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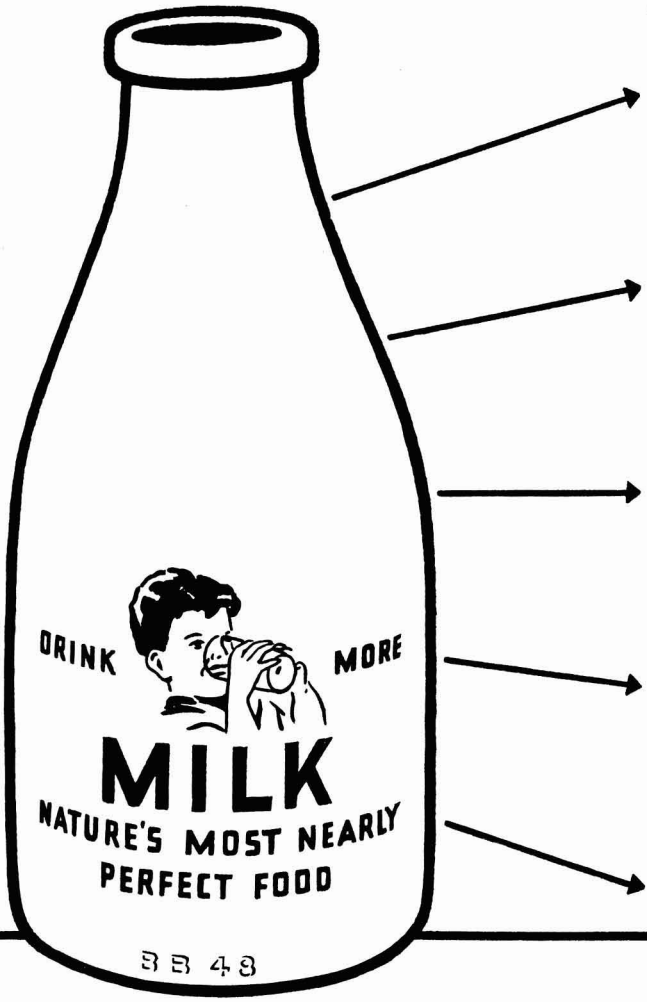

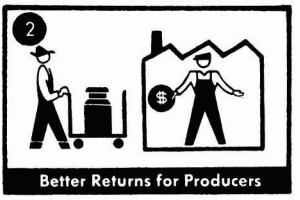

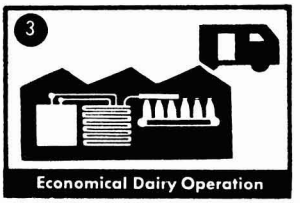

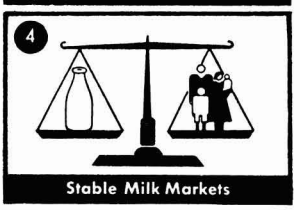

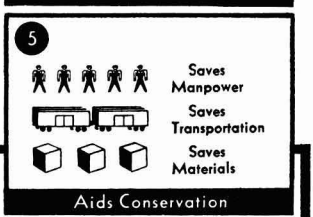

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JOURNAL OF DAIRY SCIENCE

VOLUME XXVI MARCH, 1943 NUMBER 3

SOME INVESTIGATIONS OF FLY CONTROL IN DAIRY BARNS[1, 2]

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Kansas Agricultural Experiment Station

Flies are such a serious pest to livestock that most stockmen make some effort to control them. On dairy farms, the problem is especially important due to the danger of contaminating milk. The United States Public Health Service has recognized the problem by prescribing regulations for approved dairy farms where Grade A milk is produced (18). Two general methods of control are usually recommended (3, 14): 1. elimination of breeding places, and 2. systematic killing of large numbers of flies. Frequent removal of manure by spreading it on field, elimination of stack bottoms, damp piles of feed or waste material together with utmost cleanliness inside the barn will greatly reduce the numbers of flies. On most farms more emphasis is placed on killing methods than the prevention of breeding. Killing methods include such recommendations as poison bait, traps, electric screens, spraying, or a combination of two or more such methods. Feeding drugs to cows to prevent flies from breeding in the droppings has even been tried (2, 6, 8). Fans or water sprays on doors and other devices have been used to keep flies out of stables. Sprays either of the killing or repellent type keep flies out of stables, and are probably more universally used than any of the other systems of reducing fly numbers.

Under practical conditions, fly control is a serious problem even when the approved methods are systematically followed. Each method has advantages and disadvantages. In this investigation an attempt was made to measure the effectiveness of a combination of various methods of fly control in dairy barns. The investigations were conducted in the dairy barn at the

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[1] This publication is a report on some phases of a cooperative investigation supported by the establishment of a fellowship at the Kansas Agricultural Experiment Station, June, 1940, by the Hercules Powder Company, Wilmington, Delaware. Associated in the general outline and supervision of the investigation were Mr. Friar M. Thompson, Jr., Entomologist, Hercules Powder Company and Dr. Roger C. Smith, Entomologist, Prof. F. W. Atkeson and Dr. A. O. Shaw, Dairy Husbandry, Kansas Agricultural Experiment Station. Graduate Assistants appointed to this fellowship were Floyd J. Holmes, Department of Entomology in 1940 and A. Russell Borgmann, Department of Dairy Husbandry in 1941.

[2] Contribution no. 143 from the Department of Dairy Husbandry, and no. 512 from the Department of Entomology.

Kansas Agricultural Experiment Station. This barn is a large modern structure, well situated, in which Grade A milk is produced. The milking barn floors and side walls are washed after each milking period. Walls are constructed of glazed tile and the ceiling is plaster coated with enamel paint. Breeding places for flies were eliminated under the supervision of staff members of the Department of Entomology. The sanitation program was followed to the extreme of picking up scattered droppings in the lots twice weekly. Since fly numbers were still a problem under these rather ideal farm conditions, the need for measuring the effectiveness of several control methods and various combinations of methods were indicated.

SPECIES OF FLIES PRESENT

Flies were caught with a sweep net in or near several barns, and the percentage of each species determined. With two exceptions, house flies represented 100 per cent of the flies caught. The problem is primarily that of controlling house flies. Throughout this paper the word "flies" will refer to house flies.

SCREENS VERSUS NO SCREENS

The Standard Milk Ordinance Code of the United States Public Health Service requires screens on the milk house windows but not on the barn (18). Even among the better equipped dairies, the question of screens on barns is controversial (1, 17). Some dairymen maintain that when the doors are opened and the cows are brought in, a large number of flies follow the cows, and the screens act as a trap to hold the flies in the barn. Others list screening of the barn as one of the first steps in controlling fly numbers in the barn.

In these investigations comparisons of screens and no screens were made in the maternity barn, calf barn, and nutrition barn. These barns were separate sections of the same building. Each of the barns was bedded with straw, changed daily. All the data were collected during August and September. Flies were counted many times on selected areas of the ceiling and wall when the screens were off, and again when on for several days. No spraying was done in the barns during the periods when data were taken. Average numbers of flies were compared on the same areas with the screens off and on (Table 1). All the flies counted were house flies.

On the areas of ceiling and walls selected for fly counting, there were 21 flies per 100 square feet of surface in the nutrition barn when the screens were off, and 5 flies on the same areas when the screens were on. In the calf barn the flies counted were 26 with the screens off and 6 when the screens were on. Counts in the maternity barn showed 16 flies with screens off and 2 with screens on. The number of flies were also counted on three selected calves in the calf barn. When screens were off the average number of flies per calf was 31, and 8 on the same calves when screens were on. In the

maternity barn two mostly white Ayrshire cows were selected for fly counts. The average number of flies per cow was 65 when the screens were off, and 28 per cow when screens were on.

Although selected areas of known size could not be used to estimate the number of flies in the entire barn, nevertheless the counts with screens off and on should measure relatively the effectiveness of screening barns as a supplmentary method of controlling fly numbers in a dairy barn. Considering the fact that different days were involved, the data obtained in the three barns show substantially the same results. When the screens were off approximately four times as many flies were counted as when the screens

TABLE 1

Comparison of screens and no screens in controlling flies in dairy barns (August, 1941)

Barns studied	Fly counts	Average flies per 100 sq. ft.	Flies on animals	
			Animals	Average flies per 100 sq. ft.
	No.	*No.*	*No.*	*No.*
Screens off				
A	24	21	.....	.....
B	27	26	3	31
C	31	16	2	65
Screens on				
A	10	5	.....	.....
B	33	6	3	8
C	20	2	2	28

A, Nutrition barn; B, Calf barn; C, Maternity barn.

were on. These results would indicate that screens are measurably effective even when cattle are turned in and out of the barn.

No spraying was done in the barns during the period of study but it is well to emphasize that this study was coupled with a systematic manure disposal program throughout the season and the barn had been sprayed daily with a killing spray previous to the period of study.

FLIES BROUGHT INTO BARN WITH CATTLE

When cattle are turned in and out of a screened barn, flies can come in through the open doors or be brought in on the cattle. An attempt was made to measure the increase in numbers of flies caused by bringing in the cattle (Table 2). The numbers of flies were measured by counts on selected areas of walls and ceiling. In the milking barn, the floors of which were washed, the increase in fly numbers was not important. However, in the calf barn, which was bedded, the fly numbers were greatly increased after the cattle were brought in, possibly due to the odor of the bedding. The flies counted were practically all house flies. Only one trial daily was conducted on each barn and each barn was comparatively free from flies before the cattle

TABLE 2

Increase in fly numbers in barns when cattle are brought in

Date	Cattle in or out	No. of fly counts*	Screens on or off	Ave. No. flies per 100 sq. ft.
	Milking barn			
8–19–41	Cows out	6	On	4
"	About ½ the cows brought in	3	On	11
"	2 hours later other cows brought in	3	On	15
	Calf barn			
8–8–41	Calves out	3	Off	36
"	Calves brought in	6	Off	186

* Approximately 45-minute intervals.

were brought in. The cumulative effect over a longer period in a screened barn might be different. In clean barns when spraying is regularly practiced to keep down fly numbers in addition to screening to keep out flies, it may be concluded that the necessary opening of doors to bring in the cattle does not defeat the value of screens by trapping flies in the barn.

EFFECTIVENESS OF SPRAYING WITH AND WITHOUT SCREENS

Many dairymen spray their barns to reduce the number of flies. The effectiveness of spraying barns with and without screens was compared with no screens or no spraying (Table 3). The results were measured by fly counts on selected areas of the walls and ceiling. Cattle were kept in both barns and the box stalls were bedded with straw, the stalls being cleaned each morning in the usual manner. Use of the barns in this way offered a better

TABLE 3

Combination of spraying barns with and without screens as fly control measures
(Cattle in barn during observations)

Date	Screens on or off	Sprayed or not sprayed	No. of fly counts	Ave. No. flies per 100 sq. ft.
		Maternity barn		
8– 9–41	On	No	1	59
"	Taken off	No	1	58
"	Off	Barn sprayed	4	85
8–11–41	Off	No	1	65
"	Off	Barn sprayed	11	67
8–14–41	On	Barn sprayed	12	2
8–19–41	On	No	6	21
"	Taken off	No	10	43
		Calf barn		
8– 9–41	On	Barn sprayed	4	30
8–13–41	On	Barn sprayed	5	21
8–28–41	On	Barn sprayed	6	0.4
8–29–41	On	Barn sprayed	1	0
"	Taken off	No additional spraying	9	51

opportunity to attract flies than if the barns had been empty and free from litter.

Although spraying the barn killed large numbers of flies, there was no advantage in spraying as a means of reducing the average number of flies in the barn unless the barn was screened. Counts a short time after spraying without screens were smaller but the advantage was soon lost due to the house flies coming into the barn. These results indicate that spraying alone is of doubtful value as a control measure. A combination of screens and spraying resulted in effective control of fly numbers in the barns over several hours. Such a combination would eliminate any trapping effect caused by screens when cattle are brought in or out. These findings are of interest as a supplement to the data in table 1, which showed that screens alone were much more effective as a control measure than no screens.

TRAPPING EFFECT OF SCREENS

It is well known that flies in the barn migrate to the light of the windows. This fact has caused some dairymen to question the efficacy of screens

TABLE 4

Comparison of numbers of flies on screens and walls
(Aug. 6 and 9, 1941)

Barns	No. of fly counts	Screens counted	Cattle in or out	Ave. No. of flies per 100 sq. ft.	
				On inside of window screens	On walls adjoining windows
(A)	20	8	Cows out	286	25
(B)	13	5	Calves out	370	79
				On inside of screens	On outside of screens
(A)	34	8	Cows out	230	10
(B)	13	5	Calves in	370	50

(A) Milking barn, (B) Calf barn.

and has also caused them to raise the question of the trapping effect of screens. To answer this, fly counts were made on the inside of the window screens and on wall areas of similar size adjoining the screens. In the milking barn while the cows were out, the average number of flies per 100 square feet was 286 on the inside of the screens and 25 on the walls adjoining (Table 4). In the calf barn with the calves in, there were 370 flies per 100 square feet on the screens and 79 on the walls. When counts were made of the number of flies on the inside as compared with the number on the outside of the screen, there were found to be 230 on the inside and 10 on the outside of the screens on the milking barn, and 370 on the inside and 50 on the outside of the calf barn screens. These findings are especially significant because they were obtained in a barn particularly well lighted by windows. These results lend credence to the claim that flies migrate to the light. They also

suggest the probable effectiveness of traps in widows, such as the Hodge fly trap, or electric screens on some of the windows as a supplement to the value of screens. The practice of darkening some or all of the windows to keep house flies out of a barn is also supported by these findings, although such a method may be criticized from the viewpoint of clean milk production because of insufficient light in the barn.

EFFECT OF CLEANLINESS OF FLOORS AND MANGERS ON FLY NUMBERS

Barn floors are usually cleaned by sweeping only, sweeping and liming, or washing. An attempt was made to measure the relative effectiveness of each cleaning method by counting the flies on the floors (Table 5).

One side of the nutrition barn was in use while the other side was not used. On the side in use the floors were bedded and kept rather dirty. The floors of the unused side had been scrubbed. When the screens were off and the cattle kept in the barn, the number of flies per 100 square feet on selected areas of ceiling and walls was 125, while the number of flies on similar areas on the clean side was only 16. Fly counts in the same barn with the cows out and the barn screened showed an average of 8 flies on the dirty side of the barn and 0.4 of a fly on the clean side. The barn had been sprayed previous to this second series of tests. These results indicate the importance of cleanliness of floors and the olfactory response of flies (7).

Effectiveness of liming floors was studied in the experimental barn with screens on and the cattle turned in and out of the barn. Numbers of flies on the floor were used as a measure of results. On the section of floor swept only, there were an average of 76 flies per 100 square feet; while on the section of floor freshly limed daily there were 27 flies. The difference might have been greater had there not been some residual lime from previous liming of the unlimed section of floor. When fresh limed floors were compared with day-old lime, partly soiled, 48 flies were counted on the day-old limed floor and 8 flies on the freshly limed. These two comparisons indicate that fresh lime on the floor acts as a repellent to flies and that flies are easily attracted by even slightly soiled floors.

Due to the fact that feed alleys are not so badly soiled as cow stalls and alleyways back of the cows, dairymen sometimes only sweep the feed alley even though they may scrub the rest of the barn floor. Comparison of sweeping with scrubbing of the feed alley was made in the milking barn, the rest of the barn being scrubbed after each milking period. The average number of flies counted on the floor was 9, when the floor was swept only, and 0.1 when the floor was scrubbed. Although the cleanliness of the floor was reflected in the number of flies counted, it is doubtful whether scrubbing the feed alley is justified, provided the floor is swept, even though silage, hay and grain are spilled in it.

TABLE 5

Barn cleanliness as a factor in fly numbers

Dates	Barns	Location of area counted	Screens on or off	Animals in or out	Comparisons	
					Ave. No. of flies per 100 sq. ft.	Ave. No. of flies per 100 sq. ft.
Aug. 9, 11, 14, 15, 1941	(A)	Ceiling and walls	off	in	125 uncleaned	16 cleaned
Aug. 19, 20, 24, 1941	(A)	Ceiling and walls	on	out	8 uncleaned	0.4 cleaned
Aug. 11, 15, 20, 1941	(B)	floor	on	in and out	76 swept only	27 freshly limed
Aug. 28, 29, 1941	(B)	floor	on	in and out	48 day old lime	8 freshly limed
Aug. 19, 20, 21, 1941	(C)	floor	on	in and out	9 swept only	0.1 scrubbed
Aug. 11, 14, 19, 21, 1941	(C)	manger	on	in and out	282 swept only	16 scrubbed

(A) Nutrition barn (B) Experimental barn (C) Milking barn

Swept mangers were also compared with scrubbed mangers in the milking barn. On the section swept only, there were 282 flies per 100 square feet, while on the scrubbed sections there were 16 flies on the same area. Accumulation of feed mixed with saliva of the cows often sours in the mangers and makes them insanitary. In these trials the practice of washing all sections of the mangers had been followed and the condition of the unwashed sections during the trial had no previous accumulations. The large numbers of flies found on the unwashed mangers compared with floors, walls and ceiling during the other trials and the small number found on the washed mangers emphasizes the importance of washing mangers to avoid attracting flies.

In all these comparisons the attraction of flies to dirty or soiled areas of the barn, depending on degree, is consistently shown.

NUMBERS OF FLIES ON DIFFERENT LOCATIONS IN THE BARNS

In most of the comparisons reported in this paper the numbers of flies in the barn were measured by counting the number of selected areas on the

TABLE 6

Number of flies in different areas of the barn

Dates and days	Barn	No. of fly counts	Location and average No. flies per 100 sq. ft.			
			Walls	Ceiling	Bottom of beams	Sides of beams
Aug. 19–21 (3)	A	22	3	6	3	66
Aug. 8–22 (8)	B	64	.....	23	27	113
Aug. 8–29 (9)	C	74	.....	39	40	132
Aug. 6–15 (15)	D	47	45	32	52	209
Aug. 8–29 (11)	E	74	70	114	142	1072

A, Milking barn, screened and scrubbed.
B, Maternity barn, screened and bedded, some counts included when screens were off.
C, Calf barn, screened and bedded, some counts included when screens were off.
D, Nutrition barn, screened and bedded, some counts included when screens were off.
E, Young stock barn, unscreened and bedded.

walls and ceilings. The same areas were counted throughout the study. It was apparent early that more flies congregated on the sides of the beams than on other areas. Therefore, sections of the beams, both bottom and sides were included in all counts.

Recapitulation of data collected in connection with various fly control methods made possible a comparison of the numbers of flies on several areas of the barns (Table 6). The average of all counts in each barn showed that there were generally a few more flies on the ceiling and bottom of beams than there were on the walls. The side of the beams, however, had many more flies than the other areas, the difference being more pronounced with larger numbers of flies present.

WALL COLOR PREFERENCE OF FLIES

Some dairymen paint the inside of the window panes with blue calcimine as a method of reducing fly numbers in the barn (10, 17). This tends to make the barn darker and probably causes the flies to migrate to open windows or window traps. Just why blue seems to be the accepted color is unknown. An attempt was made to determine whether flies showed any significant preference in wall color or perhaps put in the reverse order whether certain colors tended to repell flies more than others. A ply board panel 4 feet high and 6 feet wide was painted in three square foot areas with seven different colors of paint and one area left unpainted. The chart was hung lengthwise about head height on the wall of an unscreened barn. Trials of two different color combinations were conducted, 132 counts being made in the first trial and 181 in the second (Table 7).

TABLE 7

Color preference of flies determined by numbers of flies on square foot areas of a wall board hung in the dairy barn

Trial I—132 counts		Trial II—181 counts	
Aug. 19 to Sept. 24, '41		Aug. 6 to Sept. 24, '41	
Color	Ave. No. flies per 100 sq. ft.	Color	Ave. No. flies per 100 sq. ft.
Silver gray	69	Orchid	75
White	76	White	106
Baby pink	81	Unpainted wood	112
Celestial blue	104	Blue	119
Royal Carmine	140	Brown	139
Unpainted wood	153	Green	150
Lettuce green	161	Yellow	163
Cream	162	Black	191

The fly counts were made between August 6 and September 24, during which time practically all the flies counted were house flies. The data were analyzed by the analysis of variance. In the second trial it was necessary to obtain the square root transformation ($y = \sqrt{x + 0.5}$) prior to the analysis since the counts were found to be distributed in a Poisson-like manner (16).

Although differences existed in the average numbers of flies counted on the various color squares, statistical analysis showed that during the first trial the differences between the first three colors (silver gray, white and baby pink) were non significant. Neither were the next five colors (celestial blue, Royal carmine, unpainted board, lettuce green and cream) significantly different from each other. The difference between the latter five and the former three colors was significant (odds of 1:19 that results were due to chance), and the difference between some of the extremes of the two groups was highly significant (odds 1:99 that the results were due to chance).

The reaction of the flies to the various colors during the second trial showed more significant differences. When analyzed statistically the differences between the colors in the order listed were all highly significant with the exception of white and unpainted which were non significant, and the same for brown and green. Again, in this trial the numbers of flies on the different colors fell into two groups, the first four lighter colors having considerably fewer flies than the four darker colors.

