CUSHMAN, South Carolina
LYMAN RICH, Utah
W. T. CRANDALL, New York

4-H Dairy Club Committee

H. A. WILLMAN, New York, *Chairman*
M. L. FLACK, Nebraska
JOE C. NAGEOTTE, Pennsylvania
H. C. BARKER, Maryland
EARL J. MEADOWS, Colorado

Quality and Marketing Committee

EVERT WALLENFELDT, Wisconsin, *Chairman*
J. A. AREY, North Carolina
J. M. JENSEN, Michigan
C. J. BABCOCK, Washington, D. C.
H. R. SEARLES, Minnesota

Dairy Cattle Health Committee

GEO. E. TAYLOR, New Jersey, *Chairman*
J. G. HAYS, Michigan
A. I. MANN, Connecticut
C. G. BRADT, New York
RAMER LEIGHTON, Minnesota

Dairy Farm Management Committee

LESTER G. GILMORE, Kansas, *Chairman*
A. O. FOLLETT, Wisconsin
GERALD HEEBINK, West Virginia
J. M. BAIN, Washington, D. C.
G. M. HARRIS, Kentucky

OFFICERS OF THE PRODUCTION SECTION

H. A. HERMAN, Missouri, *Chairman*
K. L. TURK, Maryland, *Vice-Chairman*
DWIGHT ESPE, Iowa, *Secretary*

For committees of the Production Section see the August 1941 issue, pages 746–748.

OFFICERS OF THE MANUFACTURING SECTION

L. H. BURGWALD, Ohio, *Chairman*
R. WHITAKER, Baltimore, Md., *Vice-Chairman*
K. G. WECKEL, Wisconsin, *Secretary*

COMMITTEES OF THE MANUFACTURING SECTION

Committee on Chemical Methods for the Analysis of Milk and Dairy Products

General Committee

L. C. THOMSEN, Wisconsin, *Chairman*
E. O. HERREID, Vermont
W. H. MARTIN, Kansas
C. W. ENGLAND, Maryland
H. C. HANSEN, Idaho
G. H. WILSTER, Oregon
W. D. SWOPE, Pennsylvania
S. T. COULTER, Minnesota

Subcommittee on Milk and Cream

E. O. HERREID, Vermont, *Chairman*
W. J. CAULFIELD, Kansas
E. L. JACK, California
S. L. TUCKEY, Illinois
L. H. BURGWALD, Ohio
B. L. HERRINGTON, New York

Subcommittee on Butter

S. T. COULTER, Minnesota, *Chairman*
G. H. WILSTER, Oregon
M. E. PARKER, Chicago, Ill.
I. A. GOULD, Michigan
L. R. DOWD, Connecticut

Subcommittee on Cheese

G. H. WILSTER, Oregon, *Chairman*
W. V. PRICE, Wisconsin
A. J. MORRIS, Utah
G. P. SANDERS, Washington, D. C.
E. F. GOSS, Iowa

Subcommittee on Skimmilk, Buttermilk and Whey

H. C. HANSEN, Idaho, *Chairman*
E. W. BIRD, Iowa
CHRIS JENSEN, North Dakota

Subcommittee on Dry Milk

C. W. ENGLAND, Maryland, *Chairman*
J. H. ERB, Ohio
J. L. HILEMAN, Syracuse, N. Y.
F. J. DOAN, Pennsylvania

Subcommittee on Condensed and Evaporated Milk

W. D. SWOPE, Penna., *Chairman*
W. C. BROWN, West Virginia
J. H. ERB, Ohio
E. O. HERREID, Vermont

Subcommittee on Ice Cream

W. H. MARTIN, Kansas, *Chairman*
L. K. CROWE, Nebraska
B. E. HORRALL, Indiana
P. S. LUCAS, Michigan

Committee on Quality Program
General Committee

W. H. E. REID, Missouri, *Chairman*

C. J. BABCOCK, Washington, D. C.
W. D. DOTTERER, Chicago, Ill.
L. K. RIGGS, Chicago, Ill.
G. E. MOONEY, Plymouth, Wis.
E. C. THOMPSON, New York, N. Y.
N. W. HEPBURN, Chicago, Ill.
R. S. FLEMING, Syracuse, N. Y.
R. C. HIBBEN, Washington, D. C.

Subcommittee on Cream

P. A. DOWNS, Nebraska, *Chairman*
W. B. COMBS, Minnesota
T. M. OLSON, South Dakota
J. R. DICE, North Dakota
B. W. HAMMER, Iowa
W. H. MARTIN, Kansas
H. C. OLSON, Oklahoma
W. H. E. REID, Missouri

Subcommittee on Butter

W. H. BROWN, Indiana, *Chairman*
L. C. THOMSEN, Wisconsin
G. H. WILSTER, Oregon,
E. S. GUTHRIE, New York
K. M. RENNER, Texas
F. H. HERZER, Mississippi
HAROLD MACY, Minnesota
W. H. E. REID, Missouri

Subcommittee on Ice Cream

W. H. MARTIN, Kansas, *Chairman*
M. J. MACK, Massachusetts
P. H. TRACY, Illinois
D. R. THEOPHILUS, Idaho
E. L. FOUTS, Florida
J. H. ERB, Ohio

Subcommittee on Cheese

W. V. PRICE, Wisconsin, *Chairman*
S. L. TUCKEY, Illinois
E. F. GOSS, Iowa
G. A. PHILLIPS, California
H. L. WILSON, Washington, D. C.
F. E. HANSON, Texas
M. W. YALE, New York
Z. D. ROUNDY, Chicago, Ill.
W. H. SPROULE, Guelph, Canada
E. L. REICHART, Nebraska

Subcommittee on Market Milk

P. H. TRACY, Illinois, *Chairman*

H. B. ELLENBERGER, Vermont
A. D. BURKE, Alabama
F. C. BUTTON, New Jersey
H. A. BENDIXEN, Washington State
C. L. FLESHMAN, Virginia
C. L. ROADHOUSE, California

Subcommittee on Condensed Milk and Powder

E. H. PARFITT, Chicago, Ill., *Chairman*

A. C. DAHLBERG, New York
F. J. DOAN, Pennsylvania
W. C. COLE, California
MARTIN KLOSER, Chicago, Ill.
W. H. SLEMMONS, Oconomowoc, Wis.
L. L. HUNT, St. Louis, Mo.

