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August 21, 22, 23, 24, 1972
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Official  **Publication**

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AUGUST 21, 22, 23, 24, 1972

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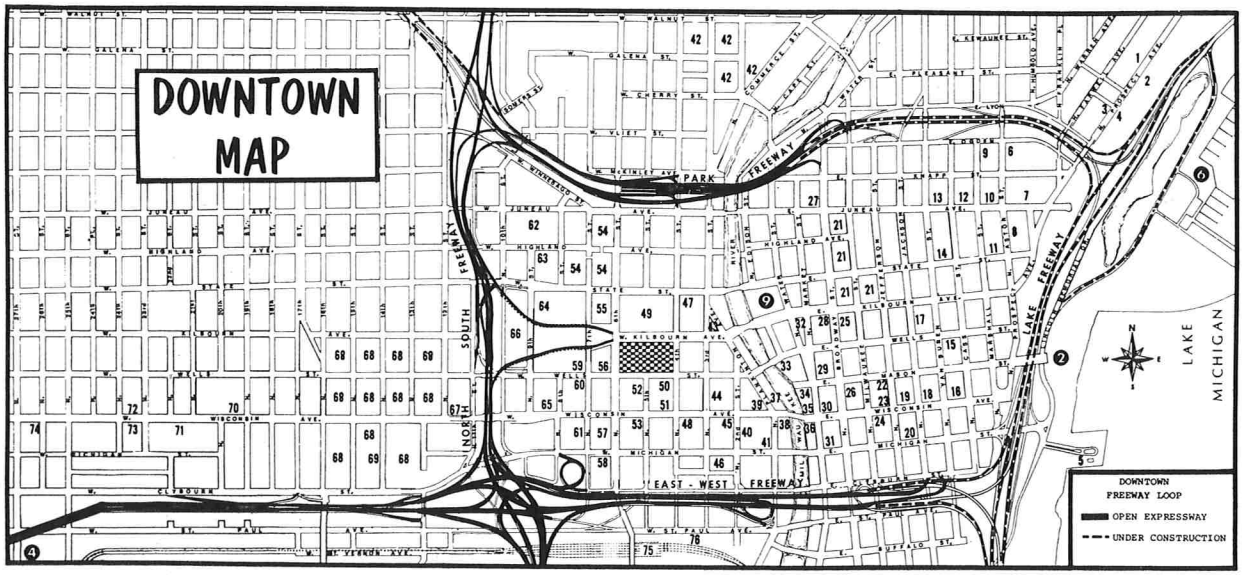

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RELATIONSHIP OF FREEZING POINT OF HERD MILK TO PRODUCTION CONDITIONS[1]

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(Received for publication October 8, 1971)

Abstract

A statistically significant relationship was found between freezing point and atmospheric temperature, season, roughage in the ration, breed of cow, and concentrate in the ration. Because of the narrow range in freezing points, however, the practical importance of these relationships appears questionable.

Results of a comprehensive survey of the freezing point of herd milk produced in Kentucky were presented in a previous report *(1)*. Methods of sampling and analysis were described in that report. The average freezing point from 509 herd samples of milk collected from five soil areas of the state over a period of one year was −0.541 C.

In connection with the above survey information was recorded at each sampling, to describe environmental, feeding, and other conditions within the herd that might influence freezing point of the milk of that herd. Factors which were analyzed statistically for relationship to milk freezing point included: (*a*) breed, (*b*) concentrates in the ration, (*c*) roughages in the ration, (*d*) environmental temperature, (*e*) rainfall, (*f*) level of management employed (above or below average) as judged by the fieldman, (*g*) fat test of herd milk, and (*h*) herd milk yield. Temperature and rainfall data were obtained from U. S. weather station records.

Results and Discussion

Data in Table 1 show correlations of milk freezing point with fat test, milk yield, and previous high temperature and rainfall. Milk freezing point, fat test, and milk yield were obtained from pooled samples of the evening and morning milk of each herd. Average high temperature and total rainfall were from the 3-day period preceding the morning milking.

High temperature was positively correlated with milk freezing point because days with higher temperature were associated with higher milk freezing points (nearer zero). A physiological explanation of the effect of atmospheric temperature on freezing point of the milk is not offered at this time. Seasonal fluctuations in milk freezing point observed in this survey are shown in Fig. 1. Since the months associated with the highest freezing points were also the

Table 1. Correlations of milk freezing point with production and environmental factors

Production and environmental factors	Freezing point
Fat test of milk	+.01
Milk yield	+.07
Average high temperatures[a]	+.30[b]
Total rainfall[a]	+.03

[a]For 3-day period prior to morning milking
[b]Probability <0.01.

Table 2. Relationship among roughage in ration, atmospheric temperature, and milk freezing point

Type of roughage	N[a]	Average high temperature, F[b]	Average freezing point of milk, C
Pasture only	178	78	−0.5397
Pasture plus other roughage	145	61	−0.5422
No pasture	110	50	−0.5435

[a]N = number of samples
[b]For 3-day period prior to morning milking

Table 3. Factors affecting the freezing point of milk

Source of variation	d.f.	Mean squares	F
Breed	3	150.95	P <.05
Concentrate	2	332.15	P <.01
Roughage	2	538.34	P <.001
Management	1	63.81	N.S.[a]
Residual	424	49.74	
Total	432		

[a]N.S. = Not significant

Table 4. Freezing point of milk from different breeds of cattle

Breed	N[a]	Average freezing point, C[b]
Guernsey	21	−0.5383
Holstein	150	−0.5425
Jersey	59	−0.5440
Mixed	203	−0.5423

[a]N = Number of samples
[b]Difference of 0.0025 would be significant by Waller-Duncan (k = 50) procedure *(2)*

[1]The investigation reported in this paper (No. 71-5-121) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

TABLE 5. FREEZING POINT OF MILK AS RELATED TO CONCENTRATE MIXTURE IN RATION

Concentrate mixture	N[a]	Average freezing point, C[b]
Grain only	56	−0.5426
Grain + commercial supplement	323	−0.5397
Commercial concentrate only	54	−0.5430

[a]N = Number of samples
[b]Difference of 0.0022 would be significant by Waller-Duncan (k = 50) procedure (2)

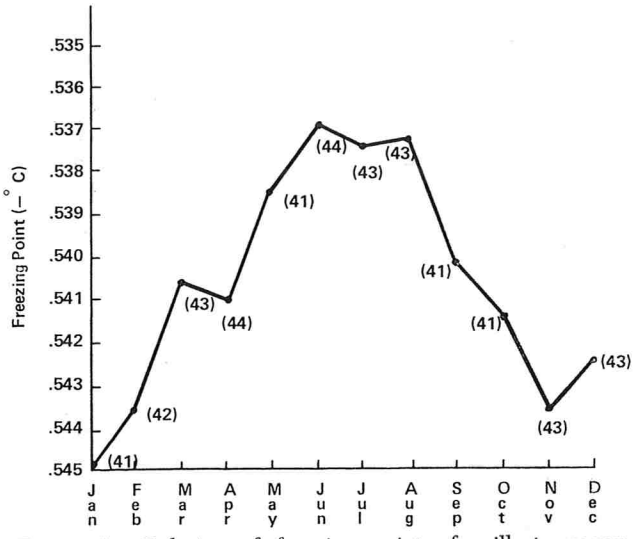

Figure 1. Relation of freezing point of milk to season. Figures in parentheses indicate number of samples involved.

months of highest atmospheric temperature, these results would be expected if high temperatures cause higher freezing points in the milk. But, the type of roughage the cows received also changed with season, and the kind of roughage in their rations might affect freezing point of their milk. Table 2 shows that the amount of pasture a cow received was directly related to temperature and to freezing point of her milk. Although cows were on pasture during the hottest days, the quality and amount of pasture available on these days might not have been ideal. Consequently, the effects of temperature, season, and type of roughage were confounded in these data. Also shown in Table 2 are the mean freezing points of milk produced with three types of roughage. The pasture-only category produced milk with a freezing point significantly higher than the two other types.

The correlation between milk freezing point and higher temperature was +0.23 after adjusting the data for differences between types of forage. Therefore, milk freezing points did vary directly with temperature fluctuations while cows were on one type of forage. But, the type-of-forage classification as reported by the fieldman after discussion with the farmer may not be reliable enough in these data for one to conclude that all variation caused by roughage was removed. Therefore, we are uncertain whether temperature, season, or roughage was the direct cause of high milk freezing point. Freezing point probably is affected by atmospheric temperature changes resulting in changes in water consumption, with some interactive effects from season and roughage. An experiment with controlled roughage and water consumption during temperature fluctuations would help resolve this confusion.

Data in Table 3 show the analysis of variance in milk freezing point according to breed of cows, type of concentrate, type of roughage, and quality of management. Two-way interactions among these factors were not significant sources of variation when tested at the ($P < 0.05$) level. Of the factors shown in this table, type of roughage was the largest source of variation; however, the factor of direct effect probably was that of associated temperatures. Also, the breed of cows (Table 4) and the type of concentrate in the ration (Table 5) were significant sources of variation but their effects were less than that from the type of roughage. Quality of management had little, if any, effect on milk freezing point.

A comment regarding the practical significance of these relationships is apropos. Values presented in Table 2 indicate that the influence of roughage, for example, on the freezing point of milk is very highly significant compared with that of the residual variance. When one looks at the range in average freezing point among roughages, however, it is seen to be quite small, the extremes being −0.5397 and −0.5435 C (Table 2). The difference between these two values is 0.0038 C, equivalent to only 0.7% added water if the regulatory standard for unadulterated milk is −0.530 C. By this standard all these samples would be considered unadulterated. This is not to say, however, that in other regions or in other years the effect of temperature and/or roughages on milk freezing point might not be of greater magnitude and therefore of practical significance.

CONCLUSIONS

Based upon data reported herein it is shown that there is a statistically significant relationship between certain production conditions and the freezing point of pooled herd milk. However, the magnitude of the freezing point variations was so small that their practical importance appears questionable.

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1. Freeman, T. R., J. L. Bucy, and D. D. Kratzer. 1971. The freezing point of herd milk produced in Kentucky. J. Milk Food Technol. 34:212-214.

2. Waller, R. A., and D. B. Duncan. 1969. A Boyes rule for the symmetric multiple comparisons problem. J. Amer. Statistical Ass. 64:1484-1503.

LOSS OF VIABILITY BY *STAPHYLOCOCCUS AUREUS* IN ACIDIFIED MEDIA

I. INACTIVATION BY SEVERAL ACIDS, MIXTURES OF ACIDS, AND SALTS OF ACIDS[1]

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(Received for publication December 10, 1971)

Abstract

Survival of *Staphylococcus aureus* (10^8 cells per milliliter) after 24 hr of incubation at 37 C in Trypticase Soy broth acidified with acetic, citric, hydrochloric, lactic, and phosphoric acids was investigated. When the organism was exposed to the medium adjusted with hydrochloric acid to pH values of 5.2-3.6, 90-99.99% of the cells were inactivated. Acetic, lactic, and phosphoric acids were more active against *S. aureus* than was hydrochloric, whereas citric was equivalent to hydrochloric. Mixtures of lactic and hydrochloric acids inactivated more cells than did either acid alone but mixtures of other acids with hydrochloric, while superior to hydrochloric acid itself, offered no apparent advantage over use of single acids. The undissociated acid molecule was responsible for enhanced inactivation of cells by partially dissociated acids since anions of these acids had no effect on cell survival. Cells were more susceptible to inactivation by hydrogens ions at high incubation temperatures (45 C) and when the number of bacteria was low. Cells of *S. aureus* were most sensitive to the effects of hydrogen ions between the 12th and 24th hr during a 120-hr incubation.

In the last few decades there has been limited interest in the survival of bacteria in acidic environments. The food industry, however, cannot afford to overlook this matter, for virtually all foods are more or less acidic in nature. *Staphylococcus aureus* and staphylococcal food intoxications, for example, have been associated with fermented foods *(2, 3, 4, 5)*, particularly when acid production during manufacture was inadequate. The rate and amount of acid development in such foods governs, in part, whether staphylococci, if present, will grow and produce enterotoxin. A direct relationship between subnormal acid development and production of enterotoxin was noted by Zehren and Zehren *(9, 10)* when they tested cheeses involved in a rather large outbreak of staphylococcal food poisoning. We studied the behavior of *S. aureus* in pasteurized milks which were gradually adjusted to various levels of acidity *(1)* and reported substantial differences in growth rates, depending on the rate of acid addition and the type of acid employed. Growth of *Salmonella typhimurium* in skimmilks similarly acidified over a period of time with several acids was investigated by Subramanian and Marth *(6)* who noted that differences in inhibition were dependent on the type of acid used. We have also examined the fate of *S. aureus* when added to market samples of cultured dairy products *(unpublished data)* and observed their rapid demise within relatively short periods of time.

The present study is concerned with inactivation of *S. aureus* in an acidic environment. This communication deals with inactivation of *S. aureus* in a Trypticase Soy broth containing separately each of five different acids, mixtures of these acids, and salts of the acids.

[1]Supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by Public Health Service Grant No. FD00009-05 from the Food and Drug Administration.

Materials and Methods

Media

Basal medium. Dehydrated Trypticase Soy (TS) broth (BBL, BioQuest) was reconstituted according to the manufacturer's directions and autoclaved (121 C, 15 min) in 30 ml quantities in 25 × 150 mm screw capped tubes. The pH of the sterilized medium ranged between 7.0 and 7.1.

Treated media. Solutions of the following acids were prepared and autoclaved (121 C, 15 min) in 10 ml quantities: 2 M acetic, glacial, A. C. S. reagent (Allied), 1 M citric, granular, analytical reagent (Mallinckrodt); 2 M hydrochloric, reagent (DuPont); 2 M lactic, USP 85% (Mallinckrodt); 2 M phosphoric (ortho), A. C. S. reagent (Allied). Solutions of the following salts were prepared in a similar manner: 2 M sodium acetate, anhydrous (Mallinckrodt); 1 M sodium citrate (Fisher); 1 M calcium lactate, NF powder (Mallinckrodt); 2 M sodium phosphate, monobasic, crystal (Allied); and, 1 M sodium phosphate, dibasic, anhydrous powder (Baker). Appropriate quantities of the above solutions were aseptically added to the previously prepared 30 ml quantities of TS broth. The volume of solutions added ranged between 0.075 and 1.35 ml per 30 ml of broth.

Culture

A culture of *S. aureus* strain 100 was obtained from Dr. K. F. Weiss (The Food Research Institute, University of Wisconsin). The organism was stocked on TS agar slants, refrigerated until used, and restocked every 2 to 3 months. Before each experiment, the organism was transferred from a stock slant to 10 ml of TS broth and incubated 24 hr at 37 C. Finally, a loopful was transferred to 16 × 125 mm screw capped tubes containing 10 ml of TS broth (two tubes were inoculated for each variable under study) and incubated 18 hr at 37 C (resulting in populations of approximately 10^8 cells per milliliter).

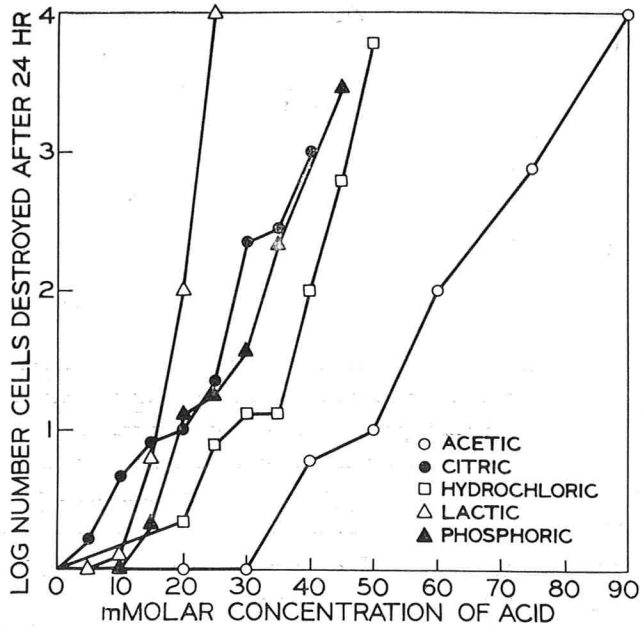

Figure 1. Inactivation of *S. aureus* after 24 hr of incubation at 37 C in a Trypticase Soy broth (BBL) acidified with acetic, citric, hydrochloric, lactic, and phosphoric (ortho) acids (cell inactivation expressed as a function of mMolar concentration). Initial cell population = 10^8 per milliliter.

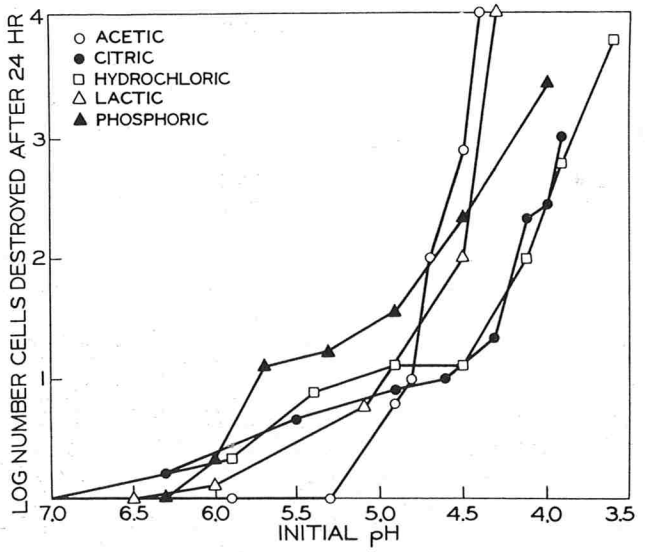

Figure 2. Inactivation of *S. aureus* after 24 hr of incubation at 37 C in a Trypticase Soy broth acidified with acetic, citric, hydrochloric, lactic, and phosphoric (ortho) acids (cell inactivation expressed as a function of pH). Initial cell population = 10^8 per milliliter.

Exposure of staphylococci to treated media

The 18-hr old cultures were cooled to 10 C and then were centrifuged at about 4000 rpm for 5 min. Supernatants were removed with sterile Pasteur pipettes and replaced, aseptically, with 10 ml of the appropriately treated broth (20 ml of a treated broth were thus used for each variable, leaving 10 ml for a pH determination). Staphylococcal cells were resuspended by means of a Vortex mixer and the tubes were incubated statically for 24 hr at 37 C. When the staphylococci were resuspended in untreated broth (control), their numbers remained fairly constant (approximately 10^8 per milliliter) during the 24-hr incubation period. Occasionally, some growth was observed in the control and during one experiment numbers of the organism reached 9×10^8 per milliliter.

Determination of cell survival

A 1.0 ml aliquot was aseptically removed from each tube after the contents were mixed with a Vortex mixer, and pour plates were prepared according to recommendations of *Standard Methods for the Examination of Dairy Products (8).* Plates were poured with TS agar and incubated at 37 C for 48 hr. An average count was calculated for the duplicate tubes of each variable and control. Each variable was compared to its control and the number of logs difference was determined.

Changes in experimental parameters

Several parameters were altered to test their effect on survival of *S. aureus* in acidified TS broths.

Time of exposure. An inoculum of 10^8 cells of *S. aureus* per milliliter was exposed for various time intervals to TS broth containing 40 mM hydrochloric acid. Survival of the organism and pH of the medium were determined after 0, 4, 8, 12, 24, 48, 72, 96, and 120 hr at 37 C.

Incubation temperature. An inoculum of 10^8 cells of *S. aureus* per milliliter was treated at 10, 23, 30, 37, and 45 C in TS broth for 24 hr with 40 mM hydrochloric acid.

Inoculum. The inoculum was varied by making several 1:100 dilutions of the 18 hr broth culture in TS broth (0.1 ml of broth culture was added to 10 ml of fresh untreated or treated broth, the contents mixed, and 0.1 ml transferred to another tube of broth, etc.). Inocula of 10^8, 10^6, 10^4, and 10^2 cells per milliliter were prepared. Two concentrations of hydrochloric acid, 30 and 50 mM, in the broth were used in these tests.

RESULTS

Inactivation of S. aureus by single acids

Figures 1 and 2 depict inactivation of *S. aureus* by five different acids in TS broth as a function of mMolar concentration and pH, respectively.

Inactivation as a function of mMolar concentration. When the acids are ranked in order of decreasing activity (as a function of mMolar concentration), they form the series: lactic > citric > phosphoric > hydrochloric > acetic. The acids can be arranged into three groups according to their performance, with lactic and acetic acids at the extremes and the remaining three acids occupying an intermediate position. A concentration of 10 mM lactic acid caused no detectable change in cell population, whereas an increase to 25 mM resulted in a four-log (99.99%) decrease in number of viable cells. In contrast, 30 mM acetic acid had no effect on cell numbers and a 90 mM concentration was required to achieve a four-log reduction in population. Citric, phosphoric, and hydrochloric acids differed only slightly from one another (the former two were slightly superior to the latter one). A 50 mM concentration of hydro-

chloric acid was required to cause a four-log decrease in staphylococcal population.

Inactivation as a function of pH. Below pH 4.8, the acids can be arranged into two groups according to their activity (as a function of pH) against staphylococci. Acetic, lactic, and phosphoric acids as a group were more effective than the other two acids (at lower pH values, phosphoric was inferior and acetic superior to lactic acid). Citric and hydrochloric acid were almost identical to each other in performance across the entire pH spectrum and as a group were inferior to the other acids (at lower pH values, hydrochloric was slightly inferior to citric).

Acetic acid had no demonstrable effect on numbers of staphylococci at pH 5.3. In contrast, citric and hydrochloric acids slightly affected cell survival at a pH just below 7, whereas the detrimental effect of lactic and phosphoric acids was first detected between pH 6.0 and 6.5. For practical purposes, none of the acids substantially inactivated (>90%) S. *aureus* above pH 5.7. Above pH 4.7, cell inactivation did not exceed 99% for any of the acids. Acetic acid inactivated 99.99% of the cells at pH 4.4, whereas a pH of <3.6 was required to achieve similar results with hydrochloric acid.

TABLE 1. SURVIVAL OF S. *aureus* AFTER 24 HR OF INCUBATION AT 37 C IN TRYPTICASE SOY BROTH ACIDIFIED WITH MIXTURES OF HYDROCHLORIC AND OTHER ACIDS.[1]

mMolar concn HCl	Other acid		pH	Reduction in population (no. logs)[2]
	Type	mMolar concn		
20	none	—	5.8	0.22
20	acetic	15	5.1	1.78
		20	5.0	0.67
20	citric	15	4.5	2.44
		20	4.3	3.56
20	lactic	15	4.4	5.22
		20	4.2	5.44
20	phosphoric	15	4.7	1.00
		20	4.5	2.33
40	none	—	4.1	2.22
40	acetic	5	4.0	4.11
		10	4.0	3.78
		15	3.8	5.11
		20	3.9	3.89
40	citric	5	3.8	2.89
		10	3.6	3.56
		15	3.3	5.56
		20	3.3	5.56
40	lactic	5	3.8	5.89
		10	3.7	7.00
		15	3.4	8.00
		20	3.3	8.00
40	phosphoric	5	3.9	4.11
		10	3.7	4.11
		15	3.4	6.00
		20	3.1	7.00

[1]Initial cell population = 10^8 per milliliter
[2]Compared to a control (no acids added)

Inactivation of S. aureus by mixtures of hydrochloric and other acids

Inactivation of S. *aureus* in media acidified with mixtures of hydrochloric and other acids is detailed in Table 1. Mixtures of hydrochloric and lactic acids were substantially more effective than were those of other acids combined with hydrochloric acid. Mixtures of hydrochloric acid and acetic, citric, and phosphoric acids were, for practical purposes, equal in effectiveness when compared to each other on the basis of pH.

All mixtures of hydrochloric and lactic acid were more effective against staphylococci at a given pH than either acid alone (see Fig. 2) at the same pH value. A 40 mM hydrochloric plus 15 mM lactic acid mixture (pH 3.4) was completely lethal (an eight-log decrease in numbers) to S. *aureus*. Mixtures of other acids with hydrochloric were, for the most part, equivalent to or more effective than hydrochloric acid alone when compared at the same pH. In many instances, the data indicated that effectiveness of acetic, citric, or phosphoric acids was impaired when combined with hydrochloric acid. In all instances, the pH of the mixture of acids was lower than the pH would have been if the acids were used alone.

Behavior of S. aureus in media containing salts of acids and acid salts plus hydrochloric acid.

Table 2 provides data on the behavior of S. *aureus* in the presence of acid salts and mixtures of these salts with hydrochloric acid in TS broth. The salts were evaluated at concentrations equivalent to those of the respective acids which inactivated S. *aureus* (Fig. 2). Acetate and citrate had no effect on the pH of the medium, whereas lactate and monobasic phosphate turned it slightly acidic, and dibasic phosphate slightly alkaline. Survival of staphylococci was unaffected by either of the phosphate salts. Citrate caused a slight decline in numbers of the organism and acetate and lactate brought about a small increase.