These results are in reasonable agreement with conclusions of Lee (9) who measured the color responses of blow flies (*Lucilia cuprina*) by the number caught in glass traps colored by different colors of cellophane. He found yellow to be the most attractive color, with blue, pink and green ranking in the order named, but the differences in the last three appeared to be of doubtful significance.

Freeborn and Berry (5) counted the fly specks accumulated on different colored squares of a dairy barn ceiling. They found graduations in numbers from light to dark with little difference among the light colors. Their results are similar to those reported in this paper except for the color carmine in the first trial.

The principal finding from these comparisons is the fact that the flies seemed to prefer the darker colors when the panel was hung in a well lighted barn. Since dairy barns are generally painted light colors for sanitary reasons, it is doubtful whether color preference of flies is important in the selection of wall colors for dairy barns.

RELATIVE EFFICIENCY OF SOME SPRAYS IN "KNOCKDOWN" AND "KILL"

Since barn spraying is extensively used as a method of controlling fly numbers, the variation in efficiency of sprays is of interest to the dairyman. Two general types of sprays are on the market, the repellent type and the killing type. Dairymen would prefer to have a spray that would fulfill both functions. Two sprays might differ in repellence but rank in opposite order in killing power. Observations indicate that sprays differ considerably in comparison of knockdown and kill. Several sprays of known composition, which had previously been tested for repellence, were tested for knockdown and kill. Three nationally advertised livestock sprays were also selected for comparison (Table 8).

The procedure used consisted of hanging cylindrical screen wire cages (about 6×9 inches) with screen wire tops and bottoms in two barns containing cattle. The cages were hung 12 feet from the center of the barn and about $1\frac{1}{2}$ feet from the wall. Four cages were equally spaced throughout the barn. A known number of flies (from 50 to 100) were placed in each cage; and the barn was sprayed with a power sprayer to get a good dispersion. The volume of each barn was about 11,500 cubic feet and 300 c.c. (about $\frac{1}{3}$ quart)

of each spray was used, or about 1 c.c. for each 38 cubic feet. Ten minutes after spraying a large number of flies were gathered off the floor and a known number placed in cages containing water and suger for feed. These cages were then taken to another building where no spraying had been done, and at the end of 24 hours the percentages of dead flies were recorded.

All data were transformed by the arc sine transformation to obtain the relative effect of the different sprays used (16).

TABLE 8

Comparison of several sprays in effectiveness of knockdown and kill (Maternity barn and Calf barn)

Sprays used		No. of trials	Average per cent flies "down" in 5 minutes[d]	Average per cent flies "down" in 10 minutes[d]	Average per cent flies knocked down which were dead after 24 hours
No.	Composition				
1	5 per cent of 20: 1 conc. of Pyrethrum in base oil[a]	10	26	31	86
2	3¾ per cent of 20: 1 conc. of Pyrethrum plus 5 per cent D.H.S. activator[b] in base oil[a]	12	57	72	94
3	Commercial Spray No. 1	9	61	63	66
4	5 per cent of Thanite[c] in base oil[a]	8	68	85	92
5	2½ per cent of 20: 1 conc. of Pyrethrum plus 5 per cent D.H.S. activator in base oil[a]	14	69	75	96
6	Commercial Spray No. 2	20	71	73	47
7	3 per cent of Thanite[c] in base oil[a]	14	78	86	91
8	Commercial Spray No. 3	8	95	94	56

[a] "Stanco" base oil, 40 seconds Saybolt Viscosity.

[b] D.H.S. activator—Ethylene Glycol Ether of Pinene (Registered U. S. Patent Office by Hercules Powder Co.)

[c] Thanite—Fenchyl Thiocyanoacetate (Registered U. S. Patent Office by Hercules Powder Co.).

[d] In general, the averages on knockdown are lower than usually reported in laboratory tests. Observations indicated that possibly better knockdown was obtained throughout the barn than in the cages. Some of the difference may have been due to the fine mesh screen covering the cages. Also it was found that in barn tests the quantity of spray per cubic foot of volume could not be more than one-half the amount usually used in the Peet-Grady Chamber. Therefore, although the average knockdown percentages obtained seem low, they probably represent barn spraying results better than do laboratory tests.

Practically the same efficacy was obtained in knockdown at the end of 5 minutes as at the end of 10 minutes, which is in agreement with other investigations (4). The number of flies which would be knocked down through longer exposure was not determined. Spray No. 1, a mixture of 5 per cent of a 20 to 1 concentrate of pyrethrum in base oil (40 seconds, Saybolt viscosity) was the least satisfactory from the standpoint of knockdown. When

the data were treated statistically the other seven sprays were not found to be significantly different in knockdown efficiency.

More difference in killing power was found between the different sprays used. Five of the sprays, Nos. 5, 2, 4, 7 and 1, were significantly better than three others, Nos. 3, 8 and 6. Some of the best sprays from the standpoint of knockdown were among the poorest as killers. For example, spray No. 8 which resulted in the best knockdown ranked next to last in killing effect. It is well to consider that the "killing" represents the relative number of flies killed among those knocked down. Therefore, a spray which showed effective killing power but poor knockdown effect would be inefficient in killing all the flies in a barn. The variation between sprays in knockdown and kill, and the relative efficiency of some sprays in both, indicate the importance of considering both factors in the development of barn sprays.

OBSERVATIONS ON SPRAYING METHODS

In preliminary trials an attempt was made to simulate the Peet-Grady laboratory method (11, 12) by using the same quantity of spray material per cubic foot of volume in the barn as was used in the Peet-Grady testing chamber (1 c.c. of spray material per 18 cubic feet of volume). It was soon found that such a ratio of spray to volume was impracticable for barn tests, because the floors became so slippery from oil coating that it was difficult to work in the barn, and the fog of spray was more than would seem necessary.

After experimenting with various ratios of spray material to barn volume a ratio of about 1 c.c. of spray material to 36 cubic feet of barn volume was adopted as a satisfactory standard procedure. It will be noted that this ratio represents one half the amount of spray used in the Peet-Grady laboratory testing methods (11, 12).

Some difficulty was also experienced in obtaining an apparently desirable fog condition in the barn when oils of more than 50 seconds Saybolt viscosity were used as the base for spray ingredients. The results are in agreement with the reports of Searls, et al. (13, 15).

CONCLUSIONS

1. Numerous counts in and near several barns showed that fly control in barns is confined almost exclusively to house flies (*Musca domestica* L.)

2. Approximately one-fourth as many flies were counted in screened barns as in the same barns without screens, when systematic manure disposal and daily spraying had been in progress throughout the summer.

3. In clean barns, when spraying was regularly practiced to keep down fly numbers in addition to screening to keep out flies, the increase in fly numbers resulting from bringing in the cows was not important. In bedded barns, the fly numbers were greatly increased after the cattle were brought in, possibly due to the odor of the bedding.

4. Spraying the barn was ineffective as a fly control measure in a bedded barn unless the barn was screened. A combination of screens and spraying resulted in effective control.

5. The trapping effect of screens and the tendency of flies to migrate to light were shown by the fact that nearly five times as many flies were found on the screens as on the wall in a bedded barn, while more than 11 times as many were found on the screens in a clean barn. This indicates the possible supplemental value of such control practices as darkened windows, screen traps and electric screens.

6. The importance of clean floors and the olfactory response of flies were shown by the fact that about eight times as many flies were counted on the ceiling and walls on the bedded side of a barn as were found on the clean side (floor scrubbed) of the same barn. The repellent effect of fresh lime on floors was shown by the fact that on a floor freshly limed approximately one third as many flies were counted as on a floor swept only in the same barn. The attraction of flies to even slightly soiled floors was shown by the fact that there were significantly larger numbers of flies on floors limed the previous day than on freshly limed floors. In a scrubbed barn the numbers of flies found on an unscrubbed (swept) feed alley and the numbers on a scrubbed feed alley did not indicate much advantage in scrubbing the feed alley. Unscrubbed mangers soiled with feed and saliva, however, had 18 times as many flies on them as were on the scrubbed mangers.

7. Numerous counts on ceilings and walls showed that flies are more numerous on the ceilings and particularly on the sides of ceiling beams.

8. Study of color preference by flies showed that the flies preferred the darker colors. Since most dairy barns are painted light colors for sanitary reasons, it is doubtful whether color preference of flies is important in the selection of wall colors for dairy barns.

9. Comparison of eight sprays for knockdown and kill when used in a dairy barn showed that seven of the sprays were not significantly different in knockdown efficiency while one was inferior. The three nationally advertised commercial sprays tested were significantly less efficient in killing power than the five mixtures prepared here composed of various combinations of Thanite or pyrethrum concentrate. Superiority of some sprays in both knockdown and kill indicated the need for considering both factors in developing sprays for barns.

10. Satisfactory results were obtained when the amount of spray used was at the ratio of 1 c.c. to 38 cubic feet of barn volume which is one half the amount used in the Peet-Grady laboratory test; amounts simulating the ratio used in the Peet-Grady test caused the floors to become slippery. Some difficulty was experienced in dispersion of sprays made with oils of more than 50 seconds viscosity.

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DETERMINATION OF MILK LIPASE[1]

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INTRODUCTION

Existing methods for the determination of milk lipase are unsatisfactory for a number of reasons. In no case has the quantitative relation between amount of lipase and degree of hydrolysis been studied. Incubation times are usually inordinately long. Very often the precautions taken to prevent bacterial growth are insufficient. The pH during incubation is never adequately controlled. Among the methods most often used are those of Rice and Markley (6), Hylynka and Hood (2) and Reder (5).

In the method of Rice and Markley, the substrate used is cream, saturated with sucrose to prevent bacterial growth. The pH is not controlled. The incubation times used are three days or more. The method, however, is undoubtedly valuable as a qualitative indication of the presence of lipase. The method used by Hylynka and Hood and others (4, 1, 3) involves incubation of the milk at a low temperature for periods of a day or more, with subsequent churning and titration of fat acidity. Aside from the inconvenience of churning and the long time required, the method in our hands is influenced by the bacterial growth which takes place during the incubation period.

In the method of Reder, lipase is estimated by measurement of the amount of hydrolysis of tributyrin added to raw, whole milk. Since incubation times of 24 hours are required, Reder uses formaldehyde as a preservative. Although pH is not controlled and incubation times are rather long, the method of Reder was chosen as the basis of the method to be described.

EXPERIMENTAL

Effect of pH on milk lipase activity. In figure 1 the pH activity curve for the hydrolysis of tributyrin by lipase of cows' milk is given. Determination for lipase activity at various pH values were carried out according to the procedure outlined under Quantitative Determination of Milk Lipase with the following exceptions. In place of 2 ml. of 0.6 molar sodium barbital, 2 ml. of a 0.6 molar phosphate, 0.6 molar sodium barbital buffer were added. By means of this composite buffer, pH was controlled throughout the pH range. Definite incubation times of 30 minutes were used throughout the experiment. In all of the experimental work presented hereafter, incubation times of 30 minutes were used unless otherwise specified.

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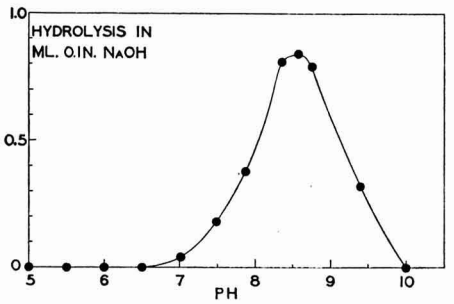

FIG. 1. Effect of pH on rate of tributyrin hydrolysis by milk lipase.

It will be seen from the figure that the tributyrin is most rapidly split at pH 8.5. It will also be noted that no enzymatic activity is evident below pH 6.5. Other experiments have shown that readjustment of the pH to 8.5 will restore only 30–50 per cent of the original lipase activity of milk which has been held at pH 4.5 for 30 minutes at room temperature. Because of the well-defined hydrolysis optimum at pH 8.5, this pH value was used in all lipolysis experiments to be described.

Variation of hydrolysis rate with amount of tributyrin present. Although tributyrin is soluble in water only in very small amounts, figure 2 shows that the rate of hydrolysis of tributyrin by milk lipase is affected by concentrations of tributyrin up to 2 per cent. At the 2 per cent level and above the hydrolysis rate appears to be constant. This effect is probably a result of formation of a tributyrin emulsion.

Effect of antiseptics on milk lipase activity. In table 1 the effect of a number of commonly used antiseptics on milk lipase activity is shown. It may be seen that of all the preservatives tried, only formaldehyde inhibited the enzyme. The activity in this case was found to be about 25 per cent of its original value.

Effect of buffers on milk lipase activity. In the development of a good method for the estimation of milk lipase, it was necessary to find a buffer

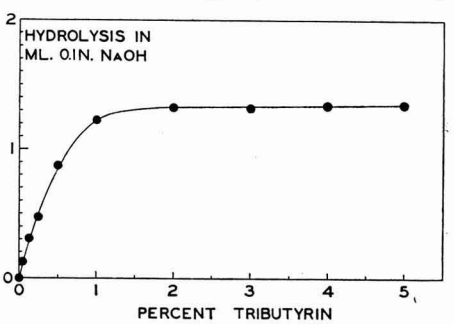

FIG. 2. Variation of hydrolysis rate with amount of tributyrin present.

TABLE 1

The effect of activators, buffers, and preservatives on milk lipase activity

Substance used	None	Zinc chloride	Potassium cyanide	Manganous sulfate	Magnesium chloride	Cysteine	Barbital sodium	Ammonium chloride Ammonium hydroxide	Phosphate	Borate	None	Formaldehyde	Chloral hydrate	Toluene	Merthiolate	Sodium fluoride	Chloroform
Use of substance		Activator					Buffer					Preservative					
*Concentration of substance	.....	0.001	0.001	0.001	0.001	0.001	0.1	0.1	0.1	0.1	.....	2.0	1.66	2.0	0.25	0.42	2.0
**Hydrolysis of tributyrin	1.11	1.06	0.83	0.95	0.99	1.02	1.14	0.29	1.01	1.08	0.78	0.19	0.75	0.77	0.77	0.78	0.77

* Concentrations of all activators and buffers expressed as molarities. Concentrations of all preservatives expressed in grams per liter.
** The figures are ml. of 0.1 N butyric acid liberated by 4.09 ml. of skim milk.

which did not affect the lipase activity and which had good buffering capacity in the range of the optimum pH of milk lipase. In table 1 are shown the effects of three buffers upon the activity of milk lipase. As may be seen, the phosphate buffer had a slight inhibitory effect on the lipase. Neither the borate buffer nor the barbiturate buffer inhibited the enzyme. Since the buffering range of the barbiturate buffer was closer to the optimum pH of the lipase than was that of the phosphate, it was chosen as the buffer to be used in the modified method.

Effect of activators on milk lipase activity. Various chemicals known to possess definite activating properties in cases of other enzymes were tested for activating effect upon milk lipase. As may be seen in table 1 none of them gave activation; most of them were inhibitors.

Activation by temperature changes. The effect of heating and cooling on milk lipase activity was also investigated. Krukovsky and Herrington (3) found definite increases in lipolysis rate in milk after warming and subsquent cooling. In the present investigation, however, it was found that warming milk from 0° to 30° over a period of 45 minutes, then recooling to 0° C. over a period of 60 minutes, had no effect on lipase activity.

QUANTITATIVE DETERMINATION OF MILK LIPASE

In the method finally adopted, the following procedure is used. The sample of milk to be tested is freed from butter fat by centrifugation. If

TABLE 2

Tributyrin and butter fat as milk lipase substrates

Milk sample number	Whole milk		Skim milk	
	Tributyrin	No tributyrin	Tributyrin	No tributyrin
	*ml. N/10 NaOH	ml. N/10 NaOH	ml. N/10 NaOH	ml. N/10 NaOH
7	0.67	0.04	0.64	0.00
8	1.21	0.06	1.15	0.01
9	0.93	0.05	0.89	0.00
10	0.81	0.03	0.78	0.01
11	1.38	0.06	1.31	0.01

* All values expressed as ml. N/10 NaOH represent the amount of hydrolysis of substrate by 4.09 ml. of milk.

the fat is not removed, a control with no tributyrin must be run in order to determine the extent of the activity of the lipase on the butter fat. As shown by table 2, there is a small but definte increase in acidity of the incubation mixture due to action of the enzyme upon the natural fats present. To 10 ml. of the resulting skim milk in a test tube is added 2 ml. of 0.60 molar sodium diethyl barbiturate (sodium barbital). This mixture is then adjusted if necessary to pH 8.5 with 5N acid or base. Very little or no adjustment should be necessary. A glass electrode is used in the pH measurement. The solution is then placed in a water bath held at 40° C. After the solution

has attained this temperature, 0.2 ml. of tributyrin are added, and the mixture is shaken vigorously for 1 minute. A 5 ml. aliquot is immediately removed for titration. After a suitable incubation period (15 to 120 minutes)[2] at 40° C., the tube is shaken for 30 seconds and another 5 ml. aliquot is titrated. The difference in titration between the two aliquots represents tributyrin hydrolysis. The titration is carried out as follows: The 5 ml. aliquot is pipetted into a 50 ml. Erlenmeyer flask containing 5 ml. of a 0.02 per cent solution of thymolphthalein in 95 per cent alcohol. After addition of 2 ml. of ether, the solution is titrated with 0.1 N alcoholic NaOH from a burette calibrated at 0.01 ml. intervals. Titration is carried to a definite blue color. It is convenient to prepare an artificial end-point comparison flask containing a dilute aqueous solution of $CuSO_4$ and $CoCl_2$ to

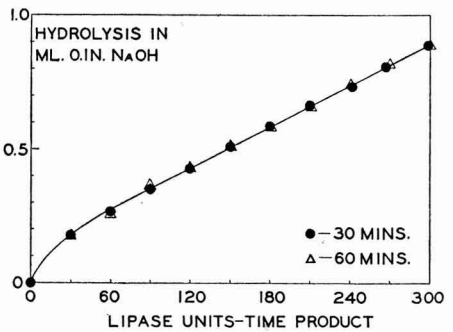

FIG. 3. Relation between quantity of lipase and amount of hydrolysis. The unit selected is arbitrary, being the quantity of lipase present in 1 ml. of the milk used. It will be noted that x ml. of milk incubated for 60 minutes gave the same amount of hydrolysis as 2 x ml. of milk incubated for 30 minutes.

which enough alumina cream has been added to give a close resemblance to the actual titration flask.

To find the total number of lipase units present in 5 ml. of incubation mixture (actually equivalent to 4.09 ml. of skim milk) the difference in titration between zero and final time of the incubation period (15 to 120 minutes) is found on the standard curve, figure 3, and its corresponding lipse unit-time product noted. Knowing the incubation time, one can determine the number of lipase units present. For example, if a titration difference of 0.51 ml. is obtained for an incubation time of 120 minutes, the corresponding lipase unit-time product is found to be 150. Dividing 150 by the incubation time in minutes, the figure 1.25 is obtained, which represents the number of lipase units contained in 4.09 ml. of raw skim milk.

The standard curve was obtained by plotting the relationship between

[2] Variable incubation periods are valuable in cases where only small milk samples are obtainable or where lipase activity in the sample to be tested is low. By increasing the length of the incubation period in such cases, larger titration differences may be obtained, thus increasing the accuracy.

quantity of lipase present and amount of hydrolysis of tributyrin obtained. The unit selected is arbitrary, being the quantity of lipase present in 1 ml. of the milk used for running the standard curve. The actual procedure was as follows: Various dilutions of a milk sample were analyzed for lipase activity. Incubation times of both 30 and 60 minutes were used. It will be noted that x ml. of milk incubated for 60 minutes always gave the same amount of hydrolysis of tributyrin as did 2 x ml. of milk incubated for 30 minutes. For routine lipase determination in milk samples, it is preferable to standardize on a 30 minute incubation period.

RELIABILITY OF THE METHOD

Tributyrin and butter fat as milk lipase substrates. From the data shown in table 2, a number of facts become apparent. It may be seen that

TABLE 3

Comparison of volatile acid distillate titration difference and direct titration difference

Milk sample number	Amt. of hydrolysis of tributyrin by titration differences of volatile acid distillates	Amt. of hydrolysis of tributyrin by direct titration differences	Sample number	Amt. of hydrolysis of tributyrin by titration differences of volatile acid distillates	Amt. of hydrolysis of tributyrin by direct titration differences
	**ml. N/10 NaOH*	**ml. N/10 NaOH*		*ml. N/10 NaOH*	*ml. N/10 NaOH*
12	0.89	0.91	4	0.76	0.78
13	1.21	1.22	5	1.03	1.04
14	0.46	0.46	6	1.49	1.48

* All values expressed as ml. N/10 NaOH represent the amount of hydrolysis of tributyrin by 4.09 ml. of skim milk.

the lipase in the milk acts much more slowly upon butter fat than it does on tributyrin. Therefore, if butter fat is used in order to obtain a natural substrate, long incubation periods are required in order to obtain appreciable increases in titration. With such long incubation periods, the use of a preservative is required. To eliminate long incubation times and the use of preservatives, it seems preferable to use tributyrin as a substrate. It also can be readily seen that there is no loss of lipase activity upon removal of the butter fat. As is apparent from the tables, the differences in hydrolysis between whole and skim milk are due to the action of the enzyme upon the butter fat.

The data in the table show that only lipolytic activity is being measured, since no activity is noted in skim milk samples without tributyrin. In whole milk without tributyrin, however, there is a small titration increase due to butter fat hydrolysis.