Committee on Judging Dairy Products

G. M. TROUT, Michigan, *Chairman*

C. J. BABCOCK, Washington, D. C.
M. J. MACK, Massachusetts
P. A. DOWNS, Nebraska
E. L. FOUTS, Florida

Committee on Score Cards

C. J. BABCOCK, Washington, D. C., *Chairman*

L. H. BURGWALD, Ohio
H. E. ROSS, New York
A. D. BURKE, Alabama
C. L. ROADHOUSE, California

Committee on Methods of Measuring Oxidation of Milk Fat

O. F. GARRETT, New Jersey, *Chairman*

E. W. BIRD, Iowa
G. R. GREENBANK, Washington, D. C.
I. A. GOULD, Michigan
W. C. BROWN, West Virginia

Committee on Methods of Measuring the Color of Milk

O. F. GARRETT, N. J., *Chairman*
P. H. TRACY, Illinois
G. A. RICHARDSON, California
F. M. SCALE, New York, N. Y.

Program Committee

E. H. PARFITT, Chicago, Ill., *Chairman*

P. F. SHARP, New York
O. F. GARRETT, New Jersey

Committee to Make Comparison of Gerber and Babcock Tests for Dairy Products

J. H. ERB, Ohio, *Chairman*
W. J. CAULFIELD, Kansas
I. A. GOULD, Michigan
H. E. ROBERTS, New Jersey

VOLUME XXV JANUARY, 1942 NUMBER 1

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
R. C. HIBBEN, 1105 Barr Bldg., Washington, D. C., Exec. Sec.

INTERNATIONAL ASSOCIATION OF MILK DEALERS
R. E. LITTLE, 309 W. Jackson Blvd., Chicago, Illinois, Exec. Sec.

CONTENTS

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Dahlberg, A. C., Elliker, P. R., Petersen, W. E., Tracy, P. H. and Weckel, K. G.

ABSTRACTORS

Anderson, E. O.
Archibald, J. G.

Babcock, C. J.
Berggren, Ruth E.
Brueckner, H. J.
Burgwald, L. H.
Bushnell, L. D.

Cole, W. C.
Cone, J. F.
Coulter, S. T.

Doan, F. J.

Dorsey, L. M.
Downs, P. A.

Erb, J. H.
Ely, Fordyce
Espe, D. L.

Frazier, W. C.

Garrett, O. F.
Glick, D. P.
Goss, E. F.

Hansen, Arne
Huffman, C. F.

Irvine, O. R.

Josephson, D. V.

Knight, D.

Lucas, P. S.
Lush, J. L.

Mack, M. J.
Macy, H.
Marquardt, J. C.
Martin, W. H.
Mueller, W. S.

Price, W. V.

Reece, Ralph P.
Riddell, W. H.
Ritter, W.

Sheuring, J. J.
Stark, C. N.
Stebnitz, V. C.

Thomsen, L. C.
Trout, G. M.

Webb, B. H.
Weckel, K. G.
White, G. C.

Yale, M. W.

JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

Biochemical Journal
Biochemische Zeitschrift

Canadian Dairy and Ice Cream Journal
Canadian Public Health Journal
Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World
Deutsche Molkerei Zeitung

Endocrinology

Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Medical Association
Journal of American Veterinary Medical Association
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Genetics
Journal of Heredity
Journal of Industrial and Engineering Chemistry
Journal of Infectious Diseases
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Kaeseindustrie
Kolloid-Zeitschrift

Lancet
Le Lait

Milchwirtschaftliche Forschungen
Milchwirtschaftliche Zeitung
Milk Dealer
Milk Industry
Milk Plant Monthly
Molkerei Zeitung
National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Oil and Soap

Pacific Dairy Review
Proceedings of Society of Animal Production
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture

Tierernährung
Tierzüchter

Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Zeitschrift für Physikalische Chemie, Abt. A and B
Zeitschrift für Untersuchtung der Lebensmittel
Zeitschrift für Züchtung. Reihe B. Tierzuchtung und Zuchtungbiologie
Zentralblatt für Bacteriologie
Züchtungskunde

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment. Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

1. **The Danger of Hydrochloric Acid Gas Poisoning When Testing Salt-Treated Cream.** H. C. HANSEN AND R. S. SNYDER, University of Idaho, Moscow, Idaho.

Samples of salt-treated cream were analyzed according to a modified method developed at the University of Idaho Agricultural Experiment Station. Salted creams of 5 to 13 per cent concentrations, when tested by the Babcock method, released high concentrations of hydrochloric acid gas dangerous to health. Single samples of 7.5, 10 and 13 per cent concentrations released hydrochloric acid gas in amounts above the maximum allowable for prolonged exposure. Sets of 12 to 24 samples in any of the concentrations tested, released hydrochloric acid gas above the maximum allowable for even a short exposure ($\frac{1}{2}$ to 1 hour). The slow rate of diffusion caused high concentration of gas near the operator thus increasing the danger.

2. **Devices for Measuring Physical Properties of Cheese.** L. A. ROGERS AND G. P. SANDERS, Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Department of Agriculture.