Mixtures of acetate, citrate, and dibasic phosphate, with 40 mM hydrochloric acid in TS broth were higher in pH than media containing just 40 mM acid (the anions, therefore, must have combined with some of the hydrogen ions and formed undissociated acid). Mixtures of lactate and monobasic phosphate with acid in TS broth were equivalent in pH to media containing acid alone. When the pH of the medium was increased by the presence of the salt, cell inactivation was almost completely prevent-

TABLE 2. BEHAVIOR OF S. *aureus* AFTER 24 HR OF INCUBATION AT 37 C IN A TRYPTICASE SOY BROTH[1] CONTAINING SALTS OF SEVERAL ACIDS AND ACID SALTS PLUS HYDROCHLORIC ACID.[2]

Salt	mMolar salt concn	mMolar HCl concn	pH	Reduction in population (no. logs)[3, 4]
None	—	40	4.1	2.00
Acetate, sodium salt	40	0	7.1	−0.22
	50	0	7.1	−0.22
	60	0	7.1	−0.22
	75	0	7.1	−0.33
	90	0	7.1	−0.44
	90	40	5.1	0.67
Citrate, sodium salt	20	0	7.2	0.11
	25	0	7.2	0.22
	30	0	7.2	0.44
	35	0	7.2	0.67
	40	0	7.2	0.67
	40	40	5.5	0.78
Lactate, calcium salt	10	0	7.0	−0.33
	15	0	6.9	−0.33
	20	0	6.9	−0.44
	25	0	6.8	−0.56
	25	40	4.2	2.78
Monobasic phosphate, sodium salt	20	0	6.5	0.11
	25	0	6.5	−0.11
	30	0	6.4	0.22
	35	0	6.4	−0.11
	45	0	6.3	0
	45	40	4.1	2.56
Dibasic phosphate, sodium salt	20	0	7.2	0
	25	0	7.3	−0.22
	30	0	7.3	−0.11
	35	0	7.3	−0.11
	45	0	7.4	0.11
	45	40	6.3	−0.22

[1]pH 7.1
[2]Initial cell population = 10^8 per milliliter
[3]Compared to a control (no salts or HCl added)
[4]Negative no. indicates growth

TABLE 3. SURVIVAL OF S. *aureus* DURING 120 HR OF INCUBATION AT 37 C IN A TRYPTICASE SOY BROTH ACIDIFIED WITH HYDROCHLORIC ACID.[1]

Hr of incubation	No acid		Acid[2]		Inactivation (no. logs)[4]
	pH	No. cells/ml[3]	pH	No. cells/ml[3]	
0	7.0	20,000	4.2	20,000	0
4	6.6	50,000	4.4	20,000	0.33
8	6.0	70,000	4.2	10,000	0.67
12	5.8	60,000	4.3	10,000	0.56
24	5.5	90,000	4.3	2,000	1.78
48	5.6	10,000	4.2	400	1.67
72	5.7	3,000	4.4	50	1.78
96	5.7	4,000	4.4	20	2.22
120	5.7	3,000	4.4	7	2.56

[1]Initial cell population = 10^8 per milliliter
[2]40 mM hydrochloric acid
[3]Divided by 10^4
[4]No. of logs difference between survivors in treated and untreated broth

ed. However, when the pH was not altered, cell inactivation was slightly enhanced by the presence of the salt in the HCl-salt mixture.

Effect of different parameters on the survival of S. aureus in an acidified medium

Time of exposure. Table 3 presents data depicting the behavior of *S. aureus* (10^8 cells per milliliter) during 120 hr of incubation at 37 C in TS broth and in broth containing 40 mM hydrochloric acid. The pH of both media was also monitored during the incubation period.

Some growth of *S. aureus* occurred in untreated broth during the first 24 hr of incubation. After this initial growth, numbers of the organism steadily declined, until 72 hr when the population stabilized. The staphylococci lowered the pH of the medium, which reached a minimum value of 5.5 after 24 hr of incubation. The pH then increased slightly and remained stable between 72 and 120 hr.

Because numbers of staphylococci changed greatly with time in untreated broth, we calculated the log difference between number of survivors in untreated and treated broth for each time interval. The greatest change in log difference with time occurred between 12 and 24 hr of incubation. The log difference in number of survivors in the two broths remained about the same from 24 to 72 hr. After 72 hr of incubation, greater loss in cell viability occurred in treated broth than in untreated broth. The pH of the acidified medium remained fairly constant throughout the 120 hr incubation.

Incubation temperature. Survival of *S. aureus* at several temperatures during 24 hr of exposure to 40 mM hydrochloric acid in TS broth is shown in Table 4. Survival of staphylococci in untreated broth was altered slightly as the temperature approached 10 C, but over 90% of the cells were inactivated in the acidified medium at 45 C. Temperatures of 10 to 37 C had no effect on inactivation of the staphylococcus by the acidic medium but loss of viability was enhanced almost 10,000-fold in the acidified medium at 45 C compared to the effect observed at all other temperatures.

Inoculum. Table 5 contains data which show the effect of hydrogen ion concentration on *S. aureus* when different sized inocula were held in untreated and acidified TS broth at 37 C for 24 hr. Staphylococci grew in untreated broth during the 24 hr period until maximum populations of 10^8 cells per milliliter were reached, regardless of the size of inoculum used. A 30 mM hydrochloric acid concentration neither allowed 10^8 cells per milliliter to grow nor be inactivated, but smaller inocula showed up to 10-20-fold growth. The 50 mM acid concentration resulted in substantial inactivation of cells

regardless of the inoculum used. Inactivation observed when inocula of 10^4 and 10^6 cells per milliliter were used was much greater than that encountered with the highest inoculum. When an inoculum of 10^2 cells per milliliter was used, *S. aureus* was completely destroyed with the high concentration of acid.

DISCUSSION

We studied growth (37 C) of *S. aureus* in pasteurized milk while the pH was gradually reduced from 6.6 to 4.0 with five different acids and several mixtures of these acids over periods of 4, 8, and 12 hr *(1)*. Data obtained during 12-hr incubation periods showed that the acids, when based on their inhibitory activity (as a function of pH), could be arranged into three groups. Acetic acid was quite distinct from the other acids and was most inhibitory to the staphylococcus. At the other extreme, the behavior of phosphoric acid was quite similar to that of hydrochloric acid and the latter was least inhibitory of all the acids tested. Lactic acid was superior but roughly comparable to citric acid and both were intermediate in activity to the other acids. These results therefore differ from data discussed in the present study (Fig. 2); but, in both instances acetic acid was superior and hydrochloric was inferior to the other acids studied (on the basis of pH) in regard to antistaphylococcal activity. Terminal pH values which were reached in milk where staphylococcal growth was reduced by ten-fold over 12 hr were in the range of 4.6-5.2, whereas growth was depressed by 100-fold in the range of pH 4.0-5.0. These values compare rather closely to pH ranges required for inactivation of staphylococci in Trypticase Soy broth (Fig. 2) (a ten-fold decrease occurred in the range of pH 4.6-5.7 and a 100-fold loss in numbers was noted between pH 4.1 and 4.7). Several mixtures of acids examined for inhibitory activity again *S. aureus* growth in milk showed neither synergistic nor antagonistic potential.

The behavior of *Salmonella typhimurium* in acidified skimmilks (pH 4.0-6.6) was investigated by Subramanian and Marth *(6)*. They employed three acids which were added gradually over a 16-hr period. Citric acid caused greatest inhibition of the organism followed in order of decreasing effectiveness by lactic and hydrochloric acids.

We have also studied the survival of *S. aureus* in several cultured dairy products (*unpublished data*). When 10^5 cells per gram were added to market samples of buttermilk and the product stored at 7 and 23 C, approximately a 100-fold decrease in numbers of staphylococci occurred within the first 24 hr. The pH of these products ranged from 4.2 to 4.5 and lactic acid was the principle source of hydrogen ions. Data in Fig. 2 indicate that inactivation of *S. aureus* in the presence of lactic acid in Trypticase Soy broth is much greater than in buttermilk.

This investigation, and the aforementioned studies, has confirmed that staphylococci are sensitive to the presence of hydrogen ion concentrations equivalent to those of the acidic environments created in many of our fermented foods. Hydrochloric acid, though, has consistently been shown to be less active against *S. aureus* than all of the other acids examined in these studies. Hydrochloric acid differs from the other acids in that it completely dissociates

TABLE 4. SURVIVAL OF *S. aureus* AFTER 24 HR OF INCUBATION AT SEVERAL TEMPERATURES IN A TRYPTICASE SOY BROTH ACIDIFIED WITH HYDROCHLORIC ACID.[1]

Incubation temp (C)	mMolar HCl concn	Reduction in population (no. logs)[2]
10	0[3]	0.56
	40[4]	1.44
23	0	0.33
	40	1.44
30	0	0.11
	40	1.44
37	0	0
	40	1.44
45	0	1.44
	40	5.22

[1]Initial population = 10^8 per milliliter
[2]Based on the 37 C control cell count
[3]pH 7.0
[4]pH 4.2

TABLE 5. SURVIVAL OF DIFFERENT INITIAL POPULATIONS OF *S. aureus* AFTER 24 HR OF INCUBATION AT 37 C IN A TRYPTICASE SOY BROTH ACIDIFIED WITH HYDROCHLORIC ACID.

mMolar concn HCl	Inoculum	No. *S. aureus* after 24 hr incubation	Difference from inoculum[1] (no. logs)
0	10^8	400,000,000	0.33
30[2]		100,000,000	0
50[3]		400,000	−2.67
0	10^6	400,000,000	2.33
30		9,000,000	0.89
50		90	−4.11
0	10^4	300,000,000	4.22
30		900,000	1.89
50		2	−3.89
0	10^2	300,000,000	6.22
30		4,000	1.33
50		0	−2.00

[1]Positive no. = growth; negative no. = cell inactivation
[2]pH 4.9
[3]pH 3.9

into ionic substances in aqueous media and its anion is a low molecular weight element. Since the anions of the partially dissociated acids had little or no effect on survival of *S. aureus* (Table 2), the enhanced antibacterial activity of these weak acids must be the consequence of undissociated acid molecules (which confirms information available in the early literature). When the strength (dissociation) and molecular weight of each acid is compared with the data in Fig. 2, it appears that neither property is associated with the ability of an acid to inactivate staphylococci (as a function of pH). That some undissociated acid molecules are more detrimental to staphylococci than are others may be related to the inherent physiology of the cell.

Since the majority of our fermented foods contain lactic acid as the principal source of acidity and a large number of foods are acidified with acetic acid, it is fortunate that these acids have demonstrated superior antibacterial activity. Food scientists, though, are working to replace natural food fermentations with direct acidification processes. Out of practical considerations, hydrochloric acid has been used in many of these applications. In the interest of food safety, it might be well to consider the use of more biologically-active acids. Mixtures of hydrochloric and lactic acid may be unusually effective against bacteria but we have been unable to demonstrate this in a food substance *(1)*.

Our data suggest that holding foods at temperatures approaching the maximum for growth of *S. aureus* (45 C), e.g. fermentation and cooking temperatures employed in yogurt and Swiss cheese manufacture, respectively, may greatly enhance inactivation of staphylococci by acids. On the other hand, Tatini et al. *(7)* have speculated that the cooking temperatures reached in Swiss cheese (50 C) may stimulate enterotoxin production by staphylococci.

Data in Table 3 indicate that there are two factors involved in the inactivation of staphylococci in acidic environments over extended periods of time, i.e. "old age" and the antibacterial activity of acid. The effect of the acid, *per se*, may be primarily operative only in the initial stage of incubation and be dominated by natural causes of death during extended storage.

Two phenomena must be considered in any discussion concerning the behavior of bacteria in acidic environments, i.e. growth inhibition and cell inactivation. We employed very high inocula in this study to severely limit opportunities for staphylococcal growth. Levels of acids which substantially inhibit growth of low and moderate cell concentrations may be insufficient to inactivate very high populations of organisms. Acidic environments which are not only bacteriostatic but also bactericidal for any population of organisms may be more effective against lower numbers.

These data, it is hoped, will again stimulate interest in the survival of microorganisms in acidic environments. A number of unanswered questions remain and concern for the safety of our foods demands that they be answered.

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VARIATIONS OF SOMATIC CELLS AND NEUTROPHILS IN MILK THROUGHOUT LACTATION[1]

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ABSTRACT

Total and differential cell counts were obtained for alternate weekly morning and evening milk from 11 Holstein cows in six different lactations. Milk from quarters suspected of mastitis were examined for presence of pathogens. Weekly cell counts for each cow showed large variations throughout lactation. The neutrophil count closely paralleled the total cell count. The average neutrophil percentage varied from 65 to 96%. No relationship was observed between cell count or type and length of lactation, age of cow, and milk yield. In addition to mastitis, unspecified stresses seemed to cause irregular sudden increases in somatic cells. Except during severe stresses, total cell counts were about 200,000 per milliliter, of which 65 to 90% were neutrophils.

Microscopic examination of unprocessed milk always reveals somatic cells. The number of these cells has long been used as an indication of irritation or inflammation of the mammary gland. Somatic cell numbers tend however to vary sharply over short periods depending on several factors, including time of sampling. Marked variations in cell counts have been reported to occur during a single milking *(18)*, at intervals during 24 hr periods *(17)*, and from day-to-day and week-to-week *(3, 15)* within the same cows. Acute infections of the mammary gland are highly associated with an increase in leucocytes. There is, however, little information on the occurence and significance of different cells, particularly of neutrophils for the duration of an entire lactation. It has been suggested *(8, 19)* that of the somatic cells only the neutrophils should be counted, because they indicate pathological disturbances in the mammary gland.

The purposes of our study were: *(a)* to amplify earlier findings on cell count variations associated with time of sampling by investigating weekly variation of somatic cells throughout a complete lactation, and *(b)* to determine the presence of neutrophils and whether certain numbers of these cells are indeed indicative of pathological conditions of the mammary gland.

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MATERIALS AND METHODS

Animals

Eleven Holsteins from the University dairy herd were used. They freshened in late August and early September and represented six different lactations. All cows met the health requirements of the veterinary control program at the start of the experiment. Three cows out of the eleven had been treated for mastitis in previous lactations.

Sampling routine

A drip sample (100 ml) was collected once a week from each cow from the metering device (approved by the D.H.I.A.) on the pipeline milker. Weekly sampling alternated between morning (7 a.m.) and evening (4 p.m.) milkings. Two tablets of the preservative Lactab *(5)* were added to the proportionally collected sample and stored at 5 C for 4 to 18 hr before making cell counts. In a previous publication *(5)* it was shown that preserved and fresh milk samples gave comparable counts. Total milk yield at sampling was also recorded.

Cell counting procedure

Differential somatic cell counts were performed using the Millipore membrane technique described previously *(4, 5)*. This procedure was shown to be superior to the Breed-type smear method *(5)*. Thirty fields per sample were counted. The microscopic factor was 81,000 (81,000 $\times$ no. of cells per field $=$ no. of cells per milliliter milk).

Bacteriological examination

When there was clinical evidence of inflammation of the udder, or when there was a two-fold increase in somatic cells for any individual cow, 0.1 ml from 30 ml of aseptically drawn foremilk from each quarter was streaked on blood agar and on phenol red mannitol agar and incubated for 24 and 48 hr at 37 C. Colonies were selected and divided into three groups: gram-negative rods, gram-positive rods, and gram-positive cocci. *Staphylococcus aureus* was identified by growth on phenol red mannitol agar and by the coagulase reaction. *Streptococcus* was identified by lack of catalase activity. Gram-positive and gram-negative rods were not found.

RESULTS AND DISCUSSION

Weekly cell count variation during a complete lactation

The rise and fall in cell numbers during a complete lactation could best be shown by graphs. Total somatic cells and number of neutrophils per 30 microscopic fields of the alternate morning and evening milk from each cow were plotted against weeks of lactation (Cow 9, 524, and 552 shown in Fig. 1). Large variations in cell counts were evident throughout the lactation in morning and evening milk. There was no indication of a regular 4-week cyclical rise and fall in cell numbers as was reported by Cullen *(3)*. The neutrophil count showed the same pattern of variation as the total cell count. When there was a rise or fall in total cell count, neutrophils generally responded accordingly. We interpreted this to mean that fluctuations in cell count were mainly increases or decreases in neutrophils. This was true of all cows except Cows 524 (Fig. 1) 72, and 75 (not shown). In those instances, the cells consisted mainly of small acidophilic epithelial cells whose morphology has been described previously *(4)*. Occasionally, these cells appeared also in increased proportions in milk of other cows. It was not known what condition in the mammary gland sometimes caused relative high numbers of these cells. The average sample-to-sample variation around the mean cell count measured as the coefficient of variation (Table 1) was smaller for the evening milk than for the morning milk which was opposite to what was generally indicated by the peaks and valleys in the graphs (Fig. 1). These two phenomena of variability are not contradictory because they represent two types of variation, sample-to-sample versus peak-to-valley. Whatever the reason for the two types of marked variation, the average cell counts over the complete lactation were higher for the evening milk in 9 cows.

Disparity between mean cell counts of morning and evening milk did not seem to be explained by differences in yield alone (Fig. 2). Of the 11 simple correlations computed between cell count and milk yield, eight were negative and not significant. Of the three positive ones only one was statistically significant and here yield accounted for less than 25% of the variation in cell count. There was thus little evidence of a relationship between cell count and milk yield. The pattern of greater peak-to-valley variability in evening milk might be related to exposure of the cows to different stresses during the day which might influence body cell secretion into the milk.

It is generally accepted that milk from cows in the terminal part of a lactation has more somatic cells, the majority of which are epithelial cells, resulting from the normal involution of the udder *(2, 3, 7, 10)*. These observations were not supported by the present study. Our findings agreed with those of Schipper *(15)* who reported no change in cell numbers with length of lactation. Although milk yield gradually decreased as the lactation progressed, it was not necessarily accompanied by a concurrent increase in somatic cells (Fig. 2), nor did a differential cell count show a higher proportion of epithelial cells. The observed irregular changes in cell counts might have been a response to chance infection, physical stress, various environmental conditions and management, particularly milking technique rather than to physiological causes. The invariably high proportion of neutrophils seemed to point to such a response.

Blackburn *(2)* showed that the somatic cell count increases with the lactation age of a cow. The effect of number of lactations was difficult to assess in our work, since four of the older cows developed mastitis in the course of the experiment and the milk had high cell counts for at least 6 weeks after successful treatment with antibiotics. Except for these periods of severe stress, the cell count was not appreciably higher than in the younger cows, except for Cow 552 which had a long history of mastitis. This suggests that increased cell counts in older cows are dependent on severity of pathological conditions which may have occurred during successive lactations rather than on a physiological process associated with stage of lactation.

The cell count in some cows was constantly higher or lower than in others under the same conditions of management and hygiene. Such differences were also reported by Schipper *(15)* and could be related to genetic factors as suggested by Afifi *(1)*.

Influence of infection or stress on cell count

Except for Cows 54, 524, and 552, there was no previous history of mastitis in the herd. During this experiment clinical mastitis was diagnosed and treated on 6 occasions, once in Cow 9, 74, 539, 553, and twice in 552. Although clinical evidence of inflammation was present, together with marked increases in neutrophils, the causative organism could not always be isolated. Only in four out of the six cases were either *Staphylococcus aureus* or *Streptococcus* or both found at the same time, in aseptically drawn foremilk of the affected quarter. Subsequent samples became negative by the 4th milking. A high neutrophil count of more than 1 million per milliliter sometimes persisted for 5 to 10 weeks after treatment with antibiotics. On several occasions during the lactations of any one cow the neutrophil count exceeded 1 million per milliliter although there was no clinical evidence of inflammation of the udder or visible abnormality of the milk, nor could any pathogens be isolated. The massive influx of

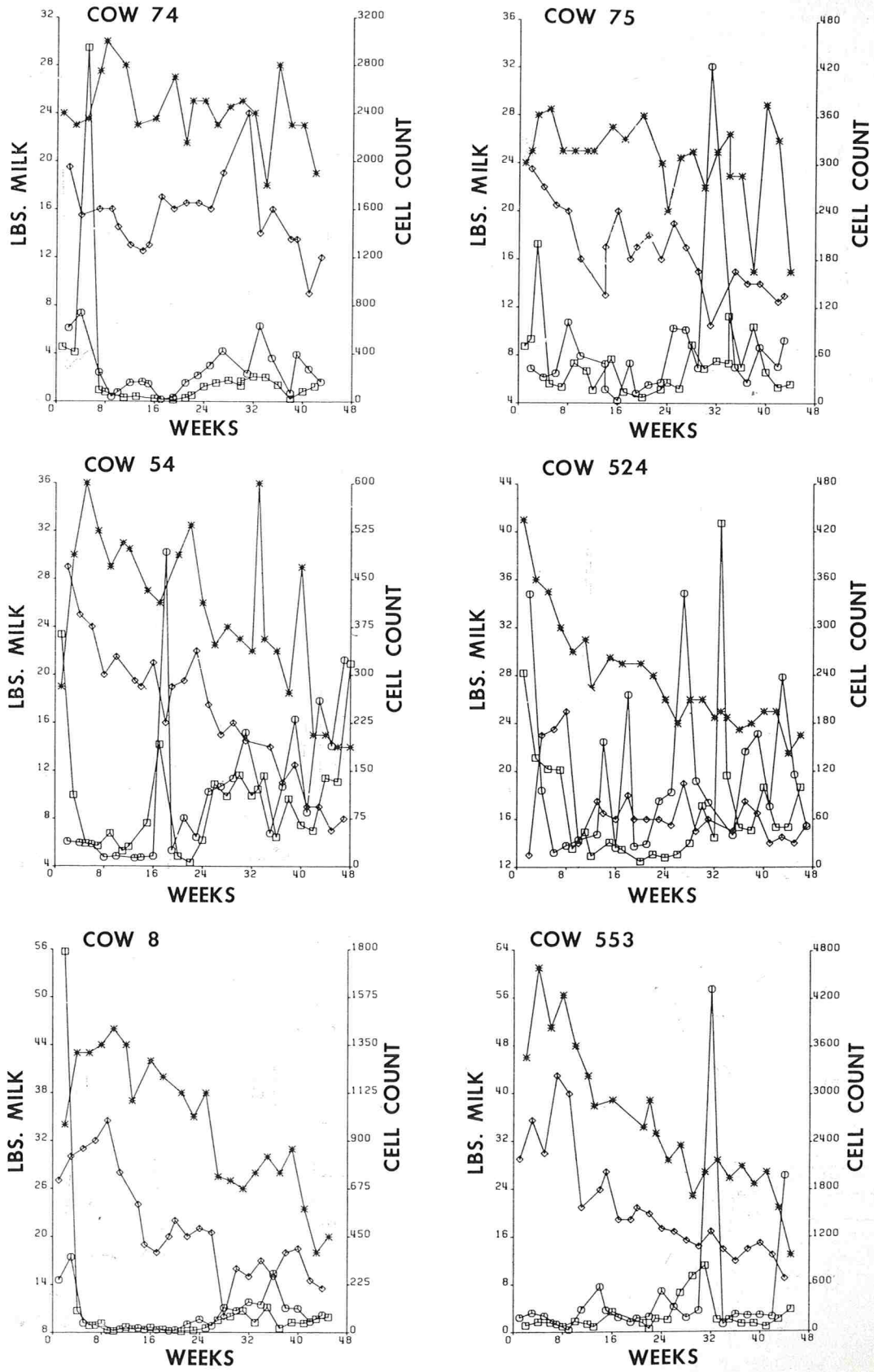

Figure 2. Weekly cell count (number of cells/30 microscopic fields) and milk yield for the whole lactation period. Yield of milk: lines with (*) = morning milk, lines with diamonds = evening milk. Total cell count: lines with squares = morning milk; lines with circles = evening milk.