Additional evidence that the titration used measures only glyceride hydrolysis is afforded by table 3, where titration figures obtained in the usual manner are compared with titrations of volatile acid distillates of equivalent aliquots. Good agreement was obtained in all cases. The volatile acid dis-

tillates were obtained as follows: a 5 ml. aliquot of the incubation mixture was pipetted into the distillation flask. The pH was then adjusted to 2 (red to thymol blue) with 5 N H_2SO_4. A constant volume distillation was then carried out until about 5 times the volume of the original sample had been distilled over. The distillates obtained were titrated to the phenol red end point.

TABLE 4

Effect of incubation time and sample size on apparent lipase content of a milk sample

Sample size	Incubation time	Titration difference	Lipase content
ml.	*minutes*	*ml. 0.1N NaOH*	*units per ml.*
5.0	30	.25	0.87
5.0	60	.39	0.88
2.5	30	.16	0.86
2.5	60	.26	0.88
2.5	120	.40	0.87
1.0	60	.14	0.86
1.0	120	.22	0.87
1.0	120	.28	0.84

* Freshly drawn milk sample held 12 hours at 0° C. before analysis.

The reliability of the method and of the standard curve was also checked by running varying concentrations of the same milk sample for varying incubation times. The results may be found in table 4.

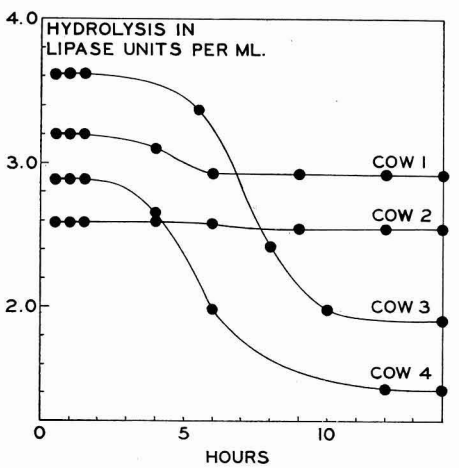

FIG. 4. Instability of milk lipase system.

DETERMINATIONS

Individual lipase determinations were made upon the milks of 25 cows in the University herd. The breeds represented were Jersey, Holstein, Guernsey, and Brown Swiss. Lipase contents varied from 0.32 units per ml. of milk to 2.98 units per ml. of milk. No obvious correlation of lipase activity with breed was noted. These determinations were made after the

samples of milk had been stored for 10 to 15 hours at 0° C. The reason for the use of held samples will be apparent from figure 4, where the time variations in lipase content of samples from four cows are given. The samples were cooled to 0° C. immediately after withdrawal and held at this temperature. It will be noted that in every case, the lipase content is constant after 12 hours, but the amount of decrease before this time varies greatly.

Lipase determinations were also made upon the milk of cows known to have mastitis. Milk lipase content was always lower in milk from an infected quarter.

DISCUSSION

There is a definite probability that more than one lipolytic enzyme exists in milk. Krukovsky and Herrington (3) reported that large amounts of formalin had no more inhibitory effect on milk lipase than did small amounts. On this basis they postulated the presence of two lipases in cows' milk, one of which is completely inhibited by small amounts of formalin. The other is apparently not sensitive to moderate amounts of formalin.

The data of figure 4 also suggest the presence in cows' milk of at least two lipases, one more stable than the other. It is also readily apparent from figure 4 that the ratio of the two enzymes seems to vary from cow to cow since varying proportions of the lipolytic activity are lost during the first few hours. If variable amounts of several lipases are present in milk, any quantitative determination of total lipase can yield only arbitrary figures.

Assuming the presence of several lipases, it is of course obvious that the rate of hydrolysis of tributyrin at pH 8.5 is not necessarily directly related to the rate of hydrolysis of the various butter fat glycerides at other pH values. This difficulty is inherent in any analytical method in which the total effect of a number of factors is measured.

SUMMARY

1. A method for the quantitative estimation of lipase in cows' milk is presented.

2. Determinations run at various incubation times and sample levels agree within 5 per cent.

3. The pH for optimum activity of cows' milk lipase is 8.5.

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A SOYBEAN AND SILAGE RATION FOR DAIRY COWS*

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In the Midwest, as in certain other sections of the country, the primary purpose of dairying is to provide a market for home grown feeds. Consequently, the usual system of feeding cows sufficient concentrates to balance a liberal ration of silage and legume hay finds its best application on those farms where a diversified system of farming is practiced.

It should be recognized, however, that there are large numbers of farms where the growing of legumes is restricted or non-existent. For example, more than half of the farms in Iowa are tenant operated, and on the average a tenant stays on one farm less than three years' time. With such a system of farming the growing of legume hay is difficult; on the other hand large quantities of corn are grown, and some of this could easily be cut and stored as silage. Also, on owner-operated farms corn silage is often the main roughage grown.

This roughage can be profitably utilized in large quantities by dairy cows without the necessity of buying hay if proper concentrates are fed with it (3). The simplest concentrate to feed which would effectively balance silage would be one carrying a high amount of protein. Soybeans are the chief home grown protein concentrate in Iowa and were therefore used in this trial.

The objection to feeding soybeans to dairy cows because of their high oil content is based largely on the results of feeding experiments with other classes of livestock. This objection is partially answered by Maynard and McCay (4, 5) who found that a medium amount of fat in the dairy ration caused a more efficient milk production than a ration of equal energy but with a lower fat content.

The maximum amount of fat that can be fed to dairy cows has not been definitely established. Though beef steers can utilize somewhat less than one pound of fat per day (9), cows in milk apparently can consume larger quantities of fat than steers, because they are constantly secreting and eliminating fat in their milk. It is probable the amount of fat that a cow is secreting in her milk would be near the limit of her intake tolerance for Buschmann (2) reports that feeding dairy cows over two pounds of fat per day each, usually caused digestive troubles.

EXPERIMENTAL

Eight fresh cows were paired into two lots on the basis of breed, age,

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TABLE 1

Information concerning cows

Pairing	Herd number	Breed	Age, Y–M–D	Weight, lbs.	Days in milk	Yield during preceding lactation		
						Milk, lbs.	% fat	Butterfat, lbs.
			Lot I. Check ration					
1	1041	J	4–1–22	912	74	5448	5.61	306
2	1081	H	3–6– 7	1247	118	13089	3.21	420
3	1072	H	4–4– 8	1253	48	10153	3.65	371
4	1073	H	4–3–25	1313	25	12098	3.61	437
Average			4–1– 0	1181	66	10197	3.76	384
			Lot II. Soybeans and silage ration					
1	1026	J	4–5– 3	772	31	6614	5.73	379
2	1058	H	3–8– 3	1140	130	12691	3.29	415
3	1071	H	4–4–12	1245	75	13095	3.27	428
4	1039	H	4–2–30	1402	30	11937	3.17	379
Average			4–2– 4	1140	66	11084	3.61	400

weight, number of previous lactations and previous yield. Data concerning them is found in table 1.

The experiment extended over a preliminary period of three weeks, which began about two weeks after the cows were removed from pasture in the fall, a one-week transition period, a twenty-two-week experimental period, and a five-week post-experimental period.

TABLE 2

Rations fed to each lot of cows during each period of the experiment

Period	Lot I	Lot II
Preliminary 3 weeks	Ration A	Ration A
Transition 1 week	Ration A	Gradually changed to Ration B
Experimental 22 weeks	Ration A	Ration B
Post-experimental 5 weeks	Same grain as above but pasture replaced roughages	Same grain as above but pasture replaced roughages

Ration A	*Ration B*
Alfalfa hay and Corn or sorghum silage (In approximate ratio of 1 lb. hay to 3 lbs. silage)	Corn or sorghum silage fed ad libitum Cracked soybeans fed at rate of 1 lb. (to the nearest ½ lb.) for each 5 lbs. of milk produced. Exception: Jersey cow received an extra ½ lb. beans daily to compensate for extra quality in milk
Grain mix 4 parts cracked yellow corn 4 parts ground oats 1 part soybean meal 1 per cent salt* 1 per cent bonemeal* Fed at rate of 1 lb. grain to each 3 lbs. milk produced	Mineral*

* Both lots had free access to a mixture of equal parts of bone meal and salt.

The cows were hand milked twice daily. Twice each week they were led to the scales and weighed, which provided their only regular exercise. The silage and grain were fed mornings and evenings after each milking, and when hay was fed, it was fed at noon. The amounts were regulated so that the cows received approximately one pound of alfalfa hay and three pounds of silage daily for each one hundred pounds of liveweight, and one pound of grain for each three pounds of milk produced until shifts were made to the experimental rations. The rations and shifts are in table 2.

No small silos were available for experimental use so the silage which was fed came from the supply of the regular herd. Corn silage was fed during the preliminary, the transition period, the first two weeks and the last six weeks of the experimental period. During the balance of the experimental period the cows were fed "Atlas" sorghum silage. After feeding sorghum silage for five weeks it was fortified with cracked yellow corn at the rate of one pound of corn (to the nearest one-fourth pound) to each twenty pounds of silage. This was done to equalize its percentage nutrients with that of corn silage and to stop a loss of weight in the cows which was occurring. One cow of one of the pairs was near to the drying-up time so only three pairs of cows were continued through the post-experimental period, during which time the cows had pasture instead of silage and hay roughages.

At weekly intervals determinations of the butterfat content (Babcock method), specific gravity (lactometer method) and nitrogen content (Kjeldahl method) were made from a composite of one day's milk from each lot of cows. Cream was also obtained from the milk produced by each lot from which samples of pure butter oil were obtained after churning.

The iodine, Reichert-Meissl and Polenske values of the butter oil were determined in duplicate for each sample. In doing this, official methods (1) were used except slight modifications were made to permit higher accuracy in a special apparatus that was used. This caused a slight divergence from standard in the results obtained but did not in any way interfere with a comparison between samples.

RESULTS AND DISCUSSION

The calculated digestible-protein and total-digestible-nutrient intake and requirements of the cows are shown in table 3. All the cows in each lot received more digestible protein than is called for in Morrison's standards (7) though the recommended total digestible nutrient (T.D.N.) requirements were practically met by the consumption of lot II. The consumption of digestible nutrients (T.D.N.) of lot I was somewhat in excess of the amount recommended for good producing cows.

The amount of protein fed to these cows more nearly meets the requirements previously given by Morrison (6) which were the ones used in designing this experiment.

The consumption of silage by lot II was high, averaging nearly 6 pounds daily per hundred pounds live weight. This would have been even higher if the appetites of the cows had not decreased with the advent of warm weather near the close of the experiment, and if the cows had been allowed all of the silage they wanted at the beginning of the experimental period. Maximum consumption by two cows weighing 1300 to 1400 pounds each was 90 pounds of sorghum silage daily and 60 pounds daily by a 750 pound Jersey. The consumption of the bonemeal-salt mixture was also somewhat

TABLE 3

Feed consumption and nutritive requirements of the cows during the experimental period*

Herd No.	Lot I					Lot II				
	1041	1081	1072	1073	Total	1026	1058	1071	1039	Total
Feed consumption in pounds										
Grain mixture**	742	1509	1966	2269	6486					
Soybeans						738	744	962	1291	3735
Corn						155	191	236	232	814
Corn silage	1343	1847	1809	1982	6981	2120	2354	3084	2809	10367
Sorghum silage	2866	4030	3953	4206	15055	5590	6948	8458	7900	28896
Alfalfa hay	1361	1940	1970	2042	7313				158	158
Digestible protein	278	446	500	547	1771	342	365	466	581	1754
T.D.N.	2018	3194	3539	3885	12636	2157	2478	3109	3318	11062
*Minimum nutritive requirements in pounds**										
Digestible protein	189	280	334	366	1169	239	242	294	361	1136
T.D.N.	1785	2527	2966	3224	10502	2142	2245	2664	3113	10164
Percent of consumption: Dig. prot.	68	63	67	67	66	70	66	63	62	65
Percent of consumption: T.D.N.	88	79	84	83	83	82	91	86	94	92
*Recommended nutritive requirements in pounds**										
Digestible protein	217	322	390	428	1357	279	280	341	409	1309
T.D.N.	1961	2770	3233	3509	11473	2329	2465	2914	3400	11108
Percent of consumption: Dig. prot.	78	72	78	78	77	82	77	73	70	75
Percent of consumption: T.D.N.	97	87	91	90	91	108	99	94	102	100

* Nutrients in feeds and nutritive requirements were calculated from tables in "Feeds and Feeding," 20th edition (7).

** Grain mixture consisted of:

4 parts corn
4 parts oats
1 part soybean meal
1% salt
1% steamed bonemeal

high, averaging about 2 pounds per week for each of the cows on the experimental ration.

At no time did cows of lot II show a disinclination to eat the soybeans. This was true even after they had been turned out to pasture. One cow, 1039, received 9 pounds of soybeans daily for a considerable period of time, and some of the others received nearly as much.

Throughout the experiment the "soybeans and silage" cows were watched closely for any indication of a nutritive deficiency. None was observed. The experimental feed may or may not have been responsible for three digestive disturbances which occurred.

The trends in live weight for the cows during the experiment is shown in figure 1. These trends show the cows of lot I gradually gained in weight as the experiment progressed while the cows in lot II first lost, then gained, and then lost in weight.

The average yields of milk and butterfat as well as the butterfat percent-

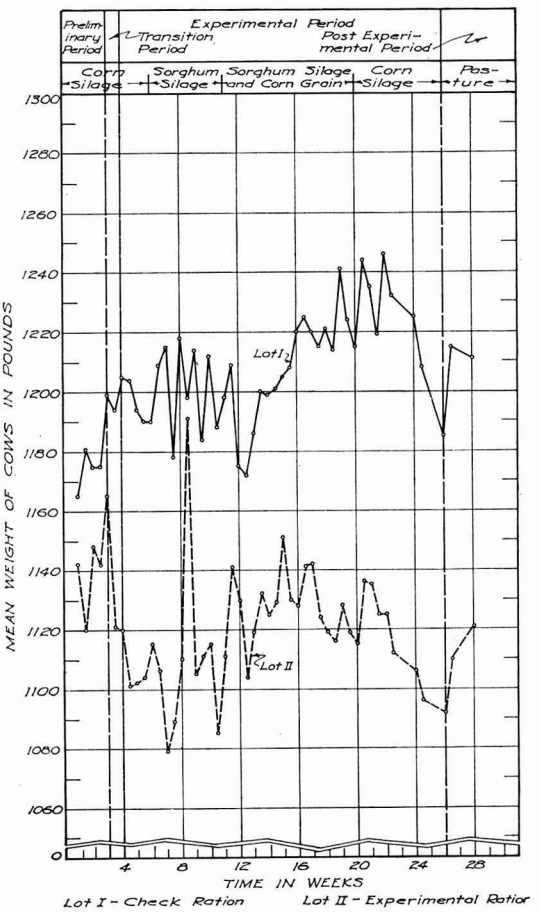

FIG. 1. Mean live weights of cows in each lot.

ages for the two lots of cows are shown in figure 2. The cows in lot I produced slightly more milk than those in lot II, while those in lot II produced milk with enough higher fat content to allow them to produce more butterfat than the cows in lot I.

Since the two lots were fairly evenly balanced in their production of milk and butterfat during the preliminary period, it might be concluded that the

feeding of silage and soybeans (lot II) decreased milk yield but increased fat production. However, since the differences could have been due to chance or to factors not under experimental control, the data were subjected to analysis of variance, following the method given by Snedecor (8). In the

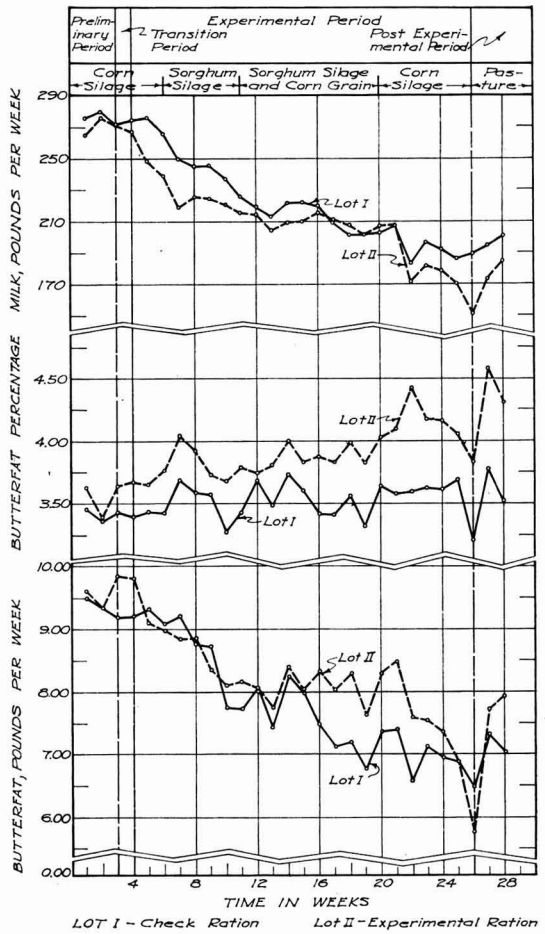

FIG. 2. Mean milk yields, butterfat percentages, and butterfat yields of cows in each lot.

analyses of the milk and fat yields, the mean squares for the variation between the lots were so little larger than the mean squares for the remainder of the variance (for milk production, $F = 1.08$ and for fat production, $F = 1.27$) that the differences in these yields were not significant, as they may have been due to chance only.

The analytical treatment of the variations in fat percentages (after the fat yields were adjusted for the differences in milk yields) showed the mean square between lots considerably larger than the mean square of the remainder (F = 12.26), indicating that feeding silage and soybeans had a significant effect on the percentage of fat in the milk.

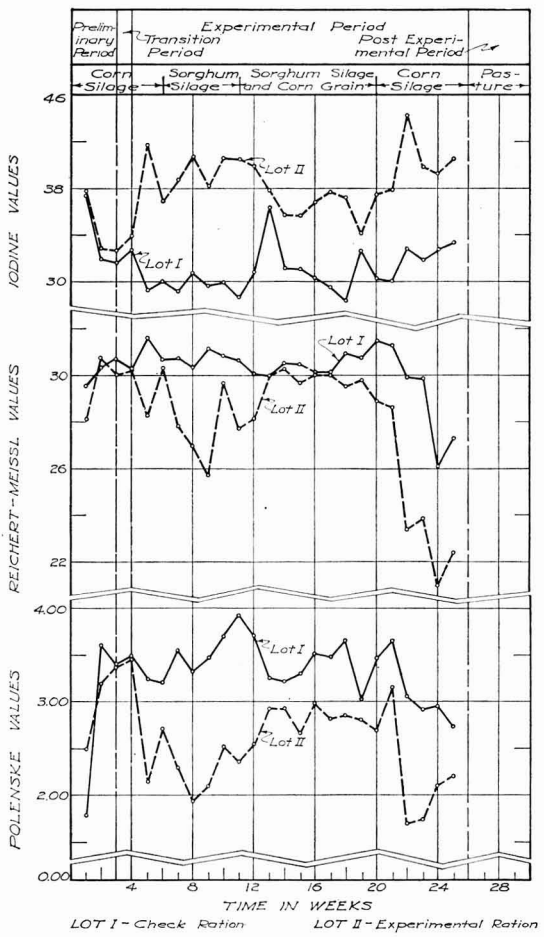

FIG. 3. Mean iodine, Reichert-Meissl and Polenske values of the butterfat yielded by cows in each lot.

Weekly determinations were made throughout the experiment of the amount of protein in the milk and the iodine, Reichert-Meissl and Polenske values of the butterfat produced by the cows in each of the two lots.

The percentage of protein in the milk rose irregularly as the experiment progressed, but did not differ between lots, hence it was not affected by the

rations being fed. This was verified by an analysis of variance which gave a value of F = 3.41.

The trends in the butterfat values are shown in figure 3. The iodine values remained fairly constant during the entire experiment except for high values during the first week of the preliminary and the ninth week of the experimental periods. With lot II the values rose abruptly some eight numbers following the change to the experimental ration and remained high throughout the period, though with one exception the value was at its highest point the first week in the experimental period. Evidently as the cows became accustomed to consuming large quantities of soybean oil (one cow received as much as 1½ lbs. of soybean fat daily, while the others received nearly as much) they were able to adjust towards normal their conversion of the unsaturated fatty acids in the feed to saturated fatty acids in the butterfat. The differences in iodine values of the butterfat produced by the two lots of cows were highly significant (F = 112.16).

The changes that occurred in both the Reichert-Meissl (F = 36.96) and the Polenske (F = 70.27) values when the cows were put on the experimental rations were found to be highly significant. Since the various values for the butterfat produced by the two lots of cows were very similar during the preliminary feeding period, with the shift in values coming after the change in ration it is evident that the significance of these changes was due to the change in feed.

Soybeans have been accused of imparting a distinct flavor to the milk produced from them. To check this, milk samples were taken from each cow and scored by competent judges. The milk from cows receiving the soybeans had a mean score less than 1 below (perfect score = 25) milk produced by cows receiving the herd ration. Scores on milk produced by cows which alternately were fed silage and then no silage indicated that silage may have contributed more to this slightly lower score than the soybeans.