Instruments are described for measuring the firmness of curd at cutting, the elasticity of Swiss cheese, and the plasticity or toughness of Swiss cheese.

The instrument for measuring firmness of curd is a modification of the Hill curd meter but may be used on a vat of milk and automatically records the firmness of the curd in terms of the time required for the cutter to move a definite distance through the curd.

Elasticity is measured by subjecting a disk of cheese of specified dimensions to air pressure. The curvature of the disk under a given air pressure for a given time is indicated by a thickness gauge graduated to read to 1/1000 of an inch.

Plasticity or toughness is evaluated by determining the force required to extrude the cheese through a small orifice in a cylinder. Force is applied to the cheese through a piston supporting a reservoir for water. Water flows into the reservoir until the first particle of cheese forced through the orifice breaks an electric circuit and closes the water valve. The reservoir is graduated to read the weight of water in pounds.

3. **Effect of Holding Cream in the Buying Station upon the Mold Content and Certain Other Quality Factors.** R. W. MORRISON, F. E.

NELSON, AND W. H. MARTIN, Kansas Agricultural Experiment Station.

The applicability of the methylene blue-borax visual mold test for grading cream held for one or two days in the cream station was studied. A preliminary survey of 75 samples showed that the visual mold score increased an average of only 0.03 units. The average increase in titratable acidity was 0.15 per cent of the 46 samples on which acidity was determined.

A more complete study on 38 cans of cream was made during the month of May, 1941. Cream temperature, titratable acidity, visual mold score, mold plate count, yeast plate count and organoleptic grade were determined before and after holding for one or two days in the station. The temperature of the cream usually was between 70° and 80° F., both at the time it was placed in holding and at the time it was shipped. Holding temperatures were maintained somewhat below maximum atmospheric temperatures by the limited use of ice. A tendency for the mold test or the plate count to decrease on samples originally high in mold content and to increase on samples originally low in mold content was observed. Changes in organoleptic grade and acidity were not paralleled by changes of similar magnitude in visual mold score, mold plate count or yeast plate count. Variation in space above the cream, within the limits of usual commercial practice, apparently did not affect the changes observed. The defects which appeared during the holding were of a variety of types, and no relationship between type of defect and other changes was observed.

The results indicate that the visual mold test used as an index of the quality of cream for buttermaking does not reflect the changes which occur during holding in the cream station.

BOOK REVIEW

4. **Refrigeration, 2nd Edition.** JAMES A. MOYER, State Director of University Extension in Massachusetts, and RAYMOND U. FITTZ, Assistant Professor of Mechanical Engineering, Tufts College. Published by McGraw-Hill Book Co., Inc., 330 W. 42nd Street, New York. 538 pages, 291 illustrations. $5.00.

A general treatment of refrigeration presented in understandable fashion and revised to cover recent refrigeration developments and applications. Contents:

1. Refrigeration Methods.
2. Systems of Refrigeration.
3. Properties of Refrigerants.
4. Compressors for Refrigerating Plants.
5. Household Mechanical Refrigeration.
6. Operation of Refrigeration Systems.
7. Thermodynamics of Refrigerating Systems.
8. Refrigeration Economics and Plant Testing.
9. Ice Making.

10. Insulation and Cold-storage Construction.
11. Small Commercial Refrigerators.
12. Air Circulation and Ventilation in Cold Storage.
13. Cold Storage of Foods.
14. Quick Freezing.
15. Production of Solid Carbon Dioxide.
16. Air-Conditioning Problems in Refrigeration Tables and Charts.

L.M.D.

BACTERIOLOGY

5. **A Synthetic Medium for the Cultivation of Streptococcus Fecalis.** ROSLYN L. SCHUMAN AND MICHAEL A. FARRELL, Penn. State College, State College, Pa. Jour. Infect. Dis., *69*, No. 1: 81–86. 1941.

A completely synthetic medium consisting of pantothenic acid, vitamin B_6, riboflavin, glucose, a salt mixture, arginine, glutamic acid, methionine, tryptophane, tyrosine, and valine; supported active growth of a stain of *Streptococcus fecalis,* as measured by means of a photoelectric nephelometer. Beta alanine could not replace pantothenic acid in the medium. The addition of other amino acids and accessory growth factors did not measurably increase growth. J. F.C.

6. **The Action of Sulfanilamide upon Hemolytic Streptococci Lancefield, Groups A and D, in Growth-promoting and Nongrowth-promoting Mediums.** ERWIN NETER, Univ. Buffalo, School of Medicine, Buffalo, N. Y. Jour. Infect. Dis., *68*, No. 3: 278–284. 1941.

Group A and group D streptococci suspended free of nutrients in a buffered solution and in a similar solution containing 1 per cent of sulfanilamide survived approximately the same length of time in both suspension media, indicating that there was no bacterial effect exerted by sulfanilamide under these conditions. When the suspension media contained growth-promoting nutrients, the medium containing 1 per cent sulfanilamide exhibited marked bacteriostatic action within 2 to 4 hours. With group A streptococci a concentration as low as 0.1 per cent of sulfanilamide in the growth-promoting medium decreased the rate of growth. J.F.C.

7. **Variation in Peroxide Production by Beta Hemolytic Streptococci.** FAITH P. HADLEY, PHILIP HADLEY, AND WILLIAM W. LEATHEN, Inst. of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa. Jour. Infect. Dis., *68*, No. 3: 264–277. 1941.

Eight type 3 and 2 type 5 strains of group A beta hemolytic streptococci that failed to give evidence of peroxide production by the usual tests, gave rise to peroxide-producing variants when aged for 4 to 10 days on benzidine

blood agar. The variants were stable. They differed from the parent strains in cell morphology, virulence, phagocytability *in vivo,* and in the amount of type- and group-specific substance. There was no difference noted in colony morphology, fermentative ability, and sensitivity to the inhibiting action of hydrogen peroxide. Neither the parent nor the variant strains produced catalase. J.F.C.