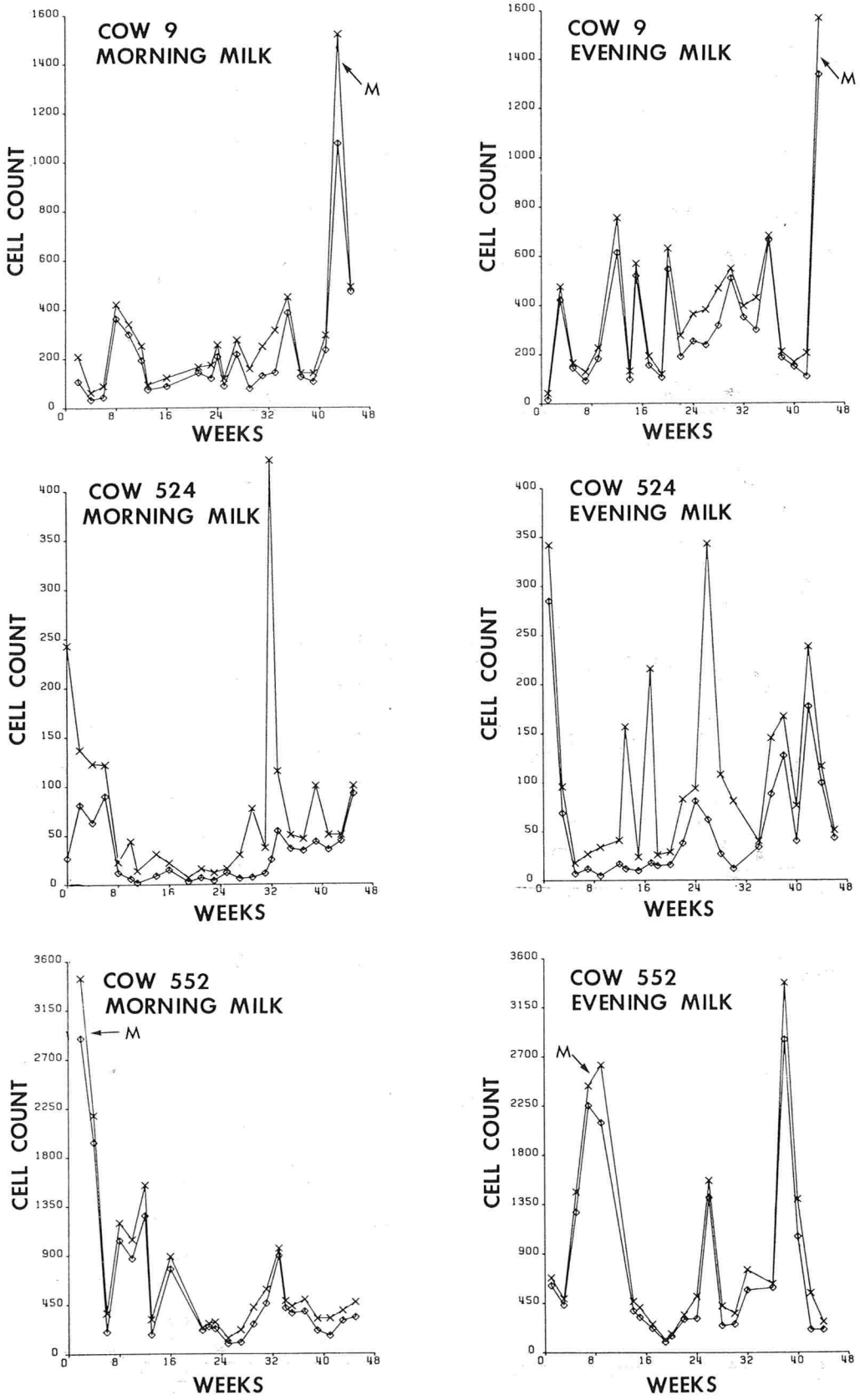

Figure 1. Weekly cell count (number of cells/30 microscopic fields) for the whole lactation period. Lines with (×) = total cell count; lines with diamonds = neutrophil count; M = mastitis diagnosed.

TABLE 1. MEAN CELL COUNT, COEFFICIENT OF VARIATION (SAMPLE-TO-SAMPLE) AND PERCENTAGE OF NEUTROPHILS FOR EACH COW FOR AM AND PM MILKING.

Lactation	Cow	Mean cell count				Neutrophils	
		AM	C.V.	PM	C.V.	AM	PM
		($\times 10^3$/ml)	(%)	($\times 10^3$/ml)	(%)	(%)	(%)
1	72	240	136	280	101	73	74
1	74[a]	280	235	478	79	88	92
1	75	97	84	110	132	65	66
2	54	190	83	180	104	79	81
3	524	140	121	210	86	75	80
3	39	470	81	750	60	95	97
4	9[a]	519	104	810	59	93	95
4	539[a]	660	146	810	78	93	95
5	8	110	301	130	106	80	82
6	552[a]	2270	103	1700	98	95	96
6	553[a]	620	102	670	190	93	95

[a]Mastitis diagnosed during lactation

neutrophils in the lactating mammary gland may have removed the invading infectious agents through phagocytosis before any symptoms of dysfunction of the gland developed. It may also be that the sudden increase in neutrophils resulted from physical stress or trauma. Usually, such a response was short and rapid recovery and return of the gland to normal occurred.

Neutrophil count

Observations at the extreme peaks in the graphs, whether associated with diagnosed udder infections or not, generally showed an influx of neutrophils which constituted invariably more than 90% of the total cells. Neutrophilia, in these instances, was clearly associated with acute abnormalities. However, relative neutrophilia was also observed in normal (or at least presumably nonpathological) secreting glands during the entire lactation. Only rarely was the neutrophil count <20% of the total, but varied from 50 to >90% at any time during the lactation. The mean cell count and the percentage of neutrophils per cow for their whole lactation period are in Table 1. Cows affected at one time or another with clinical or subclinical mastitis had the highest average cell count. That such condition had occurred was also reflected in a higher neutrophil percentage (more than 90%).

Blackburn (2) reported an average of 56% of polymorphs in uninfected quarters and 61 to 75% in infected quarters, depending upon the type of organism. Paape and Tucker (9) found 66 to 69% of granulocytes in their fraction-collected milk. These were lower neutrophil percentages than we observed. The invariably high proportion of neutrophils in normal milk was intriguing, particularly since concetrations of more than 20% were considered by Galli and Guallini (6) as certain infection. A preexisting neutrophilia may actually serve the cow well by increasing the resistance against infection as shown by Schalm et al. (12, 13).

CONCLUSION

Large irregular sample to sample variation occured in the total somatic cells and neutrophils of alternate morning and evening milks of these 11 cows. Frequently variability in counts was further reflected in marked peaks and valleys, particularly for the evening milks. There was little evidence of a relationship between cell counts and milk yield. The somatic cell count of milk from cows free of clinical mastitis were within the generally accepted levels of 300,000 to 500,000 per milliliter. A high proportion of neutrophils (70 to 95%) was common even in the absence of diagnosed clinical mastitis.

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REPORT OF THE COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS, 1970-1971

The IAMFES Committee on Food Equipment Sanitary Standards, known hereafter as the Committee, is charged with the responsibility of cooperating with other interested health organizations and related industries in the formulation of sanitary standards and educational materials for the fabrication, installation, and operation of food equipment and to present to the membership those standards and educational materials which the Committee recommends be endorsed by the Association.

The purpose of this cooperative program is to aid industry in improving the design, construction and installation of equipment so that it will lead to easy cleaning and proper functioning when placed into service in food establishments. It is the Committee's further purpose to cooperate with industry in the preparation of standards or guidelines which public health agencies will accept, thereby securing uniformity in the manufacture and nationwide acceptance of such equipment.

The following report outlines the Committee's activities during the past year in working with two health and industry organizations (National Sanitation Foundation's Joint Committee on Food Equipment Standards and the National Automatic Merchandising Association's Automatic Merchandising Health-Industry Council) and progress in meeting its purposes and objectives. It is expected these organizations will be the two groups that the Committee will work with during the coming year.

National Sanitation Foundation (NSF)

The Committee was represented at the 1971 meeting of the National Sanitation Foundation's Joint Committee on Food Equipment Standards, where action was taken on several proposals; and prior to the meeting, the Committee reviewed and submitted comments on each draft of these proposals. Since the meeting, the Committee has also reviewed and submitted comments on proposed changes to standards.

Standard for soda fountain equipment

Prior to the recent revision of the Standard for Soda Fountain Equipment, properly labeled bobtail soda fountain equipment has been exempted from complying with the requirement for separate drainboards, since this small equipment normally is installed in places dispensing food in and with single service articles and with adequate facilities to wash and sanitize any multi-use equipment or utensils. Consequently, the following addition to Item 5.21 of Standard No. 1 was approved by the public health representatives:

> "This provision for separate drainboards shall not apply to bobtails; provided however a label stating the follow-in shall be affixed in a conspicuous position on each unit: This unit is intended for use with single-use service and the sink and drain section DOES NOT COMPLY with Standard No. 1 as it relates to multi-use customer service."

Standard for food service equipment

The current provision limiting the size of cutting boards to 24 × 36 inches and not heavier than 50 lb. was reviewed by the public health members and was amended to permit a 36-inch maximum dimension in any one plane and a weight not to exceed 50 lb.

Standard for spray-type dishwashing machines

The Foundation staff brought to the Joint Committee's attention that the current edition of Standard No. 3 on Spray-Type Dishwashing Machines failed to provide adequate specifications for some machines, as to spray patterns and proportion of spray jets for the lower and upper wash arms. The Joint Committee deemed that more specificity was needed in order to enable the manufacturer and evaluator to carry out their responsibilities to the user and consumer and in-

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BACTERIAL COUNTS OF RAW MILK AND FLAVOR OF THE MILK AFTER PASTEURIZATION AND STORAGE

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Abstract

A total of 216 raw milk samples with a variety of Standard Plate Counts and psychrotrophic bacteria counts were laboratory-pasteurized, stored at 7 C, and then evaluated for flavor after 1 and 2 weeks. Results showed that milk with counts of >1,000,000/ml before heating frequently developed objectionable flavors after pasteurization and subsequent storage. The most common defect was a bitter flavor which appeared within 2 weeks after pasteurization in nearly all samples which as raw milk had counts exceeding 10,000,000/ml. This off-flavor developed in spite of small numbers of organisms in the pasteurized product and in the absence of post-pasteurization contamination.

Alternate day bulk handling of raw milk and less frequent home deliveries of processed products have resulted in prolonged storage of milk at low temperatures. Sometimes this causes a build-up of large bacterial populations from growth of psychrotrophic bacteria *(3, 4, 11, 17, 20)*. This group of organisms has been associated with the deterioration of flavor in both raw and pasteurized milk *(1, 3, 6)*. Although in a few instances the existence of heat resistant psychrotrophs has been reported *(5, 12, 16)*, it is generally accepted that most of these organisms are destroyed by pasteurization *(4, 7, 20)*. Flavor defects of microbial origin in pasteurized and properly stored milk, therefore, are usually considered to be the result of post-pasteurization contamination *(2, 7, 13)*. Johns *(8)* has suggested that certain compounds produced by microorganisms in raw milk might be responsible for off-flavor development in pasteurized milk. Other authors *(9, 10, 15)* have found some fairly heat-stable bacterial enzymes which were not inactivated by pasteurization temperatures.

The purpose of this study was to determine the effect of large numbers of microorganisms in raw milk on the flavor of the pasteurized product after storage for up to 14 days.

Materials and Methods

Initially, 72 raw milk samples were collected from farm bulk tanks, from storage tanks of processing plants, and from a few individual cows. All samples were held at 5 C or lower and analyzed within 18 hr.

Each raw milk sample was divided into three portions, resulting in a total of 216 samples. One portion was plated immediately for Standard Plate Count (SPC), coliform plate count (CPC), psychrotrophic bacteria count (PBC), laboratory-pasteurization count (LPC), and then laboratory-pasteurized at 62.3 C for 30 min *(18)*, cooled, and stored at 7 C. Portions 2 and 3 of the raw milk samples were held at 7 C for 2 and 4 days, respectively, before the various plate counts were determined, then laboratory-pasteurized, and stored as the first portion. The purpose of the additional holding time in the raw state was to obtain samples with larger bacterial populations to have a wider range of counts. After pasteurized milk was stored for 1 week, each sample was plated for SPC and PBC and the flavor was determined by a panel of three or four judges. This was repeated once more with the milk stored for 2 weeks after pasteurization.

Bacteriological analyses of all 216 samples were performed according to the procedures recommended by *Standard Methods for the Examination of Dairy Products (18)*. However, in a few instances it was necessary to count plates with <30 colonies.

Results and Discussion

The SPC of the raw milk samples ranged from <100/ml to nearly 100,000,000/ml. Psychrotroph counts were generally higher than SPC, especially in those samples that were held for 2 or 4 additional days before pasteurization. This was expected since any increase in bacterial populations resulted from growth of psychrotrophs, many of which failed to form visible colonies on SPC plates incubated at 32 C. Results indicated that additional holding of raw milk either on the farm (e.g. every-third-day pick-up) or in the processing plant (e.g. on weekends) may cause significant increases in numbers of psychrotrophs. Any unnecessary delay before analyzing samples would have a similar effect, possibly resulting in counts that may exceed the limit of a given standard.

Coliform counts ranged from <1/ml to 20,000,000/ml. Coliform bacteria multiplied rapidly at 7 C, and consequently it is reasonable to assume that these organisms may represent a significant portion of the psychrotroph count of raw milk.

Thermoduric counts in most samples were <1,000/ml, and generally these numbers remained at the same level or even decreased slightly during the 2- and 4-day holding period before pasteurization. However, there were a few exceptions where the numbers of thermoduric bacteria increased in the raw

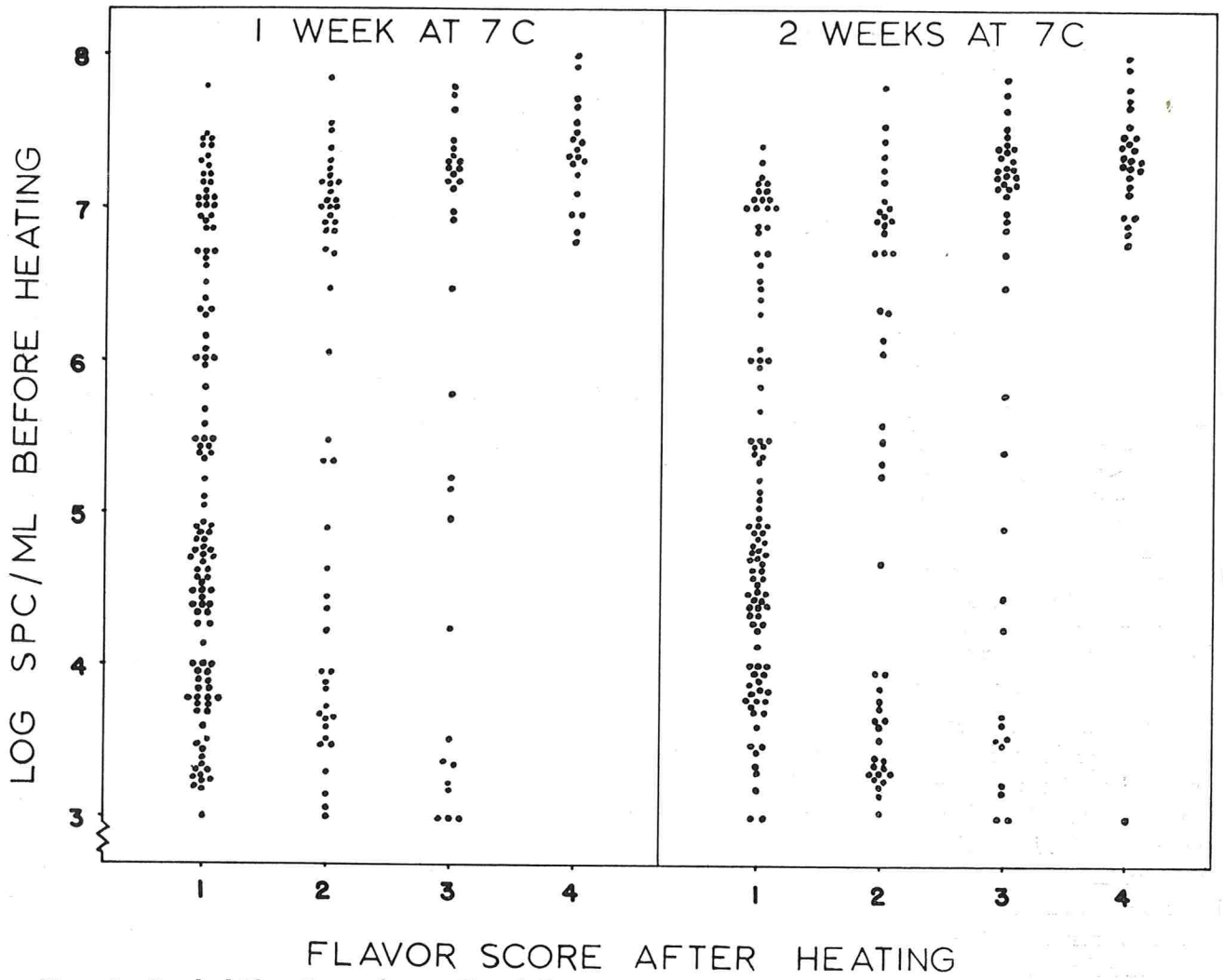

Figure 1. Standard Plate Count of raw milk and flavor scores after pasteurization and storage for 1 and 2 weeks at 7 C. (1 = good; 2 = fair; 3 = poor; 4 = very poor)

milk stored at 7 C. The same samples also showed increases in SPC and PBC during storage *after* pasteurization, indicating the presence of heat-resistant psychrotrophs. Most of these were found to be gram-positive rods.

In Fig. 1, the SPC of the milk immediately before heating are plotted against the flavor scores of the pasteurized product after 1 and 2 weeks of storage at 7 C. In most instances the flavor was good to fair. Several lots of milk with raw SPC of up to about 50,000,000/ml were still acceptable 14 days after pasteurization, indicating that the predominant types of organisms in these samples apparently had little effect on the milk. This again emphasizes that *numbers* of bacteria, the basis of nearly all our standards, are far less important than the *types* present and their ability to cause changes in the product.

Milk which did develop defects during storage generally had SPC of >1,000,000/ml before pasteurization. A typical example is Sample No. 2 in Table 1. When received, the raw milk had a SPC of 6,000/ml and the flavor after pasteurization and storage for up to 2 weeks was good. However, when the raw milk was held for 4 days before heating, the SPC increased to 27,000,000/ml and, although no differences were found in counts after heating and storage, the flavor of the pasteurized product deteriorated within 7 days.

In spite of eliminating post-pasteurization contamination by heating in sealed tubes, a few samples showed significant increases in SPC and PBC during storage after heating. The existence of thermoduric psychrotrophs has been demonstrated by several authors *(5, 12, 16)*. In this study no bacterial growth was observed in the pasteurized milk during the first week of storage at 7 C. This agrees with a recent investigation by Watrous et al. *(19)* who pointed out the importance of a sufficient length of time for the recovery of heat-resistant psychrotrophs. During the second week of storage, however, both SPC and PBC occasionally increased to such an ex-

TABLE 1. FLAVOR CHARACTERISTICS OF SOME PASTEURIZED MILK SAMPLES WITH VARIOUS SPC BEFORE AND AFTER HEATING AND STORAGE

Sample no.	Raw SPC/ml	Pasteurized		
		Weeks at 7 C	SPC/ml	Flavor
1	16,000,000	1	400	Slightly bitter
		2	27,000	Very bitter
2[a]	6,000	1	300	Good
		2	100	Good
2[b]	27,000,000	1	100	Very bitter
		2	300	Very bitter
3	11,000,000	1	200	Good
		2	100	Good
4	5,000,000	1	1,500	Good
		2	30,000	Unclean

[a]Pasteurized immediately
[b]Held for 4 days at 7 C before pasteurization

tent that flavor defects occurred. Because of the logarithmic rate of heat inactivation, large numbers of organisms in raw milk are more likely to result in psychrotrophic survivors than are smaller numbers. Commercial pasteurization, which usually employs higher temperatures, may be more effective in the destruction of many of these types of organisms. However, since psychrotrophic spore formers have been found *(5, 16)*, the possibility of heat resistant, cold-tolerant bacteria must be taken into consideration, especially in milk stored for extended periods of time.

In Fig. 2, all 216 samples were divided into 4 groups of equal size arrayed according to SPC of the raw milk. Group A contained the 25% of the milk with the lowest SPC, whereas group D comprised the 25% of the samples with the highest SPC. The most common off-flavor encountered was bitterness. Approximately 23% of all samples showed this defect after the 2-week storage period. It may be seen in Fig. 2 that no bitterness developed in the low count samples (A). Raw milk with higher SPC (B, C, D) resulted in increasing numbers of bitter samples. Nearly all milk with SPC of >10,000,000/ml before heating (D) showed some degree of bitterness after pasteurization and storage for 2 weeks.

Oxidized flavor appeared in 6.5% of the samples. Although oxidized flavor is generally a defect of non-microbial origin, its intensity may be lowered by large numbers of reducing bacteria. Consequently, this off-flavor was noticed more frequently in low-count milk (<5,000/ml). Data in Fig. 2 indicate that no oxidized samples were found in high count milk (D).

Other defects such as staleness, fruitiness, and unclean flavors occurred in about 14% of the samples after 2 weeks at 7 C.

It is suggested that one or more of the following may be the reasons for the development of flavor defects: *(a)* end products of microbial metabolism in raw milk may become apparent in the pasteurized product; *(b)* constituents of large numbers of heat-inactivated and lysed bacterial cells may impart off-flavors to the pasteurized milk; *(c)* heat stable microbial enzymes produced in raw milk may remain active after pasteurization and then cause changes in some constituents of the pasteurized milk during storage; and *(d)* presence of thermoduric psychrotrophs and their growth in pasteurized milk during prolonged storage. In fact, during this investigation one proteolytic enzyme was found which was not inactivated by laboratory-pasteurization or even by boiling for a short time and which was very active at 7 C. (Various characteristics of this enzyme, such as temperature and pH optima, heat stability, and other factors are now under investigation).

SUMMARY AND CONCLUSIONS

When raw milk was held at 7 C for up to four additional days, the bacterial populations increased significantly. Although at the time of heating no objectionable flavors were detected in such milk, the pasteurized product developed flavor defects during subsequent storage at 7 C. Counts of >1,000,000/ml

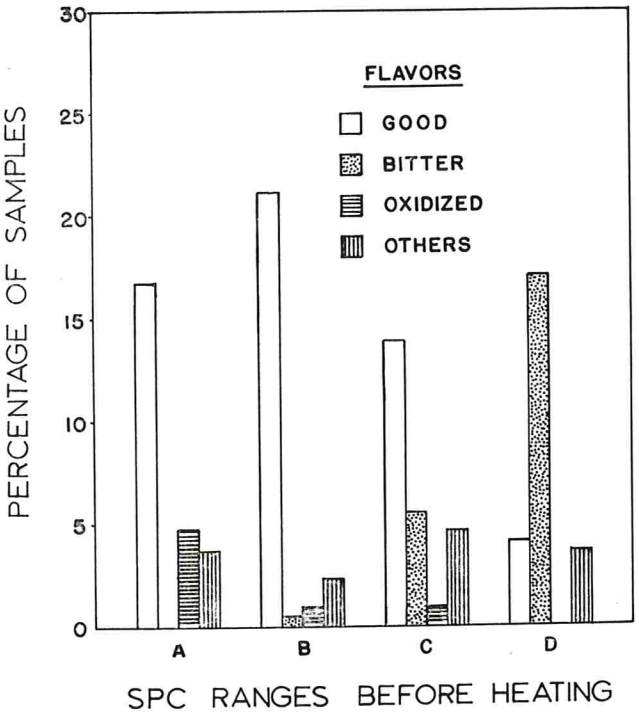

Figure 2. Relationship between raw milk SPC and the distribution of various flavors in the pasteurized milk after 14 days at 7 C. Each group (A, B, C, D) contains 25% of the samples. The approximate SPC were: A = <10,000/ml; B = 10,000 to 300,000/ml; C = 300,000 to 10,000,000/ml; D = >10,000,000/ml.

in raw milk frequently resulted in off-flavors of various types, and when SPC exceeded 10,000,000/ml, bitterness accounted for nearly all of the flavor defects.

There is no doubt that in commercially pasteurized and packaged milk, post-pasteurization contamination is by far the most common cause of flavor defects of microbial origin. However, even in the absence of contaminants, off-flavors may be encountered if the raw milk contained large populations of psychrotrophs.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT

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structed the Foundation staff to establish a Standard Task Committee to study this problem with particular attention to test procedures.

Standard for cooking and warming equipment

A member of the industry reviewed some of the new technology and developments and possible health hazards as they related to deep fat fryers and fat filtering systems. Based on this presentation and subsequent discussions, the Foundation was requested to establish a Standard Task Committee to consider the question of technical and scientific factors and to prepare either a proposed amendment to Standard No. 4 or a new standard covering fat filter systems.

However, in order to provide the Foundation staff with some interim guidelines until the desired specificity could be achieved, the Joint Committee recommended that Item 4.404 of Standard No. 4 be amended as follows:

> "Fat filters when operated in accordance with the manufacturer's instructions shall be capable of removing, from the fat or oil being filtered, particles of the micron size, and larger, specified by the manufacturer. The manufacturer's literature shall state the micron rating for his filters. Further, the manufacturer shall stipulate the procedures used to determine said rating."

Standard for hot water generating equipment

The proposed revision of Standard No. 5 on Hot Water Generating Equipment was approved by the Joint Committee with some modifications and with the understanding that the Foundation staff would review this Standard with the manufacturers of Pot, Pan, and Utensil Washers with the object of encompassing hot water generating equipment for such washers under this Standard. Some of the changes in the amended Standard included the deletion of Tables I and IA on hot water requirements for particular dishwashing machines from the technical information and all of the references to 40 F temperature rise data in Tables II and IVA, since this assumed that the standard line temperature of hot water in a building was 140 F or higher. The Foundation was also requested to recalculate Tables II and IVA deleting the references to the type of dish machines and replacing same with gallons of water per hour.

Standard for dispensing freezers

It was reported during the 1970 meeting of the Joint Committee that it might be advisable to label dispensing freezers with a precaution that they should not be used in connection with other than milk products, particularly acid-

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PROGRAM FOR QUALITY ASSURANCE OF FINISHED DAIRY PRODUCTS[1]

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ABSTRACT

Specifications concerning quality of milk products from raw ingredients to finished products are now often required by many distributors and food processers. Problems concerning plant sanitation, post-pasteurization contamination, and product temperature still persist despite an abundance of practical remedial knowledge available. The manifestations of many current problems are the same as they were 10 to 15 years ago. Two examples of these problems are induced rancidity in silo tanks as a result of excessive air agitation and thermoduric organisms in pasteurized milk which may proliferate to excessive numbers at refrigeration temperatures.