SUMMARY

Cows yielding as much as 50 pounds of milk daily (one cow averaged 40 lbs. daily during experimental period) were successfully carried over a period of 22 weeks on silage, fed *ad libitum,* and soybeans, fed at the rate of 1 pound per 5 pounds of milk produced. The silage was corn silage, sorghum silage or sorghum silage supplemented with 1 pound of corn per 20 pounds of silage. Bonemeal and salt were also provided. Silage consumption averaged about 6 pounds daily per hundred pounds live weight. Up to 9 pounds daily of soybeans were consumed by a single cow. No indications of nutritional deficiencies were observed. The cows practically maintained their weight while being fed soybeans and silage.

As compared with paired cows on a normal grain and roughage ration the cows on the experimental ration produced slightly less milk but more

butterfat. These differences were not statistically significant. The cows receiving the experimental ration produced milk with a significantly higher percentage of butterfat in it than did the cows on normal feed.

No differences were found in the protein content of the milk produced by the two lots of cows, but there were significant differences in the iodine, Reichert-Meissl, and Polenske values. The iodine number was raised and the Reichert-Meissl and Polenske numbers were lowered by the experimental feeding.

The experimental ration of soybeans and silage caused no marked change in milk flavor.

ACKNOWLEDGMENT

The chemical analyses, other than percentage of butterfat, were made by Jack Frame under the supervision of Dr. E. W. Bird, Associate Dairy Chemist.

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THE OPTIMUM TEMPERATURE OF GROWTH OF 22 CULTURES OF *OOSPORA LACTIS*

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INTRODUCTION

The determination of the amount of mold as a criterion for cream quality for buttermaking has been studied by several investigators (1, 4, 6, 7, 9, 11, 12). Their findings show that there is a definite relationship between the quality of the cream and the quantity of mold present. The usual method of determining the quantity of mold was the methylene blue-borax test developed by Wildman (12) and modified by Parsons (8).

Macy and Gibson (5) found that the optimum temperature of growth of 61 cultures of *Oospora lactis* isolated from butter was from 15° to 25° C. Adams and Parfitt (1) showed that, in cream, it took less time to obtain the same quantity of mold at 80° F. (26.7° C.) than it did at 75° F. (23.9° C.) or at 70° F. (21.1° C.). They also determined that agitation favored mold growth. This latter discovery is of significance if the cream is agitated excessively during handling.

An experiment carried on by Vandaveer and Wildman (9) showed that a higher percentage of moldy cream is found in lots stored above 70° F. (21.1° C.). However, White and Hood (10) found that the mold and yeast count of cream stored at 37° C. was nearly always lower than at 30° C. or 25° C. The mold count at 30° C. was not significantly lower than at 25° C.

It was thought that perhaps the temperature requirements of the various strains of *O. lactis* had not been studied as thoroughly as they should have been. There was the possibility that certain strains of *O. lactis* may have an optimum temperature of growth which would make a quality program relatively unsatisfactory from the standpoint of the mycelium test, if such mold became more active at lower temperatures of holding the cream. Therefore, this work was carried out to determine the rates of growth at various temperatures of cultures of *O. lactis* isolated from cream.

METHODS AND MATERIALS

The cultures used were isolations from farmers' commercial cream during the hot summer months.[1]

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[1] Thanks are tendered to J. D. Wildman, Microanalyst, Microanalytical Division, Food and Drug Administration, Washington, D. C., for supplying these cultures.

The procedure used in this experiment was identical with the procedure used by Golding (2) except for the following:

Culture: The cultures used for the water dispersion were grown for 10 days at a temperature of 75° F. (23.9° C.) to 78° F. (25.6° C.).

Incubation: Weston metallic thermometers were fitted into the desiccators for the temperature reading of incubation.

Incubation Chamber: Pyrex vacuum desiccators were used as incubating chambers for the molds. Four cultures were grown in each desiccator. Since five plates of each culture were grown at each temperature, a total of twenty plates was held in one desiccator.

The temperature and humidity of the desiccators were controlled by the method used by Golding (3), but using a continuous flow of humidified air. Previous tests have shown that this method very greatly reduced evaporation from the plates and that the temperature of the agar is the same as that of the desiccator.

EXPERIMENTAL

Figures 1, 1a, 1b, and 1c are seven-day growth curves for the twenty-two cultures of *O. lactis* used. The various temperatures at which the cultures

TABLE 1

The rate of growth of cultures of O. lactis as affected by temperature

Culture number	Optimum growth		Temperature at which growth is one-half of the diameter of the optimum			
			Below optimum		Above optimum	
	Diameter	Temperature	Temp.	Dif.	Temp.	Dif.
	mm.	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*
1	58.0	77.5	64.0	13.5	91.5	14.0
2	53.5	78.0	65.0	13.0	90.0	12.0
3	55.0	78.0	62.0	16.0	91.0	13.0
4	57.5	78.0	61.5	16.5	90.5	12.5
5	58.0	78.5	61.5	17.0	89.5	11.0
6	57.0	81.0	64.5	16.5	91.5	10.5
7	58.5	81.0	63.0	18.0	91.0	10.0
8	80.5	83.0	66.0	17.0	91.5	8.5
9	75.5	83.5	67.0	16.5	92.0	8.5
10	80.5	83.5	68.5	15.0	92.0	8.5
11	81.5	83.5	67.5	16.0	92.5	9.0
12	70.5	84.0	66.0	18.0	91.5	7.5
13	79.0	84.0	64.5	19.5	93.0	9.0
14	78.0	84.5	64.5	20.0	92.0	7.5
15	81.5	84.5	65.0	19.5	93.5	9.0
16	60.5	85.0	66.0	19.0	91.5	6.5
17	61.0	85.0	63.5	21.5	92.0	7.0
18	64.5	85.0	65.5	19.5	91.5	6.5
19	70.5	85.0	65.5	19.5	91.5	6.5
20	77.0	85.5	68.5	17.0	92.0	6.5
21	77.5	85.5	69.0	16.5	92.0	6.5
22	75.5	86.0	68.0	18.0	93.0	7.0
Average	68.7	82.7	65.3	17.5	91.7	9.0

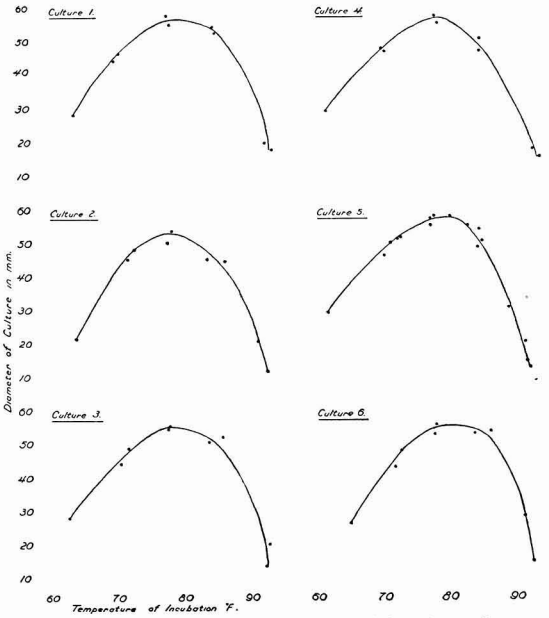

Fig. I - Growth Curves of *O. lactis* Cultures Isolated from Cream During Warm Weather.

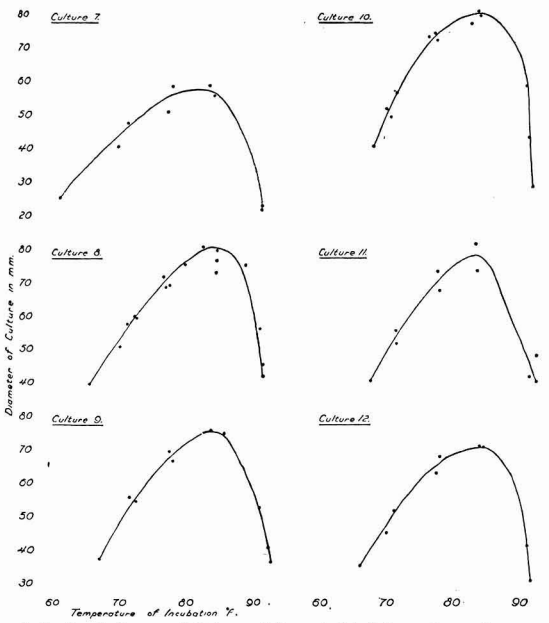

Fig. Ia - Growth Curves of *O. lactis* Cultures Isolated from Cream During Warm Weather.

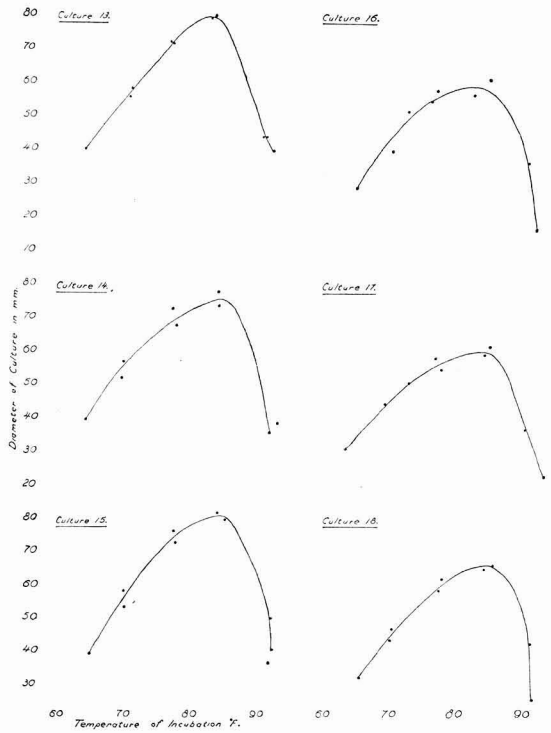

Fig. Ib-Growth Curves of *O. lactis* Cultures Isolated from Cream During Warm Weather.

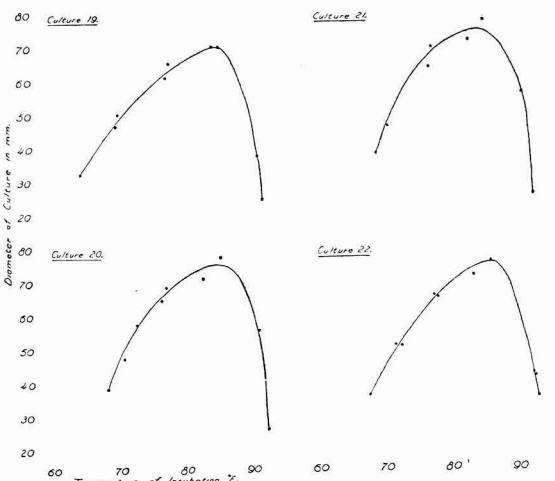

Fig. Ic-Growth Curves of *O. lactis* Cultures Isolated from Cream During Warm Weather.

were grown range from 69.6° F. (20.9° C.) to 93.3° F. (34.1° C.). The curves have been extended wherever necessary by extrapolation in order to derive parts of table 1, namely; the figures for the temperatures at which growth was one-half of the diameter of the maximum.

Table 1 shows the various cultures arranged as nearly as possible in the order of their optimum temperature of growth. These temperatures were derived from the growth curves shown in figures 1, 1a, 1b and 1c. The temperature above and below the optimum at which the amount of growth of each culture is equal to one-half of the diameter of each culture was derived, when necessary, by extrapolation. The differences from the optimum of these two temperatures are also given.

Figures and table 1 show:

1. The optimum temperature of growth for the twenty-two cultures of *O. lactis* ranges from 77.5° F. (25.3° C.) to 86° F. (30° C.).

2. As the optimum temperature increased, the difference between the optimum temperature and the temperature above the optimum, at which the rate of growth is one-half of the diameter of the optimum, decreased.

3. The average temperature change necessary to inhibit the growth to the extent of one-half of the diameter of the optimum is 17.5° F. (9.7° C.) below the optimum and 9.0° F. (5.0° C.) above.

4. The temperature above the optimum temperature of growth of all cultures, at which the growth was one-half of the diameter of the optimum, varied only 4° F.

5. The optimum diameter of growth of the individual cultures varies from a maximum of 81.5 mm. to a minimum of 53.5 mm.

DISCUSSION OF RESULTS

The optimum temperature of growth of 61 cultures of *O. lactis* reported by Macy and Gibson (5) was found to be from 15° C. to 25° C. Our experiments show a higher optimum temperature for the 22 cultures of *O. lactis* used, ranging from 77.5° F. (25.3° C.) to 86° F. (30° C.). The latter results are in closer agreement with the findings of Adams and Parfitt (1) and White and Hood (9) both working directly with cream.

The definite disagreement in optimum temperatures of growth for strains of *O. lactis* as recorded by Macy and Gibson (5) and in our data must be accounted for by using different cultures of *O. lactis* or differences in technique. It is to be noted that Macy and Gibson (5) obtained their cultures as isolations from butter which had been manufactured in Canada or Domestic butter (probably Minnesota butter), the time of year the butter was made is not mentioned. However, it is safe to conclude that our isolations were made under warmer temperatures, which would tend to select organisms having higher optimum temperatures of growth. On the other hand, the chief difference in technique was the humidifying of the air in our

experiment. It is suggested that the higher optimum temperature of growth for the cultures of *O. lactis* recorded in our experiment is in part due to less evaporation from the agar plates and thus a higher temperature of the medium was maintained. Further it must be pointed out that a higher humidity in the air is closer to the conditions found in a can of cream.

The mycelium test is a measure of the quantity of mold mycelium in the cream and is used as an indication of the undesirable fermentation in cream, a considerable part of which fermentation does not reach an optimum until 37° C. Therefore, the mycelium test increasingly loses its value between the optimum temperature of the growth of the molds in the cream and a temperature approximating 37° C. In other words, the same cream held the same time at a temperature of 28.2° C. would be a better quality cream and have a poorer mycelium test than the same cream held the same time at 35° C.

If a supply of cream was inoculated with an equal quantity of each of these cultures of *O. lactis,* it could be assumed that the critical temperature above which the mycelium test would not give positive results would be 82.7° F. (28.2° C.). However, in actual practice, cream would not receive an equal inoculation of all cultures of *O. lactis;* and, therefore, the critical temperature would be between 77.5° F. (25.3° C.) to 86° F. (30° C.).

All cultures, except number 1, required less variation in temperature above the optimum than they did below the optimum to inhibit the culture an equal amount. This shows that variations in the higher temperature range are of more importance than in the lower temperature range. In most cases the average optimum temperature for *O. lactis* is probably high enough to discard the possibility of cream being held at a temperature above the optimum of mold growth and thus destroy the value of the mycelium test.

CONCLUSIONS

1. The optimum temperatures of growth of twenty-two cultures of *O. lactis* have been determined on malt agar and were found to range between 77.5° F. (25.3° C.) and 86° F. (30° C.).

2. The optimum diameter of growth of the individual cultures varies from a maximum of 81.5 mm. to a minimum of 53.5 mm.

3. Slight variations in temperature above the optimum of a culture are of more importance in inhibiting growth than variations below the optimum.

4. Unless cream is held at a temperature above 82.7° F. (28.2° C.) the temperature requirements of *O. lactis* should not materially affect the usefulness of the mycelium test.

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THE RELATIONSHIP BETWEEN THE CURD TENSION OF MILK AND GASTRIC EMPTYING TIME IN CHILDREN

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INTRODUCTION

Cow's milk forms a much tougher curd in the stomach than does human milk. In infant feeding it is a common practice to modify cow's milk so that, among other things, the curd that is formed in the stomach will be soft and finely divided. In 1928, and later in 1931, Hill made some observations on infant feeding, reporting desirable results obtained in feeding milk with a low curd tension (4, 5) as measured by a test which he had devised in 1923 (6). This curd tension test, somewhat modified and amended, has been widely adopted. The belief is widely prevalent that the curd tension of milk is indicative of its digestibility, and directly associated with the rate at which the milk leaves the stomach of normal individuals. In 1932, Espe and Dye reported that "doubling the curd tension of milk increases the length of the digestive period from 30 to 65 per cent" (1). Using seven children for a study Reynolds, Macy and Souders in 1939 compared the gastric motility of bariumized milks* having a high curd tension with others having a low curd tension. They reported that the average gastric emptying times were 227, 214 and 193 minutes, respectively for pasteurized, evaporated and base exchange milks (9).

In 1939 Hadary and Sommer reported that chocolate milks have the curd tension characteristics of "soft curd" milk (3). The reduced curd tension of chocolate milks was attributed to the chocolate flavoring ingredients. It appears from some animal studies by Mueller that low curd tension chocolate milk is more difficult to digest than high curd tension untreated whole milk (7). Therefore, this study was undertaken to shed more light on the relationship between the curd tension measurement of milk and the rate of elimination of the milk from the stomachs of normal individuals as indicated by roentgenograms.

PROCEDURE

The course of the milk meal under investigation was followed through the digestive tract with the aid of roentgenograms. For this purpose it was

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* Two ounces of barium sulphate to four ounces of milk.

necessary to add barium sulphate to the milk. Preliminary investigations showed that two ounces of barium sulphate by weight per eight fluid ounces of milk sufficed to indicate the position of the milk meal in the system. The test milk meal was prepared in accordance with these findings and suspension was accomplished with the aid of an electric mixer. The meal was served at body temperature.

To determine the relative stomach emptying time when milks with varying curd tensions were fed, milk was treated in different manners to reduce the curd tension. With the exception of two soft curd milks, (viz. base exchange and evaporated milk), all the milks used in the feeding tests originated from the same source. Consequently they originally had approximately the same curd tension. The herds of the University of Wisconsin were surveyed to select cows having milk with a curd tension of 70 to 80 grams. Two Guernsey cows were selected. The butterfat content of their milk ranged from 4.6 per cent to 4.9 per cent. The milk from these cows was treated in the laboratory, duplicating commercial conditions, to produce the following:

(1) Control,—unmodified whole milk was pasteurized at 143.5° F. for 30 minutes and cooled to 45° F.

(2) Homogenized milk,—pasteurized at 150° F. for 30 minutes, homogenized at 2000 pounds per square inch and cooled to 45° F.

(3) Chocolate milk,—commercial chocolate syrup was added in the ratio of 1:12 to milk at 110° F., the chocolate milk was pasteurized at 160° F. for 15 minutes, and cooled to 45° F.

The following were taken from commercial sources:

(4) Base Exchange milk—A commercial product made in a licensed local dairy.

(5) Evaporated milk,—A commercial evaporated milk (non-irradiated) diluted in a ratio of 1:1 with tap water.

The curd tensions of all milks were determined in duplicate before and after the barium sulphate was added, using the procedure as tentatively recommended by the American Dairy Science Association in 1938.

The different bariumized milks were fed to children between the ages of four and thirteen. All these children were "milk-drinkers" and did not indicate any intolerance toward milk. They were patients in the Wisconsin State General Hospital, admitted for orthopedic treatment or plastic repair of relatively minor conditions. Otherwise, they were in good health, with no evidence of present or past gastro-intestinal disturbances. They were interested in the experiments being conducted, and were uniformly cooperative. Because there is no reason to expect a relation between any digestive idiosyncracies and the presence of these subjects in the hospital, and because all of the subjects which were available for the purpose were studied, it is

believed that the results may be considered typical for children of this age group.

The test milk meals were given to each child at intervals sufficient for the elimination of the residues from any previous feeding. The control milk was not given first to all the subjects, since it was desired to eliminate the effect of fear and nervousness which might be present at the first feeding but not at subsequent examinations as the child became acquainted with the roentgenographic procedure. Therefore, the order in which the milks were tested varied with each subject. Each milk was introduced into the child's diet some four days before the roentgenograms were taken, to eliminate any question of adjustment to the particular milk. This preliminary feeding consisted of one quart of the particular milk to be tested per child per day. The preparation of the subjects consisted of the withholding of all food and fluid after the evening meal on the day preceding the examination so that the stomach would be in the fasting state at the time the milk was given. After the test milk meals were given at 7 : 00 A.M., no further food or fluid was taken until the gastric emptying study was completed.

With the first two children examined, fluoroscopic examination was carried out during the taking of the meal and at frequent intervals thereafter until the stomach was empty. Roentgenograms were made as indicated. In subsequent cases fluoroscopy was omitted, but roentgenograms were made at intervals of two, four, five and in most cases six hours after the meal was given. These intervals were found satisfactory in indicating the time required for total gastric emptying. In a few cases the stomach still contained a small amount of the meal at the end of six hours, but the examination was not carried out to complete emptying since the children complained of hunger and became restless.

In most instances, a roentgenogram was made the following day, 24 hours post cibum, and in a few cases, another was made 48 hours after intake of the meal since, as a secondary consideration, it was desired to study the motility of the colon and the rapidity of colonic emptying with the different milks.

The roentgenograms were reduced photographically, and the per cent of stomach or colonic emptiness was estimated in each case by the roentgenologist without knowledge as to the type of milk involved. The records of these estimates were tabulated and analyzed statistically.

EXPERIMENTAL

From November, 1940, through July, 1941, over 350 roentgenograms were taken of some sixteen children. Of these, two received one milk; two children received two milks; four, three milks; one, four different milks; six, five different milks; and one, six milks. For purposes of the statistical analysis, only seven subjects who received five or more milks were used.