8. **Influence of Sulfanilamide on Mucoid and Smooth-phase Cultures of Hemolytic Streptococci in vitro.** PHILIP HADLEY AND FAITH P. HADLEY, Inst. of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa. Jour. Infect. Dis., *68,* No. 3: 246–263. 1941.

In broth media a type 5 beta hemolytic streptococcus of Group A in mucoid phase was markedly inhibited at 37° C. in concentrations of sulfanilamide of 1:40,000 or greater. When the incubation temperature was raised to 40° C. the 1:10,000, 1:20,000, and 1:40,000 concentrations were often germicidal for the mucoid phase in 24, 48, and 72 hours respectively. Serial passage at 37° C. and at 40° C. in neopeptone broth containing increasing concentrations of sulfanilamide resulted in progressive transformation from mucoid to smooth phase. The small percentage of remaining mucoid forms continued to possess virulence, but the derived smooth forms were lacking in virulence and did not regain it either by cultivation procedures or by mouse passage. The possible importance of such modification *in vivo* during sulfanilamide therapy is discussed. J.F.C.

9. **Dissociative Aspects of the Bacteriostatic Action of the Sulfonamide Compounds.** RUTH A. MCKINNEY AND RALPH R. MELLON, Inst. of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa. Jour. Infect. Dis., *68,* No. 3: 233–245. 1941.

Mice with experimental pneumococcic peritonitis were treated with sulfonamide compounds in less than maximally efficient doses. From such mice a series of intermediate variant pneumonococci or "modulations" was isolated. This series of "modulations" ranged from a slightly modified mucoid colony to a minute colony composed of unencapsulated, avirulent organisms. It represented a gradient of diminishing metabolic activity and increasing phagocytability. J.F.C.

10. **An Additional Growth Factor Needed by Some Hemolytic Streptococci.** A. BASS, SAM BERKMAN, AND FELIX SAUNDERS, Univ. Chicago. Jour. Infect. Dis., *68,* No. 3: 220–225. 1941.

Using two strains of Lancefield's Group A streptococci as test organisms, the authors tested for necessary accessory growth requirements with a basal medium consisting of hydrolyzed gelatin supplemented with amino acids,

inorganic salts, 0.2 per cent dextrose, and the accessory growth factors nicotinic acid, B-alanine, hemin, i-inositol, cocarboxylase, and riboflavin. The further addition of glutamine, ascorbic acid, pantothenic acid, and B_6 was not sufficient for growth of the test organisms. The addition of an especially prepared extract of yeast resulted in good growth, with less activity resulting from the addition of extracts of spleen, liver, fresh tomato juice, green pepper, banana, and potato. The necessary factor in these extracts was soluble in water and glacial acetic acid, but not in anhydrous solvents or fat solvents. It withstood autoclaving in neutral solutions, but was rapidly destroyed by heat in dilute alkali or acid. It could be precipitated almost completely from concentrated extracts with silver salts. Charcoal was the only effective adsorbent found. Elution from charcoal was difficult and incomplete. Attempts at purification for identification have been unsuccessful. J.F.C.

11. Isolation of Haemolytic Streptococci from Wounds. A. E. FRANCIS, London. Lancet, *241*, No. 6154: 159–160. 1941.

In a series of 300 wound specimens the gentian violet agar (1: 500,000) of Garrod showed 50 per cent more positive for hemolytic streptococci than plain blood agar. In a series of 60 specimens containing proteus or *Pseudomonas pyocyanea* but giving negative results for hemolytic streptococci on plain blood agar, 18 yielded hemolytic streptococci when the inoculated plates were covered with a second layer of agar and treated with alcohol according to a modified method of Fry. Also, the phenol agar (1: 1,000) of Braun and Schäffer yielded positive results in 11 of 120 specimens when plain blood agar was rendered useless by the presence of proteus or *Pseudomonas pyocyanea*. The use of sodium azide for the suppression of the gram negative organisms was not satisfactory. J.F.C.

12. The Distribution of Hemolytic Streptococci, Groups A, B, and C, in Human Infections. LOWELL A. RANTZ AND CHESTER S. KEEFER, Thorndike Memorial Lab., Second and Fourth Med. Services (Harvard), Boston City Hospital, and the Dept. of Med., Harvard Med. School, Boston. Jour. Infect. Dis., *68*, No. 2: 128–132. 1941.

Eleven hundred and fifty-nine strains of hemolytic streptococci isolated from human sources were grouped serologically. Of these strains, 1,104 (95.2 per cent) belonged to group A; 19 (1.6 per cent) were group B; 14 (1.2 per cent) were group C; and 22 strains were not classified except to determine that they did not belong to groups A, B, and C. Of the 19 group B strains, 4 appeared to be the primary etiological agents involved in human infections. Some of the remaining 15 group B strains were found in conditions which suggested their etiological relationship, whereas the strains iso-

lated from throats and sputums were of doubtful significance. None of the group C strains appeared to cause any pathological condition. J.F.C.

13. **The Bacteriology and Sanitation of Quick Frozen Foods.** N. H. SANDERSON, JR., Cascade Frozen Foods, Inc., Seattle, Wash. Refrig. Engin., *42*, No. 4: 228. 1941.