A program for quality assurance should include a raw ingredient program, methods for evaluation, effective plant sanitation, care of finished products, human motivation, and follow through. The area of most neglect is the last aspect. Plant cleaning and sanitation have improved remarkably in the last few years. Many dairies have capitalized on proven refinement of automated systems and have incorporated fine points of sanitation. The most effective procedure for trouble shooting problem areas is to follow product processing backwards using aseptic sampling techniques. Data accumulated in successfully pinpointing problem areas the past 5 years have demonstrated the value of this approach.

Quality assurance is a term now frequently encountered in the dairy and food industry. It has a broader meaning to consumers since they feel that quality is assured to them from raw ingredients to the ultimate finished products. In-plant quality assurance, which is synonymous with quality control, will be the main area of discussion.

In the last few years there has been a great increase in the need for chemical and bacteriological analyses on raw and finished food products. Results of these tests are intended to provide information to manufacturers, distributors, or buyers concerning the specifications they require. For instance, the meat industry, jointly with U.S.D.A. is presently engaged in setting bacteriological and chemical standards on ready to eat products such as wieners and bologna. The canning industry requires bacteriological specifications on starch which deal with the numbers of thermophilic spores and hydrogen sulfide producing and non-hydrogen sulfide producing anaerobes. Large super market chains frequently require a specified shelf life for all finished dairy products.

At the I.A.M.F.E.S. conferences of 1963 and 1966 Elliker et al. *(1, 2)* presented papers which discussed post-pasteurization contamination of milk and dairy products. Possible causes and suggested procedures to minimize the problem were mentioned. Attempts to isolate and pinpoint the problem (8 or 9 years ago) were accomplished by aseptic sampling procedures and equipment. The data collected indicated that the major post-pasteurization contamination areas were the fillers. As further effort was made, some contaminating areas were traced back as far as the HTST unit. It was common knowledge that normal pasteurization (HTST unit) would destroy most organisms able to grow at 45 F. These organisms were then referred to as psychrophiles but a more accurate term used today is psychrotrophs. Other areas that were found to be potential sources of contamination included joints, valves, manifolds, pumps, and air supply. Suggested procedures for correction, such as on-the-spot sanitizer spraying of the filler area before and during operation, were found to be effective. The culmination of this work realized an increase of 14 to 20 days shelf life for many dairy products.

In the past few years there have been many changes in dairy operations throughout the country. It is of interest to visit plants to note new changes and to evaluate them for their contribution to post processing contamination. As a result, we have made several conclusions.

1. The small independents generally lag behind the larger plants (majors, captives, and large co-ops) with respect to basic design and plant improvements made. Yet ironically, the small plants may show a better profit picture than the large up-to-date dairies. Being behind oftentimes may be in their favor since they can update their plants and take advantage of modern technology. They certainly capitalize on all the refinements of automated systems. On the other hand, the CIP systems (in their infancy 15 years ago) are now being converted and brought up to date.

2. Those actively pursuing cleaning and sanitizing

[1]Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Cedar Rapids, Iowa, August 17-20, 1970

and quality control programs continue to be on top, saleswise and profitwise. The question most frequently asked now is—are all plants taking full advantage of the accumulated knowledge in this field? Unfortunately not. Those plants that are more conscious of pricing than of quality will not be in business long. However, those who feel that quality has marketable value will continue to grow and make a profit.

3. New equipment and concepts have been beneficial in many ways, however, as a result new problems also are introduced. Aseptic systems, silo storage, high speed fillers, and air control are examples of a few such areas.

In discussing a program for plant quality assurance of finished dairy products, five main points of concentration should be considered. These may be listed as follows:

1. Raw ingredient program.
2. Methods for evaluating quality assurance.
2. Methods for evaluating quality assurance of finished products.
3. Effective plant sanitation procedures.
4. Care of post-processed (finished) products.
5. Human motivation and follow through.

In discussing each of the above points, it is of value to illustrate or discuss problems that have been recently encountered. It is surprising that the manifestations of many current problems are the same as they were 10 to 15 years ago.

Raw Ingredient Program

Important considerations in this program are flavor, bacterial content (raw and pasteurized count), raw storage, adulterants (water and antibiotics), pesticides, and composition (butterfat and solids). Flavor in a raw ingredient program is considered the most important of all the aspects described above.

The most critical problem is developed flavor as the result of excessive bacterial growth. In most instances these flavors are the result of prolonged storage, either on the farm or in the plant, at temperatures which are favorable for proliferation of bacteria. Temperatures in excess of 40 F could cause rapid multiplication of bacteria which will cause flavors such as putrid, rancid, rotten, or fruity. Most of these developed flavors in the raw product cannot be eliminated by pasteurization. On the other hand, flavors that develop in the finished, pasteurized product are generally the result of post-pasteurization contamination.

In discussing various aspects of the flavoring program, current trends towards large storage capacity such as silo tanks, have been beneficial. The greater commingling of milk has resulted in dilution of undesirable flavors. However, one of the problems encountered with large storage capacities is insufficient turn-over of raw product. In many instances, silo tanks for raw milk may not be washed for 3 to 4 days. If raw product is continuously added at favorable temperatures, psychrotrophic growth will perpetuate off-flavors.

Another problem that has been encountered concerning silo tanks is development of rancidity. The problem is more pronounced in silo tanks with capacities >30,000 gal. Rancidity develops because of excessive air agitation, especially when the tank is partially filled. The smaller silo tanks generally have mechanical impellers that will maintain proper agitation. The larger tanks, by necessity, have relied, primarily, on air agitation. Many of these large tanks are air agitated with constant air pressure rather than constant volume. Data in Table 1 illustrate what happens in a 40,000 gal silo tank that is agitated by constant air pressure. When the tank was full, acid degree values (ADV) were satisfactory. As the tank was emptied, the ADVs increased. At 18,000 gal the ADV was 0.76, whereas at 2,500 gal it was 1.48. Many silo tank installations that are agitated using constant air pressure are finding similar results. A remedy for this situation is the installation of a constant volume air regulator that insures proper agitation at any given volume of liquid in the tank.

Despite development or rapid tests and sophisticated instrumentation in the last 10 years to detect raw ingredient problems, these problems are still frequently encountered. Some of these problems are induced rancidity, high thermoduric counts, antibiotics, pesticides, added water, and mastitic milk. Ironically, these are, basically, the same problems that we faced many years ago.

A suggested in-plant raw milk program is as follows:

1. Grade raw products at the tanker level by taste and smell. It would be desirable that two people be involved, for example, the receiving man and a person in quality control. It is important that people concerned with tasting milk are trained and are able to detect off-flavors. To avoid flavoring raw milk, these samples may be heat shocked at 150 F for 2 or 3 min and cooled, prior to tasting.

2. All milk in raw storage tanks should be tasted just before processing. This step is to insure that raw milk going to the HTST unit has an acceptable flavor.

3. Laboratory tests should be performed periodically. These should include standard plate counts, laboratory pasteurized counts, leucocyte counts, and tests for adulterants, such as antibiotics, added water, and pesticides. Other quality tests such as flavor

of milk from individual producers and pre-incubation prior to standard plate counts have proven to be beneficial. Some plants also conduct culture activity tests on the milk to make certain that it will support active growth of lactic acid bacteria in production of cottage cheese, buttermilk, and yogurt.

To illustrate that some problems which existed in the past are still persisting today, refer to Table 2 which shows that high thermoduric counts still persist in some operations. If you will compare the fresh count to the shelf life count, you will note that the high count was not as a result of post-pasteurization contamination but a raw ingredient problem. Some thermodurics have been found that will grow at refrigeration temperatures. Recently reported was a carton of pasteurized milk exhibiting a very bitter flavor that had a count of 58,000,000/ml. These organisms were not the typical gram-negative contaminants but were large spore-forming rods. This product was picked up 7 days after processing and the milk was held at refrigeration temperatures. These large spore-forming rods have often been found to cause sweet coagulation in finished products that may be 2 or 3 weeks old. Data as indicated in Table 2 are important from the standpoint that companies buying on specific standards for pasteurized milk will not tolerate high thermoduric counts even though proper processing procedures have been used.

METHODS FOR EVALUATING QUALITY ASSURANCE OF FINISHED PRODUCT

This topic concerns procedures for evaluating quality assurance of finished products. These procedures are quality tests performed in the laboratory and methods for determining in-plant problem areas. The following discussion will be limited to present application of prior techniques described in past publications *(1, 2)*.

The finished product coming directly from each packaging line should be tasted at various intervals during the production day. It would be desirable that the final product be tasted by the operator and a person in quality control. Finished products taken from each line for this purpose also may be used for checking weights and leakage. It must be pointed out that tasting the final product at this point is extremely important since often this is the last opportunity before the product is distributed.

Randomly selected samples of all pasteurized products should be taken for bacteriological and flavor tests. These samples should be taken at periodic intervals during production and the number of samples taken should be proportional to the quantity of any given item processed. Each of the samples should include pertinent information regarding its production such as HTST unit, tank, line, filler, sequence of production and time. These samples of finished products are to be subjected to keeping quality tests.

A number of laboratory tests for determining shelf-life characteristics are available. It is important to select a test that will provide accurate data concerning shelf life characteristics. One of the most suitable tests for shelf-life is the Moseley 5-day, 45 F test. In recent years the test has been made more sensitive by extending the number of days at 45 F. In doing keeping quality tests for either flavor or bacteria count, it is most important to simulate marketing conditions to which the product will actually be subjected. A minimal test for keeping quality would be holding the product for a given number of days at 45 F and tasting it to detect bacteriological spoilage. If the collected data do not show satisfactory results based on bacteriological counts and flavor, then spot check procedures should be implemented to determine problem areas.

Various sampling devices are available to aseptically obtain line samples. Recommended areas for installation of in-line sampling devices are:

1. Outlet of each HTST unit
2. Outlet of each pasteurized surge tank
3. Pasteurized surge manifold
4. Above the bowl of each filler

A suggested procedure of line sampling to pinpoint the area of contamination is to work backwards from the finished product. Sampling prior to the filling machine is a good location to monitor. Many plants will take daily routine samples at this point. If results are satisfactory here, there is no need to trace back beyond this point. This approach has been proven to be extremely effective.

In collecting data from numerous plants, it has been found that the major problem areas are as follows. Filling machines, by far, cause most contamination problems. This is followed by the pasteurized surge line system if it contains many sanitary thread joints and plug valves. If the surge line system contains air valves and welded lines, these areas contribute little contamination if satisfactorily cleaned and sanitized. Pasteurized surge tanks that are cleaned by automation cause few problems. Hand washed storage tanks are still major problem areas. The HTST unit and related pipe lines often have been a source of contamination. The specific defect most often encountered is accumulation of soil in the cooling section or in dead-end fittings such as air eliminators and thermometer probes.

A present-day plant containing standard equipment should be able to produce products from the outlet side of each HTST unit which have a count of $<3{,}000$/ml after 14 to 21 days storage at 45 F.

If the plant has all welded lines and automatic valves, there should be only minimal pick-up of contaminants between the HTST unit and the point above the filler bowl of any given filling machine.

Lastly, evaluation of any plant requires thorough records concerning the operation. These records should include daily inspection reports, noting cleanliness of equipment and potentially dangerous areas; time and temperature recording charts; and procedural changes in cleaning and sanitizing. Monitoring data also are important. For example, data should be obtained from the outlet side of each HTST unit and the area above the filler bowl of each filling machine.

Effective Plant Sanitation Procedures

This discussion pertains to procedures used in cleaning and sanitizing and also covers some of the fine points of sanitation. Observations made in the past few years have shown specific points to be important. These are as follows:

1. All product contact surfaces and associated surfaces should be cleaned to the surface of the equipment material. Associated surfaces are areas that may not come in direct contact with the product but may become potential sources of contamination through their relation to the product. Examples of these areas are the interior surface of lids and tops of tanks, and the exterior surface of fillers. There is only one criterion for determination of a clean surface, and that is whether it is bare surface clean. It is also important that we learn to recognize a clean surface and to provide cleaning procedures to insure that this criterion is met.

2. It is necessary for quality control and plant supervisors to be present during the clean-up. As cleaning systems become more complicated and more automation is installed, it becomes increasingly important that highly trained people supervise the clean-up operation. It is felt that one day cleaning and sanitizing will be accepted as the most important functions in any plant operation.

3. Proper maintenance of all facets of the cleaning system is an absolute necessity. These include periodic changing of all joint gaskets; insuring proper functioning and seating of automatic or manual valves and electronic components of the CIP cleaning system; checking proper flow rate of CIP and return pumps; and removal of all extraneous material blocking jets of spray balls.

4. Daily routine checks of concentrations of cleaning agents and sanitizers are required to insure the performance of CIP systems as well as manual cleaning operations.

In sanitizing, we have found procedural methods to be the most important.

1. To insure proper sanitizing, the system should be, for the most part, closed. By this we mean that all pasteurized surge tanks are closed, jumpers to the pasteurized manifold in place, jumpers to filler bowls of machines in place, and all fillers assembled. The pasteurized distribution manifold also should be closed with all jumper hook-ups connected to all tanks. It has been found that if spray balls are permanent installations, and sanitizing through spray balls is programmed into the systems, this is the most ideal way to sanitize. Fogging of tanks with high concentration of sanitizers, in contrast, is a poor practice.

2. The suggested procedure for sanitizing the HTST unit would be to start with a sanitizing solution made up in a processing tank and then pumped to the balance tank. Sufficient sanitizing solution of the proper concentration should be made up to insure proper contact time. The sanitizing solution from the HTST unit is then distributed throughout the pasteurized distribution system into the surge tanks. Again, sufficient solution should be available in each surge tank to sufficiently flood the pasteurized surge tank outlets and lines to the machines. To properly flood filler bowls, many plants have devised a tool to hold down the floats to insure complete contact. Finally, machines are operated to insure sanitizing of the internal parts of assembled machines. Prior to sanitizing, filler machines are assembled using a sanitizer while building up the machine. Most companies now have systems which provide a sanitizer in key areas in the plant for sanitizing on the spot. The most common fault in most plant operations is the indiscriminate use of untreated water in all post-pasteurized areas. Procedures such as flushing lines with untreated water and rinsing pasteurized surge tanks with untreated water are bad practices.

3. Procedures to insure proper contact time, concentration, and pH of sanitizers also are important. The manufacturer's directive for use of various types of germicides should be closely followed.

In discussing fine points of sanitation, some of the points to be stressed have already been mentioned. In most instances, fine points of sanitation are procedural changes or use of special equipment that may be suitable only to a particular operation. Procedures are implemented before and during the operation. It is important to recognize that equipment does not remain clean and sanitized all day. Present day operations require extremely long runs, therefore, failure to recognize that this equipment will not remain sanitary all during the operation will lead to poor results.

TABLE 1. EFFECTS OF EXCESSIVE AIR PRESSURE AGITATION IN RAW SILOS ON RANCIDITY

Tank		ADV[a]
Raw silo #7	(18,000 gal)	.76
Raw silo #7	(2,500 gal)	1.48
Tank #7 to balance tank (recycling)		1.73
Normal	ADV	0.4
Unsatisfactory	ADV	1.5

[a]ADV = Acid degree value

TABLE 2. EFFECTS OF HIGH THERMODURIC COUNTS ON FRESH AND SHELF LIFE TESTS

Sample	Fresh	After 5 days at 45 F
Qt. homo	140,000	150,000
½ Gal homo.	24,000	24,000
Half and half	90,000	>3,000,000
	SPC	Lab. Past.
Raw tanker	1,200,000	72,000

Two effective procedures have been implemented in the past years. First, be assured that all filling machine parts are sanitized prior to assembly and sanitize while assembling. Secondly, spraying the exterior parts of filling machines with an appropriate sanitizer periodically during the day's operation has helped to reduce post-pasteurization contaminants at these sites. The two most common and effective sanitizers used in the industry today are iodophors and chlorine compounds. Iodophors are non-corrosive acid sanitizers that are most effective at fairly low concentrations. The central distribution iodophor systems that are piped throughout the plant also have been extremely beneficial for use as on-the-spot sanitizers for filler parts, exterior of fillers, jumper hook-ups, plug valves, valve clusters, and other areas which may contribute post-pasteurization contamination. Sodium hydrochlorite solution is still the most effective general sanitizer for tanks, lines, and HTST units. In some instances, consideration must be given to the pH of the water used to make up the sanitizer. It has been found that water with a high pH, in some instances, has negated the sanitizing properties of certain iodophors when used at low concentrations. When low concentrations of chlorine are employed for cottage cheese wash water, their activity is enhanced by controlled acidification of the water. Chlorine sanitizers are more active if a final acid rinse is used in plant cleaning programs. Acid rinsings also have been beneficial in reducing milk stone formation and hard water and detergent spotting.

In recent years in-plant environmental conditions have been demonstrated as affecting the finished product. Air and condensate have been found to be sources of post-pasteurization contamination. Implementing fine points of sanitation have helped control these areas. In addition, manufacturers of air control systems and dairy equipment have produced new designs with sanitation in mind. Good examples of these concepts are use of HEPA type filters for the air supply for critical filling room areas often combined with a positive pressure air system for specific rooms. Also, the location of laminar flow, filtered air units over filling units insures essentially sterile air in the filler environments.

CARE OF POST-PROCESSED (FINISHED) PRODUCTS

In view of current distribution systems, it is extremely important to properly care for finished products. Finished products from processing plants, in many instances, are being trucked or shipped to other distribution complexes. As many as three re-handling procedures could be made from point of origin to final destination. For this reason, close control of storage temperatures is essential to meet the requirement for longer shelf-life. Points to stress are as follows:

1. Processing plants must practice maximum sanitation as discussed earlier. Temperature control to keep the product as cold as possible will also be necessary. Many plants are putting in additional cooling capacities, for example, glycol systems are becoming more common in the industry. Product leaving the HTST unit may be at temperatures close to freezing. Many plants will conduct their products through insulated lines and store in refrigerated holding tanks.

2. Storage vaults for finished products also should be as cold as possible. Maintenance of temperatures at 34 to 38 F is not uncommon. The importance of maintaining these temperatures despite re-handling of the product to different coolers cannot be overstressed.

3. Display cases in merchandising areas are areas of temperature abuse. Numerous studies have been made on display cases showing temperatures in many cases to be far above 45 F. Another poor practice often encountered is stacking of finished products above the recommended load line of display cases.

It has been demonstrated that by virtue of temperature control alone, without any changes in plant processing, the shelf-life of finished products can be increased. The point of this discussion is to stress that with the combination of good sanitation practices and temperature control optimum shelf-life characteristics can be attained.

HUMAN MOTIVATION AND FOLLOW-THROUGH

Of the five points discussed, the combination of motivation and follow-through is the most important.

Unfortunately, this also is the area of most neglect. Most dairy plant operations have all the physical needs for producing a long shelf-life product, however, failure to meet this goal is most often attributed to human weakness. Some of the reasons why we do not accomplish the total job of quality assurance may be:

1. Lack of emphasis from management to incorporate improved programs. The success of quality assurance depends upon management passing down or approving the necessary requirements to attain their goals.

2. Often there is a lack of a properly defined quality control department. In many instances this department is a part of production and not directly under management. Under this arrangement the administrative responsibilities are such that the job will never be accomplished. If quality control people are bogged down with production problems, there is no opportunity for success of any quality assurance program.

3. Supervisory people in production or quality control must be trained in human relations otherwise they will certainly not be able to motivate others.

4. Careful screening of employees should be done to obtain personnel that are experienced or that can be trained to do specific jobs.

All successful endeavors are the result of people. Despite increased technology, automation, new sophisticated systems, and up-to-date equipment, an operation functions only as well as the individuals make it perform. As operations become more complex and additional knowledge is required, it becomes necessary to assist control and plant personnel in every way possible to develop the skill to carry out programs or manipulate costly equipment. Results of care are successful endeavor and pride. This will insure continuance of a job well done.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT

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type foods, since such action might produce a toxic product. According to the Foundation staff, currently dispensing freezers are being manufactured with component parts which have been found to be suitable for use with either dairy products or with slush ice products; thus, any precautionary labeling for new equipment would be useless.

Standard for walk-in and reach-in refrigerators and storage freezers

Following presentations by various manufacturers and a lengthy discussion by the members of the Joint Committee, a revision in the errata to Item 5.02, as it related to the requirements for joints and seams in walk-in refrigerators, was recommended as follows:

> "The provisions of this specification are interpreted to require that gaskets meet the material requirements for splash zone. Interpretation further precludes the use of sealing compounds applied in the field during or after erection of the unit except for effecting repairs."

Further presentations were made regarding the revision of Standard No. 7 to permit the use of plastic coving strips in walk-in refrigerators and freezers to effect the required radii in two and three plane intersections in lieu of the present requirements which specify that the radii shall be smooth and continuous, i.e., of the parent material. After discussing these new materials and techniques, it was concurred that the subject should be sent to the Standard Task Committee for study and recommendations with a subsequent review and ballot by the Joint Committee. A recommendation was made also by members of the industry that the Standard Task Committee review the current provisions which require a flush door sill for walk-ins.

Standard for manual dispensing equipment

The potential hazard of backflow of carbon dioxide and carbonated water in manual dispensing equipment was discussed. The Foundation staff indicated that it was their plan to propose an amendment to Standard No. 18 (Manual Dispensing Equipment) in the same manner as Standard No. 25 (Food and Beverage Vending Machines), is being amended to prevent carbonated water from coming in contact with copper lines.

Standard for commercial cooking equipment exhaust

The public health members briefly considered the need for further study of commercial cooking equipment exhaust systems with particular attention to units other than canopy hoods. They noted that at the present time it is strictly a performance standard and there are no specifications for exhaust systems other than of the canopy-type, thus making field evaluation of such equipment very difficult. The NSF Testing Laboratory staff recommended that changes be held pending the results of the Laboratory's testing of a number of these units in order to provide a baseline for the design and construction parameters.

Requirements for covers over food zones

The Foundation staff reviewed a survey of requirements for covers over food zones as now contained in Standards 1, 2, 4, 6, 7, 8, 12, 18, and 25 and Criteria C-2; and the survey revealed that some required a cover or other protection, some specified that the cover overlap the opening, some specified protection of port openings, and some required a 3/16 inch raised rim for covers over food zones. In view of the survey findings, the Joint Committee requested the Foundation to establish a Standard Task Committee to review the present requirements relative to covers over food zones and to make a recommendation to the Joint Committee. Specifically, the

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BACILLUS CEREUS: FOOD POISONING ORGANISM. A REVIEW

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Abstract

This paper is devoted to a review of information pertinent to the role of *Bacillus cereus* as a food-borne pathogen. Primary emphasis is on the properties of *B. cereus* and published accounts of its involvement in animal and human disease. Methods for isolation, identification, and enumeration are discussed. Research needs pertaining to *(a)* recognition of the potential public health hazard and *(b)* further investigation of the food poisoning syndrome are presented.

It has become increasingly clear that *Bacillus cereus* is involved in foodborne illness. The scope of the problem in this country is unknown. This review of the literature pertinent to *B. cereus* is presented with the hope that it may serve as a springboard from which investigations into *B. cereus* food poisoning may be launched. Obviously, the quantity of information that exists on this single microorganism is so voluminous that all could not possibly be reviewed in this limited space. With this in mind, we have based our selection of data to those areas which seem today most pertinent to the problem of *B. cereus* as a food-borne pathogen.

The Organism

Taxonomy

Before 1946, classification of the genus *Bacillus* could best be described as chaotic. In that year, the first of two excellent monographs in which a rational and orderly approach to the taxonomy of *Bacillus* spp. was published *(158)*. In 1952, the second monograph (largely an expansion of the first) was published and the taxonomic scheme proposed therein is the basis for separation and identification of bacilli today. Smith et al. *(158, 159)* proposed division of the genus *Bacillus* into three groups (I, II, and III) based on spore and sporangium morphology. Group I bacilli consisted of those genera without definitely swollen sporangia (by the spore). Group II and III organisms evidenced swollen sporangia and spore morphology (i.e. oval spores for group II and round spores in group III) was employed to differentiate the two groups. Within group I, further subdivision was made on the basis of vegetative cell size and presence or absence of vacuoles within vegetative cells grown on glucose-nutrient agar. Thus, the large-celled species (vegetative cell diameter $\geq 0.9\ \mu$; also vacuolated) included *Bacillus megaterium* and *B. cereus* and varieties while the so-called small-celled species (cell diameter $< 0.9\ \mu$; not vacuolated) of group I bacilli included *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilis, Bacillus coagulans,* and two rarely encountered species *Bacillus firmus* and *Bacillus lentus.*

In these extensive treatises, Smith et al. chose to consider *Bacillus mycoides, Bacillus thuringiensis,* and *Bacillus anthracis* as varieties of *B. cereus* rather than as separate species. This position has evoked favorable response from some *(26, 170)* and criticism from others *(9, 66, 85, 100)*. In this review, we shall refer to these organisms as separate species purely for simplicity without intent to support either the species or variety taxonomical position. These organisms are very closely related and tests designed to differentiate them have been the subject of numerous papers (see Section on confirmatory tests).