This was necessary because the variation between subjects was rather great, and the same subjects had to be used for all milks, so that each calculated average emptying time would be of similar composition.

ADEQUACY OF THE SAMPLE

The observations made in this study were taken with the view to determining whether or not differences in stomach emptiness of bariumized milks with different curd tension is a universal phenomenon, applying to normal children. Of necessity the problem is one of statistical inference, that is, from differences between the observed per cents of stomach emptiness for the different milks in the cases of the seven subjects studied it is desired to infer whether real differences exist between the average per cent of stomach emptiness for the different milks in the "population" comprised of all possible subjects. This being the situation, it is clearly of interest to know how large such real differences would have to be in order to have a reasonably good chance of detection by observations on only seven subjects which vary as much among themselves as did those in the present study. Using graphs and tables developed to answer such questions (10), it was found that with only seven subjects a difference of 20 per cent or more in average per cent stomach emptiness would be detected in about nine out of ten cases, and a difference of 10 per cent or more in about fourteen out of twenty cases. Therefore, it was concluded that if differences in average per cent stomach emptiness of biological importance existed between bariumized milks with different curd tensions, it is very likely that the seven subjects studied would have detected them.

RESULTS AND DISCUSSION

Table 1 shows the curd tension of the control and the four different soft curd milks fed to the seven subjects before and after the barium sulphate was added. The table shows that the addition of the barium sulphate to the milks did not increase the average curd tension of any of the milks more than 9.5 grams. In spite of the increase in the curd tension, the chocolate, base exchange, homogenized and evaporated milks were "soft curd" milks by the commonly accepted standards, and the control (unmodified whole milk) was a "hard curd milk." Inasmuch as the curd tension of the control milk used in the feeding tests was relatively high, and that of the soft curd milks relatively low, it was concluded that the barium sulphate in the quantities used in the feeding tests did not distort the curd tension relationships between the control and the soft curd milks. A distinct difference existed between the curd tension of the control and the soft curd milks. In fact, the curd tension of the bariumized control milk was over 55 grams higher than that of any of the soft curd bariumized milks, whereas the maximum difference between any two of the soft curd milks did not exceed 24

grams. The curd tension of the bariumized homogenized milk was the highest of all the soft curd milks, followed in a descending order by chocolate milk, base exchange, and evaporated milk.

Table 2 records the sex, age, and degree of stomach emptiness for the bariumized milks fed to the seven children at two, four, five, and six hours after feeding. The average per cent of stomach emptiness for each of the milks, and the estimated standard errors of each average were calculated and recorded in the table. The table indicates that as the time after feeding advances, the difference between the average stomach emptying time for the different milks declined. Thus, at two hours, the maximum difference between the seven-subject average emptying time was 18.6 per cent, at four hours 5.7 per cent, and at five hours 5.0 per cent. The maximum difference

TABLE 1

Curd tension of control and soft curd milks fed to seven children, before and after the $BaSo_4$ was added. November, 1940, through July, 1941. Wisconsin General Hospital

Subject	Control		Chocolate		Base exchange		Homogenized		Evaporated	
	Before	After	Before	After	Before	After	Before	After	Before	After
	Gr.	*Gr.*	*Gr.*	*Gr.*	*Gr.*	*Gr.*	*Gr.*	*Gr.*	*Gr.*	*Gr.*
G.B.	75–77*	81–81	10–11	15–15	0–0	8–8	18–20	24–24	0–0	4–3
W.C.	75–77	81–81	10–11	15–15	0–0	8–8	18–20	24–24	0–0	4–3
G.G.	75–77	81–81	10–11	15–15	0–0	8–8	18–20	24–24	0–0	4–3
M.N.	72–72	80–80	0–0	9–8	0–0	10–12	14–14	21–21	0–0	0–0
E.R.	72–72	80–80	0–0	9–8	0–0	10–12	14–14	21–21	0–0	0–0
F.D.	78–78	79–80	2–1	9–9	2–1	12–12	25–25	26–27	0–0	0–0
M.P.	78–79	79–80	2–1	9–9	2–1	12–12	25–25	26–27	0–0	0–0
Ave. curd tension	75.5	80.2	4.8	11.4	0.4	10.0	19.3	23.8	0.0	1.5

* Duplicate determinations.

between the average emptying time of the five subjects at the end of six hours after feeding was 4.2 per cent. Hence, it becomes apparent that the average percentage emptiness for the milks with the various curd tension becomes more and more uniform as the time after feeding advances, which is to be expected since all of the values are converging towards 100 per cent. It should be noted that the estimated standard errors of the averages are, to within fluctuations of random sampling, of the same magnitude at two hours as at four hours, whereas the averages differ more widely at two hours than at four hours. Therefore, if differences do exist in the rate of stomach emptying for milks with different curd tensions, they stand greatest chances of detection previous to four hours after feeding.

In table 3 the percentage of elimination from the system at twenty-four hours after feeding is summarized in terms of per cent emptiness for five

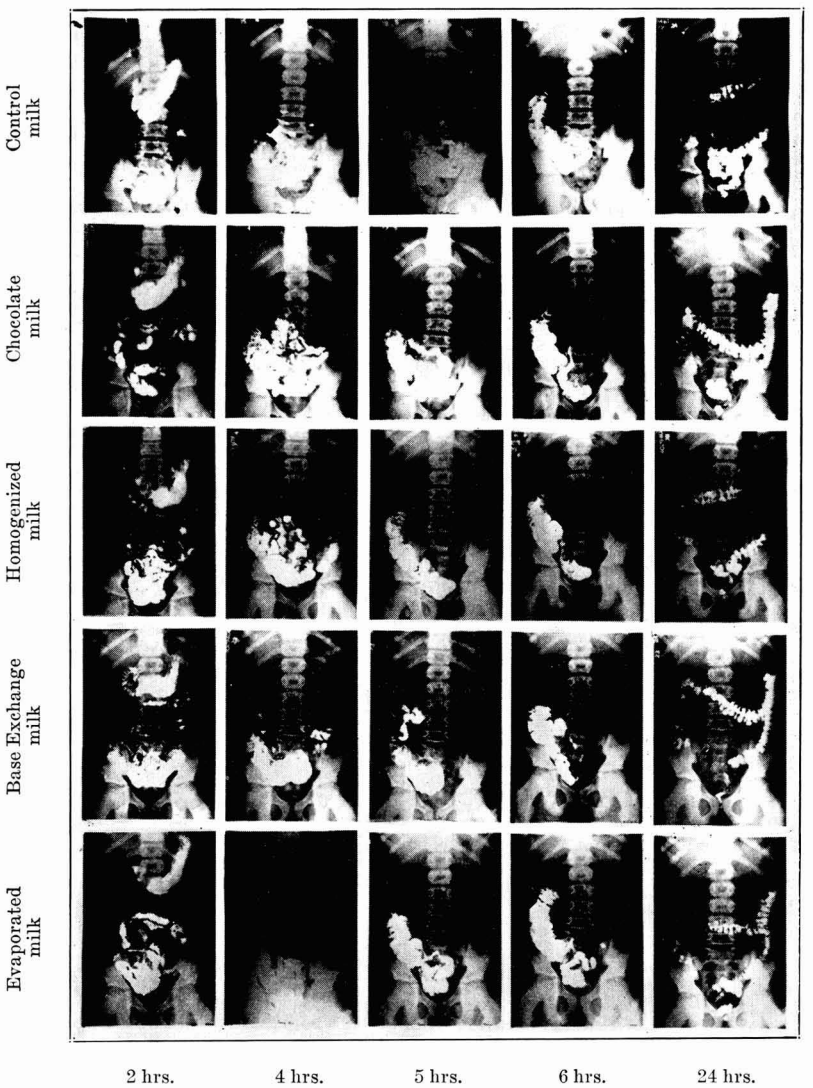

FIG. 1. Roentgenograms of one subject, 2, 4, 5, 6 and 24 hours after ingestion of five different types of milks. (Subject W. C. See Tables 1, 2 and 3.)

TABLE 2

Stomach emptiness as indicated by roentgenograms in response to bariumized hard and soft curd milks. November, 1940, through July, 1941. Wisconsin State General Hospital

Subject	Age	Sex	Type of milk																	
			Control				Chocolate				Homogenized				Evaporated				Base exchange			
			Hours after feeding																	
			2	4	5	6	2	4	5	6	2	4	5	6	2	4	5	6	2	4	5	6
G.B.	10	F	50*	75	90	99	50	75	90	99	50	75	95	100	50	90	100	100	50	80	100	100
W.C.	13	M	50	100	100	100	50	100	100	100	50	100	100	100	50	100	100	100	50	100	100	100
G.G.	7	F	50	80	100	100	25	75	99	100	75	90	100	100	40	80	100	100	40	90	100	100
M.N.	5	M	50	100	100	.....	25	60	90	.....	50	90	100	.....	50	80	100	.....	50	99	100	.....
E.R.	10	M	20	60	100	.....	20	50	60	.....	20	60	100	.....	20	60	99	.....	20	25	60	.....
F.D.	5	M	30	80	100	100	30	80	99	100	60	75	85	99	30	80	90	100	30	80	90	100
M.P.	13	M	30	40	50	80	25	90	100	100	50	75	80	90	30	80	85	90	25	80	100	100
Average at 2 hrs.			40.0				32.1				50.7				38.6				37.9			
Standard error at 2 hrs.			4.9				4.7				6.2				4.6				4.9			
Average at 4 hrs.				76.4				75.7				80.7				81.4				79.1		
Standard error at 4 hrs.				8.1				6.4				5.1				4.6				9.6		
Average at 5 hrs.					91.4				91.1				94.2				96.3				92.8	
Standard error at 5 hrs.					7.0				5.5				3.2				2.3				5.7	
Average at 6 hrs.						95.8				99.9				97.8				98.0				100.0
Standard error at 6 hrs.						4.0				0.02				2.0				2.0				0.0

* The figures in the table represent the per cent emptiness, 100 indicating that the stomach is completely empty.

subjects. The five children-average percentage eliminations for each of the milks are given.

ANALYSIS

To determine the statistical significance of the differences between the average percentage of stomach emptiness for the seven subjects on the five test milk meals recorded in table 2, the data were analyzed statistically, (See Appendix Table 4). On the basis of this analysis it was concluded that as the time after feeding advances, the difference "between subjects" and "between milks" decreases. At two and four hours after feeding the variation "between subjects" was rather great; this variation was considerably less at five hours after feeding. The differences between the average per cent of stomach emptiness in the case of four of the milks—control, chocolate, base exchange, and evaporated milks—two hours after feeding

TABLE 3

Percentage elimination from the system at twenty-four hours after feeding as indicated by roentgenograms in response to bariumized hard and soft curd milks. November, 1940, through July, 1941. Wisconsin State General Hospital

Subject	Type of milk meal				
	Control	Chocolate	Homogenized	Evaporated	Base exchange
G.B.	0	0	0	0	80
W.C.	30	0	80	80	30
G.G.	80	75	25	80	90
F.D.	75	0	0	25	25
M.P.	25	80	50	75	50
Mean	42	31	31	52	55

were not statistically significant. The average per cent of stomach emptiness in the case of homogenized milk did appear to be significantly greater than for the other milks at the two hour interval. However, this difference was not evident at four and five hours after feeding, all milks showing substantially similar degrees of stomach emptiness. Similarly, no statistically significant difference in elimination from the system was evident at twenty-four hours after feeding for the five test milk meals.

The results at twenty-four hours after feeding indicated that there was not a significant difference between the average per cent elimination for the test milks having different curd tensions. None of the subjects studied indicated an unusual response to chocolate milk which was consumed for four days before the test meal,—a constipated or laxative state was not detected as a result of drinking the chocolate milk. This was true at the end of forty-eight hours, as well.

The analysis of the data failed to indicate any relationship between the curd tension of bariumized milk and stomach emptying time as indicated

by roentgenograms. Curiously enough, bariumized-homogenized milk which has a relatively high curd tension as compared with the other soft curd milks, was the only milk which had a significantly greater per cent of stomach emptiness two hours after feeding than did the bariumized control.

The data obtained in this study are essentially in agreement with the results obtained by Reynolds *et al.* When the data of Reynolds *et al.* were subjected to statistical analysis as employed for data reported in the present article, it was found that the differences between the average stomach emptying times of the three test milks for the seven subjects cited by them were not statistically significant.

CONCLUSIONS

On the basis of the data accumulated in this study, it appears that roentgenographic examinations do not indicate that any correlation exists between the curd tension of bariumized milks and stomach or colonic emptying time of children. Soft curd milks did not leave the digestive tract more rapidly than the hard curd milk. Chocolate milk behaved in this respect as did all other soft curd milks.

ACKNOWLEDGMENT

The roentgenographic work was done in the Department of Radiology and Physical Therapy of the Medical School of the University of Wisconsin.

The authors are indebted to Professor C. Eisenhart, Station Statistician of The Wisconsin Agricultural Experiment Station for suggesting the mode of the analysis employed, and for valuable aid in the statistical interpretation of the results obtained.

APPENDIX—STATISTICAL ANALYSIS

By analysis of variance three sources of variation were segregated: (a) that introduced experimentally by the five milks (designated as "between milks"), (b) that introduced by the individuality of the subject (designated "between subjects") and (c) the remainder which can be neither controlled nor estimated in advance.

Every mean square "between milks" was compared with the error, making it possible to compute F-values to judge whether the characteristics of each of the milks had a significant effect on the degree of stomach emptiness. Similarly, every mean square "between subjects" was compared with the error, making it possible to compute F-values to judge whether the individuality of the subjects had an effect on the degree of stomach emptiness.

Lacking duplicate determinations for each subject for each type of milk, there is no alternative but to employ this remainder as the basis for estimating the experimental error. In so doing it is necessary to assume that there are no underlying inconsistencies among the responses of the subjects to the several milks, *i.e.*, that the underlying differences, if any, between the

milks as regards rate of stomach emptying are the same from subject to subject. If this assumption is false—and the present data provide no means of testing it,—the estimates of experimental error obtained from this residual variation will tend to be excessive so that detection of differences between milks will be handicapped. This limitation, which could be removed if duplicate determinations for each subject for each milk were at hand, should be kept in mind.

The F-values for "between milks" exceeded its 5 per cent point (2.78) only at two hours after feeding. Therefore, it was concluded that underlying differences between the milks existed only at the end of two hours

TABLE 4

Analysis of variance of stomach emptying time of five milks by seven subjects. Wisconsin General Hospital

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
		Two hours after feeding		
Total	34	6,724.29		
Between milks	4	1,138.57	284.64	3.631*
Between subjects	6	3,704.29	617.38	7.876**
Discrepance (error)	24	1,881.43	78.39	
		Four hours after feeding		
Total	34	10,475.55		
Between milks	4	180.35	45.09	0.293
Between subjects	6	6,611.75	1,019.58	6.643**
Discrepance (error)	24	3,683.45	153.48	
		Five hours after feeding		
Total	34	12,959.6		
Between milks	4	127.3	31.82	0.0676
Between subjects	6	1,543.2	257.20	0.5468
Discrepance (error)	24	11,289.1	470.38	

* Indicates significance at a 5% level.
** Indicates significance at a 1% level.

after feeding. A test suggested by Newman (8) indicated that only one of the milks, *i.e.*—homogenized milk, differed from the control and the other soft curd milks.

Observed differences between subjects were statistically significant at the 1 per cent level at two and at four hours after feeding. At five hours after feeding the "residual" mean square was significantly larger than that for "between milks" ($F = 470.38/31.82 = 14.78$; 1 per cent point = 13.93), which suggested that this "residual" mean square was excessively large as a result of some underlying inconsistency of the type mentioned earlier. An examination of the data for five hours after feeding shown in table 2 revealed such an irregularity. Subject M.P. registered a stomach emptiness at five hours after feeding far below the others with control milk and in excess of all but

one of the others with chocolate milk. On account of this erratic behavior no further statistical interpretation of the data for five hours after feeding time was attempted.

A similar analysis was made using the data reported by Reynolds *et al.* (Table 5). The F-values for "between milks" and for "between subjects" did not reach the corresponding 5 per cent point, 3.88 and 3.00 respectively.

TABLE 5

Analysis of variance of stomach emptying time of three milks by seven subjects. (Data reported in study by Reynolds et al.)

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	20	29,095.3		
Between milks	2	4,173.8	2,086.9	1.687
Between subjects	6	10,078.6	1,679.8	1.358
Discrepance (error)	12	14,842.9	1,236.9	

Therefore, it was concluded that no differences in stomach emptying time existed between Reynolds' three milks.

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THE USE OF A DIRECT READING pH METER FOR ROUTINE EXAMINATION OF MILK AT THE DAIRY PLANT INTAKE*

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Many dairy plants use certain, so called, "quick quality tests" to determine the acceptability of milk delivered to their receiving rooms from dairy farms. Perhaps the most commonly used test is the "acid grading" test which determines whether milk is above or below a previously selected acidity level, often 0.18 per cent. Another common test is the alcohol test which classes as inferior any milk exhibiting flocculation when mixed with an equal volume of alcohol, usually about 70 per cent by volume. The prevailing belief among the users of these tests is that they will detect milk which has undergone bacterial fermentation or milk which has been affected by mastitis infections of the udder and which would otherwise escape notice. It is well known that these tests are not particularly accurate for the purpose intended and the proportion of borderline cases and false judgments is relatively large. Nevertheless their use continues, possibly because they are easily made, the results are immediately available and they do not interfere with nor retard the efficient handling of the milk at the intake.

Inasmuch as it appears that some plants will persist in using some form of quick grading test in the receiving room for detecting milk deemed inferior and unacceptable, it was thought that a quick acting, direct reading pH meter might be used for this purpose with some advantage.

It is realized of course that the pH of normal herd milk is not sufficiently constant to be employed as a perfect index of abnormality. Nevertheless this value would appear to be a more valid one than titrable acidity or protein stability toward alcohol. Furthermore a potentiometric measure of pH would be less subject to error than a colorimetric method, sometimes employed in connection with alcohol tests.

The sole purpose of this study was to determine the adaptability and the accuracy of a standard make of pH meter when used for the purpose of determining the pH of herd milk as received at a dairy plant; and to ascertain whether readings can be made with sufficient rapidity and ease not to interfere with normal receiving operations. Incidental to this, some observations were made on milk quality but not enough to be conclusive.

All observations were made in the receiving room of The State College Creamery, where milk was received from about 50 farms daily during the period of the study.

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DESCRIPTION OF POTENTIOMETRIC EQUIPMENT

The instrument used for determining the pH of incoming milk in the receiving room was a Beckman, Industrial Model M, pH Meter, manufactured by the National Technical Laboratories, Pasadena, California, and loaned through the courtesy of the Arthur H. Thomas Company of Philadelphia, Pennsylvania. This instrument is a portable, self-contained, direct reading, unit capable of intermittent or continuous readings on samples at the instrument or at distances of several feet. The electrode assembly consists of a sealed and internally shielded glass electrode and a companion factory-filled calomel electrode. Fluid contact with the test liquid is made by the calomel electrode through a small hole protected by a ground glass

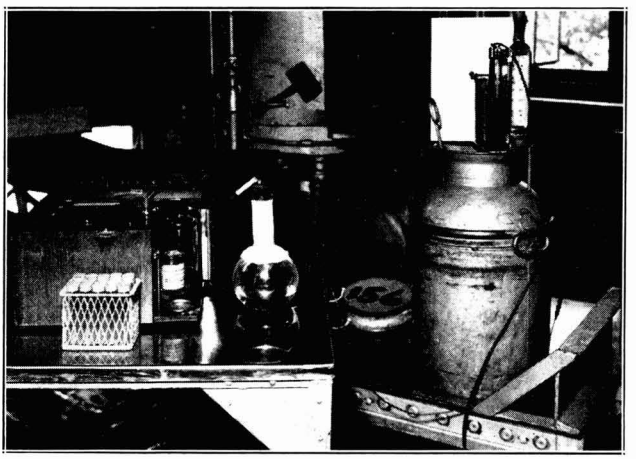

FIG. 1. View of the potentiometer connected by long shielded leads to the protected electrode assembly which is resting on a can preparatory to immersion.

sleeve, the medium being saturated KCl which is replenished from time to time. This arrangement effectively prevents contamination of the electrode and minimizes the manipulations required between readings.

The particular instrument used in this study was equipped with shielded lead wires about six feet long which enabled the remote electrode assembly to be immersed in the cans of milk as they came along the conveyor line to the dump tank. The instrument itself was placed on a table, at a convenient location, beside the conveyor. The remote electrode assembly furnished with the instrument was found unsatisfactory and a special fixture was constructed. This consisted of stainless steel screening soldered to a rod support in the form of a box open at the top and one side. The supporting frame for the electrodes (as normally used at the instrument) was attached to the screen box in such a way that the electrodes were protected from damage

and at the same time readily accessible. This arrangement also caught the ground glass sleeve of the calomel electrode if, for any reason, it dropped off in the course of making the determinations. The arrangement of the instrument and the remote electrode assembly is illustrated in figure 1.

MANIPULATIONS IN DETERMINING PH OF INCOMING MILK

The pH meter was adjusted and standardized against a buffer of known pH (about pH 6.6) according to directions furnished by the manufacturer.