Quick frozen foods, unlike canned foods are subjected to no positive means of sterilization. The act of quick freezing only slightly reduces the number of bacteria in most of the foods frozen while prolonged storage at 0° F., although effecting a gradual reduction in the total viable cells, in no way brings about the destruction of all bacteria present. Bacterial investigations of quick frozen foods may be divided into two groups. 1. Those dealing with the effect of freezing on pathogenic bacteria which are commonly considered to be of public health significance. 2. Those dealing with the effect of quick freezing on the relatively inactive saprophytic bacteria which are normally associated with food spoilage. Emphasis is placed on careful sanitary control in the preparation of various foods for quick freezing in order to prevent the possibility of their acting as disease vendors. The author further points that methods for enumerating bacteria in frozen foods do not solve the problem of pathogenic microorganisms as possible contaminants and advocates the establishment of some standard by the frozen foods industry which will adequately cover the public health phase. Total bacteria count and *Esch. coli* count would be more desirable than total count alone.

In dealing with processing plant sanitation the processing lines should be operated on continuous flow principle free from any time consuming stoppages. Metals such as stainless steel should be employed, eliminating wood, canvas, and improperly protected metals. A plentiful supply of clean cold water for rinsing surfaces used for food preparation and handling prior to freezing is indicated, and that this cleaning be of continuous automatic nature limiting manual control to a minimum. L.M.D.

14. **Accessory Growth Factor Requirements of Some Representatives of the Brucella group.** STEWART A. KOSER, BEVERLY B. BRESLOVE, AND ALBERT DORFMAN, University of Chicago. Jour. Infect. Dis., *69*, No. 2: 114–124. 1941.

In synthetic media consisting of amino acids, glucose and inorganic salts, 7 of 8 strains of *Brucella* grew when certain accessory growth factors were added to the medium. The growth factors studied were nicotineamide, diphosphopyridine nucleotide (coenzyme I), thiamin hydrochloride (vitamin B_1), diphosphothiamin (cocarboxylase), beta-alanine, calcium pantothenate, vitamin B_6 hydrochloride, riboflavin, inositol, glutamine, adenine, sodium

pyrophosphate and biotin. The significant accessory factors were thiamin, nicotineamide, pantothenic acid and biotin. When only thiamin and nicotineamide were added to the basic media, 4 of the 8 strains grew in serial culture, although growth was slow in some cases. The further addition of pantothenic acid accelerated growth. The addition of a biotin concentrate as a fourth accessory factor produced growth in 3 of the 4 remaining strains, but produced no marked stimulation with the strains that were able to grow without it. Other accessory factors did not substitute for these required factors and caused no greater stimulation when included with them. In these studies it was found that the optimum concentration of sodium chloride in the medium was 0.6 to 1.0 per cent. J.F.C.

CHEESE

15. The Control of Acid Development in Cheddar Cheesemaking. R. M. DOLBY, Dairy Res. Inst., N. Z. New Zealand Jour. Sci. and Technol., *22*, No. 4A: 289A–302A. 1940.

The effect of type of starter culture, percentage of culture, cooking temperature, "running acidity," amount of dry stirring and time of salting on rate of acid production was followed by means of pH determinations. When the percentage of starter and acidity at draining were adjusted to give the same rate of acid development in later stages of the process, cheeses of the same pH resulted. The rate of increase in acidity in the early stages was influenced by the percentage of starter and in later stages by the acidity at draining, which also controlled the acidity of the cheese. The pH of the curd at salting did not greatly affect the acidity of the cheese, but did influence the amount of salt retained and the body of the cheese. The cheese was most highly buffered between pH 4 and 5. Measurements of pH are useful in controlling acid production during cheesemaking. W.C.F.

DISEASE

16. Laboratory Infections Due to Brucella. K. F. MEYER AND B. EDDIE, George Williams Hooper Foundation, University of California, San Francisco, Calif. Jour. Infect. Dis., *68*, No. 1: 24–32. 1941.

From data obtained by questionnaire on 74 cases of laboratory infection occurring in the United States, the authors discuss the factors causing the greatest hazard in laboratory procedure; the frequency with which the caprine, porcine, and bovine strains, respectively, are involved; diagnostic procedures employed; and the course of the disease. Handling of cultures or specimens and inhalation of dust containing Brucella organsims appeared to cause the greatest hazards. J.F.C.

17. A Cytophagic Reaction Employed in the Diagnosis of Brucella Infection. MOGENS JERSILD, State Seruminstitute, Copenhagen, Denmark. Jour. Infect. Dis., *68*, No. 1: 16–19. 1941.

The author describes a modification of Huddleson's opsono-cytophagic test. The chief difference between the two tests is that Huddleson employed citrated blood of the patient to furnish both opsonin and phagocytes, whereas Jersild employs the patient's serum and citrated, freshly drawn blood not containing Brucella opsonin, taken from a previously tested donor. The advantages claimed are: 1. The blood specimens can be taken in the usual manner without citrate. 2. The test need not be done within 6 hours as with Huddleson's test. 3. The citrated blood is collected just prior to use, so that the greater activity of the leucocytes increases sensitivity of the test. 4. Only one control slide with the donor's blood is required for an entire series of serum tests instead of one for each patient, as with Huddleson's test.

J.F.C.

18. Studies on the Detection of Mastitis in New Zealand Dairy Herds. I. A Field Outfit for the Bromthymol Blue Test for Mastitis. C. M. HUME, New Zealand Dairy Board, New Zealand Jour. Sci. and Technol., *22*, No. 6A: 322A–327A. 1941.

A field testing outfit for the bromthymol blue test and method of its use are described and a form for recording results is given. W.C.F.

19. Studies on the Detection of Mastitis in New Zealand Dairy Herds. II. Factors Influencing the Bromthymol Blue Test for Mastitis. F. H. MCDOWALL, Dairy Res. Inst., N. Z. New Zealand Jour. Sci. and Technol., *22*, No. 6A: 328A–337A. 1941.