Bacillus cereus was originally isolated and described by Frankand and Frankland *(57)* in 1887. It is characterized as a gram-positive and catalase-positive long rod. Various biochemical, serological, toxicological, and bacteriophage susceptibility patterns have been ascribed to the species but, by and large, intensive investigation has shown that great variability in properties is characteristic of this organism. The major properties of the organism are presented, but the reader is warned that aberrant and deviant strains are not uncommon.

Biochemical properties

As a species, *B. cereus* strains are able to utilize glucose, fructose, and trehalose. Xylose, arabinose, galactose, sorbose, sorbitol, mannitol, and dulcitol are not attacked *(85, 101)*. Sucrose, salicin, maltose, mannose, glycerol m-inositol, and lactose [strains utilizing lactose are usually named *Bacillus albolactis (159)*] are attacked by some but not all members of the species *(26, 85, 101)*. Urease is produced by few strains *(85)*. Acetylmethylcarbinol (AMC) production is characteristic of most *B. cereus* strains *(29, 66, 101, 159)* but is not produced by all *(26, 78, 85)*. Nitrates are reduced to nitrites *(101, 159)*, but nitrate reductase negative strains have been isolated *(26, 29, 78, 85)*. Most strains attack soluble starch but a few

amylase-less mutants have been described *(78, 85)*.

Most strains produce a neutral metalloprotease *(52, 60, 152)* and actively dissimilate such substrates as casein and gelatin *(26, 78, 85, 101, 159)*. Certain strains have been reported to synthesize a dehydropeptide reductase that catalyzes cleavage of the antibiotics nisin and subtilin *(72, 73)*. Virtually all strains produce a penicillinase *(149)* which enables them to grow on agar containing 10 International Units of penicillin, a test which aids in differentiating *B. cereus* from *B. anthracis*. In contrast to *B. megaterium*, few strains (if any) are sensitive to lysozyme *(86)*. Reportedly, *B. cereus* is not able to grow in a medium in which ammonium ion is the sole source of nitrogen *(83)*.

Recent reports by Knisely *(84)* and Mossel et al. *(119)* have indicated that *B. cereus* grows well on chloral hydrate agar and phenethylalcohol agar. Kim and Goepfert *(78)* could not substantiate this with tests of strains freshly isolated from food products.

One of the most important aspects of the biochemical pattern of *B. cereus* is synthesis and excretion of certain toxins. At least three important extracellular toxins are produced by *B. cereus;* these products have received extensive study.

Production of lecithinase by *B. cereus* was recognized by McGaughey and Chu *(109)* and Colmer *(38)* in 1948. Chu *(34)* later reported that culture filtrates of *B. cereus* also possessed hemolytic, dermonecrotic and lethal (for mice) activity. At the time, and for some years after, it was tempting to speculate that all of these activities resided in a single molecular species as was true of *Clostridium perfringens* α-toxin *(105)*. But Molnar *(117)* provided evidence that lethal toxin and phospholipase were separate entities and could be physically separated. Ottolenghi et al. *(142)* reported that hemolysin and phospholipase could be differentiated on the basis of thermal stability.

There is some evidence that more than one phospholipase is produced by *B. cereus*. Slein and Logan *(156, 157)* reported separation of two phospholipolytic enzymes on columns of DEAE-cellulose. The enzymes were different with respect to digestion by trypsin, thermal stability, and substrate specificity. Similar evidence for a multiplicity of phospholipases was presented by Kushner *(91)*, who stated that phospholipase and an egg yolk turbidity-producing factor differed in thermal stability. Recent studies on the lecithinase C of *B. cereus* have shown that it has a molecular weight of 20,000 and an isoelectric point of 8.0-8.1 *(140)*. Some argument has arisen as to the requirement for Zn^{2+} ions for full enzymatic activity *(75, 81, 141)*. In contrast to the substrate specificity of the *C. perfringens* enzyme *(104)* the lecithinase C of *B. cereus* is able to hydrolyze phosphatidyl-ethanolamine and phosphatidyl serine *(157)*. The enzyme is formed and secreted by cells at the end of the log phase (on complex media) and is somewhat susceptible to denaturation if vigorously shaken *(92)*. Although there are reports of lecithinase-negative strains of *B. cereus*, such strains in our hands always produce turbidity on egg yolk agar. For the most part, the typical reaction of these latter strains on egg yolk agar is the 'restricted' form defined by Proom and Knight *(150)*. Synthesis, but not activity of the enzyme from lecithinase positive strains, can be inhibited by growing the culture in the presence of C_1 - C_3 alcohols *(90)*. Many questions remain regarding the role of the enzyme in pathogenicity, but at present, use of egg yolk containing agar as a presumptive medium for isolation and identification of *B. cereus* is the most efficient procedure.

Hemolysin, or "cereolysin" as it has been termed *(11, 12)*, is similar to streptolysin 0 in that it is activated by cystine, inhibited by cholesterol, and is neutralized by horse serum containing antistreptolysin 0 antibodies *(12)*. Although some reports *(21, 75)* have suggested that "cereolysin" is heat labile, others *(56)* have said that the purified enzyme is relatively stable. Fossum *(56)* has presented evidence that lability of hemolysin in crude preparations is caused by enhanced activity of proteases as the preparations are heated. Removal of the proteases allowed a more definitive determination of thermal stability of the hemolysin. Purified hemolysin does not show phospholipase activity *(12)*.

The lethal toxin of *B. cereus* differs considerably from the toxin formed by *B. anthracis (117)*. Not only is the skin lesion resulting from intradermal inoculation different, but the *B. cereus* toxin is rapidly lethal for mice (intravenous injection) while the *B. anthracis* toxin is not *1(9)*. Moreover, the *B. cereus* toxin rapidly destroys monolayers of guinea pig spleen cells while the *B. anthracis* toxin is without effect *(20)*. Johnson and Bonventre *(75)* have obtained partially purified preparations of lethal toxin which failed to exhibit hemolytic or phospholipolytic activity. Recently, Krieg *(88)* has reported that *B. cereus* produces a toxin which is lethal for mice and insects. Properties of this toxin were not described, so a comparison with the lethal toxin studied by Johnson and Bonventre *(75)* was not possible. The possible role of the lethal toxin in food-borne disease is currently under investigation.

Bacteriophage sensitivity

A number of bacteriophages have been isolated which will lyse strains of *B. cereus* and one or more closely related species *(39, 59, 106, 107, 144, 161, 168)*. Several phages have attracted the attention of in-

vestigators interested in a definitive diagnostic test for *B. cereus* and *B. anthracis*. Phage W was isolated by McCloy *(106, 107)*, and its specificity has been examined by various investigators *(25, 26, 70, 106, 107)*. Their findings indicate that all strains of *B. anthracis* and most strains of *B. cereus* are susceptible to W. phage.

Phabe γ, a clear plaque mutant of W, has been reported by many workers *(25, 26, 69, 86, 100)* to be specific for *B. anthracis*. Buck et al. *(27)* stated that strains of *B. subtilis* and *B. megaterium* were also susceptible to γ phage but this has remained unconfirmed. The apparent specificity of this phage resides in the absence of specific receptor sites on the surface of *B. cereus* and other *Bacillus* spp. *(69)*.

Smith et al. *(158)* isolated phage 201 which was purported to be specific for *B. cereus*. However, Brown et al. *(26)* and Kim and Goepfert *(78)* reported that not all strains of *B. cereus* were susceptible. The species specificity of 201 is questionable since the phage was able to attack *B. thuringiensis* ATCC 10792 *(78)*. At present, there does not appear to be a reliable test employing bacteriophages for the identification of *B. cereus*.

Serology

The serological relationships within the genus *Bacillus* are not as well defined as for other genera, e.g., *Salmonella*. There have been numerous attempts over the years to develop serological procedures for use as diagnostic aids, but these have met with little success. There are several excellent reviews of the serology of the *Bacillaceae* and *B. cereus* in particular *(6, 135)* The reader is referred to these articles for details additional to those presented below.

When considering the antigenic content of *B. cereus*, it must be kept in mind that, except in occasional mutant strains, flagellar, vegetative cell somatic, and spore antigens are present.

Flagellar antigens

Although it is well documented that *B. cereus* is motile by means of peritrichously arranged flagella *(8, 26, 145)*, some variation in motility (e.g., diminished, absence of, etc.) of certain strains has been reported *(86, 100, 101, 159)*.

Although flagellar antigen analysis has resulted in a serotype classification of *B. thuringiensis (8, 9, 17)*, attempts at such a classification of *B. cereus* have not met with equal success. One of the most comprehensive investigations was described by Lemille *(102)*. He was able to group 33 strains of *B. cereus* into 17 H serotypes based on flagellar agglutination patterns. When sera prepared against these serotypes was tested against 60 strains representing 8 species of *Bacillus*, cross reactions were obtained with 2 strains of *B. megaterium*. Conversely, a test of the 33 *B. cereus* strains against flagellar sera prepared against 6 other species of *Bacillus* revealed certain cross reactions with *B. subtilis* antisera.

Krieg *(86)* reported that B. cereus strains cross-reacted with sera containing antibodies to flagellar antigens of *B. thuringiensis*. Norris and Wolf *(135)* demonstrated that flagellar antigens of *B. cereus* were strain rather than species specific.

Somatic antigens

An early attempt to determine the specificity of vegetative somatic antigens of *B. cereus* was described by Sievers and Zetterberg *(155)*. Cross reactions of *B. subtilis* and *Bacillus vulgatus* in anti-*B. cereus* sera were observed. Some years later, Ivanovics and Foldes *(69)* evaluated the precipitin test on extracted polysaccharides from vegetative *B. cereus* cells. They concluded that two broad groups of *B. cereus* existed based on cross reactions (and lack of such) with anti-*B. anthracis* sera. Tomcsik and Baumann Grace *(169)* evolved 13 0 groups of *B. cereus* from 23 strains examined. However, extracted polysaccharides from vegetative cells reacted in anti-*B. anthracis* sera *(10)*. Norris and Wolf *(136)* failed to demonstrate the presence of a species-common vegetative cell precipitinogen in strains of *B. cereus*. Presence of vegetative cell somatic antigen in spores has been demonstrated *(174)*. However, these antigens are located along the cortical membrane and are not readily available for reaction with immune serum. At present, it seems that serological reactions involving cell wall antigens are of little value as an aid in identifying *B. cereus*.

Spore antigens

The antigenic nature of spores was described by Defalle *(47)* in 1902. Since then, numerous attempts have been made to use the serology of spores as an analytical tool. Much of the older work was beset by technical problems involving contamination of spore preparations with vegetative cell debris or germination of spores within the inoculated animal leading to synthesis of anti-vegetative cell immunoglobulins.

In 1940, Lamanna *(94)* attempted to differentiate the 'large-celled' bacilli by use of spore agglutinins. Although differentiation from *B. megaterium* was possible, *B. mycoides* would cross-react in anti-*B. cereus* sera. Later, Doak and Lamanna *(48)* showed that *Bacillus brevis* and *Bacillus sphaericus* would cross-react in *B. cereus* sera. The multiplicity of spore surface antigens in a single strain of *B. cereus* was also demonstrated. Later work by several investigators *(95, 96)* revealed that extensive cross agglutination occurred with spores of *B. cereus, B. an-*

thracis, and *B. thuringiensis.*

In their comprehensive survey of *Bacillus* spp., Norris and Wolf *(136)* showed that heat resistant agglutinogens and precipitinogens were present in spores but absent in vegetative forms. No single heat stable agglutinogen could be found in all strains of *B. cereus.* However, a spore precipitinogen common to all strains of *B. cereus* was found. Cross-precipitin reactions with *B. thuringiensis* spore extracts were not infrequent.

More recently, work has begun on the antigenic structure of the exosporium of *B. cereus* and the potential use of this structure in serodiagnostic tests. Results with fluorescein labeled anti-exosporium globulins have indicated that this structure may possess more species-specific antigens than are present in the underlying structures (H.U. Kim, *unpublished data*).

At present, it appears that serological tests are available to differentiate the *B. cereus* group from other bacilli but intragroup speciation is not possible with any degree of certainty.

HUMAN AND ANIMAL PATHOGENICITY NOT RELATED TO THE INGESTION OF FOOD

Bacillus cereus is commonly and justifiably considered to be a harmless saprophyte under most circumstances *(22),* but the number of reports implicating this organism in various human infections is large enough to be significant.

As mentioned above, the early literature contains a great deal of confusion concerning the taxonomy of the genus *Bacillus.* Many authors identified their isolates as *B. subtilis,* but the published descriptions more closely resemble that of *B. cereus.* Isolates described as "anthracoid" or "anthrax-like" should also be considered to be *B. cereus (22).*

The scientific literature before 1920 contains many accounts of *B. cereus* or *B. cereus*-like organisms causing various eye infections, e.g., acute conjunctivitis *(5, 53, 112);* purulent iridocyclitis (Baenziger and Silberschmidt, cited in ref. *62*); and hemorrhagic panopthalmia (Steuber, cited in ref. *167*).

Bronchopneumonia has been associated with *B. cereus* infection *(166, 167).* In one case, the pneumonia occurred along with sepsis after a hernia operation, and was ultimately fatal.

Bacillus cereus is often a secondary invader, commonly associated with trauma. Sweany and Pinner *(167)* isolated a *Bacillus sp.* from most of the lesions and principal body fluids of a patient suffering from tuberculosis. Bais *(7)* reported isolating a large, motile *Bacillus* sp. from blood of a patient dying from gangrene of the lung. Sheen and Klein *(154)* described an ulcerated wound from which they isolated motile, anthrax-like bacilli.

Lazar and Jurcsak *(99)* demonstrated *B. cereus* in two separate cases of ear infection. It has also been implicated in gall bladder and urinary infections *(110),* and in five cases of bacteremia associated with intermittent hemodialysis *(42).*

Pearson *(143)* reported on infections in 48 patients, seen in a general hospital over a period of two years, which were possibly caused by organisms of the genus *Bacillus.* Unfortunately, he made no species identification, but from the statements of other authors *(36, 110)* one is led to believe that a large fraction of the infections were caused by *B. cereus.*

Reports on naturally occurring infections in animals caused by *B. cereus* are rare. Biancardi (cited in ref. *162)* described eight cases of bovine mastitis attributed to this bacterium. Recently, an autopsy following the fatal infection of an adult tiger at the Cincinnati zoo revealed *B. cereus* in the blood and viscera of the animal (Bell, cited in ref. *21).* But evidence that *B. cereus* is pathogenic for animals comes chiefly from laboratory investigations.

Confusion in the older literature regarding taxonomy of the genus *Bacillus* clouds the issue of animal pathogenicity of *B. cereus* as well.

Grierson *(62)* demonstrated that 30 of 65 isolates of "*Bacillus anthracoides*" caused death of mice within 24 hr after intraperitoneal injection. Clark *(36)* recognized that the bacilli described in some earlier accounts were probably *B. cereus.* In one study, he proved that 10 isolates lethal for guinea pigs, classed as *Bacillus siamensis,* were in fact culturally indistinguishable from *B. cereus.*

Both Burdon *(28)* and Brown, et al. *(26)* reported that stock cultures of *B. cereus* had low virulence for mice, guinea pigs, and rabbits, but that virulence could be increased by several passages on blood agar plates at 3-hr intervals.

In a later paper, Burdon and Wende *(31)* showed that as few as 3×10^5 actively growing cells were able to kill mice when injected subcutaneously. They suggested that the illness produced by virulent *B. cereus* cultures might be called "cereobacillus disease" *(32).* In addition to killing mice, virulent cultures also produced local hemorrhagic and granulomatous lesions in rabbits and guinea pigs.

Using washed vegetative cells from cultures incubated overnight, Lamanna and Jones *(97)* found that the LD_{50} of three strains of *B. cereus* injected intraperitioneally into mice ranged from $1.05 - 3.20 \times 10^7$. Burdon, Davis, and Wende *(30)* confirmed this finding by observing that intraperitoneal injection of 2.2×10^7 brain heart infusion grown-bacilli caused 84-100% mortality in mice. Others *(99, 131),* have also

confirmed the mouse lethality of *B. cereus* vegetative cells. Spore suspensions were lethal for mice, but much higher numbers were required *(97)*.

Several groups have demonstrated the toxic nature of sterile culture filtrates for mice, rats, and golden hamsters *(18, 19, 75, 87, 162)*. The primary pathological change that occured after intravenous injection of the toxic filtrates was appearance of many fibrous blood clots in arteries and capillaries of the lungs *(19, 30)*. Death occurred rapidly after intravenous or intranasal inoculation, usually in less than 2.5 hr *(19, 162)*. Stamatin and Anghelesco *(162)* stated that "*Bacillus cereus* toxin inoculated by respiratory route seem(ed) to be the strongest of the so far known bacterial toxins."

Nikodemusz and Gonda *(132)* subjected both dogs and cats to challenge with saline suspensions of vegetative cells of *B. cereus* (no numbers given). Dogs, injected subcutaneously with 1-4 ml of cell suspension developed fever, tender swelling at the site of injection, and loss of appetite. All symptoms disappeared by the fourth day. Intraperitoneal injection of 5 ml of cell suspension into each of five cats led first to vomiting, diarrhea, and weakness; then to shock; and within 12 hr, to death. Autopsy of the cats revealed *B. cereus* in the spleen and heart blood.

The failure in many cases to isolate *B. cereus* from the blood or spleen of animals dead from cereobacillus disease *(30, 31)*, suggests that the illness is a toxemia. Lethal toxin is not associated with washed cells at the time they are injected into the test animal *(21)*. Presumably, it is produced *in vivo* before the cells are destroyed by the host's defense mechanisms. When a generalized infection does occur, it is possible that the host is in a weakened condition and unable to defend against the invading cells.

OUTBREAKS OF *Bacillus cereus* FOOD POISONING

Although there are few reports of *B. cereus* food-borne illness in the United States, the European literature since the turn of the century contains numerous accounts of food poisonings caused by *B. cereus* or *B. cereus*-like sporeformers. In 1906, Lubenau *(103)* described an outbreak in a sanitorium in which 300 of 400 inmates and staff became ill with profuse diarrhea, stomach cramps, and vomiting. From meatballs remaining after the incriminated meal, he isolated a sporeforming bacillus he called *Bacillus peptonificans*. From his description (strongly hemolytic, rapidly peptonizes milk, rapidly forms spores), the isolate closely resembles *B. cereus*. Some years later, Seitz *(153)* reported isolating a *cereus*-like bacillus from the feces of a young man suffering severe enteritis and profuse diarrhea. Brekenfeld implicated aerobic sporeformers similar to *B. cereus* in food poisonings involving vanilla sauce *(23)* and a jellied meat dish *(24)*; and Trub and Wundram *(172)*, later Krehnke (cited in ref. *128*) reported that food contaminated with aerobic sporeformers and stored for long periods of time at improper temperatures was able to cause illness when consumed.

During the period 1936-1943, 117 of 367 cases investigated by the Stockholm Board of Health, were suspected of being caused by aerobic sporeformers *(148)*. Foods most often involved were boiled beef or pork, sausage, and meatballs. Meat and meat products were reported to be the major vehicles of food borne *B. cereus* poisoning *(77)*. Early reports on *B. cereus* food poisoning shared several features: they rarely gave a complete description of the organism, usually classified it as a member of the *subtilis-mesentericus* group or as an "*anthracoid*" bacillus, and failed to give the numbers of bacteria in the incriminated foods.

Hauge *(64, 65)* presented the first classic descriptions of *B. cereus* gastroenteritis in his discussion of four Norwegian outbreaks involving 600 persons. In all four outbreaks, the food implicated was a vanilla sauce prepared and stored at room temperature for one day before being served. Since the outbreaks were similar, only one, involving the patients and staff of an Oslo hospital, was described in detail. Samples of the sauce taken after the outbreak contained 25-110 million *B. cereus* per milliliter. The average latent period for patients was 10 hr; for the staff, 12-1/2 hr. They suffered abdominal pains; profuse, watery diarrhea; rectal tenesmus; moderate nausea; and, rarely, vomiting. Fever was uncommon. Symptoms did not usually last more than 12 hr.

From 1950 to 1960, reports of *B. cereus* food-borne illness came from many European countries. A yellow pudding dessert with 1.3×10^7 *B. cereus* per gram produced abdominal pains and diarrhea in 15 of 18 adults and 106 of 150 children who consumed it *(33)*. Chicken soup containing 6.0×10^7 bacilli caused similar symptoms in six persons in an Italian outbreak (Pisu and Stazzi, cited in ref. *128*). Clarenberg and Kampelmacher (cited in ref. *120*) attributed *B. cereus* outbreaks in The Netherlands to consumption of contaminated mashed potatoes, vegetables, minced meat, liver sausage, certain rice dishes, puddings, and soups. In eleven specifically cited cases, the level of *B. cereus* contamination in the incriminated foods ranged from $0.5\text{-}200 \times 10^6$ cells per gram. Meyer *(111)* reported two cases of gastroenteritis blamed on canned herring and liverwurst. Both foods yielded members of the "*subtilis-mesentericus*" group of bacilli which were culturally and biochemically identical to *B. cereus*.

Hungarian investigators, particularly Nikodemusz and co-workers, did extensive work on the problem of *B. cereus* food poisoning. Nikodemusz *(120)* first described a foodborne illness in which vegetable soup served as a vehicle for the organism. Sixty-five of 175 persons eating the meal developed the typically mild symptoms. *Bacillus cereus* was not isolated from the feces of victims. Nikodemusz and Csaba later published accounts of seven outbreaks, encompossing 90 individuals in which *B. cereus* was directly involved *(40, 121, 130)*. The incriminated foods were liver sausage, pumpkin greens, cream pastry, and a mutton and egg dish. Contamination with *B. cereus* ranged from 1.6-16 million cells per gram. Kiss *(80)* implicated an improperly stored sausage containing 1×10^5 *B. cereus* per gram in the illness of three people.

A summary of food poisoning in Hungary caused by aerobic sporeformers *(122, 128)* lists 51 outbreaks, 35 attributed soley to *B. cereus*. These 35 outbreaks affected about 800 people. Foods most often incriminated were sausage, vegetable dishes, cream pastries, and meat or vegetable soups. The level of *B. cereus* contamination ranged from 3.6×10^4 to 9.5×10^8 cells per gram. Symptoms, identical to those described by Hauge, were generally mild. In at least four outbreaks, examination of feces from victims revealed *B. cereus*.

In relation to other food-borne pathogens, *B. cereus* ranked as the third most common cause of food poisoning in Hungary during the period 1960-1966 *(127)*. This organism was responsible for 88 outbreaks (4.4% of total) and 3,560 stricken individuals (11.0% of total). Other statements on the significance of *B. cereus* food poisoning in Hungary came from Nikodemusz et al., Novotny et al. *(134)*, Csaba and Nikodemusz *(41)*, Bojan and Lakatos *(16)*, and, more recently, from Ormay and Novotny *(138, 139)*, who reported that between 1960 and 1968, *B. cereus* was responsible for 8.2% of all outbreaks and 15.2% of all cases of food poisoning of known etiology. They further stated that three out of every five *B. cereus* food poisonings involved meat or meat products. This high incidence of meat related illness was explained by the fact that Hungarian meat and meat dishes are highly seasoned with spices that often contain large numbers of aerobic sporeformers. The heat treatment given the food was usually insufficient to kill spores, and improper holding after cooking allowed the spores to germinate and the resulting vegetative cells to multiply. Other authors also recognized the significance of improper holding conditions in outbreaks of the illness *(33, 65, 129)*.

Bacillus cereus gastroenteritis is traditionally associated with mild symptoms, yet in certain circumstances it may be more serious. Heinertz (cited in ref. *137*) documented the case of a hospitalized patient in a pre-ileus condition who, having eaten a pork casserole containing 20 million *B. cereus* per gram, developed acute ileus. Three-hundred sixty other patients in the hospital were stricken with gastroenteritis after eating the same casserole. The possibility that young children may be more severely affected by *B. cereus* was raised by Bodnar *(15)*. He described two separate outbreaks in which adults and children ate the same *B. cereus*-contaminated food. Adults suffered only mild symptoms, but children (5 and 9 years old) required hospitalization for 3 days.

Other reports concerning *B. cereus* food poisoning have appeared in the European literature in the last few years *(51, 146, 147, 176)*, including an excellent case-study by Birzu, et al. *(14)*. In contrast, there have been few well-documented outbreaks in the United States *(2, 3, 46)*. The only extensively studied case to be reported thus far *(113)* occurred in 1969 and affected 15 members of a fraternity who ate meat loaf contaminated with *B. cereus*. Samples taken from the meat loaf contained 7.0×10^7 *B. cereus* per gram. The victims suffered the classic symptoms of *B. cereus* food poisoning and recovered within 24 hr.

Bacillus cereus food poisoning remains an unknown quantity in the United States. Symptoms are usually not severe enough to force a victim to seek medical attention, so the cause of illness in individual cases may easily be overlooked. Only when several people are sick at the same time do those involved suspect food poisoning.

THE NATURE OF *Bacillus cereus* PATHOGENICITY

Isolation of large numbers of *B. cereus* from foods implicated in gastrointestinal illness is strong presumptive evidence that this organism was the responsible agent. At present, its enteropathogenicity can only be conclusively proven by eliciting symptoms in human volunteers fed pure cultures of the organism *(43)*.