TABLE 1

Comparison of pH readings obtained with milk samples using the Beckman instrument at 77° F. and at various other temperatures employing correction factors. Also, comparisons of the Beckman with the Coleman pH meter and a type K potentiometer utilizing a quinhydrone electrode

Sample	Beckman reading at 77° F.	Readings at different temperatures			Error due to table	Coleman reading at 77° F.	Quinhydrone potentiometer reading at 77° F.
		Temperature	Reading	Corrected reading from table			
No.	*pH*	*°F.*	*pH*	*pH*	*pH*	*pH*	*pH*
1	6.68	48	6.71	6.63	−.05	6.67	6.64
		89	6.62	6.68	.00		
2	6.63	55	6.70	6.64	+.01	6.61	6.60
		91	6.55	6.63	.00		
3	6.59	48	6.67	6.59	.00	6.59	6.54
		91	6.55	6.63	+.04		
4	6.63	60	6.69	6.64	+.01	6.62	6.60
		83	6.59	6.62	−.01		
5	6.73	52	6.78	6.71	−.02	6.70	6.69
		92	6.62	6.71	−.02		
6	6.59	41	6.69	6.61	+.02	6.59	6.60
		91	6.52	6.60	+.01		
7	6.62	49	6.73	6.66	+.04	6.61	6.60
		89	6.57	6.63	+.01		
8	6.61	56	6.67	6.61	.00	6.62	6.59
		90	6.49	6.56	−.05		
9	6.57	48	6.65	6.57	.00	6.57	6.55
		91	6.53	6.61	+.04		
10	6.51	38	6.59	6.50	−.01	6.50	6.47
		88	6.45	6.50	−.01		

Average deviation of corrected reading from reading at 77° F. (Beckman) ±.0175 pH.
" " of Beckman reading at 77° F. from Coleman +.008 pH.
" " of Beckman reading at 77° F. from quinhydrone +.03 pH.

The electrodes were then rinsed with distilled water and were ready for use in the cans of milk. As each can of milk came along the conveyor, the lid was removed, the odor noted and a long handled stirring rod inserted along with a quick acting thermometer. The milk was quickly agitated, the stirring rod put in the next can and the remote electrode assembly immersed in the milk. A pH reading was obtained from the instrument dial, the temperature noted and a temperature correction immediately applied. The electrode assembly was then removed, the electrodes rinsed with a fine stream

of distilled water from a wash bottle and the assembly was ready to be immersed in the next can. It was found that satisfactory results could be obtained by rinsing after every third or fourth can at which time the ground sleeve of the calomel electrode was loosened momentarily to permit flushing of the contact surface with fresh KCl. If the instrument was in use for more than 10 to 15 minutes, or if it stood idle for some time, it was found advisable to readjust the setting by means of the amplifier control. Every

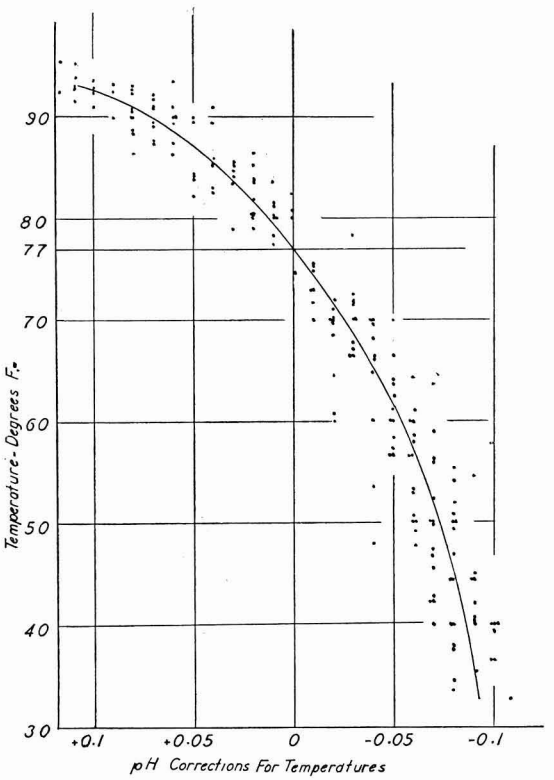

FIG. 2. Temperature correction curve for milk.

half-hour or so the asymmetry potential was checked against the buffer but adjustments were needed only occasionally.

At frequent intervals during the conduct of the studies the Beckman instrument was checked against a Coleman pH meter, which operates on a somewhat different principle, and against a potentiometer employing a quinhydrone electrode using selected samples of milk. The results with the direct reading meters were always very close. The quinhydrone electrode consistently gave slightly lower readings which seldom varied more than 0.05 pH (table 1). On the whole the instrument was found to be very accurate and astonishingly stable.

CORRECTION FOR TEMPERATURE

The Beckman pH Meter was standardized by the manufacturer for operation at a temperature of 77° F. (25° C.). Since the temperature of the incoming milk varied from 38° F. to 86° F. and since temperature adjustments of the milk were out of the question, it was necessary to determine whether a temperature correction could be applied to the pH readings and whether such corrections would be consistently accurate. Consequently, preliminary studies were made using 33 different samples of milk the pH being determined at seven different temperatures, one of which was 77° F. The data obtained are plotted in figure 2, the curve of which indicates the correction in pH applicable to milk when tested at various temperatures

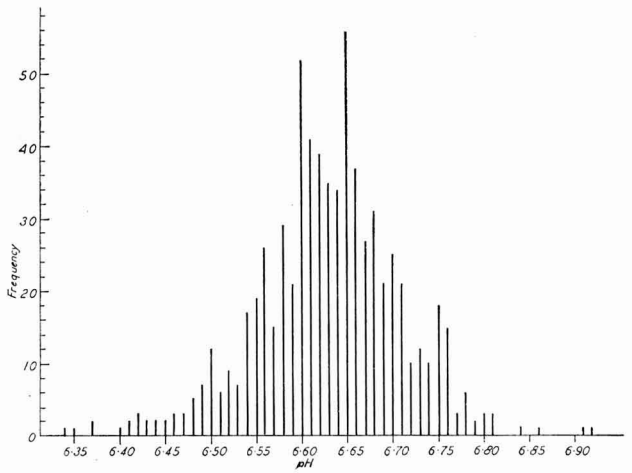

FIG. 3. Frequency distribution of pH values of herd milk.

above or below the standard. The amount of correction required was found to be less for a given number of degrees under standard than for the same number of degrees above standard. The temperature correction curve was accurate to within ± 0.05 pH, which was considered quite satisfactory.

After the temperature correction curve had been established a table was prepared from it for use in the receiving room, which enabled the operator to make corrections immediately after the readings were obtained. Table 1 presents data showing the relative accuracy obtained with the table of correction factors when milk temperatures varied from about 40° F. to 90° F. It also shows comparisons of pH determinations made with other instruments, the Coleman pH meter and a type K potentiometer employing a quinhydrone electrode.

pH VALUES OF MILK RECEIVED

In the course of the study 702 pH determinations were made of milk as it was being received. These were distributed through all four seasons of

the year and came from the same 50 herds, although every herd was not represented the same number of times. The pH values ranged from 6.34 to 6.92 with 97 per cent between 6.45 and 6.80, inclusive, 92 per cent between 6.50 and 6.76, inclusive, and 78 per cent between 6.54 and 6.71, inclusive. Frequency distribution of these values is shown in figure 3.

Milk with pH values of 6.8 or higher showed evidences of mastitis in the herds in 7 of the 10 cases, as judged by high leucocyte counts, high chlorides or the presence of chain streptococci. Eight of the 10 coagulated in the phosphate boiling test of Ramsdell, Johnson and Evans (1). It might therefore be concluded that a pH of 6.79 is the upper limit for normal herd milk. Milk exhibiting pH values of less than 6.40 showed evidences of bacterial activity as indicated by high bacterial counts. Three of the four samples in this category coagulated in the phosphate boiling test. Thus a minimum value of pH 6.40 might be taken as the limit for normal herd milk. It must be admitted, however, that these limits are somewhat arbitrary for not all the samples outside the range showed evidences of inferiority and on the other hand some samples having pH values within the range did exhibit inferior characteristics, particularly those associated with infections of the udder.

In so far as could be detected from the data available, seasonal factors seemed to have no influence on the pH values of herd milk although some indications of better buffering was noted in the case of milk produced in the spring and summer.

CONCLUSIONS

A Beckman direct reading pH meter, equipped with a remote electrode assembly connected with the instrument by six feet long shielded lead wires, was found suitable for use in determining the pH of incoming milk in a dairy plant receiving room.

The instrument was employed without appreciably interfering with or slowing the work of dumping, weighing or sampling of the milk.

Immediate results were obtainable by using a temperature correction table. This was found to be accurate to within about .05 pH by comparisons with results obtained at 77° F. and by comparisons using other pH instruments on the same samples.

Observations made on 702 samples of herd milk at different seasons of the year indicated a normal range of from pH 6.40 to 6.80. Only 14 (2 per cent) of the samples fell outside this range and eleven of these showed evidence of bacterial action or the presence of mastitis infections in the herds.

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THE VALUE OF CHLORINE IN PRODUCING LOW BACTERIAL COUNT MILK*

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The germicidal value of chlorine has long been recognized by health authorities and its use in dairy plants as a sterilizing agent is quite general. While the use of chlorine has gradually increased in the production and handling of milk, it is not yet generally used by dairymen except as a sterilizing agent for milking equipment.

With the increased demand for improvement in the quality of dairy products, it becomes more and more necessary that greater emphasis be placed upon sanitation at the very beginning of the milking procedure. While bottle-milk producers generally recognize and practice sanitary measures, it is believed that these precautions should be more commonly used by other classes of producers.

The use of chlorine in udder wash water and in milking machine teat cup rinse water is one of the suggested sanitary measures that might well be used by dairymen.

A survey of literature gives no data on the difference that might be expected in the bacterial count of milk produced with and without the use of chlorine in the manner suggested. Rather the literature seems confined to the use of chlorine in the washing and sterilizing of dairy utensils. Prouty and Hill (2) recommend that for sterilization of dairy utensils the solution of chlorine should be of 75 p.p.m. strength. Burgwald and Grant (1) recommend for washing milking machines that the chlorine solution be of 200 p.p.m. strength. These authors suggest that the cow's udder be washed with a clean damp rag but say nothing of using a chlorine solution for the washing.

In an effort to obtain data on the value of chlorine in keeping down the bacterial count of milk, the following study was made: Experiment 1 dealt with the bacterial count of milk when water alone was used to wash the udders and rinse the teat cups and experiment 2 with the bacterial count of milk when chlorine was added to the wash and rinse water.

EXPERIMENTAL PROCEDURE

Experiment 1: Seven cows from the college herd were selected for this work. These cows were free from mastitis on the basis of both a microscopic and bacteriological test. The animals were placed at the head of the milking string and were washed and milked in succession, beginning with cow No. 1.

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The milking was carried out with the use of two Surge milking machines. All equipment was sterilized with flowing steam at 180° F. for five minutes or longer between each milking. The same operator washed the cows and handled the milking machines during all of the experimental work. The udders of the seven cows were washed with the same two gallons of warm wash water using one wash cloth of heavy outing flannel of approximately 1 square foot in area. The cloth was sterilized after each milking period.

The procedure in obtaining samples was as follows: After a cow's udder was thoroughly washed, each of the four teats was dipped to its base in one sample of sterile water. The number of bacteria per cc. in this sample was used as a measure of the thoroughness of the washing procedure. A sample of the wash water was taken between the washing of each cow. The cow was then milked and a sample of milk taken with a sterile pipette directly from

TABLE 1

Average number of bacteria per cc. in various samples from individual cows when washed with water

Cow No.	No. of samples	Wash water	Teat rinse	Milk	Teat cup rinse
1	70	14,500	1,400	1,000	2,200
2	70	18,900	2,600	1,300	2,000
3	70	21,500	2,300	1,600	1,500
4	70	24,000	2,000	2,000	2,900
5	70	26,000	2,600	2,600	3,800
6	70	29,300	3,000	2,800	3,900
7	70	42,700	4,300	2,900	5,700

the milking machine. The milk was then weighed and the teat cups were immersed once in a bucket of two and one-half gallons of warm water. The four teat cups were then rinsed with one sample of sterile water as a measure of the value of rinsing the teat cups with water after each cow milked.

Plate counts were made of all water samples taken. All plating of water was done according to the Standard Methods for the Examination of Water and Sewage (3). The milk samples were plated according to the Standard Methods for the Examination of Dairy Products (4).

Experiment 2: Experiment 2 was identical with experiment 1 with the exception that chlorine was used in the udder wash water and in the teat cup rinse water. This water was chlorinated to the strength of 150 p.p.m. Bacterial counts of each sample of milk and water were made. Titrations to determine the amount of available chlorine in the water were run after each cow was washed and on the teat cup rinse water after each time the teat cups were rinsed. The titrations were made using a standard solution of sodium thiosulfate with potassium iodide and starch as an indicator.

RESULTS

Table 1 gives the actual number of bacteria per cubic centimeter as an increasing number of cows were washed with the same water.

From the data it is evident that when water only is used to wash the udders and rinse the teat cups the number of bacteria and the increase in the number of bacteria as each successive cow is washed and milked is quite large. It appears that as the number of cows milked increased, the bacterial count of the various water samples and milk samples increased. Such an increase presents a formidable threat to contamination of the milk produced.

Table 2 shows the number of bacteria per cc. when the seven cows were washed and the teat cups rinsed with a chlorine solution.

TABLE 2

Average number of bacteria per cc. in various samples from individual cows when washed with a chlorine solution

Cow No.	No. of samples	Wash water	Teat rinse	Milk	Teat cup rinse
1	70	500	900	600	1,300
2	70	1,000	1,300	900	600
3	70	1,100	1,400	1,200	1,000
4	70	1,200	1,600	1,300	1,200
5	70	1,400	2,300	1,600	2,300
6	70	1,900	2,400	2,100	2,500
7	70	3,600	2,600	2,300	3,700

Table 2 shows that when chlorine is used in the wash water and the rinse water the number of bacteria in the samples from Cow No. 1 are appreciably lower than in the case of the same cow when only water is used. The number of bacteria in the samples from Cow No. 7 are also considerably lower. The greatest difference is shown in the number of bacteria in the wash water samples. The use of chlorine in the wash water reduced the number of bacteria in these samples as much as 93 per cent. While there is an increase in the number of bacteria from the time the first cow was washed to the time the last cow is washed, the average bacterial count in each instance is considerably lower when chlorine is used than when water alone is used to wash the udders and rinse the teat cups.

Table 3 gives the average number of bacteria per cc. for the four types of samples taken using both systems of washing.

Because of the number of samples examined being large there appears to be justification in saying that the reduction in number of bacteria by

TABLE 3

Comparative number of bacteria per cc. by the two methods of washing

	No. of samples	Wash water	Teat rinse	Milk	Teat cup rinse
With water	490	25,271	2,800	2,171	3,142
With chlorine solution	490	1,528	1,785	1,428	1,800
% decrease in bacteria per cc.		93.8	36.2	34.2	42.7

the use of chlorine of 36.2 per cent in the teat rinse water, 93.8 per cent in the udder wash water, 34.2 per cent in the milk and 42.7 per cent in the teat cup rinse water is significant.

Figure 1 shows a negative correlation that exists between the chlorine content of the wash and rinse waters and the bacterial content of the various samples.

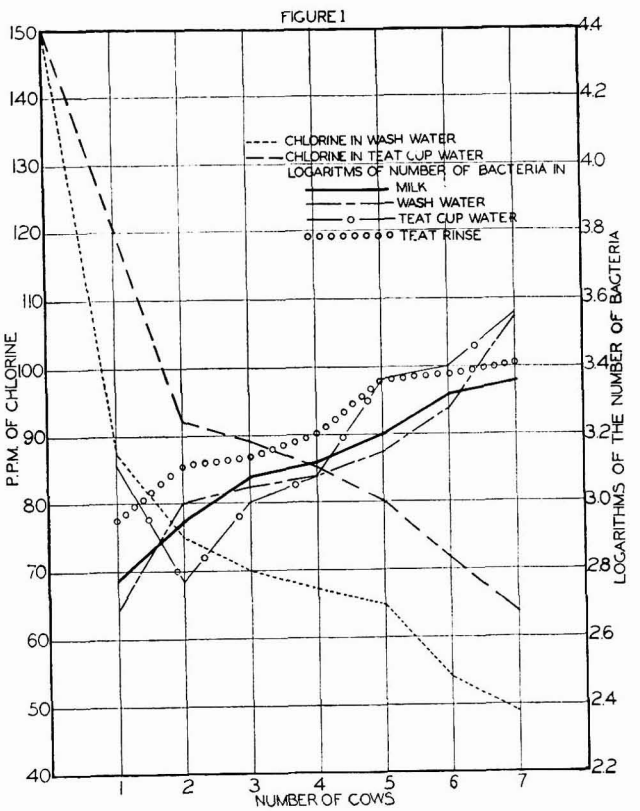

FIG. 1.

The figure shows that when the first cow was washed the chlorine content of the waters declined rapidly and as each successive cow was washed the decline continued but at a more gradual rate. With the reduction in chlorine strength was an increase in bacterial contamination of the various samples.

From the data it does not seem advisable that when starting with a wash water of 150 p.p.m. of chlorine that more than 7 cows should be washed without a change in the washing solution. With the washing of more than

seven cows under average conditions, it was found that the chlorine solution would be reduced to below 50 p.p.m., which is recognized as the minimum amount of available chlorine for germicidal value. The number of cows washed by a given quantity of water must be limited if the number of bacteria are to be kept at a minimum and when chlorine is used if the available chlorine content is going to be kept at a beneficial level. For maximum sanitary conditions the cow's udder should be washed in a chlorine solution of not less than 50 p.p.m. available chlorine.

The justification for dipping the teat cups in the chlorine solution after each cow has been milked is clearly evidenced in the data. It is evident that failure to carry out this practice may result in a greater chance of carrying organisms from one cow to the next and also multiplying the number of bacteria that may come in contact with the milk. By the use of chlorine in the rinse water the number of bacteria in the teat cups was reduced on the average 42.7 per cent.

SUMMARY

Seven cows were used to determine the value of using a chlorine solution for washing udders and rinsing milking machine teat cups. Bacterial counts were made as follows on (1) water in which the teats had been dipped, (2) samples of wash water, (3) milk samples, and (4) samples of water used to rinse the teat cups after they had been dipped. The use of chlorine in the wash water and teat cup rinse water reduced the number of bacteria in the wash water 93.8 per cent, in the teat rinse water 36.2 per cent, in the milk 34.2 per cent, and in the teat cup rinse water 42.7 per cent. While the bacterial count of the milk produced without the use of chlorine is fairly low and well within the limits set by the Standard Milk Ordinance, the value of chlorine under less sanitary conditions is clearly evidenced. The use of chlorine in udder wash water and in teat cup rinse water in the milking procedure is beneficial in reducing the number of bacteria in the milk produced.

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A RAPID METHOD OF CHURNING CREAM INTO BUTTER FOR MOLD MYCELIA DETERMINATIONS

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Early in June, 1940, the Federal Food and Drug Administration announced that it would vigorously pursue action against cream and butter producers who held, handled or manufactured cream and butter in violation of the law. This agency announced that it would use the mold mycelia test (1) as a guide for determining the quality of cream from which butter was manufactured. Cream that has been produced under insanitary conditions, improperly cooled and held too long on the farm will develop mold growth on the surface of the cream. *Oospora lactis* is the most common of the many genera of molds which contaminate cream. The vegetative mycelium is the part of the mold that is used as the basis of the mold mycelia test, which is a modification by Wildman (4) of the test being used for analyzing catsup and tomato products. Although pasteurization destroys mold, the Federal authorities contend that the presence of the dead mold mycelia in fairly large numbers in the butter is indicative of filthy, putrid or decomposed cream having been used in its manufacture.

REVIEW OF LITERATURE

Since the mold mycelia test is relatively new to the dairy industry, there is a demand for information concerning factors related to mold mycelia in butter. The standard mold mycelia test is used only for the determination of mold mycelia in butter. This test has not been designed for mold mycelia determinations of cream. No standard method has yet been published for the determinations of mold mycelia in cream. A method has been developed by Wildman (4) by which the mold content of cream may be estimated. In this method the mold is clumped with a methylene blue-borax (MBB) solution and then filtered from the cream and measured. A modification of this test has also been suggested by Parsons (2) in which the estimation of mold in cream may be done visually. This MBB test is an estimation of the relative amounts of mold in cream. These estimations are not expressed in terms of per cent positive fields of mold mycelia as in the mold mycelia test for butter. Therefore there is a need for a method of expressing the amount of mold in cream in the same terms as used to express the amount of mold mycelia in butter.