The pH values obtained electrometrically were compared with those estimated with bromthymol blue as an indicator in tests of milk of individual cows for mastitis. The colorimetric method is subject to variations or errors. The size of the sample of foremilk is important, because the pH of the milk decreases, as does the chlorine content, with successive streams of milk. Due chiefly to a loss of carbon dioxide, the pH of the milk rises on standing and also on shaking. A high fat content of the milk renders the reading of the blue color of a positive test more difficult, and a variation in the quantity of indicator affects the reading. It is recommended that a uniform technique for making the test be adopted. W.C.F.

FOOD VALUE OF DAIRY PRODUCTS

20. Calcium and Phosphorus Studies in Normal People, Including Old Age. J. DOUGLAS ROBERTSON. Lancet, *241*, No. 6152: 97–100. 1941.

Blood serum studies on 60 normal people under age of 60 showed the

calcium content to be 9.9 to 11.1 mg. per 100 ml. (mean 10.393) and the phosphorus content 3.1 to 4.8 per 100 ml. (mean 3.831). In 15 healthy people aged 60 to 78 the serum calcium and phosphorus were not significantly higher than in the younger subjects. A study was made of the calcium and phosphorus balances of 9 normal people. On a calcium intake of 0.1 g. daily or on a phosphorus intake of 0.37 g. daily, all subjects were in a negative balance. For calcium the point at which equilibrium between intake and output took place was 0.45 g. daily for a 70 kg. subject. J.F.C.

MILK

21. **Experiments on the Use of Certain Antioxidants for Control of Oxidized Flavor in Dairy Products.** W. J. CORBETT AND P. H. TRACY, Univ. Illinois. Food Res., *6*, No. 5: 445. 1941.

The control of oxidized flavor in dairy products through the use of ascorbic acid, certain amino acids, concentrated water extracts from cereal flours, and a pancreatic enzyme are reported.

Tyrosine and the more soluble esters of tyrosine were found to be very effective antioxidants when used in milk in concentrations of .02 to .04 per cent. The normal amyl ester of leucine was also an effective antioxidant but imparted an objectionable off-flavor to the milk. The di-ethyl ester of glutamic acid did not produce a noticeable antioxidant effect and it gave a rather objectionable off-flavor. In copper-contaminated milk the ascorbic acid first retarded the development of the oxidized flavor and then after a certain point was reached, the development of the oxidized flavor was accelerated. In cases where 50 to 100 milligrams of ascorbic acid were added, an oxidized flavor developed in the copper-contaminated milk before all the reduced ascorbic acid disappeared.

The addition of pancreatic extract in the proportion of one part of extract to 25,000 parts of milk effectively prevented the development of an oxidized flavor. The addition of concentrated water extracts of the cereal grains was found to delay the development of an oxidized flavor in milk. The most effective product was made by drying a mixture of a water extract of a cereal flour and concentrated skim milk on a roller drier.

The addition of the various antioxidants which retarded the development of the oxidized flavor was found to have no effect on the oxidation of ascorbic acid.

The addition of the water extracts prepared from the cereal flours had only a slight antioxygenic effect when used in ice cream. A more effective product was the dried water-extract and concentrated skim-milk mixture. The addition of a commercially prepared Avenized sugar was found to give a slight antioxidant effect.

The addition to churning cream of a concentrated water extract of the

cereal flours or addition of an Avenized salt to the butter was found to retard the development of oxidized flavors in butter. P.A.D.

22. The Phosphatase Test for Control of Efficiency of Pasteurization. H. D. KAY, R. ASCHAFFENBURG, AND F. K. NEAVE, Tech. Commun. 1 of Imp. Bur. Dairy Sci., pp. 54. 1939. 2 shillings.

A review. W.C.F.

23. A Note on the Influence of High Temperature Short Time Pasteurization on the Phosphatase Reaction and Creaming of Milk. W. J. WILEY, Council Sci. and Indus. Res., Australia. New Zealand Jour. Sci. and Technol., *22,* No. 1A: 42A–43A. 1940.

Milk, pasteurized in a regenerative plate-type machine, with 39 seconds to reach the heating section, 29 seconds in the heating and holding sections, and 39 seconds again in the regenerator section, gave a negative phosphatase test when pasteurization was at 155° F. and a positive reaction at 153° F. Pasteurization temperatures above 155° F. reduced creaming. W.C.F.

MISCELLANEOUS

24. The Effect of Frozen Mass Formations on the Freezing Rate of Foods. WM. J. FINNEGAN, Consulting Engineer, Los Angeles, Calif. Refrig. Engin. *42,* No. 4: 233. 1941.

The author reports original data on tests comparing freezing rates obtained by various methods of freezing foods. The report is illustrated with diagrams, graphs, and photographs, the latter of feeding end and harvesting end of a spiral tubular freezer employed in freezing one gallon cans of orange juice. A few outstanding points in the report are listed.

1. In freezing large masses, the use of internal heat conductors will accelerate freezing rate.

2. Each food has an optimum point of final solidification which will give the highest freezing rate at a given temperature, regardless of the freezing method employed.

3. When a fluid is used as a secondary heat transferring vehicle, reversing the direction of flow at frequent intervals will increase the freezing rate and produce a more uniform product.

4. Uniform freezing may be assured by determination of the form and location of the final freezing point.

5. The point or points of "final solidification" should be selected as the point for temperature observation when arriving at a freezing rate.

6. Consideration must be given to methods of refrigeration application in arriving at freezing rate determination in conjunction with the above factors. L.M.D.

25. **Bibliography on Refrigeration.** From the Report of A.S.R.E. Technical Committee on Agricultural Products Refrigeration under D. F. Fisher, U. S. Dept. of Agr.