Hauge *(65)* inoculated vanilla sauce with a strain of *B. cereus* isolated from an outbreak of gastrointestinal illness. After incubation at room temperature for 24 hr, the sauce contained 92 million bacilli per milliliter. Hauge consumed 200 ml. Thirteen hours later he experienced severe abdominal pain, diarrhea, and rectal tenesmus. The symptoms persisted for 8 hr. During this time, fecal samples revealed few *B. cereus* cells. In a previous experiment *(64)*, six human volunteers drank 155-270 ml of vanilla sauce containing 30-60 million *B. cereus* per milliliter. Four developed symptoms of *B. cereus* food poisoning.

Dack et al. *(45)*, could not confirm Hauge's findings. Human volunteers who ingested washed cell suspensions, skim milk cultures, or vanilla sauce cultures in 47 test feedings failed to develop any definite symptoms. The number of viable cells administered per volunteer ranged from 0.45-47.6 $\times$ 10^9. Dack et al. used four strains isolated from cheese spreads in these experiments since they were unable to obtain Hauge's strains. Though these strains appeared biochemically identical to those isolated by Hauge, the fact that they were not isolated from a food poisoning outbreak limits the significance of these negative results. Using one of Hauge's strains, Johannesen *(74)* demonstrated that ingestion of cell extracts by human volunteers led to headache, dizziness, and meteorism (gas accumulation causing distension of the intestine).

Nikodemusz et al. *(133)* had only partial success in eliciting a response in human volunteers. Ingestion of 1.8 $\times$ 10^{10} bacilli washed from a 24-hr nutrient agar slant culture failed to produce symptoms in any of the four volunteers; broth cultures containing 5.6 $\times$ 10^8 cells were similarly innocuous. When the volunteers ate 200 ml of pudding containing 1.4 $\times$ 10^{10} cells, one developed diarrhea lasting 24 hr. In all cases, *B. cereus* could be isolated from the feces of the volunteers on the second and third day of the test.

Without question, the need for further human feeding experiments remains. But the results of the previously described investigations intimate that, given the proper conditions, certain strains of *B. cereus* can elicit gastrointestinal illness in human volunteers.

The enteric response of certain animals to cultures of *B. cereus* has been more consistently positive than that of human volunteers. It gives some promise of providing a suitable experimental model for the study of *B. cereus* gastroenteritis.

Feeding cultures of *B. cereus* or *B. cereus*-like sporeformers to rodents has caused enteritis *(153)*, decreased intestinal passage time *(137)*, or has elicited no symptoms at all *(55, 131)*. Mice injected intraperitoneally with sterile culture filtrates *(111)* and guinea pigs injected subcutaneously or intraperitoneally with whole cultures *(148)* developed severe diarrhea. Korentschewsky (cited in ref. *133*) reported that intrarectal doses of *B. cereus* produced intestinal illness in rabbits. In our laboratory, we have had some preliminary successes in attempting to elicit fluid accumulation in the ligated ileal loop of rabbits by injection of whole cultures of *B. cereus* (*unpublished data*).

Carnivores appear to be quite sensitive to the enteropathogenic activity of *B. cereus* cultures administered *per os*. Dogs given milk or other food containing large numbers (greater than 1 $\times$ 10^5 cells/ml) of *B. cereus* consistently developed diarrhea *(55, 103, 126)*.

Cats are also sensitive to *B. cereus (123)*, possibly even more so than dogs *(124)*. Nikodemusz *(125)* gave a striking example of how seriously these animals are affected by oral administration of the organism. For several weeks, six cats were fed food containing 1 $\times$ 10^5 - 1 $\times$ 10^8 of *B. cereus* per gram. All six developed a thin, watery diarrhea and within 3 weeks, one-half had died. The remaining cats lost over 10% of their original body weight by the end of 30 days.

An important consideration in laboratory attempts to demonstrate *B. cereus* enteropathogenicity is whether the syndrome is a result of infection or an intoxication. The relatively sudden onset of symptoms and short duration of illness suggest an intoxication. The characteristic lack of fever in outbreaks supports this suggestion.

Nygren *(137)* postulated that phosphorylcholine, produced from the hydrolysis of lecithin by *B. cereus* phospholipase C, was the toxicant in *B. cereus* food poisoning. But other investigators could not confirm his results *(44, 175)*. Hauge *(65)* demonstrated that though whole cultures of *B. cereus* produced symptoms in human volunteers, Seitz filtrates of the same cultures would not. Since phosphorylcholine would be expected to pass this filter, it does not appear to have been the active agent.

In the study by Johannesen *(74)*, two volunteers drank 150 ml of a 48-hr broth culture of *B. cereus* (isolated by Hauge) filtered through a Berkefeld filter. Neither became ill. They then drank 150 ml of a cell extract from a similar culture also sterilized by Berkefeld filtration. After 6 hr, one volunteer complained of headache and dizziness; after 4 hr the other suffered from meteorism. Neither experienced diarrhea, nausea, or vomiting. The author suggested that the responsible agent for *B. cereus* food poisoning is cell-associated. His results imply that this agent can be formed outside the intestine, but the premise that growth or sporulation in the intestine must occur in order to produce a food poisoning syndrome cannot be overlooked. Presently, data are not sufficient to justify any definite conclusion concerning the nature of the enteropathogenic factor. The idea of a cell-associated enterotoxin, released upon lysis of the cell, agrees with the other known characteristics of *B. cereus* food-borne illness, e.g., *(a)* the requirement in outbreaks for large numbers of cells to elicit a response *(44, 65)*; *(b)* rapid onset and short duration of the illness; and *(c)* lack of fever coupled with inability of investigators to isolate *B. cereus* from fecal samples in many outbreaks,

indicating that neither invasion of tissue nor multiplication within the intestine occurs to any great extent.

Bacillus cereus IN FOODS

Bacillus cereus strains have been related to food spoilage and to the production of certain types of food. In 1952, Stone and Rowlands *(165)* and Stone *(163, 164)* showed that *B. cereus* and *B. mycoides* were responsible for a defect in milk called 'broken' or 'bitty' cream. Their studies demonstrated that the lecithinase produced by the microorganisms disrupted the fat-globule membranes, resulting in the irreversible dispersion of cream into various size particles when shaken. Lecithinase-negative strains of *B. cereus* failed to produce this defect. The defect occurred in both raw and pasteurized milk that was held at 15 C or higher. Donovan *(50)* and Billing and Cuthbert *(13)* found that the major source of *B. cereus* in milk was cans that were rinsed and left standing overnight prior to reuse. Rinsing diluted the milk nutrients and thereby enhanced sporulation of vegetative cells of *B. cereus* that remained in the cans.

Bacillus cereus produces a penicillinase, and this organism has occasionally been used as a commercial source of the enzyme *(4)*. Because the organism produces a protease readily, investigations on the use of this enzyme as a clotting agent in cheese manufacture have been conducted *(160)*. These trials were rather limited since the *B. cereus* enzyme was not as efficient as certain other microbially derived enzymes.

In the past, at least one baking concern has employed *B. cereus* in the manufacture of certain types of bread (Dr. W. Bradley; *personal communication)*. In this procedure, flour is fermented with *B. albolactis* (a lactose fermenting *B. cereus*) prior to drying, pulverising, and adding to dough before baking. The baking treatment destroys vegetative cells so that the aerobic plate count on the final product rarely exceeds 3×10^3/g.

There are relatively few papers describing surveys of food products for *B. cereus* contamination. In 1957, Kjellander and Nygren (cited in ref. *137*) examined 514 food samples and found 26% of the meats, 77% of the milk samples, and 51% of the fruits, nuts, and vegetables to contain *B. cereus*. Two years later, Andersson and Storgards (cited in ref. *137*) reported that 129 of 486 pasteurized milk samples, 147 of 161 cream samples, and 157 of 161 whipping cream samples contained viable *B. cereus*. The relatively high frequency of *B. cereus* in milk products was reiterated by a study conducted in Rumania *(68)* in which 72.4% of the fresh raw milk, 86.7% of bottled pasteurized milk, and 100% of milk samples taken directly from the pasteurizer were found to contain *B. cereus* at levels ranging from 1×10^{-1} to 1×10^4 organisms per milliliter.

Perhaps the most comprehensive study of the incidence of *B. cereus* in food materials was carried out by Nygren *(137)*. The examination of 3,888 samples revealed that 51.6% of 1,546 food ingredient samples, 43.8% of 1,911 cream and pudding samples, and 52.2% of 431 meat and vegetable products contained *B. cereus*. In most instances, the level of contamination was less than 1×10^2 organisms per gram.

More recently, a survey of selected dry foods purchased at retail outlets in Madison, Wisconsin, resulted in isolation of *B. cereus* from 25.3% of the products *(79)*. Items most frequently containing *B. cereus* were seasoning mixes, spices, dry potatoes, dry milk, and spaghetti sauces. In a study conducted in Russia, Akimov *(1)* reported that 13.6% of canned foods, 7.7% of sausage products, and 6.7% of confectionery items contained *B. cereus*.

From these reports, it is evident that *B. cereus* organisms are widely distributed and better deserve the label 'ubiquitous' than do the salmonellae.

GROWTH AND DESTRUCTION OF *Bacillus cereus*

The optimum temperature for growth of *B. cereus* is reported to be 30 C *(22)*. This same source lists the maximum temperature for growth as between 37 C and 48 C. However, Kim and Goepfert *(78)* reported that 15 of 51 isolates from food products were able to proliferate at 49 C. Larkin and Stokes *(98)* described a culture of *B. cereus* that grew at 50 C, whereas Iyer and Bhat *(71)* stated that 48 C was the upper limit for most strains and that a minority failed to multiply at 40 C. There appears to be more general agreement on the minimum temperature for vegetative cell growth. Larkin and Stokes *(98)* reported 10 C as the lower limit for a single strain of *B. cereus*, whereas Mol *(115)* demonstrated that strains would grow at 12 C in a few days but not at 8 C when held as long as 4 months. Larkin and Stokes *(98)* described the isolation of several group I bacilli among the 90 cultures of psychrophilic bacilli obtained from soil, mud, and water. Since further taxonomic investigation was not attempted, it is not known whether any of these isolates were *B. cereus*.

Studies by Halvorson, et al. *(63)* and Knaysi *(82)* have demonstrated that the temperature limits for germination are somewhat different than for vegetative cell growth. Knaysi *(82)* reported that −1 C, 30 C, and 59 C were the minimum, optimum, and maximum temperatures, respectively, for the 'initial stage' of germination. Halvorson et al. *(63)* stated that the initial stage would proceed at temperatures

as low as −6 C. Stage two of germination was characterized as proceeding between 10 C and 44 C *(82)*.

The range of pH permitting growth of *B. cereus* in laboratory media has been reported to be pH 4.9 *(78)* to 9.3 *(54)*. However, the media were adjusted with mineral acids and alkalis, so the influence of organic acids commonly used as food acidulents is unknown. Since the nature of the acid molecule plays a significant role in determining the growth response of other organisms, e.g., *Salmonella (35)*, it probably is pertinent to the growth of *B. cereus* and should be investigated more fully.

Data on interaction of *B. cereus* with other food borne microorganisms are scant. McCoy and Faber *(108)* observed that *B. cereus* significantly stimulated staphylococcal growth and enterotoxin production in inoculated meat slurries. Contrastingly, Troller and Frazier *(171)* noted that *B. cereus* produced a substance inhibitory to *Staphylococcus aureus*. More recently, Kafel and Ayres *(76)* demonstrated an antagonistic effect on *B. cereus* by Lancefield Group D streptococci isolated from canned hams. The suggestion has been made that the rather low (observed) incidence of *B. cereus* enteritis may be due to the inhibitory action on the growth of *B. cereus* by common saprophytes in the food *(118)*.

Certainly a major concern to food microbiologists is the destruction of microorganisms. As *B. cereus* forms spores, most of the concern revolves about destruction of the spore rather than the vegetative cells. The reader is referred to the excellent chapter by Roberts and Hitchens *(151)* for a review of the resistance of bacterial spores to various chemical and physical agents.

Of most practical concern is the heat resistance of the *B. cereus* spore. Much of the work on the heat resistance of *B. cereus* in food products has evolved from studies on the defect 'bitty cream' and its prevention. Clegg et al. *(37)* showed that heating cream to 90 C for 30 min prevented bittiness provided the initial spore level was 20/ml or less. Obviously, the product could not tolerate such a treatment. A $D_{100\,c}$ value of 2.7-3.1 min for *B. cereus* in skim milk was reported *(114)*. Ingram *(67)*, citing data from various sources, has estimated the $D_{100\,c}$ value in low acid foods (>pH 4.5) to be 5 min. D values at 85 C, 90 C, 95 C, and 100 C in phosphate buffer (pH 7.0) of 220 min, 71 min, 13 min, and 8 min, respectively, have been published *(116)*. In contrast, the $D_{121\,c}$ values in soybean oil and olive oil were 30 and 17.5 min *(116)*.

In 1957, Vas and Proszt *(173)* showed that destruction of *B. cereus* spores was not strictly logarithmic when plotted against time on a linear scale. They demonstrated that approximately one spore in 10^7 - 10^8 possessed an unusually high heat resistance. These findings were reiterated by Franklin *(58)* who described the isolation of a strain of *B. cereus* from cream that had been heated at 140 C for 2 sec. When this strain was cultured and retested, the author demonstrated that one spore in 10^5 - 10^6 would survive 135 C for 4 hr. Observations of such deviations from logarithmic kill curves have in the past largely been attributed to experimental error or clumping, rather than to a distribution of heat resistance values in a given population. However, data to the contrary have accumulated and may well necessitate reconsideration of the problem.

It would appear that *B. cereus* possesses heat and radiation resistance *(61)* similar to most mesophilic sporeformers and is not extraordinary in this property.

DETECTION AND CHARACTERIZATION OF *Bacillus cereus*

Several methods have been described to detect and enumerate of *B. cereus* in food products. These simple and rapid procedures are presumptive in nature, and colonies that appear on the agar media should be confirmed by further testing. The presumptive methods are based on an expression of the hemolytic and lecithinase properties of *B. cereus*.

In the initial work on food poisoning *B. cereus*, Hauge *(65)* employed surface plating on blood agar followed by incubation at 37 C for 18 hr. Colonies surrounded by a clear zone were then picked and tested for lecithinase activity.

Some years later, Donovan *(49)* devised a peptone-beef extract-egg yolk agar that contained lithium chloride and polymyxin B as selective agents. Typical *B. cereus* colonies were surrounded by an opaque zone after 18 hr at 30 C. Mossel et al. *(119)* expanded this procedure and added mannitol to aid in differentiating non-*B. cereus* from the mannitol-negative *B. cereus* organisms. The authors reported good recovery of *B. cereus* from food products, but cautioned that biochemical confirmatory tests should be made in certain instances. Recently, Kim and Goepfert *(79)* formulated an equally sensitive egg yolk containing medium that was designed to enhance sporulation by *B. cereus* within the 24-hr incubation period. Colonies would then be ready for serological confirmation by using fluorescein-labeled anti-exosporium sera.

Other quantitative methods e.g., single tube dilution series, millipore filtration, multiple tube dilution (Most Probable Number), and centrifugation followed by plating *(93)* have been described, but these procedures are either *(a)* too sensitive for routine needs, *(b)* too laborious, or *(c)* relatively inaccurate.

Procedures that rely on lecithinase production as the identifying characteristic of *B. cereus* can be at best presumptive indicators of the *B. cereus* population in a given substrate. Lecithinase-negative strains of *B. cereus* have been reported *(86, 101)* and despite the theory of Nygren, these organisms must be considered potentially pathogenic. In addition, there are other species of bacilli, e.g. *B. thuringiensis, B. mycoides, B. laterosporus,* and certain members of the *polymyxa-macerans* group that are also lecithinase-positive and will evoke a *B. cereus*-like reaction on egg yolk agar. For these reasons, confirmatory tests are required to establish the identity of an isolate as *B. cereus.*

It is safe to say that no single biochemical test is 100% effective in identifying a culture as *B. cereus.* Most of the past work has been directed at differentiating *B. cereus* from the very closely related species *B. anthracis, B. thuringiensis,* and *B. mycoides.* Procedures evolved for establishing a culture as *B. cereus* may include biochemical tests, cell and spore morphology, and animal and insect pathogenicity. Obviously, the typical food laboratory is not equipped to perform all of these tests and the need exists for simple, rapid, and precise methods.

In general, most laboratories are able to examine microscopically for swelling of the sporangium by sporulating cells [not characteristic of *B. cereus (22, 159)*] and for the presence of a crystal or parasporal body characteristic of *B. thuringiensis.* Rhizoidal growth on solid media is quite readily identified and is a simple test for *B. mycoides.*

Differentiation of *B. cereus* from *B. anthracis* is somewhat more difficult. Various properties such as motility, growth on penicillin-containing agar, failure to form a capsule on bicarbonate-containing agar, and insensitivity to γ phage have been ascribed to *B. cereus.* Production of mouse-lethal toxin and failure to cause anthrax in animals is also characteristic of *B. cereus.*

More commonly, biochemical tests are employed. Usually, anaerobic dissimilation of glucose, liquefaction of gelatin, nitrate reduction, hemolytic activity, acetylmethylcarbinol production, and failure to utilize mannitol and xylose are characteristic of *B. cereus* and represent readily testable properties *(26, 29, 31, 65, 83, 86, 89, 101, 119, 137).* It should be remembered that these tests are subject to limitation in that variant strains are relatively common. In doubtful cases, the aid of laboratories able to perform the more definitive tests should be sought.

NEEDED INFORMATION

There are several major needs relating to the problem of *B. cereus* food poisoning. Certainly, in Europe, the role of this organism in food borne disease is well documented. In this country, this is not true. Little information is available concerning the involvement of *B. cereus* in the overall picture of food poisoning. When the magnitude of the *B. cereus* problem in eastern Europe is appreciated, it seems rather unusual and somewhat surprising that there are virtually no reported occurrences in the United States. It is conceivable that outbreaks of *B. cereus* poisoning are not significant in number in the United States, but this should be established by investigation of suspect vehicles rather than by ignoring the possibility and proclaiming its absence. As an example, the Annual Summary of Food-borne Outbreaks for the Year 1969 *(3),* published by the Center for Disease Control, lists 16 outbreaks caused by *Clostridium perfringens.* The etiology of 19 outbreaks is listed as "probably *C. perfringens.*" But, *C. perfringens* was isolated from afflicted individuals in only two of the latter 19 outbreaks and from none of the foods involved. It is known that *B. cereus* poisoning elicits the same symptoms and disease pattern as *C. perfringens.* Were these outbreaks investigated in sufficient detail to rule out the possibility of *B. cereus* prior to being classified as "probable *C. perfringens?*" This would seem doubtful since to the authors' knowledge very few clinicians and laboratory people are aware of the potential pathogenicity of *B. cereus,* and fewer still are cognizant that simple and rapid presumptive methods of detection are available. In addition to the outbreaks just mentioned, the Annual Summary also lists 50 outbreaks as "etiology unknown." Several of these fit the pattern (i.e. symptoms, incubation period, duration) expected if *B. cereus* were the causative agent. One can only wonder how many outbreaks would be removed from the "probable" and "etiology unknown" classification if a simple quantitative *B. cereus* examination were added to the battery of tests routinely performed on suspected food poisoning vehicles. If it is decided that incidence of *B. cereus* outbreaks is insignificant, it would be comforting to know that this decision was based on investigatory data rather than on speculation. Clearly, one major need is for greater cognizance of the pathogenic nature of the organism and increased surveillance of suspect food vehicles.

Recognizing that a problem exists is a big step, but only a small part of the overall picture. We must have a more knowledge of the nature of the disease and the organism so that preventative measures may be taken. At this writing, the nature of *B. cereus* poisoning is a mystery. Because of its similarity to *C. perfringens* poisoning, it is attractive to speculate that similar, if not identical, mechanisms of pathogen-

icity exist. Answers to questions on pathogenicity cannot be obtained until a suitable assay system is developed. Only then can the nature (i.e. intoxication or infection) of the disease be established. Should a toxin be indicated, further questions develop: What is the nature of the toxin? Do all strains produce toxin? Under what conditions is toxin produced? Is there more than one toxin? How can toxin be inactivated or destroyed? These are but a few.

More information is needed on the properties of the organisms themselves with special reference to their behavior in food environments. Conditions which support growth and/or toxin production in foods must be delineated so that these may be avoided (if possible) by food service establishments.

It is our hope that this review has called the attention of the appropriate people to a potentially significant problem. Moreover, we hope that the literature review presented will provide the informational basis from which investigations of *B. cereus* in foods can be initiated.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT

(Continued from Page 212)

Task Committee will be instructed to consider the function as well as the public health aspects. In addition, consideration will be given as to whether the equipment is designed for long or short term storage or holding, or for service only, and whether the covers are provided on a protection or performance basis, i.e., pressure vessels or similar use.

General and future projects

Standard Task Committees have been formed and instructed to review for possible revision of Standard No. 12, relating to Automatic Ice Making Equipment; Standard No. 20, relating to Commercial Bulk Milk Dispensing Equipment; and Basic Criteria C-2, relating to the Evaluation of Special Equipment and/or Devices.

The need for in-position cleaning for some NSF Standard No. 4 equipment was reviewed by the NSF Testing Laboratory staff, who noted that several large items of equipment had been encountered which did not lend themselves to disassembly for cleaning and sanitizing nor were they capable of being cleaned in-place by normal methods, thus requiring in-position cleaning with special facilities and procedures. It was suggested by the staff that a provision similar to the one in Standard No. 8 which permits in-position cleaning and sanitizing be added to Standard No. 4. After considerable discussion, it was the decision of the public health representatives that it would be much more logical to develop a new standard or criterion for large equipment which is not capable of being disassembled for cleaning or cleaned in-place. This standard or criterion should encompass similiar types of food service equipment covered in all standards.

The Foundation staff advised the public health representatives that Standard No. 26 covering Pot, Pan, and Utensil Washers is currently being implemented. Further, there had been no efforts during the past year to include cart washers or the large aircraft container washers in this Standard. It was the staff's opinion that it would not be feasible to develop a standard for aircraft container washers since they were of custom design and made for specific types of containers. However, with regard to cart washing equipment, the representatives requested the Foundation to invite manufacturers of such equipment to participate in development of a standard for cart washers, since it did not seem feasible to include such equipment in the Standard for Pot, Pan, and Utensil Washers.

According to some of the public health representatives, carpeting is being used on an ever increasing basis in food service establishments, as well as in institutions; and there appeared to be a lack of guidelines or standards covering same. The Foundation indicated that if a request were received from either the manufacturers, user groups, or regulatory agencies it would be willing to explore the feasibility of developing such a standard.

The NSF is to be commended for setting aside a day for the public health representatives to meet and discuss agenda items and other points of interest and to arrive at a mutual understanding on many matters of public health significance before the Joint Committee sessions officially commenced.

NATIONAL AUTOMATIC MERCHANDISING ASSOCIATION (NAMA)

The National Automatic Merchandising Association's Automatic Merchandising Health-Industry Council (AMHIC) held its 15th annual meeting during November 1970, and this Association and other public health organizations and the affected industries were represented and participated in AMHIC's discussions.

The afternoon of the first day was reserved solely for a meeting of the public health representatives and was used by them to discuss and clarify their view on public health objectives and policies to be followed in their work with the entire membership of the AMHIC. The Chairman of the IAMFES Food Equipment Committee was re-elected Chair-

(Continued on Page 244)

STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCAL FOOD INTOXICATIONS. A REVIEW

IV. STAPHYLOCOCCI IN MEAT, BAKERY PRODUCTS, AND OTHER FOODS[1, 2]

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Abstract

Staphylococcal intoxications involving foods, other than dairy products, have been associated predominantly with meats and bakery items. Outbreaks involving Genoa sausage, corned beef, barbecued chicken, baked ham, fish, pastries, pie fillings, and other foods are reviewed. Because meats have caused food-poisoning outbreaks, commercial samples have been tested for incidence of staphylococcal contamination. Several surveys have revealed that approximately 40% of the samples tested contained coagulase-positive staphylococci. These and other surveys, including studies of some frozen foods, are discussed. Finally, results of research concerning the behavior of staphylococci in several foods are discussed. Investigations on raw, cooked, canned, cured, and smoked meats, and meat pies have been reported in the literature. Studies on pastry and pie fillings have also been conducted and are summarized in this review.

Part I of this review (*223*) discussed the nature of the staphylococci, the bacterial agents of staphylococcal food intoxication. Part II (*224*) reviewed some properties of the enterotoxins, the toxic moieties of these intoxications, and epidemiology of staphylococcal food poisoning. Part III (*225*) summarized research results on staphylococci in dairy foods. In this paper we will discuss studies of staphylococci in meat, bakery products, and other foods; covering, (*a*) outbreaks of staphylococcal food poisoning traced to such foods, (*b*) surveys of these foods for staphylococci, and (*c*) research on behavior of staphylococci in various foods.

Outbreaks

Staphylococcal food poisoning outbreaks with meat as the vehicle include one in a defense plant traced to corned beef and involving 81 employees (*305*), another in which barbecued chicken containing 408 million coagulase-positive staphylococci per gram was involved (*256*), and two related incidents involving baked hams (*104*). Fish were involved in an outbreak aboard a Navy ship in which a shrimp salad was implicated (*190*) and in a rather large outbreak which occurred in a hospital in Puerto Rico, affected 539 persons, and was caused by salt-preserved codfish (*213*).