EXPERIMENTAL

In order to determine the percentage of mold mycelia in cream, it is necessary to first churn the cream into butter, and then determine by stand-

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TABLE 1

Statistical analysis of malt mixer method of churning small samples of cream

Sample No.	Per cent mold mycelia		Malt mixer deviation		Square of deviation
	Plant churn	Malt mixer	Minus	Plus	
1	40	44		4	16
2	48	42	− 6		36
3	40	32	− 8		64
4	52	52			0
5	36	42		6	36
6	36	28	− 6		36
7	44	34	− 10		100
8	68	68			0
9	24	18	− 6		36
10	64	66		2	4
11	50	60		10	100
12	32	42		10	100
13	46	54		8	64
14	32	32			0
15	24	34		10	100
16	28	22	− 6		36
17	20	20			0
18	36	30	− 6		36
19	52	50	− 2		4
20	64	70		6	36
21	56	48	− 8		64
22	42	38	− 4		16
23	30	32		2	4
24	22	30		8	64
25	60	52	− 8		64
26	46	54		8	64
27	46	32	− 14		196
28	28	24	− 4		16
29	0	2		2	4
30	72	62	− 10		100
31	68	62	− 6		36
32	32	34		2	4
33	32	32			0
34	56	48	− 8		64
35	56	54	− 2		4
36	24	28		4	16
37	24	22	− 2		4
38	28	26	− 2		4
39	44	48		4	16
40	32	34		2	4
41	50	48	− 2		4
42	84	86		2	4
43	68	62	− 6		36
44	50	44	− 6		36
45	32	30	− 2		4
46	32	36		4	16
47	20	18	− 2		4
48	52	50	− 2		4
49	40	36	− 4		16
50	30	32		2	4
51	34	26	− 2		4
52	28	22	− 6		36
53	12	12			0
54	26	30		4	16
55	32	32			0
56	48	42	− 6		36

TABLE 1—(*Continued*)

Sample No.	Per cent mold mycelia		Malt mixer deviation		Square of deviation
	Plant churn	Malt mixer	Minus	Plus	
57	58	54	− 4		16
58	20	18	− 2		4
59	38	40		2	4
60	36	42		6	36
61	22	32		10	100
62	42	46		4	16
63	98	86	− 12		144
64	52	48	− 4		16
65	62	58	− 4		16
66	42	46		4	16
Totals	2742	2678	−188	126	2196

ard methods the mold mycelia in the butter thus obtained. By using this procedure it is possible to express the mold content of cream in terms of per cent positive fields of mold mycelia in butter. A method of churning comparable to actual commercial churning conditions on the basis of mold mycelia could be considered satisfactory. Therefore the authors developed a method by which small samples of cream could be rapidly churned into butter with a malt mixer and then counted by the standard methods of counting mold mycelia in butter. This method has been named the "Malt Mixer Method" which is as follows:

1. Obtain a representative sample of cream by use of a stirring rod.
2. Transfer about 20 ml. of cream by means of a pipette into a two ounce sample jar.
3. Add an equal amount of ice water to the cream. It was found that the most convenient way to keep the water was in a one liter pitcher containing ice cubes.
4. With the malt mixer (Hamilton Beach No. 18) set at low speed, churn the cream into butter by carefully holding the glass sample jar so that the malt mixer blade extends about half way to the bottom of the jar. Care must be taken to prevent loss of the cream over the top of the jar due to excessive whirling.
5. The time required to churn a sample should be from $1\frac{1}{2}$ to 2 minutes. The temperature of the mixture partially determines the length of time required. If the cream breaks too soon the temperature is apparently too warm; so add a little more ice water to a new sample and repeat the churning process. However if too much water is added, the cream will not break; so the samples of cream must be cooled in an ice water bath.
6. As soon as the cream has broken, lower the jar away from the malt mixer blade. The butter granules should be about the size of a pin head. By carefully watching through the glass jar, the breaking can be observed by a color change from white of cream to a grayish, watery colored liquid containing butter granules.
7. Hold a wire mesh over the end of the jar and pour off the buttermilk.
8. Wash the butter with about one ounce of ice water. Stir thoroughly with a spatula, being careful to keep each granule separate. Pour off this wash water and repeat the washing process with another ounce of ice water.

9. After removing the second wash water, work the granules with a spatula until thoroughly lumped together. This working may be done in the same jar in which the butter was churned.

10. When sufficiently worked, store the butter in the same sample jar in which the butter was churned. The jar should be sealed, numbered, and immediately placed in an ice water bath or under refrigeration. The mold count on this butter should be determined as soon as possible after it has been churned, since the cream has not been pasteurized, and there is a possibility of mold growth on its surface even at refrigeration temperatures.

In order to determine whether this method was comparable to actual commercial churning conditions, a series of comparisons were made of the two methods. Cream samples were obtained in a commercial butter manufacturing plant directly from the churn before churning. These samples were then churned by the "Malt Mixer Method" and mold mycelia determinations were made on the butter obtained. Samples of butter were also obtained from the plant churn after the cream had been churned. There were 66 duplicate determinations made in this manner. The mold mycelia counts of these corresponding samples of butter are shown in table 1.

A difference of 8 per cent in the mold mycelia counts of various analysts working on the same sample of butter is generally considered satisfactory by Federal authorities. Considering 8 per cent as a tolerable amount of error, a statistical analysis by Student's method (5) was made on the data obtained in order to determine whether the "Malt Mixer Method" was comparable to actual commercial churning conditions. The positive deviations, those in which the malt mixer showed more than the plant churn, totaled 126. Only four of the positive deviations shown were in excess of 8 per cent. In each of these four cases the deviation was positive 10. The negative deviations, those in which the malt mixer showed less than the plant churn, totaled – 188. Four of the negative deviations shown were in excess of – 8 per cent. These four deviations were – 10, – 14, – 10 and – 12 respectively. The difference of the two sums (– 62) divided by the total number of samples gave a mean difference of – 0.94. The sum of the squares of the deviations was found to be 2196. The square root of the sum of the squares of the deviations divided by the number of samples gave a standard deviation of differences of 5.768. Dividing this standard deviation of differences by the square root of the number of samples (8.124) a standard error of mean differences of 0.71 was secured. The mean difference (– 0.94) divided by the standard error of mean differences (0.71) gave a significant number of only 1.3239. Fisher's table (5) shows the least significant number would be 1.95996. Hence it was concluded that there was no statistically significant difference between the mold mycelia counts of butter churned by the "Malt Mixer Method" and that churned in commercial butter plants. The previous assumption that the rapid speed of the malt mixer would break the mold mycelia too much proved false. The mold mycelia appeared just as

long as those appearing in the butter churned in a commercial churn under commercial churning conditions.

DISCUSSION

The "Malt Mixer Method" offers a new approach to the studies of mold in cream. Small cream samples obtained from the farm, cream station, factory or laboratory may be rapidly churned into butter by this method, and then counted by standard methods of counting mold mycelia in butter. In this way the mold content of a sample of cream may be expressed as per cent positive fields of mold mycelia. Results of this kind may be interpreted as per cent positive fields of mold mycelia in the butter churned from a particular sample of cream.

The "Malt Mixer Method" has been used by the authors in an investigation (5) of a number of factors related to mold in cream. This method has been found to be very applicable to studies of this type. By having results from both cream and butter studies expressed as per cent positive fields of mold mycelia, the interpretation of the results is aided materially.

SUMMARY

A rapid method of churning cream into butter for mold mycelia determinations has been developed. This method consists of churning cream into butter by use of a malt mixer and the butter obtained then counted by the standard method of counting mold mycelia in butter. It was found that there was no statistically significant difference between the mold mycelia counts of butter churned by the "Malt Mixer Method" and that churned in commercial butter plants. The "Malt Mixer Method" was found to be rapid, simple, accurate and applicable to the study of mold in cream and the mold mycelia in the butter made from the same cream.

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American Dairy Science Association Announcements

MISSOURI WELCOMES YOU

January 29, 1943

OFFICERS AND MEMBERS OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION:

The University of Missouri is looking forward with pleasure to your meeting scheduled to be held on this campus beginning on June 21. The University of Missouri is the oldest State University west of the Mississippi River and its College of Agriculture has had, as you well know, a long and distinguished career. Several of the graduates of the College have served as President of your organization and they occupy positions of prominence in the field of the dairy industry. We take great pride in the work of these men.

We all recognize that the entire dairy industry is confronted with many acute problems in connection with its contribution to the nation's war efforts. I trust that your deliberations on our campus will contribute materially to the solution of these problems and to our nation's victory.

FREDERICK A. MIDDLEBUSH,
President

AMERICAN DAIRY SCIENCE ASSOCIATION
THIRTY-EIGHTH ANNUAL MEETING (TENTATIVE PROGRAM)
UNIVERSITY OF MISSOURI, COLUMBIA, MISSOURI,
JUNE 22–24, 1943

Tuesday, June 22

10:00–12:00	Opening General Session
1:30– 3:30	Section programs (papers or symposium)
3:30– 5:30	Committee meetings
7:00	Social Period
8:00	Reception and Entertainment

Wednesday, June 23

8:00– 9:30	Committee meetings
9:30–11:45	Section programs (papers or symposium)
11:45–12:00	Group picture
1:30– 3:00	Section programs (papers or symposium)
3:00– 4:00	Section Business meetings
4:00– 5:30	Tour and Demonstrations—University Campus, Laboratories and Barns

6:00	Lakeside barbecue
9:00	Entertainment

Thursday, June 24

8:00– 9:00	Committee meetings
9:00–11:00	Section programs (papers or symposium)
11:00–12:00	Section Business meetings
1:30– 3:00	Section meetings
3:00– 5:00	General Association Business meeting
6:00	Association Banquet

CALL FOR TITLES

Titles of papers to be presented at the 38th annual meeting should be in the hands of the program committee not later than April 1, 1943.

Program Chairmen are as follows:

H. F. JUDKINS, *General Chairman,* 230 Park Ave., N. Y.

E. C. SCHEIDENHELM, *Extension Section Chairman,* Michigan State College.

P. F. SHARP, *Manufacturing Section Chairman,* 317 Ramona Ave., Piedmont, California.

K. L. TURK, *Production Section Chairman,* Univ. of Maryland.

Abstracts of papers to be presented should be in the hands of the program committee by *June 1.* These will be published together with the proceedings of the business meetings in the August issue of the Journal.

VOLUME XXVI MARCH, 1943 NUMBER 3

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

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

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Canadian Public Health Journal
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United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEWS

102. Rassenkunde des Rindes. SCHMID, A. (Eidg. Techn. Hochschule, Zurich, Switzerland.) Two volumes. Published by Bentelli A. G., Bern. Volume 1 (10 francs) of 205 pages is not illustrated; volume 2 (15 francs) consists entirely of pictures (about 360) without printing except for legends and table of contents.

Volume 1 opens with some twenty pages of general remarks concerning the taxonomy of cattle, the economic place and importance of cattle production, and the various ways in which the breeds of cattle may be classified. The countries are then considered one by one. First for each come some introductory remarks about natural conditions, industries, and animal production. Then come descriptions of the cattle husbandry and breeds. The description of each breed includes its place of origin; its general racial characteristics such as conformation, hair coat, kind of horns, color of hair and skin, etc.; then its typical productiveness as indicated by live weight, milk yield, percentage of fat in milk; whether it is well or poorly suited to meat and work production; and finally any notes of special interest.

Because of its brevity the account of each breed will no doubt be considered incomplete by the enthusiastic admirers of that breed. Doubtless in each country those who are well acquainted with the details of the local cattle husbandry will find some errors of omission or emphasis concerning the cattle of their own country. Yet this comprehensive survey of the cattle races of the whole world will be very welcome to the man who seeks only a general knowledge on this subject or who intends to learn in detail about the cattle husbandry of some country with which he is unacquainted but wants to begin with a simple and general survey.

Many of the breeds pictured and described will be new to most Americans. The author's intention was to describe and picture typical individuals of the breed rather than the best representatives or the ideal. Yet a few pictures of "true type" paintings or of show winners are included. A few landscapes are included to show the nature of the agriculture in the region in which the breed being described is kept. J.L.L.

103. Virus Diseases. THOMAS M. RIVERS, WENDELL M. STANLEY, LOUIS O. KUNKEL, RICHARD E. SHOPE, FRANK L. HORSFALL, AND PEYTON ROUS. The Rockefeller Institute, New York. Published by Cornell Univ. Press, 124 Roberts Place, Ithaca, N. Y. 170 pp., illustrated, $2.00.

In this book the 1942 series of Hiram J. Messenger lectures are brought together under one cover. The titles of these lectures are as follows: Virus

Diseases with Particular Reference to Vaccinia; Chemical Structure and Mutation of Viruses; New Hosts as a Key to Progress in Plant Virus Disease Research; Swine Influenza; Human Influenza; Viruses and Tumors. Each lecture is accompanied by a bibliography of references cited.

Interesting and important facts about these peculiar disease-producing agents are discussed by six virus workers in the fields with which they are familiar. T.S.S.

BACTERIOLOGY

104. Studies on Staphylococci. I. Occurrence of Bacteriophage Carriers Among Strains of *Staphylococcus aureus*. ROY T. FISK, School of Med., Univ. South. Calif., Los Angeles. Jour. Infect. Dis., *71*, No. 2: 153–160. Sept.–Oct., 1942.

Forty-three strains of *Staphylococcus aureus* were tested for the presence of phage by spotting broth cultures of each strain onto streaks of a susceptible strain growing on an agar medium and noting which spots developed lysed areas in the streak. Of the 43 strains, 44.2% were found to be phage carriers. Twenty-four different phages were identified by their selective action on various strains of the aureus group. Lysogens could not be demonstrated in cultures of nonpathogenic *Staph. aureus*, and these strains were not lysed by phages isolated from lysogenic strains. J.F.C.

105. Studies on Staphylococci. II. Identification of *Staphylococcus aureus* Strains by Means of Bacteriophage. ROY T. FISK, School of Med., Univ. South. Calif., Los Angeles. Jour. Infect. Dis., *71*, No. 2: 161–165. Sept.–Oct., 1942.

In a study of the susceptibility of 95 strains of *Staphylococcus aureus* to 27 different strains of phage, it was found that aureus strains isolated from related sources reacted to the same phages and could be differentiated from other cultures by this method. J.F.C.

106. Oxidation-Reduction Potentials in Salmonella Cultures. IV. A Note on the Relation of Observed Potentials to pH. WILLIAM BURROWS, Univ. Chicago, Jour. Infect. Dis., *71*, No. 2: 106–109. Sept.–Oct., 1942.

Using three Salmonella cultures differing widely in characteristic oxidation-reduction potential, the author measured the potential for each strain at various pH levels in a medium in which the pH was closely controlled by phosphate buffer. The change in potential per unit pH was observed to be 61 millivolts for *Salmonella cholerae-suis*, 55 m.v. for *S. typhi*, and 62 m.v. for *S. enteriditis*. This is very close to the theoretical relationship of 60 m.v. per unit pH which would exist if the O–R system involved a 2 electron transfer with no new cations formed by the hydrogenation. J.F.C.

107. Egg Mediums for the Isolation of All Three Types of Tubercle Bacilli. JANET R. MCCARTER AND ELIZABETH M. KANNE, Univ. Wis., Madison. Jour. Infect. Dis., *71*, No. 2: 102–105. Sept.–Oct., 1942.

Whole egg media and egg yolk media prepared in various ways were compared as to their ability to support growth of avian, bovine, and human types of tubercle bacilli. Inoculation was made both from laboratory cultures and from infected tissue specimens. Egg yolk media without glycerine were found to be the most satisfactory. Since none of the nutritive factors in the egg yolk appeared to be heat labile, autoclaving was preferred to inspissating or to no heat treatment. J.F.C.

BREEDING

108. British Animal Husbandry. ANON. Food Mfr., *27*, No. 19: 350–351. Dec., 1942.

The ideas in this article are those of Dr. John Hammond. Details on production tests are given for Ayrshire and Jersey cattle. Breeding to develop stock for beef, mutton, lamb, and milk fat production are discussed and explained. J.C.M.

BUTTER

109. Effect of Different Pastuerization Temperatures on Keeping Quality of Butter Made from Cream Containing Naturally Active Lipase. E. L. JACK, N. P. TARASSUK, AND E. L. SCARAMELLA, Univ. Calif., Davis. Natl. Butter and Cheese Jour., *33*, No. 12: 16. Dec., 1942.

Incipient rancidity of butter is a winter defect caused by naturally active lipase enzyme sometimes present in milk produced late in lactation and on green-feed deficient rations. Such milk was selected, separated, and the cream subjected to various pasteurizing and holding treatments. The cream was churned and the butter stored at 20°–22° F., scored and analyzed for acidity. The flavor defect is associated with acidity increases in the fat. Deterioration did not occur during storage for 60 days but rather in the cream before pasteurization. Surface tension measurements demonstrated the lipase activity in the raw cream. Heat treatment of cream at 150° F. for 30 minutes apparently inactivated the lipase but it is possible that lipase was only partially inactivated by the heat treatments and that lipase activity in the butter would have occurred at higher storage temperatures. The study is being continued. W.V.P.

CHEESE

110. War Demands Call for Quick Curing Cheese. J. C. MARQUARDT. Food Indus., *15*, No. 1. 1943.

This article deals with the manufacture of high moisture, quick curing cheese varieties. Procedures for making Tilsiter, Hollander and Wilstermarsh cheeses are given. These varieties have a characteristic cheese flavor without strong odors. These varieties are successfully made from raw or pasteurized milk. Procedures for making Tilsiter cheeses from 1.0, 1.8, and 3.0% fat milk are given.

Directions for rapidly curing cheddar cheese based upon laboratory observations are given. These procedures have been adequate to achieve rapid curing under practical conditions.

A report is also given of the possibilities of making soft cheese spreads of the above cheese types with a standardized high salt percentage.

The article is a brief review of the author's knowledge of Continental European cheeses of the quick curing type. It also includes information regarding quick curing of cheddar cheese and the making of high moisture cheese spreads based upon laboratory and field experiences. J.C.M.

111. Cheese Grading Clinics. WALTER V. PRICE, Univ. Wis., Madison. Natl. Butter and Cheese Jour., *33*, No. 12: 12. Dec., 1942.

Four cheese grading clinics where state and licensed cheese graders criticized and graded identical lots of cheese were held at the request of the Wisconsin cheese industry. The cheese were specially selected to include many "border-line samples." An average of 3 out of 10 graders failed to agree with the majority on individual samples; 2 of the graders always agreed with the majority; about one-third agreed with the majority on 12 or more out of 15 samples; while three-fourths of them picked 9 or more out of 15 samples. Cheese clinics can help graders attain greater uniformity if they are held frequently, if cheese are properly selected, if inexperienced graders are encouraged to attend and if proper educational methods are used. Grading clinics can serve to identify outstanding graders. W.V.P.

112. Pasteurization (of Milk for Cheddar Cheese). H. P. NIELSEN, Waterloo, Wis. Natl. Butter and Cheese Jour., *33*, No. 12: 14. Dec., 1942.

A practical discussion of plant practice and experience. W.V.P.

DISEASE

113. Human Tuberculosis of Bovine Origin in England and Scotland. Jour. Amer. Vet. Med. Assn. *101*, No. 789: 468. Dec., 1942.

Available statistics indicate that "in Wales the bovine bacillus accounts

for 1%, in England, 1.6%, and in Scotland 7% of pulmonary tuberculosis." The bovine type is responsible for about 50% of lupus and cervical gland tuberculosis in England, 25% of the meningeal and 15% of the bone, joint and genito-urinary tuberculosis. Corresponding figures for Scotland are considerably higher. S.A.F.

114. Calfhood Vaccination in a Brucellosis Control Program in Certified Dairies. C. W. BONYGNE, Los Angeles Medical Milk Commission, Los Angeles, Calif. Cert. Milk, *17*, No. 199: 5. Nov., 1942.

This article deals with experiences which were obtained during 14 years of effort in Brucellosis control in herds which are among the largest in the country. Attempts to eliminate Brucella infection by tests and removal was found to be economically unsound and offered no prospect of permanent eradication unless supplemented by calfhood vaccination. Also, the practice of establishing herds composed of unvaccinated heifers raised under disease free environments was unsuccessful, because such animals were found to be highly susceptible to the disease when induced into milking strains. It is pointed out that the possibility of dairy herds consisting of animals having definite immunity to brucellosis may eventually be realized through calfhood vaccination. W.S.M.

115. Toxic Effects of Tyrothricin, Gramicidin, and Tyrocidine. CHARLES H. RAMMELKAMP AND LOUIS WEINSTEIN, Evans Memorial, Mass. Memorial Hospitals and Boston Univ. School of Med., Boston. Jour. Infect. Dis., *71*, No. 2: 166–173. Sept.–Oct., 1942.

Under the conditions of the experiment hemolysis was observed in a concentration of 0.00007 mg. of tyrocidine per ml., whereas the concentration of gramicidin required to produce hemolysis was 0.01 mg. per ml. Leucocytolysis and supression of phagocytosis were much more pronounced with tyrocidine than with gramicidin, with tyrothricin occupying an intermediate position with respect to these reactions. Intravenous administration of small amounts of tyrothricin to mice caused death. With intraperitoneal injections gramicidin was somewhat more toxic than tyrothricin, but it was possible to inject either in amounts sufficient to sterilize local infections without producing general toxic effects.

In intradermal injections tyrothricin caused a greater degree of induration and inflammation than did gramicidin. Oral administration of tyrothricin to mice caused no toxic symptoms but the gram-positive flora of the intestines of the subjects was not altered. With local applications, tyrothricin was effective as a bactericide and its use was not attended with toxic symptoms even when large amounts were used. J.F.C.