1. Refrigeration of fruits and berries. H. J. Read. Refrig. Engin., *39,* No. 5 : 303. May, 1940.

2. Cold storage locker plants spread. American Builder, *62,* No. 6, 72–73, 102–103. June, 1940.

3. Cold storage lockers. Nebraska Farmer, *82,* No. 6 : 8. March 23, 1940.

4. Preparation of early fruits and vegetables for locker storage : Table. Locker Patron, *1,* No. 10 : 10–11. May, 1940.

5. Preservation of fruits and vegetables in refrigerated food lockers. H. L. Seaton and R. M. Griswold. East Lansing, Mich. Folded chart. Michigan State College, Extension Division. Extension Bul., No. 208. 1940.

6. Fruits and vegetables for refrigerated locker plants. H. H. Plagge. Locker Patron, *1,* No. 7 : 10–11. Feb., 1940.

7. Quick-frozen foods. S. R. Winters. Southern Agriculturist, *70,* No. 3 : 46. March, 1940.

8. Cooling of milk for cheese making. Dominion of Canada, Dept. of Agr., Dairy Products Div., Marketing Service. Pub. 687, Circ. 158, 9 p. 1940.

9. Precooling tests of Indiana strawberries, cantaloupes and peaches. T. E. Hienton and K. I. Fawcett. La Fayette, Ind. Purdue Univ. Agr. Expt. Sta. Bul., No. 439, 36 p. "Literature cited": p. 36. 1939.

10. Minnesota cold storage locker plants. A. A. Dowell and others. Univ. Farm, St. Paul, Minn. Univ. of Minnesota Agr. Expt. Sta. Bul., 345, 39 p. 1940.

11. Refrigerated food lockers in Michigan. H. L. Seaton. In Mich. Agr. Expt. Sta. Quarterly Bul., *22,* No. 3 : 153–159. Feb., 1940. "References" p. 159.

12. Freezing fruits and vegetables in the Southwest. J. L. Heid, Refrig. Engin., *38,* No. 5 : 286–288. Nov., 1939.

13. Railroad service and frozen foods. Willis R. Woolrich. Refrig. Engin., *38,* No. 5 : 277–278. Nov., 1939.

14. Farm milk house. A. J. Bell and J. M. Jensen. East Lansing, Mich. Mich. State College Extension Division, Ext. Bul., 206, 11 p. 1940.

15. Dry-ice as a transport refrigerant. N. E. MacLean. Ice and Refrig., *97,* No. 5 : 330. Nov., 1939.

16. Polar chest locker system. E. C. Lloyd. Refrig. Engin., *38,* No. 5 : 308–309. Nov., 1939. L.M.D.

AMERICAN DAIRY SCIENCE ASSOCIATION

INCORPORATED IN THE DISTRICT OF COLUMBIA

Officers

President ..... HENRY F. JUDKINS, New York, New York
Vice-President ..... HERBERT P. DAVIS, Lincoln, Nebraska
Secretary-Treasurer ..... R. B. STOLTZ, Columbus, Ohio
Editor ..... T. S. SUTTON, Columbus, Ohio
Director ..... G. MALCOLM TROUT, East Lansing, Michigan
Director ..... J. C. KNOTT, Pullman, Washington
Director ..... HOWARD B. ELLENBERGER, Burlington, Vermont
Director ..... ARTHUR C. DAHLBERG, Geneva, New York
Director ..... C. N. SHEPARDSON, College Station, Texas
Director ..... FORDYCE ELY, Lexington, Kentucky
Director ..... HARRY W. CAVE, Stillwater, Oklahoma

Officers of Sections

DAIRY PRODUCTION SECTION

Chairman ..... H. A. HERMAN, Columbia, Missouri
Vice-Chairman ..... K. L. TURK, College Park, Maryland
Secretary ..... DWIGHT L. ESPE, Ames, Iowa

DAIRY MANUFACTURING SECTION

Chairman ..... L. H. BURGWALD, Columbus, Ohio
Vice-Chairman ..... R. WHITAKER, Baltimore, Maryland
Secretary ..... KENNETH G. WECKEL, Madison, Wisconsin

DAIRY EXTENSION SECTION

Chairman ..... GLEN W. VERGERONT, Madison, Wisconsin
Vice-Chairman ..... J. F. KENDRICK, Washington, D. C.
Secretary ..... E. C. SCHEIDENHELM, East Lansing, Michigan

Officers of Divisions

SOUTHERN

Chairman ..... C. D. GRINNELLS, Raleigh, North Carolina
Vice-Chairman ..... R. B. BECKER, Gainesville, Florida
Secretary-Treasurer ..... J. P. LAMASTER, Clemson, South Carolina

EASTERN

Chairman ..... K. S. MORROW, Durham, New Hampshire
Vice-Chairman ..... H. C. MOORE, Durham, New Hampshire
Secretary-Treasurer ..... L. R. DOWD, Storrs, Connecticut

WESTERN

Chairman ..... D. L. FOURT, Moscow, Idaho
Vice-Chairman ..... N. S. GOLDING, Pullman, Washington
Secretary-Treasurer ..... H. P. EWALT, Corvallis, Oregon

The American Dairy Science Association was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

Membership. Any person is eligible to membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person filling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order. The membership fee is $5.00.

The dues are $5.00 a year, $3.00 of which is for a year's subscription to the *Journal of Dairy Science*. Correspondence regarding membership and dues should be addressed to R. B. Stoltz, Ohio State University, Columbus, Ohio.

NOTICE TO CONTRIBUTORS

Authorship of Original Articles and Reviews.—Space in the Journal is reserved for the publication of original research voluntarily submitted by members of the association to the JOURNAL and review articles by invitation. In the case of joint authorship the membership ruling applies to one author only.