Several outbreaks of staphylococcal food poisoning were recently reported (*238*) in Colorado and Wisconsin in which Genoa sausage produced by a major manufacturer was implicated. Analyses revealed 1,000 to 100,000 coagulase-positive staphylococci per gram in plant samples of the sausage and upwards of 1 million per gram in some of the food-poisoning samples. Enterotoxin A was recovered from two samples. Shortly after this report, another series of outbreaks traced to Genoa sausage were recorded (*239*) but the product in this instance was produced by another company. Some of the samples, which were tested, again contained upwards of 1 million coagulase-positive staphylococci per gram.

Bakery products have also been implicated in outbreaks of staphylococcal food poisoning. Coughlin and Johnson (*70*) reported 17 outbreaks involving 1,246 cases within a 5-year period (1935-1939) caused by cream-filled pastries in New York State exclusive of New York City. Chocolate eclairs and cream puffs were most commonly involved, rarely creamfilled pies. Other incidents include a 12-case outbreak in which a custard filling was implicated (*69*), two cases caused by butterscotch pie (*82, 368*), and an outbreak caused by a doughnut filling which contained 215 million staphylococci per gram (*199*).

Other food-poisoning outbreaks reported in the literature which involved various foods contaminated with staphylococci, include: 38 cases caused by a pineapple pudding (*97*), 59 cases caused by minced egg and olive sandwiches (*119*), and 57 cases which involved potato salad (*250*).

We have not included in this discussion the cases of staphylococcal food poisoning reported in local and state medical journals. Those outbreaks which have been cited are sufficient to demonstrate that a variety of foods can, under the right circumstances, become hazardous if they are contaminated with enterotoxigenic staphylococci and if the bacteria grow in the food. The reader who wishes to have

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[2]Parts I, II, and III appeared in previous issues of this Journal.

current information of food poisoning outbreaks is advised to read the weekly *Morbidity and Mortality* reports issued by the National Center for Disease Control in Atlanta, Georgia.

SURVEYS FOR STAPHYLOCOCCI IN FOODS

Meat and fish

Jay (*166*) studied four types of market meat and concluded that both human sources of *Staphylococcus aureus* (based on the phage types encountered and characteristics of the hemolysins produced) and nonhuman sources of the organism (based on their resistance to several antibiotics) were involved. In another study, Jay (*167*) obtained 209 samples of 16 types of unfrozen market meats and found 39% of 173 samples from 27 stores contained coagulase-positive staphylococci, whereas 36 samples from 7 stores were free of staphylococci. Meats from which staphylococci were recovered, in decreasing order of frequency, were: chicken, pork liver, fish, spiced ham, round beef steak, hamburger, beef liver, pork chops, veal steak, and lamb chops. Meats found to be free of the organisms were: bologna, shucked oysters, olive and pickle loaf, salami, wieners, and chopped ham. A study of these isolates (*168*) led the author to conclude that the staphylococci were of animal origin.

Commercial chicken tested by Messer et al. (*216*) contained 2 to 620 *S. aureus* per milliliter of rinse solution in 80% of the samples. Market hamburger tested by these authors contained 50 to 3500 staphylococci per gram in 35% of the samples. Studies by Surkiewicz et al. (*332*) revealed 20% of samples of frozen, raw-breaded fish produced under good and poor conditions of sanitation contained coagulase-positive staphylococci and 10% of frozen, fried, breaded fish contained the organisms. Virgilio et al. (*352*) reported 140 of 392 samples of precooked frozen shrimp from two Chilean manufacturers contained staphylococci but only one-half of these had populations exceeding 100 cells per gram. Shewan (*300*) found up to 29% of the cocci recovered from dehydrated fish were coagulase-positive.

Canale-Parola and Ordal (*37*) encountered 160 to 390,000 coagulase-positive staphylococci per gram in all brands of precooked frozen chicken and turkey pies manufactured by five companies.

Data by Chou and Marth (*59*) showed that about 40% of samples of frozen meat by-products and liver used in the diets of mink contained coagulase-positive staphylococci. None were recovered from frozen fish or dried mink feed.

Other foods

A survey of frozen "TV dinners" conducted by Ross and Thatcher (*277*) in a Canadian city revealed that foods contaminated with staphylococci contained 14 to 334,000 coagulase-positive *S. aureus* per gram. Staphylococci were found in 0 to 60% of samples of a variety of precooked frozen meals tested by Huber et al. (*158*). Splittstoesser et al. (*309*) discovered that 31 to 64% of samples from several kinds of frozen vegetables contained coagulase-positive staphylococci but large numbers were not encountered. The highest counts were obtained from frozen peas (7.3 per gram), whereas the greatest number of positive samples (64%) were associated with corn. Casman (*40*) isolated staphylococci from frozen foods and determined that 8.5% of these strains were enterotoxigenic.

Green beans were tested by Messer et al. (*216*) who found that 10% of the samples contained staphylococci. Counts of 100 to 105,000 per gram were observed. According to studies by Davison and Dack (*84*), staphylococci grew in canned corn and elaborated enterotoxin.

Christiansen and King (*62*) reported 39% of salads and 60% of sandwiches obtained from commercial outlets contained coagulase-positive staphylococci. Populations were encountered which exceeded 10^5 organisms per gram for a few samples of salads and 10^3 per gram for one type of sandwich. Jopke and Riley (*173*) collected food samples (meat, fruits and vegetables, soups, salads and salad dressings, dairy products, pies, and other foods) from serving lines in cafes and cafeterias. Bacteriological analyses revealed that most of the foods were free of staphylococci, but the organisms were found in egg salad (<10-240 per gram), chicken salad (<10-380 per gram), potato salad (<10-10), and carrot and raisen slaw (150 per gram).

RESEARCH ON BEHAVIOR OF STAPHYLOCOCCI IN DIFFERENT FOODS

Meat and fish

Raw and cooked meats, and meat juices. Little or no growth of *S. aureus* in raw ground beef was observed by Casman et al. (*45*), but when surfaces of raw and cooked meats were inoculated (250 cells per cm^2), good staphylococcal growth and enterotoxin A production occurred.

Pivnick et al. (*257*) reported populations of staphylococci inoculated on the surface of barbecued chicken increased 10,000-fold within 8 hr and maximal numbers were reached in 16 hr. Temperatures in the range of 35.5-42.5 C did not appreciably alter the growth rate or maximum population. At 45 C, numbers of staphylococci increased 1,000-fold within 8 hr, but at 47 C they did not grow. Enterotoxin was detected after 2.5 days of incubation at 35 C but none was detected after 24 hr at 40 C and above.

Luxuriant growth and survival of staphylococci was reported by Surgalla and Dack (*327*) in canned meats held 60 days at 22 and 37 C when loss of moisture was prevented. When the organism was inoculated at a point on the top surface or in the geometrical center, it rapidly spread throughout the contents of the can.

Temperatures employed to cook, broil, or bake meats are sufficient to destroy any staphylococci which may be present in the meat, provided time is allowed for the interior of the meat to cook. Dack and Surgalla (*79*) determined that holding pork for 2 hr and 40 min in a roaster set for 260 C was sufficient to destroy staphylococci in the meat. The time required for complete destruction of *S. aureus* in chicken a la king was reported by Angelotti et al. (*9*) to be 40-47 min at 60 C. Heating of enterotoxin in the presence of meat proteins, according to Satterlee and Kraft (*282*), resulted in a rapid loss of toxic activity. The slope of the thermal inactivation curve (z value) for enterotoxin A in beef bouillon (initial pH 6.2) was reported by Denny et al. (*86*) to be approximately 27.8 C for toxin concentrations in the range of 5 to 60 μg per milliliter.

Vacuum packaging was noted by Christiansen and Foster (*61*) to markedly inhibit growth of staphylococci on sliced ham. Staphylococcal growth was prevented by Greenberg and Silliker (*132*) in ham and domestic sausage containing 3 and 5 ppm tylosin, respectively. Gomutputra and Fabian (*130*) employed 1% organic acid solutions to sanitize the surface of pork and beef inoculated with *S. aureus* and reported the following effectiveness (in decreasing order): acetic > monochloracetic > dehydroacetic (sodium salt) > dihydroacetic.

Staphylococci can be controlled in meat pot pies by the natural microflora of the pies. Frozen chicken pot pies were selected by Peterson et al. (*251*) for study because of their high total populations of microorganisms (staphylococci numbered between 10 and 39,000 per gram). It was not possible to enhance growth of staphylococci under any conditions of defrost although the pies spoiled under these conditions. Dack and Lipputz (*78*) found the natural microflora of pot pies (chicken, turkey, and beef) exerted an inhibitory effect on growth of staphylococci after incubation at 35 C for 18 hr. The predominating organism isolated from the microflora of the pies was a lactobacillus. When the pH of a meat pie slurry was adjusted with phosphoric acid, staphylococci failed to grow at pH 4.0, 4.1, and 4.3, but grew slightly at pH 4.5. Good growth occurred at pH 5.0 and above.

The minimum temperature for growth of *S. aureus* in chicken gravy was reported by Kereluk et al. (*185*) as between 5 and 10 C. The maximum stationary phase of growth was reached in 48 hr at 37 C, in 6 days at 20 C, and in 15 days at 10 C.

Cured and fermented meats. Growth of *S. aureus* in 39 different processed meats at different pH levels and with several combinations of NaCl and $NaNO_2$ was evaluated by Genigeorgis et al. (*124*). The meat environments were more conducive to growth of the staphylococcus than Brain Heart Infusion broth. Elevated yields of enterotoxins B and C were encountered at high pH levels coupled with low brine concentrations.

Food-poisoning staphylococci were discovered to grow vigorously in ground pork containing any permissable and palatable combination of curing ingredients (NaCl, $NaNO_3$, and $NaNO_2$) in studies by Lechowich et al. (*193*). However, if the pH of the meat was lowered to 4.8-5.0, anaerobic growth of the staphylococci was prevented even in the absence of nitrite. The organisms died rather rapidly in curing pickle but were protected by addition of meat juices. Most, if not all, staphylococci injected into hams with the pickle solution survived normal curing until the smoking operation. Heating meat in a smokehouse to an internal temperature of 58.3 C killed all surviving staphylococci. Heating at 48.9 C for 48 hr slowly killed the organisms in the interior of the meat, but they occasionally multiplied on the surface.

Enterotoxin B was detected by Genigeorgis et al. (*123*) in laboratory cured hams inoculated with 10^3-10^5 staphylococci per gram and held anaerobically for up to 16 weeks [initial pH >5.3, up to 9.2% NaCl (brine), and 0.54 ppm undissociated nitrous acid]. Greater toxin production occurred at 30 C than at 22 or 10 C. Toxin was detected at 10 C after 2 weeks in a few samples and in most instances after 8 weeks when the pH was >5.6. Toxic hams contained $>4 \times 10^6$ staphylococci per gram. In contrast, Kross and Vinton (*133*) could not produce enterotoxin in meat held as long as 2 weeks at 6.1 C.

Staphylococcus aureus was reported by Eddy and Ingram (*100*) to occur and survive in bacon curing brines and on shop-sliced and vacuum-sliced bacon. Under anaerobic conditions, the staphylococci competed with the natural microflora of the bacon but staphylococcal growth was favored by low numbers of the other organisms and by a high storage temperature.

Metcalf and Deibel (*217*) suggested that lysostaphin (an enzyme which specifically lyses staphylococci) might be an effective control agent for *S. aureus* in susceptible foods. In a simulated sausage process, the substance appeared to have no practical value though an initial inhibitive effect was noted.

Fish. Davison and Dack (*84*) reported staphy-

lococci could elaborate enterotoxin in cans of oysters but toxin could not be detected in canned salmon. Staphylococci were added by Hall et al. (*137*) to slurries of shrimp, scallops, lobster, and crabmeat. Enterotoxin was consistently recovered in quantities of 4 to 68 μg per milliliter and toxin was occasionally found in lake perch similarly inoculated with *S. aureus*.

Bakery products

Commercial dry-mixed puddings reconstituted with milk were noted by Cathcart et al. (*50*) to support growth of *S. aureus* but growth was inhibited when water was substituted for the milk. Vanilla, pumpkin, squash, sweet potato pie, cheese cake fillings, and whipping-cream mixes supported growth of staphylococci. Addition of sufficient citric acid to vanilla filling to lower the pH to 3.43-3.65 effectively checked growth of *S. aureus* and a decrease to pH 5.12 gradually retarded growth. Lactic acid was effective at pH 4.42 and 4.67.

Crisley et al. (*74*) studied seven synthetic cream fillings made with water and found that growth of *S. aureus* decreased slightly in three, decreased markedly in two, and increased significantly in two during 72 hr of incubation (22-25 C). Increasing the number of staphylococci in the inoculum and neutralizing the pH of the fillings did not significantly alter the pattern of growth but increased the ability of the staphylococci to survive in larger numbers. Substitution of milk for water in preparing the fillings, addition of minute quantities of whole egg, and combination with pie crusts increased the ability of fillings to support staphylococcal growth. Natural chocolate and cocoa fillings (custard base) were observed by Cathcart and Merz (*49*) to have a definite inhibiting action on growth of *S. aureus*. Ryberg and Cathcart (*280*) reported that lemon, orange, pineapple, apricot, and strawberry fruit fillings effectively inhibited staphylococcal growth.

Staphylococcus aureus was inoculated by Preonas et al. (*259*) on the top and on cut surfaces of freshly baked Southern custard pies. The top surface inoculum showed a 24-36 hr lag because of the protective action of a cakelike consistency (acting as a barrier to moisture migrating from the interior to the surface). The cut surface supported better growth of staphylococci than the top surface.

Staphylococci were reported by Post et al. (*258*) to be inhibited to some extent by naturally-occurring competitors in a whipped cream used in cream puffs.

The heat treatments to which many bakery products are exposed during their preparation are usually sufficient to destroy any staphylococci they may contain. The minimum temperatures for thickening egg (78-86 C) were found by Kintner and Mangel (*186*) as insufficient to destroy all *S. aureus* inoculated into the dried egg in puddings. However, the organisms were reduced to a minimum and growth was inhibited in all instances for at least 3 hr. When a temperature of 91-93 C was employed to prepare stirred and baked custards, none of the inoculated staphylococci remained viable. Cathcart et al. (*51*) found that the act of merely bringing custards to a second boil after addition of the thickening mix rendered them free of staphylococci. Baked custard pies inoculated with *S. aureus* prior to baking were free of the organisms as they left the oven. *Staphylococcus aureus* (10^5 per gram) cells were destroyed in the custard filling of puffs and eclairs subjected by Stritar et al. (*317*) to an oven temperature of 190.6 C for 25 min. A similar study by Gilcreas and Coleman (*129*) showed that staphylococci could be destroyed in the custard filling of eclair shells by exposing the product to oven temperatures of 215.6-220 C for 15 min. Angelotti et al. (*9*) reported 53-59 min were required to destroy all staphylococci in a custard filling heated at 60 C.

Cathcart et al. (*52*) effectively reduced staphylococci in the air and on smooth surfaces of custard and cream with ultraviolet irradiation (2,000-2,950A), but the treatment was not as effective on irregular surfaces. Ozone was ineffective. Both potassium sorbate and sodium benzoate were noted by Schmidt et al. (*288*) to be effective against *S. aureus* at pH values of 4.5-5.0 when these chemicals were incorporated at a concentration of 0.1% in a synthetic cream filling or a pie shell or when 0.1% solutions of the chemicals were sprayed on the surface of the pie shell (and the products incubated at 22 and 37 C). The amino acids serine, glycine, and cysteine were noted by Castellani (*48*) to have an inhibitory effect on food poisoning staphylococci in a cream pastry.

Other foods

According to Segalove et al. (*293*), growth of *S. aureus* in canned foods was best in low-acid foods (peas, corn) but growth was not affected by the kind of food in this category. Growth did not occur in high acid canned foods (tomato juice, peaches). In almost all instances, growth was better at 22 than at 37 C.

Mayonnaise and salad dressings were noted by Wethington and Fabian (*365*) to be improbable sources for staphylococcal food poisoning because of their acid content. Decreasing the quantity of acetic acid increased the survival time of staphylococci in these foods. Incomplete destruction of staphylococci inoculated by Kintner and Mangel (*187*) into cooked salad dressing made with dried eggs occurred

at the pH of the basic formula and any palatable variation, but these pH levels do not provide a favorable medium for growth of the organism.

Smith and Iba (*307*) found *S. aureus* on Pistachio nut meats held at 4 C die slowly but still survive longer than 141 days. At 16-26 C, *S. aureus* increased in numbers reaching a maximum count in 9 days. The population then decreased and viable cells disappeared in 99 days. At 37 C, the maximum number of staphylococci was reached within 48 hr after which there was a rapid inactivation which was complete in 63 days.

CONCLUDING REMARKS

Methods of food processing, food distribution, and food preparation are undergoing constant change. Sometimes these changes are made without a full understanding of their impact on the presence and growth of microorganisms in a food product. Most commercial food processors recognize this problem and attempt to institute processes and procedures which will minimize or eliminate potential hazards. That they are largely successful is borne out by the relatively few reported outbreaks of staphylococcal food poisoning caused through mishandling of foods by processors (Table 2, Part I of this review).

Inspite of this excellent record, processors must be constantly vigilant to identify products and processes with the greatest degree of potential hazard and then take the appropriate action to minimize or eliminate the risks. More often than not critical products are nonsterile foods, frequently of animal origin, which are subjected to substantial handling by people. Although we now have methods to detect staphylococcal enterotoxins in foods, processors and epidemiologists both would benefit if simplified and shorter procedures could be developed.

The real problems with staphylococcal food poisoning are not associated with food processors, but instead are associated with mishandling of food in food service establishments and in the home (Table 2, Part I of this review). Undoubtedly the true incidence of this disease is far greater than is reflected by the number of outbreaks that are actually reported. Olson (*245*) has speculated that there are several million cases of staphylococcus food poisoning annually in the United States.

We have all the information which is needed to prevent outbreaks of staphylococcal food poisoning that originate in food service establishments and in the home. If these problems areas could be eliminated, the number of persons suffering from this illness would drop to negligible levels. Even though the information is available, it must be applied before it is beneficial. Management, supervisors, and food handlers in food processing plants and in food service establishments must all recognize the situations which can lead to trouble and then must avoid them. The consumer must be offered better training in the way foods must be handled to keep them safe. Perhaps this should be done in grade schools and high schools. Surely the handling of food to keep it safe must be as important as driving an automobile. We offer high school training in the latter but what about the former?

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A Research Note

EFFECT OF INCUBATION TEMPERATURE ON ACID AND FLAVOR PRODUCTION IN MILK BY LACTIC ACID BACTERIA[1]

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Abstract

The effect of incubation temperature on acid and flavor production by 8 lactic starter cultures was studied. Higher titratable acidity was produced at 22 than at 30 C by *Streptococcus cremoris*, while a reverse trend was observed with *Streptococcus diacetilactis*. With *Streptococcus thermophilus*, *Lactobacillus casei*, and *Lactobacillus helveticus*, increasing the incubation temperature from 37 to 42 C retarded acid production by 8.54, 31.8, and 37.4%, respectively. Volatile acidity increased at higher incubation temperatures in some cultures, while in others, a significant decrease was observed. Higher diacetyl production was noted when *S. diacetilactis* was incubated at 22 C, as compared to 30 C, while the reverse occurred with *S. thermophilus*. Acetoin production by *S. diacetilactis* was enhanced 7-fold by increasing the incubation temperature from 22 to 30 C. No significant difference was noted in proteolytic activity of all cultures, regardless of incubation temperature.

Among the factors that affect growth and activity of starter cultures, incubation temperature is known to play an important role *(9)*. Hammer and Babel *(8)* stressed the need of an optimum incubation temperature for maintaining a proper balance between acid producing and aroma bacteria. The present communication reports the effect of incubation temperature on biochemical changes produced in milk by selected lactic cultures.

Experimental

Eight cultures, including *Streptococcus lactis* (C-10), *Streptococcus cremoris* (C-1), *Streptococcus diacetilactis* (DRC-1), *Streptococcus thermophilus* (HST), *Lactobacillus bulgaricus* (LBW), *Lactobacillus casei* (L-8), *Lactobacillus helveticus* (L-23), and *Lactobocillus plantarum* (L-24) were studied.

Reconstituted nonfat milk steamed for 30 min was used. Streptococci were incubated at 22 and 30 C, while lactobacilli were incubated at 37 and 42 C. The incubated milk samples were analysed after 24 and 48 hr. Total acidity was determined by titrating 10 g of sample with 0.1 N NaOH. Volatile acidity was determined by the method of Hempenius and Liska *(10)* by titrating 100 ml of distillate from 50 g of the sample with 0.01 N NaOH. Diacetyl was determined by Owades and Jacovac's method as modified by Pack et al. *(13)*, while acetoin was measured by the method of Anderson and Leesment *(1)*. Proteolytic activity was determined by Hull's method *(11)* and expressed as milligrams of tyrosine liberated per gram of the sample.

Results and Discussion

Although the role of incubation temperature on acid and flavor production by starter cultures has been studied by several workers *(2, 14, 15)* a wide range of incubation temperatures, viz. 22, 30, or 37 C, have been reported to be optimum for acid production *(2, 3, 6, 9)*. Golding et al. *(7)* reported that propagation at 21-22 C gave an active starter, though the optimum temperature for acid development by starters was 30 C. Variation in acid production by starter cultures in response to incubation temperature is evident from the data presented in Tables 1 and 2. Whereas *S. cremoris* produced higher titratable acidity at 22 than at 30 C, the reverse occurred with *S. diacetilactis*. The higher incubation temperature of 42 C retarded acid production by *S. thermophilus*, *L. casei*, and *L. helveticus*, while with *L. bulgaricus*, increased titratable acidity was noted at that temperature. More volatile acids were produced at higher incubation temperatures by *S. lactis*, *S. cremoris*, and *S. thermophilus*. Folda *(5)* reported similar observations with single and mixed strain starters. In normal commercial practice in India and elsewhere, the cooking temperature during cheese manufacture is about 40 C which is appreciably higher than the optimum incubation temperature for growth and activity of starter cultures. This results in a slow rate of acid production during cooking of the cheese curd. Further, increased amounts of volatile acids are produced at higher incubation temperatures. These observations suggest the need for selecting cheese starters with high temperature optima for uniform acid development and improved flavor production during cheese ripening. In the yogurt starters, namely *L. bulgaricus* and *S. thermophilus*, the higher titratable acidity by the former and increased total and volatile acid production by the latter at 42 C may be viewed with interest, since the above incubation temperature is normally used during the manufacture of yogurt.

[1]N.D.R.I. Publication No. 71-63.

TABLE 1. EFFECT OF DIFFERENT INCUBATION TEMPERATURES ON ACID AND FLAVOR PRODUCTION IN MILK BY SELECTED STREPTOCOCCI[1]

	Streptococcus lactis		*Streptococcus cremoris*		*Streptococcus diacetilactis*		*Streptococcus thermophilus*	
	22 C	30 C	22 C	30 C	22 C	30 C	37 C	42 C
Total acidity (per cent lactic)	0.85	0.86	1.00	0.92	0.80	0.90	0.82	0.75
Volatile acidity (ml of 0.01 N NaOH/ 50 g of curd)	6.4	8.2	6.0	8.4	22.5	22.7	5.0	7.3
Diacetyl (ppm)	0.00	0.00	0.00	0.00	25.0	14.0	6.0	12.0
Acetyl methyl carbinol (ppm)	0.00	0.00	0.00	0.00	13	87	0.00	0.00
Proteolytic activity (mg of tyrosine liberated/g of curd)	0.25	0.27	0.40	0.41	0.37	0.42	0.16	0.17

[1]Cultures were grown in reconstituted nonfat milk and examined after 18 hr. Results represent an average of three trials.

TABLE 2. EFFECT OF DIFFERENT INCUBATION TEMPERATURES ON ACID AND FLAVOR PRODUCTION IN MILK BY SELECTED LACTOBACILLI[1]

	Lactobacillus bulgaricus		*Lactobacillus casei*		*Lactobacillus plantarum*		*Lactobacillus helveticus*	
	37 C	42 C	37 C	42 C	37 C	42 C	37 C	42 C
Total acidity (per cent lactic)	1.40	1.85	0.22	0.15	0.25	0.23	0.24	0.15
Volatile acidity (ml of 0.01 N NaOH/ 50 g of curd)	23.0	26.0	6.5	4.5	6.8	7.4	4.8	2.5
Proteolytic activity (mg of tyrosine liberated/g of curd)	0.38	0.42	0.15	0.18	0.19	0.20	0.17	0.18

[1]Cultures were grown in reconstituted nonfat milk and examined after 18 hr. Results represent an average of three trials. These cultures did not produce diacetyl or acetoin in milk.