116. An "Infection-Prevention" Test for the Evaluation of Skin Disinfectants. W. J. NUNGESTER AND ALICE H. KEMPF, Univ. Mich., Ann Arbor. Jour. Infect. Dis., *71*, No. 2: 174–178. Sept.–Oct., 1942.

An "infection-prevention" test has been devised for the evaluation of skin disinfectants. This test depends upon the ability of the disinfectant, applied to skin contaminated with a virulent organism, actually to prevent infection when the skin is placed in the peritoneal cavity of the animal. A number of disinfectants have been tested by this method in mice. The results are discussed. (Authors' summary.) J.F.C.

FEEDS AND FEEDING

117. Feeding Vitamin A to Dairy Cows to Increase Milk Production. L. T. WILSON, Walker Gordon Lab. Co., Inc., Plainsboro, N. J. Cert. Milk, *17*, No. 197: 5. Sept., 1942.

The feeding of a commercial vitamin preparation (Synergol) in amounts furnishing 125,000 U.S.P. units of vitamin A and 35,000 U.S.P. units of vitamin D per cow per day resulted in increases in milk production equivalent to approximately 2 quarts of 4% milk per cow per day. These results were obtained in a number of tests with small groups of cows, and in two large scale trials involving 50 and 600 cows, respectively. However, a test with two small groups in the summer time failed to show any increase. A set of tests on small groups of cows designed to see whether the feeding of other products furnishing vitamin A would produce an increase in milk production similar to Synergol failed to give conclusive results. The following vitamin A supplement—Synergol, shark liver oil and preparations put out by the Special Products Division of the Bordon Company, when fed at levels of 500,000 and 1,000,000 U.S.P. units per cow per day brought about definite increases in the vitamin A potency of the butterfat produced. When fed at the 500,000-unit level all three resulted in butterfats of the same potency. W.S.M.

FOOD VALUE OF DAIRY PRODUCTS

118. The Variability of the Vitamin Content of Milk, Part I. I. J. WOLMAN, Philadelphia Pediatric Society. Cert. Milk, *17*, No. 197: 3. Sept., 1942. Part. II. Cert. Milk, *17*, No. 198: 5. Oct., 1942.

A review article covering the important vitamins found in milk and emphasizing those features which can be made subject to control by the producers of milk. It is pointed out that the content of vitamin A and riboflavin in milk can be maintained at a high and uniform level by more scientific feeding of the cows. Also, fortification of milk with vitamin D

merits greater attention than it now receives. Fresh milk contains appreciable amounts of ascorbic acid (vitamin C) and of thiamin (vitamin B_1). Since these two vitamins are partly destroyed or inactivated by pasteurization or handling, every effort should be made to conserve them. Vitamin A, Vitamin D, riboflavin and niacin are stable substances not destroyed or inactivated by pasteurization or handling. W.S.M.

ICE CREAM

119. Ice Cream Ingredient Procurement. E. C. Scott, Swift & Co. Res. Lab., Chicago. Ice Cream Field, *40*, No. 5: 12. Nov., 1942.

The restrictions on sugar have been met by the greater use of corn sugar, corn syrup and corn syrup solids, and an increase in sweetness of 23 to 25% is gained as the result of inverting sucrose. The inversion of sucrose can be accomplished in stainless steel or glass pasteurizing vats, by adjusting the syrup to pH 2.0–2.5 with either tartaric, citric, phosphoric or hydrochloric acids and then heating for 1 to 1¼ hours at 190 to 200° F.

Chocolate or cocoa flavoring can be reduced 25 to 30% without materially changing the character or flavor intensity, provided the sugar content is also reduced. Mention is made also of the fact that especially processed domestic oils have been successfully used to replace cocoanut oils as chocolate thinners. Butterscotch and carmel flavored, as well as unflavored coatings, are being used to replace the deficiency of chocolate coating.

There is an adequate supply of gelatin; also certain gums of vegetable and marine origin. There is a critical shortage of vanilla beans, vanillin also is short, and coumarin has been withdrawn from the market. It is stated also that it is likely there will be a sizeable increase in alcohol tax and the possibility that alcohol will be withdrawn from the market competely.

W.C.C.

120. Wartime Sanitation Safeguards. J. J. Sampey and R. K. Lawhorn, Abbots Dairies, Inc., Philadelphia. Ice Cream Field, *40*, No. 5: 43. Nov., 1942.

The advisability of checking the condition of ice cream equipment at the time it is cleaned is stressed and it is stated that poorly cleaned equipment can contaminate properly pasteurized mix.

Brief mention is made of washing compounds and a tabulation given in which meta silicate, caustic soda, phosphates and soda ash are listed on the basis of their relative effectiveness for (1) wetting, (2) emulsifying, (3) dissolving, (4) deflocculating, and (5) germicidal properties. The authors also mention that soap, linseed oil, pine oil, rosin, borax, ammonium sulfate, phosphoric acid, bichromate of sodas, caustic potash, ammonium chloride, sodium silicate, methyl orange and phenolphthalein are used in washing compounds by various manufacturers.

The important of wetting agents is stressed, and the use of acid washing preparations, especially in washing milk cans, is also mentioned. It is claimed that in removing milk stone best results are usually obtained by first treating with acid and then with alkali solution. In the final analysis good results can be obtained only when the surfaces are properly washed and then sterilized and the authors point out that this can be accomplished only by the intelligent use of efficient washing compounds plus the conscientious effort on the part of both the person responsible for, and the men actually doing the work. W.C.C.

MILK

121. Keeping Qualities of Milk. JOHN E. NICHOLAS AND T. G. ANDERSON, Pa. Agr. Expt. Sta., State Col., Pa. Refrig. Engin., *44*, No. 6: 370–371. 1942.

The experimental evidence obtained by the authors demonstrated that bottled pasteurized milk and homogenized pasteurized milk may be kept in the home refrigerator at 40° F., undisturbed, from 12 to 28 days without detectable spoilage occurring. While oxidized flavor developed in both milks it was not of sufficient intensity to class the samples as spoiled. Similar samples of milk when taken from the refrigerator daily and shaken and then allowed to stand for one hour before being returned, withstood spoilage from 9 to 20 days. Tests conducted with raw milk undisturbed and disturbed resulted in spoilage in from four to seven days. Bacteria counts of the samples ranged from one thousand to ninety-four thousand per cc. The variation in keeping time at 40° F. of the same and different types of milk is attributed to the differences in the type of initial contamination of the milk. Raw milk containing a large percentage of low temperature organisms spoil in a relatively short time. Because of inhibition organisms in pasteurized milk of even relatively high bacterial content bring about spoilage slowly. In the raw milk little or no change in the bacterial content occurred on initial refrigeration. These findings point to the feasibility of every other day deliveries to householders but it also emphasizes the necessity for maintaining the home refrigerator temperature at 40° F. which is lower than that at which many are kept. L.M.D.

122. Recent Developments in the Resazurin Test. Part II. JOHN G. DAVIS. Food Mfr., *27*, No. 19: 344–349. Dec., 1942.

This is a very detailed article on the subject and tables and references are given. The use of a comparator and where it can be obtained are listed. Preparation of the Resazurin solutions are given. Data on measurements and temperature are also discussed.

Mastitis as a cause of failure in the test is discussed and explained. Six different Resazurin tests are given in detail and some comparisons are made. Two pages of references are given. J.C.M.

123. Proper Mixtures for the Can Washer and Types of Sequestering Agents. S. T. COULTER, Univ. Minn., St. Paul. Amer. Butter Rev., *4*, No. 6: 248. 1942.

Scale accumulation in the can washer may be prevented by adding to the washing solution used, if satisfactory otherwise, tetra sodium pyrophosphate or sodium tetraphosphate or sodium hexametaphosphate, ordinarily in 10% amounts of the alkaline content although varying with the hardness of the water. The last named proved least efficient but was cheapest. A satisfactory washing powder mixture for the can washer consists of 85% sodium metasilicate, 10% tetra sodium pyrophosphate, and 5% Nacconol N.R. added to water in such amount as to give an alkalinity of .07% expressed as sodium. Scale formation is encouraged by use of too concentrated alkali solutions. Use of briquets aids in this respect. Insufficient experimental data are available as yet in the acid cleaner composed of organic acids, wetting agents, corrosion inhibitor and microstatic agent to check its efficiency as a scale remover. P.S.L.

124. Factors Affecting the Phosphatase Test. FRANKLIN W. BARBER. Univ. Wis., Madison, Wis. Amer. Butter Rev., *4*, No. 6: 206, 208, 210. 1942.

Beside his own observations on the phosphatase test the author gives a good review of the literature. Among the observations made regarding the test were these: phosphatase concentrated in the cream layer of separated milk; pasteurized cream, giving a negative test when fresh, often gave a positive test when held a few days at ice box temperatures; the time elapsing before the test changed from negative to positive varied with the source of milk, suggesting that milk varies in phosphatase content. Two theories were advanced to explain the reaction. Of these two, a bacteriological explanation seemed the more plausible. Several colonies of a spore forming rod bacteria were isolated from the cream reacting positively. These resemble *B. mesentericus* and were capable of producing phosphatase but not phenol or phenol like substances. From his deduction that these bacteria may explain the reaction the author has two theories as to the production of sufficient enzyme to cause a change in the test by the small numbers of the bacteria present in pasteurized cream. These observations, the author emphasizes, should cause no less reliance on the test except in the interpretation of results from cold held pasteurized cream. P.S.L.

125. Timely Test of Public Sentiment. LELAND SPENCER AND H. ALAN LUKE. Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., *4*, No. 10: 250–254, 263; No. 11: 292–296. 1942.

By questionnaire methods the authors interviewed the residents of Jamestown, N. Y., as to their (1) purchases of dairy products, (2) experi-

ences with every-other-day delivery of milk, and (3) attitude towards unified delivery and establishment of a municipal milk plant. The average family income per week was $40. Ninety-eight per cent were users of fresh milk, 52% had their milk delivered at the door, while 30% secured their supply from milk depot or store. Of families interviewed 72% believed a saving would result from unifying routes; 45% were willing to change milkmen if a saving amounting to ½ cent or more could be effected. Racial groups varied appreciably from each other in their opinions on these matters. One-fourth of the families voiced objection to unified delivery, their chief concern being effect on quality of milk, injury to the dealer's business, and possible losses of positions for drivers.

Of 1025 families, 62% were in favor of a municipally owned milk plant, with 13% definitely opposed to the idea. Of families at present buying from stores or depots, 70% favored establishment of a municipal milk plant. It was noted that the percentage favoring the proposal was greater among Scandinavian groups. Of those in favor 67% thought milk would sell for less. Lower quality product and injury to present dealers were considered the chief disadvantages to the idea. Store customers seemed more concerned with price than quality. If a municipal plant were operated in competition with private dealers, 40% said definitely they would patronize the former and only one-third of those now buying from retail routes said they would support it. Fifty-eight per cent only, of those favoring establishment of a municipal plant, stated they would support it, and 83% of those opposing the establishment stated they would continue dealing with private distributors. Well-to-do families generally seemed to favor less than others any radical changes in milk distribution. This was true also for the English-Scotch-Irish and Italian groups as compared with the Scandinavian.

P.S.L.

126. Evaluation of Some Milk Tests. M. P. BAKER, Iowa State College, Ames, Iowa. Amer. Milk Rev., *4*, No. 8: 186–189. 1942.

The sediment test for milk has psychological value in improving milk supply but its interpretation must depend upon the manner in which it was taken and upon the treatment of the milk from which the sample was taken—whether or not strained at the farm. The plate count, despite shortcomings is still extremely valuable in milk work. The direct microscopic count has several of the disadvantages of the plate count, but also many additional advantages. The value of a coliform count of raw milk is doubtful except for high quality product. The methylene blue test is satisfactory as a plate count substitute. The resazurin test, very similar in principle to the methylene blue test, is gaining steadily in usage as a quality test. Like any of the above tests the phosphatase test for pasteurization efficiency has limitations. It is recommended that two laboratory tests at least be used

for evaluating quality, because each has particular advantages, and that these be supplemented with inspection at the receiving depots as well as at the farm. P.S.L.

127. The Care of Milking Machines. H. J. BRUECKNER, Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., *4*, No. 6: 130–132. 1942.

Milking machines may be efficiently and adequately cleaned with as little equipment as two washtubs and proper brushes. No milk should be allowed to dry on any unit, and to avoid this, all units should be rinsed thoroughly in lukewarm water immediately after milking the last cow. A few gallons of water at 130°–140° F. containing a small amount of dairy cleaner and wetting agent should be then forced through the units, followed by "sterilization" with 200 p.p.m. of chlorine solution or 180° F. "sterilizing" water and dry storage. The latter method is gaining in popularity. For solution storage, a solution rack, rather than a crock, is preferable for several reasons. Lye solutions of 0.5% strength for "sterilizing" teat cup assemblies are very efficient and economical. They are not harsh in their effects upon rubber but obviously must not be used in metallic milker parts. Outer surfaces of rubber tubing may be cleaned by immersing 5 to 10 minutes in a 2% lye solution at 150° F., and vacuum lines by drawing a 3 to 4% lye solution through them from the stall cocks to the moisture traps. Frequency of disassembling the teat cup assembly for cleaning depends upon the care given the machine. If handled carefully it may be unnecessary to take this apart after each milking in order to produce clean milk. P.S.L.

128. Pasteurization Control, Part I. A. J. POWERS, Borden's Farm Products Res. Lab., New York City. Cert. Milk, *17*, No. 199: 7. Nov., 1942. **Part II,** Cert. Milk, *17*, No. 200: 7. Dec., 1942.

This article deals with high-temperature-short-time pasteurization. The application of the phosphatase test for determining whether the proper time and temperature relationship has been attained by this method of pasteurization is described. Some possible reasons for faulty pasteurization and some of the routine precautions that may be taken to assure proper pasteurization are given. Control of thermophilic and thermoduric organisms which survive pasteurization is also discussed. W.S.M.

129. Deterioration of Milk by Bacterial Growth Under Refrigeration at 40° F. F. E. MOTT AND H. MAZER, Boston Health Dept. Cert. Milk, *17*, No. 199: 3. Nov., 1942.

Certified milk pasteurized, Grade A milk pasteurized, and Grade B milk pasteurized were stored in a refrigerator at 40° F. for five days. The samples of milk were obtained from vehicles on the streets of Boston engaged in

the delivery of milk during the period March 3 to May 10, 1942. After three days, both Grade A and Grade B milk had bacterial counts so great that their sale would be in violation of the law and standards of the Commonwealth of Massachusetts. Even after four days in the refrigerator the Certified milk pasteurized, still conformed to the bacterial standards and was safe for use. These findings suggest that the present conception of the inhibiting effect of refrigeration on bacterial growth in milk must be revised.

Editor's comments: It should be kept in mind that the possibility of the increase in numbers of bacteria in Grade A and B milk stored at 40° F. for 4 days being a cause of intestinal disturbances will depend upon the type of organisms present. W.S.M.

130. False Positive Phosphatase Test from a Thermophil in Pasteurized Milk. THEODORE C. BUCK, JR., Baltimore City Health Dept., Baltimore, Md. Amer. Jour. Pub. Health, *32*, No. 11: 1224–1236. 1942.

A thermophilic organism, for which the name *Lactobacillus enzymothermophilus* is suggested, was found to produce a bacterial phosphatase responsible for false positive phosphatase tests in properly pasteurized milk. The organism was isolated from the products of four Baltimore dairies.

A phosphatase test of 0.20 Gilcreas units from 0.5 ml. of the fluid was obtained by washing approximately 50 million organisms in 10 ml. of distilled water. This bacterial enzyme was destroyed after exposure to 185° F. for one minute.

Production of the bacterial phosphatase in milk at pasteurizing temperatures of 142° to 144° F. begins after a 90-minute holding period. Two hours after the start of the enzyme production at 142° F. a phosphatase test of 0.20 Gilcreas units was obtained. At the 144° F. temperature a lag in the enzyme production is noted for 180 minutes; and the production thereafter is rapid.

The factors governing the production of the bacterial phosphatase are the inoculum number, the temperature and the time period. In one pasteurizing trial at 143° F. for 150 minutes, microscopic and plate counts were 3,200,000 per ml. and the phosphatase test 0.08 Gilcreas units per 0.5 ml. of milk. The maximum direct microscopic count of 150 million organisms per ml. of milk was obtained after 5½ hours of pasteurizing milk at 143° F. and the phosphatase test gave 0.25 Gilcreas units per 0.5 ml. of milk.

The practice of separating route return pasteurized milk for standardizing the butter fat content represents a continuous reinoculation process and may explain the marked difficulty of ridding some dairies of thermoduric or thermophilic bacteria. M.W.Y.

MISCELLANEOUS

131. Food Packing Today. R. L. ROWNEY. Food Mfr., *27*, No. 19: 340–343. Dec., 1942.

The author briefly reviews the position of food packing after 3 years of war and discusses probable post-war developments. The government has to protect the food packing industry but fresh foods because they keep only so long do not need this protection.

Tin supplies are small and although Germany is using lacquered blackplate, England thinks their needs will not result in its use. Orders of the Ministry of Food are listed. Progress in the use of plastics has not been striking and substitutes are not promising. An advantage of glass packing is that it is reusable. The glass industry is already overtaxed and labor is scarce.

A warning against hoarding is given and the average life of canned goods recorded. Post-war factors are discussed in detail. Good will and other factors concerned with the future of packaging are discussed and many interesting ideas are presented. J.C.M.

132. Can You Add a Locker Plant. FRED D. MOSHER. Ice Cream Field, *40*, No. 5: 16. Nov., 1942.

The possibility of curtailing ice cream production for the duration may mean complete idleness for some plants and partial idleness for others. According to the author, the most logical use of the available refrigeration equipment is the conservation of food—that is the conversion to refrigerated locker storage.

Government agencies are encouraging the use of locker plants as a means of conserving tin and steel used in the canning industries. There were between 4000 and 5000 locker plants in 1942 serving over 1,000,000 customers but this will be inadequate for 1943 needs.

Many features of ice cream plants lend themselves to easy conversion to the processing of other foods. The limiting factor in the expansion of a locker plant is refrigeration equipment, but it is expected that limited expansion in certain localities will be permitted. The Middle West leads in the use of lockers, but the Southwest, Northwest, and West have shown rapid growth. In areas of large home consumption of food commodities, regardless of location, a well-organized locker plant should be a sound and profitable investment. A locker plant operated as a sideline may show a profit with as few as 100 to 200 lockers whereas 500-locker installations would be desirable if it were operated as the sole enterprise. A 15-ton refrigeration system will be sufficient for a 1000-locker plant, whereas a 7½ horsepower Freon unit would handle a 400-locker plant. Approximate operating costs and incomes also are given. W.C.C.

133. A Low-Temperature Test Cabinet for Industrial Use. R. E. BORDEN, Harding & Gross, Cambridge, Mass. Refrig. Engin., *44*, No. 6: 372. 1942.

A description of a low-temperature testing cabinet of the immersion type for testing of instruments, chilling of small parts and the calibration of controls. Three units are manufactured, No. 1—Temp. range of −50° F. to +10° F.; Unit No. 2—Temp. range of −10° F. to +50° F.; Unit No. 3—Temp. range of +15° F. to +90° F. Units No. 1 and No. 2 are refrigerated by a standard Freon −12 condensing unit, while Unit No. 3 has a heating unit in addition to that for refrigeration. These cabinets are of table height and may be worked at from both sides and are portable, being mounted on casters. The various compartments are filled with water or antifreeze solution depending upon the required temperature. To insure even distribution of temperature throughout the solution a means of agitation is provided. Access to the thermostats and electrical relays is had by means of a hinged cover over the control compartment. The cabinet frame and enclosure are of all-steel construction, 6 ft. long, 21 in. wide and 34 in. high. Operates by plug-in connection to standard 110-volt, 60 cycle outlet. These units would seem to have fine possibilities of application in several types of dairy products research. L.M.D.

134. Preparing Dairy Plants for Blackouts. Southern Dairy Products Jour., *32*, No. 4: 11. Oct., 1942.

The first actual air raid alarm occurred in Southern California on February 25. This first experience has resulted in many blackout installations in that section of the country.

The cheapest, in the first cost, and quickest method of obscuring light is painting but has the disadvantages of being difficult to remove, possibly causing breakage from heat absorption, and it must be replaced immediately if glass is broken.

The following formula for industrial blackout paint will cover 700 to 800 square yards at a material cost, less than three cents per square yard: 100 pounds of Black Ground in oil; 50 pounds of Paste Dryer; two gallons Turpentine; one-half gallon of Boiled Linseed Oil; and one pint of Terebene.

This paint can be removed by the following mixture: five gallons Benzine; 3.3 gallons Acetone, and 15 pounds Paraffin Wax.

A satisfactory coat of interior paint may be made with carbon black and filler, 57% by weight and four parts gum and oil to six parts of volatile thinner, 43% by weight.

Some plants have painted their windows black on the outside and white on the inside. "Elastic" paints on the market which may be removed by peeling, cost an average of approximately 25 cents per square yard.

Shades and curtains are more expensive but have the advantage of feasibility. They also offer some protection against flying glass.

Panels may be constructed of plywood, masonite, fibre board or similar material. Panels or sections should overlap about six inches.

Phosphorescent paint, which when exposed to light will glow for about 45 minutes, may be used for painting fire exits, door knobs or other objects which may be located in the darkness. F.W.B.

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