Papers that have already appeared in print or that are intended for simultaneous publication elsewhere, will not be accepted.

Manuscripts.—Manuscripts should be submitted in double spacing on one side of suitable $8\frac{1}{2}'' \times 11''$ paper. The original copy—not the carbon—should be furnished, packing it flat—not rolled or folded. All illustrative and tabular material should accompany the manuscript. The position of each illustration and of each table should be clearly indicated in the text.

Each manuscript must be accompanied by an Author's Abstract not to exceed 300 words which will appear in the advance abstract section of the JOURNAL.

In the case of manuscripts, other than review articles prepared by invitation, that contain more than 12 printed pages, the author is charged at the rate of $5.00 per page for all pages in excess of twelve. This charge is omitted in the case of articles of extraordinary merit.

Manuscripts voluntarily submitted, when approved for publication, will be published in the order of their receipt. Manuscripts should be sent to the Editor, T. S. SUTTON, Plumb Hall, Ohio State University, Columbus, Ohio.

Drawings.—Drawings, diagrams and charts for illustrations should be prepared for reproduction as line drawings or halftone engravings. The original drawings should be done in India ink on white or blue-white tracing cloth, tracing paper, or Bristol board and neatly lettered in India ink. Legendary material on the drawing should be neatly lettered in India ink—not typewritten.

The original drawings—not photographs of the drawings—should accompany the manuscript. Illustrations not in proper finished form will be prepared for publication and the author charged for the cost of the work.

Photographs.—Photographs for halftone reproductions should be glossy prints, free of all imperfections.

Legends.—All illustrative materials, both drawings and photographs, should be accompanied by appropriate legends, typewritten on a separate sheet of paper.

Tabular Material.—Tabular material in the manuscript should be clear, concise and accurate. Simple tables are more effective than complicated ones. If possible, tables should be so organized that they may be set crosswise of the page. In many instances it is possible to materially improve the appearance and usefulness of tabular material without sacrificing completeness of information, by condensing detailed data and presenting them in a simple table summarized form.

References.—References should be listed alphabetically as to authors and numbered; and citations in the text should be made by the number in parentheses, corresponding to the number in the reference list.

Each reference should contain the following data in the following order: Name and initials of author or authors; title of the article referred to; principal words in the titles of all articles should be capitalized; name, volume, number, page number and year of publication.

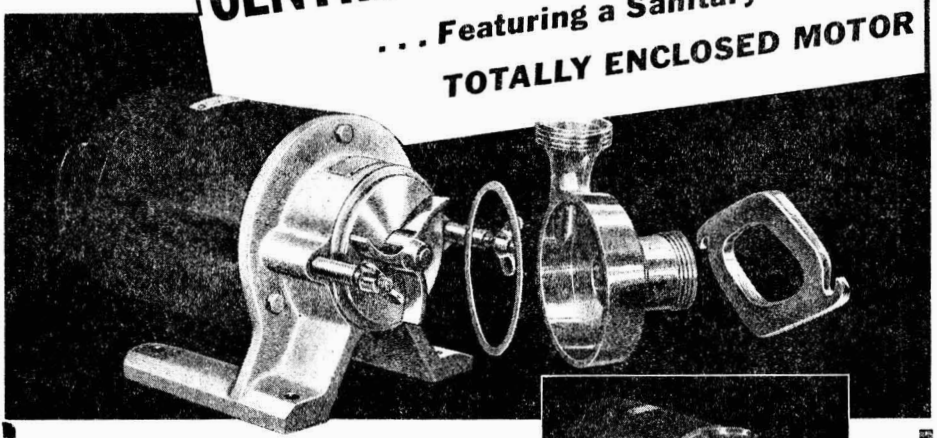
Abbreviations of the titles of publications should conform to the standard set by the United States Department of Agriculture given in U. S. Dept. Agr., Misc. Pub. 337, April, 1939.

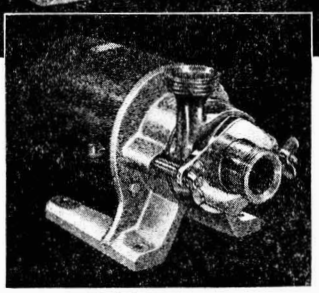
For uniformity of punctuations the references should conform to the following example: (1) JONES, L. W., AND SMITH, J. D. Effect of Feed on Body of Butter. JOUR. DAIRY SCI., **24**: 4, 550–570. 1941.

References should be carefully checked for accuracy by the author.

New—IMPROVED CP ROTO-SEAL CENTRIFUGAL *Milk-Pump*

. . . Featuring a Sanitary TOTALLY ENCLOSED MOTOR





Simple . . . Sanitary . . .

. . . Compact . . . Efficient . . .

A brilliant successor to a popular performer. Represents a distinct advance in sanitary pump design. New, totally enclosed Westinghouse motor eliminated dirt and bacteria hazards.

The new CP Roto-Seal Pump delivers 10,000 lbs. per hour at 12½ ft. head . . . meets every requirement for proper handling of milk, cream and liquid food products. Totally enclosed motor and simplified construction reduce maintenance and cleaning costs. Pump can be assembled or disassembled in thirty seconds. No tools required. Write for bulletin.

Three Improved Features

1. Improved, totally enclosed motor: SANITARY—No crevices or cracks to collect dirt.

2. Improved, enclosed motor mounting.

3. Stainless steel pump shaft.

THE CREAMERY PACKAGE MFG. COMPANY

1243 W. Washington Blvd., Chicago, Illinois

Sales Branches in 18 Principal Cities

CREAMERY PACKAGE MFG. CO. OF CANADA, LTD.,
TORONTO, ONTARIO