As regards incubation temperature and flavor production by starter cultures, reducing it from 30 to 22 C enhanced diacetyl production by S. *diacetilactis*, while the reverse was true of S. *thermophilus*. In the former organism, a 7-fold increase in acetoin production was observed by incubating at 30 rather than at 22 C. Pack et al. *(14)* observed earlier initiation of diacetyl synthesis as well as better growth at 30 C by a strain of S. *diacetilactis*. Increased accumulation of diacetyl by S. *thermophilus* at 42 C noted in these results may be considered significant since this culture has been reported as one of the predominant streptococci occurring in dahi samples in almost all parts of India *(12)*. No significant differences were noted in proteolytic activity of all cultures, regardless of incubation temperature.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT

(Continued from Page 227)

man of the Public Health Group and also served as Co-Chairman of AMHIC during 1970-1971.

Evaluation manual for food and beverage vending machines

Carbonation backflow prevention. The former Item 603.1 (1), (2), (3), and (4) on general requirements to prevent carbonation backflow in post-mix beverage machines is being replaced by the new and more stringent Item 603.1 which is described in the following three paragraphs:

> "In those vending machines in which the accidental backflow of carbon dioxide or carbonated water from the carbonator could come into contact with potentially-toxic tubing or components upstream, an air gap, atmospherically-vented valve or other positive venting device (in addition to the check valves or other devices necessary to maintain CO_2 pressure) shall be installed between the carbonator and any such potentially-toxic tubing or components upstream.
>
> *NOTE*: A brass pump housing shall not be considered 'potentially-toxic' in a system which is otherwise free of copper or other materials listed in 602.3.
>
> In machines designed to be directly connected to the location water supply (no air-gapped water reservoir), such supply lines shall be considered to be potentially-toxic. Machines designed for such direct connection shall be equipped with an air gap, atmospherically-vented valve, or other positive venting device between the carbonator and the water connection fitting, even if no potentially-toxic tubing or components are present in the internal water system of the machine."

In addition, a Committee was appointed at the 1970 meeting to explore the need to rule out the use of copper pre-cooler (or other) tubing in the carbonated water systems of post-mix beverage vending machines. At the present time this Committee and the Carbonation Backflow Committee are considering the following available options: *(a)* evaluate the design and operational features of vented solenoid-type valves; *(b)* recommend tests for such valves to determine if they fail safe under all circumstances and, if not, what corrective measures are needed; *(c)* Repeat *(a)* and *(b)* for the new Watts No. 9BD non-electric vented valve; and *(d)* Recommend to the Manual Revision Committee: (i) adoption of a "no internal copper tubing" requirement; *or,* (ii) new design or testing standards for vented valves; *or,* (iii) recommend no action.

Furthermore, it is considered commendable that the NSF and NAMA have voluntarily agreed to maintain uniform specifications for the evaluation of food and beverage vending machines. Furthermore, NSF has already arranged to adopt the new provisions of the aforementioned Item 603.1 in NSF Standard No. 25 relating to Food and Beverage Vending Machines.

Cup turret covers. During the 1969 meeting, Item 505.2 concerning covers for cup turrets was amended to require that covers be made available but not installed on the machine, since in the opinion of several public health representatives the top of the machines afforded reasonable protection to the cups. Nevertheless, at the request of the members of the vending industry, Item 505.2 was amended during the 1970 meeting to require covers on all cup turrets.

Icemaker standards. The present *Evaluation Manual* has not provided specific items on icemaker design and construction. Icemakers, by implication, would be required to meet applicable specifications for food and water contact surfaces. This has been deemed to be inadequate, and based on the 1968-1969 NAMA icemaker research project and field observations, consideration is being given to adding a new item to the *Manual on Icemaker Systems.* This new item would include construction and design specifications relating to accessibility, materials and fabrication, water reservoirs, water tubing, ice storage hoppers, icemaker harvesters, ice dispensing chutes, and water filters.

Icemaker sanitation bulletin. At the request of the members of AMHIC, the Committee on Education and Training in cooperation with the NAMA staff has developed a first draft of a bulletin on icemaker sanitation for vending operators. This proposed bulletin is being designed to pose significant questions and then to provide appropriate answers on subjects such as: Is it algae? Where does growth occur? How should icemakers be cleaned? What cleaners should be used? What cleaning procedures should be used? How often should icemakers be cleaned? The application of the information proposed in this bulletin and in the icemaker

(Continued on Page 251)

3-A SANITARY STANDARDS FOR AIR ELIMINATORS FOR MILK AND FLUID MILK PRODUCTS

Serial #2900

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Air eliminator specifications heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion are equivalent or better, may be submitted for joint consideration of the IAMFES, USPHS, and DIC, at any time.

A.
SCOPE

A.1
These standards cover the sanitary aspects of air eliminators for milk and fluid milk products.

A.2
In order to conform with these 3-A Sanitary Standards, air eliminators shall comply with the following design, material, and fabrication criteria.

B.
DEFINITIONS

B.1
Product: Shall mean milk and fluid milk products.

B.2
Surfaces:

B.2.1
Product Contact Surfaces: Shall mean all surfaces which are exposed to the product and surfaces from which liquid may drain, drop, or be drawn into the product.

B.2.2
Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.3
Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C.
MATERIALS

C.1
All product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See Appendix, Section E.), or equally corrosion resistant metal that is non-toxic and non-absorbent, except that:

C.1.1
Rubber and rubber-like materials may be used for gaskets, seals, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #1800".

C.1.2
Plastic materials may be used for inner liners, valve parts, gaskets, seals, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000", as amended.

C.2
All non-product contact surfaces shall be corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. All non-product contact surfaces shall be relatively nonabsorbent, durable and cleanable. Parts removable for cleaning having both

[1]The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April, 1963, Table 2-1, pp. 16-17. Available from: American Iron and Steel Institute, 633 Third Avenue, New York, New York 10017.

[2]Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, Ohio 44116.

product contact and non-product contact surfaces shall not be painted.

D.

FABRICATION

D.1

All product contact surfaces shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix, Section F.)

D.2

All permanent joints in product contact surfaces shall be welded. All welded areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3

All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.4

Means for mechanically cleaning the air eliminator, when provided, shall clean the product contact surfaces of the air eliminator and all nonremovable appurtenances thereto except those areas requiring manual cleaning.

D.5

All product contact surfaces shall be self draining except for normal clingage.

D.6

All internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch except the radii of vent seal parts may be 1/8 inch.

D.7

There shall be no threads on product contact surfaces.

D.8

Sanitary pipe and fittings shall conform with "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #0809", as amended and supplements thereto and /or the applicable provisions for welded sanitary product pipelines found in the "3-A Accepted Practices for Permanently Installed Sanitary Product-Pipelines and Cleaning Systems", effective June 9, 1966, Serial #60500, as amended.

D.9

Air vents shall be designed or protection provided to prevent foreign material from entering the air eliminator through the air vent.

D.10

Gaskets shall be removable. Gasket retaining grooves shall be no deeper than their width. The minimum radius of a gasket retaining groove shall not be less than 1/8 inch, except that the radius may be 3/32 inch where a standard 1/4 inch O-Ring is used and the radius may be 1/32 inch where a standard 1/8 inch O-Ring is used.

D.11

A pressure transmitter, if used, shall be installed so that it is relatively flush with the product contact surface if the air eliminator is designed to be mechanically cleaned.

D.12

If legs are used, they shall be smooth with rounded ends and no exposed threads. Legs made of hollow stock shall be sealed. The clearance between the lowest part of the air eliminator (excluding legs) and the floor shall be one of the following:

D.12.1

Not less than four inches if the horizontal area of the air eliminator is more than one square foot.

D.12.2

Not less than two inches if the horizontal area of the air eliminator is not more than one square foot and the air eliminator is designed to be portable.

D.13

Non-product contact surfaces shall be free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI[2] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel equivalent to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are coverer by ASTM[3] specifications A296-68 and A351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

These Standards are effective June 23, 1972.

[3]Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.

SURVIVAL OF POLIOVIRUS AND ECHOVIRUS DURING SIMULATED COMMERCIAL EGG PASTEURIZATION TREATMENTS

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Abstract

Fresh liquid whole egg and egg white were inoculated with poliovirus and echovirus and subjected to various process conditions of egg pasteurization. The process conditions studied included *Salmonella* destructive time-temperature exposures for whole egg and egg white, and various heat-peroxide treatments for egg white. Viruses did not survive time-temperature exposures that are effective against *Salmonella* in whole egg or egg white, but survived at various levels the heat-peroxide treatments applied to egg white. Although commercial equipment was not used in these studies, times, temperatures, and peroxide levels closely approximated those currently used commercially.

Currently in the U. S. and certain other countries all forms of liquid egg used commercially must receive a pasteurization treatment to render the egg *Salmonella*-negative. Regulations with respect to time-temperature exposures are not rigid, however, and the processor currently may choose from several treatments that have been shown to be effective against *Salmonella (1)*. All commercial pasteurization treatments for egg are considerably milder in terms of heat exposures than are used for milk and various other low acid foods because of the high order of sensitivity of the functional properties of eggs to heat *(5, 7, 8, 10)*. With egg white, which is still more sensitive than yolk containing products, temperatures as low as 51.7-54.4 C for 3.5 min or less combined with hydrogen peroxide addition constitutes effective pasteurization against *Salmonella (1)*.

Egg products, of course, are subject to contamination by agents other than *Salmonella*, and among these are viruses able to cause human infection. In the commercial production of shelled egg in the U. S., egg breaking is mostly done by machine. There are, however, many small operations that still use hand labor to break eggs, thus creating the possibility of human viral contamination. Egg spoilage, even when machine breaking is used, is tested for by sniffing broken out eggs at regular intervals, introducing another possible source of human viral contamination.

Many studies on heat treatment of viruses and their survival have been made. These are quite often in disagreement because of different measuring techniques and conditions. One report *(9)* shows that poliovirus survives at 55 C for 60 min while another *(11)* indicates that 56 C for 5 min is sufficient to kill poliovirus. Infectious hepatitis virus was found to withstand heating at 56 C for 30 min *(6)*. A study on inactivation of foot and mouth disease virus compared heating of cell-free inoculum and heating in tissue culture suspensions *(4)*. The latter imparted considerable protection to the virus, which was still active within cells after 4 hr at 80 C. Viruses often are more stable in food than in artificial media, however, the effects of food processing on virus destruction have received relatively scant attention *(2, 3)*. A search of the literature has revealed a dearth of information on the viricidal effects of commercial egg pasteurization processes. Treatments used in the present investigation were modeled after commercial processes with regard to times, temperatures, and peroxide levels in the case of egg white. One strain of poliovirus and one of echovirus as inoculum were studied in both whole egg and egg white.

Materials and Methods

Viruses

Echovirus type 6 strain D'Amori and Poliovirus type 1 strain chat were obtained from the American Type Culture Collection. They were propagated on green monkey kidney cells obtained from the Veterinary Virus Research Institute at Cornell University.

Media and reagents

All media and reagents were obtained from the Grand Island Biological Company, Grand Island, N.Y. The following reagents were used: *(a)* fetal calf serum (GIBCO 614), *(b)* gamma globulin-free calf serum (GIBCO 624), *(c)* minimal essential medium (MEM) (Eagle) (1 ×) with L-glutamine (GIBCO 109G), *(d)* lactalbumin hydrolysate solution (GIBCO 164), *(e)* penicillin-streptomycin solution (10,000 units penicillin and 10,000 mcg streptomycin/ml) (GIBCO 514), and *(f)* trypsin-EDTA solution (1 ×) (GIBCO 530).

The above reagents were mixed in solution to form the following media: *Medium* A: 78% Eagle's MEM, 10% lactalbumin hydrolysate, 10% fetal calf serum, 2% penicillin-streptomycin; *Medium B*: 86% Eagle's MEM, 10% lactalbumin hydrolysate, 2% fetal calf serum, 2% penicillin-streptomycin; *Medium C*: 86% Eagle's MEM, 10% lactalbumin hydrolysate, 2% gamma globulin-free calf serum, 2% penicillin-streptomycin; and *Medium D*: 58% Eagle's MEM, 10% lactalbumin hydrolysate, 20% fetal calf serum, 2% penicillin-streptomycin, 10% glycerol.

Cell maintenance

Cells were grown at 37 C in glass Roux bottles containing

80 ml of Medium A. When fully sheeted (2-3 days after passing), this medium was poured off and replaced with 80 ml of Medium B. Cells could be maintained 2-3 weeks on the medium.

Cell passing

The medium was poured from the Roux bottles and 40 ml of trypsin-EDTA added. Bottles were incubated at 37 C for 5 min and then shaken to dislodge and separate cells. The trypsinized cells were then centrifuged and the pellet resuspended in 240 ml of Medium A.

Virus titrations were done in 16 × 150 mm tissue culture tubes. One milliliter of cell suspension was added to each tissue culture tube, which was then stoppered and incubated at a 5° angle.

Virus propagation

Viruses were propagated on cells in disposable, plastic, 75 cm^2 surface tissue culture flasks. The flasks received 30 ml of suspended cells which were grown to a full cell sheet in 2-3 days at 37 C. The medium was poured off and 0.2 ml of virus suspension was added. Flasks were incubated at 37 C for 30 min to permit virus adsorption to the cells. Thirty milliliters of Medium C were added and cells were incubated at 37 C.

At the first sign of cytopathic effect (CPE), flasks and contents were frozen. Generally, CPE appeared after 2 days with echovirus and 4 days with poliovirus. It was at this point that maximum titers were obtained. Cells were frozen and thawed three times to rupture them and free virus particles. When allowed to incubate further, viruses were inactivated by metabolic products and low pH produced by the cells.

Virus titration

Virus was titrated following dilution in Medium C. Two-tenths milliliter portions were added to tissue culture tubes (cells growing, medium removed) prepared by the cell passing method described above. Tubes were then incubated at 37 C for 30 min. Following incubation, 1.5 ml of Medium C was added to each tube. Tubes were then incubated at 37 C and results read after the appropriate incubation period (2 days for echovirus, 4 days for poliovirus). Titrations were done in triplicate using the most probable number method for enumeration. Three control tubes (no virus) were used for each titration to assist with CPE detection.

Reproducibility of titration method

Reproducibility of the titration method was determined in Medium C and in egg white (pH 8.5). In egg white, this included preparing a homogeneous virus suspension.

For titration reproducibility in Medium C, five identical portions of poliovirus suspension were titrated. With egg white, 5 ml of poliovirus suspension in Medium C were added to 25 ml of egg white in a 250 ml Erlenmeyer flask and stirred until the pH indicator of Medium C was uniformly spread throughout the mixture. Another 75 ml of egg white were added and stirred for 10 min. Thick and thin protein were completely intermixed and there was no foaming. Three identical portions were taken, one from the center, one from the top, and one from the bottom of the flask. Titration results are shown in Table 1.

Heat pasteurization treatments

Pasteurization studies were carried out in a controlled temperature water bath. Virus inoculated fresh shelled egg was injected into stainless steel tubes of 3/16-inch outside diameter with 10 mil thick walls with a syringe. Tubes of 8-inch length held about 1 ml of egg. Come up time to 60.0 C was 15 sec measured by a temperature sensor in the tube held in the water bath. These 15 sec were added onto the processing times listed in subsequent tables. Tubes of heated egg white-virus mixtures (pH 8.5) and whole egg-virus mixtures (pH 7.0) were chilled in an ice bath to 10 C immediately after processing. Portions of 0.2 ml were then titrated as previously described.

Heat-hydrogen peroxide pasteurization treatments

The combined bactericidal effects of heat and hydrogen peroxide enable the commercial processor to use lower pasteurization temperatures than would be effective with heat alone. Hydrogen peroxide pasteurization is used commercially for egg white only. The following treatments differed in the amount of catalase destruction (natural catalase of the egg) and the amount of peroxide added. In each treatment the temperatures studied (see Results) were the same.

Heat-peroxide pasteurization treatment A consisted of heating 100 ml of egg white at 51.7 C for 30 min to inactivate the natural catalase of the egg. The egg was cooled and 5.0 ml of virus suspension added. The egg-virus mixture was made homogeneous and 10 ml portions were heated in test tubes to the pasteurization temperature of 54.4 C or 51.7 C. Come up time was approximately 2.5 min. Hydrogen peroxide was added at a level of 0.07% (0.23 ml of 3% H_2O_2). The egg was held at the respective pasteurization temperature for 2.0 min. At the end of the holding period, the egg was immediately cooled in an ice bath to 10 C and catalase added to decompose any remaining peroxide. To observe the effect of the peroxide alone on the viruses, the above procedure was also followed using room temperature as the holding (pasteurization) temperature.

Heat-peroxide pasteurization treatment B was a more realistic test of the egg pasteurization method as presently practiced commercially. One hundred milliliters of egg white were inoculated with virus and made homogeneous. Ten milliliter portions of egg-virus suspension were heated in test tubes to 54.4 C or 51.7 C and held for 1.5 min. This was the catalase destruction period. Peroxide at the 0.07% level was added and egg held at 54.4 C or 51.7 C for 2.0 min of pasteurization. Egg was cooled and catalase added. After 1.5 min of peroxide decomposition, the egg was titrated.

This treatment differed from treatment A in two ways. In treatment A, all the catalase in the egg was destroyed before addition of the virus or peroxide. Thus, there was no peroxide decomposition during processing from this enzyme. In treatment B, however, catalase destruction may have been incomplete after the 1.5 min allowed for this purpose. Some peroxide might then have been decomposed before being able to fully affect the viruses. Also in treatment B, virus was present during the 1.5 min catalase inactivation period before the pasteurization period, thus receiving heat for 3.5 min rather than 2.0 min as in treatment A.

Heat-peroxide pasteurization treatment C disregarded the natural catalase of the egg and compensated by using a higher level of peroxide. Ten milliliter portions of virus-inoculated, mixed egg white were heated in test tubes to the pasteurization temperature of 54.4 C or 51.7 C and 0.09% hydrogen peroxide (0.3 ml of 3% H_2O_2) was added. The egg was held at pasteurization temperature for 3.5 min. It was then cooled in an ice bath and catalase added. After 1.5 min of peroxide decomposition the egg was titrated.

RESULTS

Effects of heat pasteurization treatments

The heat pasteurization of whole egg resulted in

TABLE 1. REPRODUCIBILITY OF VIRUS RECOVERY FROM MEDIUM C AND FROM EGG WHITE

Titration	Most probable number	
	Medium C	Egg white
1	9.3×10^4	2.4×10^5
2	2.4×10^5	9.3×10^4
3	2.4×10^5	4.6×10^5
4	2.4×10^5	—
5	2.4×10^5	—
	$\mu = 2.1 \times 10^5$	$\mu = 2.6 \times 10^5$
	$\sigma = 6.5 \times 10^4$	$\sigma = 1.8 \times 10^5$

TABLE 2. VIRUS SURVIVORS OF PASTEURIZATION OF WHOLE EGG

Temp.	Time	Poliovirus		Echovirus	
°C	Min	Initial count	Survivors	Initial count	Survivors
56.7	20.0	2.4×10^6	<.03	2.4×10^4	<.03
57.8	11.0	2.4×10^6	<.03	2.4×10^4	<.03
58.9	7.0	2.4×10^6	<.03	2.4×10^4	<.03
60.0	3.5	2.4×10^6	<.03	2.4×10^4	<.03

complete inactivation of both viruses at all temperatures tested (Table 2). It should be noted that there was coagulation of egg protein in the samples processed at 60.0 C.

In the heat pasteurization of egg white (Table 3), echovirus was completely inactivated at all temperatures studied. Poliovirus was destroyed at 56.7 C, 57.8 C, and 58.9 C, but survivors were found at 60.0 C and 61.1 C. It again was observed that coagulation of egg protein occurred at both of these higher temperatures, which could be responsible for the lesser kills, because of decreased heat transfer.

Effects of heat-peroxide pasteurization treatment A

As seen from data in Table 4, echovirus in egg white was destroyed at 54.4 C but survived at 51.7 C. Poliovirus survived at both temperatures, indicating that in egg white it is the more resistant of the two viruses. Virus titers of the room temperature treatment did not differ significantly from the initial virus counts of the unprocessed egg, indicating that peroxide without heat is not viricidal. This is not true with *Salmonella* which is quite sensitive to peroxide. Reductions in virus titer at 51.7 C and 54.4 C in treatment A were two logs or more in all instances. Thus, this treatment does give significant reductions in virus populations but is not as effective as the more severe time-temperature pasteurization conditions without peroxide (Table 3).

Effects of heat-peroxide pasteurization treatment B

Results of treatment B are shown in Table 5. Echovirus was completely inactivated at both temperatures, probably as a result of the additional 1.5 min of heat received by the virus. The more resistant poliovirus was able to survive in large numbers at the lower temperature of 51.7 C. Here catalase destruction probably was incomplete after only 1.5 min allowed for its destruction. If present in appreciable quantity it could have lowered the concentration of the peroxide sufficiently to explain the negligible effect on the virus at 51.7 C. Quite possibly, at 54.4 C there was enough catalase destroyed so that the peroxide concentration was not significantly lowered and the peroxide was able to act fully on the viruses. At 54.4 C the poliovirus titer was reduced by more than three logs.

Effects of heat-peroxide pasteurization treatment C

Results of treatment C are given in Table 6. Poliovirus reduction was > two logs at 54.4 C but < one log at 51.7 C. Echovirus also survived at both temperatures but with at least a log greater reduction than the poliovirus at both temperatures. This again indicates that poliovirus is the more resistant of the two viruses in egg white. Results of treatment C indicate that it may be the least effective of the heat-

TABLE 3. VIRUS SURVIVORS OF PASTEURIZED EGG WHITE

Temp.	Time	Poliovirus		Echovirus	
°C	Min	Initial count	Survivors	Initial count	Survivors
56.7	3.5	9.3×10^6	<.03	9.3×10^3	<.03
57.8	2.0	9.3×10^6	<.03	9.3×10^3	<.03
58.9	1.1	9.3×10^6	<.03	9.3×10^3	<.03
60.0	0.62	9.3×10^6	7.5×10^4	9.3×10^3	<.03
61.1	0.35	9.3×10^6	9.3×10^3	—	—

TABLE 4. SURVIVORS OF HEAT-PEROXIDE TREATMENT A

Pasteurization temp.	Poliovirus		Echovirus	
°C	Initial count	Survivors	Initial count	Survivors
54.4	9.3×10^4	21	2.4×10^3	<.03
51.7	9.3×10^4	150	2.4×10^3	23
Room temp.	9.3×10^4	4.3×10^4	2.4×10^3	4.3×10^3

TABLE 5. SURVIVORS OF HEAT-PEROXIDE TREATMENT B

Pasteurization temp.	Poliovirus		Echovirus	
°C	Initial count	Survivors	Initial count	Survivors
54.4	4.3×10^4	15	2.9×10^4	<.03
51.7	4.3×10^4	1.1×10^4	2.9×10^4	<.03

TABLE 6. SURVIVORS OF HEAT-PEROXIDE TREATMENT C

Pasteurization temp.	Poliovirus		Echovirus	
°C	Initial count	Survivors	Initial count	Survivors
54.4	4.3×10^4	2.4×10^2	2.9×10^4	9.1
51.7	4.3×10^4	9.3×10^3	2.9×10^4	43

peroxide pasteurization treatments studied with respect to the test viruses. This treatment disregarded heat inactivation of the natural catalase present in the egg white before peroxide addition and a higher concentration of peroxide was added to offset decomposition from catalase. However, too much peroxide probably was decomposed before it was able to act on the viruses. Since the heating time was similar to that of heat-peroxide treatment B which destroyed the echovirus, the difference appears to lie with decomposition of the peroxide.

DISCUSSION

Throughout these studies, eggs were inoculated with high titers of virus to facilitate measurement of the effects of pasteurization treatments on reduction of virus populations. In commercial egg handling where virus infection may occur, it is not likely that levels of contamination would be as high. Further, should virus-contaminated egg be processed industrially, viruses would become greatly diluted.

Results of the heat pasteurization of whole egg show that the two viruses selected for this study were completely inactivated over the entire time-temperature range tested. With egg white pasteurized by heat alone, poliovirus, which was completely inactivated at temperatures from 56.7 to 58.9 under the test conditions, survived the heat treatments at the higher temperatures of 60.0 C and 61.1 C. This survival is not attributed to resistance of the virus but to coagulation of egg white protein that occurred. The coagulation caused a drop in the heat transfer rate which, in turn, minimized the heat treatment that was received by the virus. This decreased heat treatment, nevertheless, still was effective against the less resistant echovirus.

Although straight heat pasteurization over a range of times and temperatures that span commercial practice appear to be quite adequate for destruction of test viruses studied in both whole egg and egg white, a similar conclusion cannot be reached with milder heat plus peroxide commonly used for commercial pasteurization of egg white.

The treatments used in this study were modeled after commercial processes with regard to times, temperatures, and peroxide levels. To be sure, the current studies carried out under small batch-heating laboratory conditions only approximate the true exposures encountered by liquid egg in commercial pasteurization equipment. However, it is not unlikely that conditions exist where current commercial pasteurization of egg white with heat plus peroxide are no more severe than in the present studies. Such egg white may be rendered *Salmonella*-negative but could retain virus. It therefore would appear that virus survival in mildly pasteurized liquid egg, especially egg white, is worthy of additional study. To be most meaningful, such studies should include pasteurization processing with industrial equipment.

ACKNOWLEDGEMENT

This study was supported, in part, by the National Institutes of Health Training Grant FD 00002 from the United States Department of Health, Education, and Welfare, Public Health Service.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT

(Continued from Page 244)

standards should assure the production and dispensing of a safe and acceptable ice product.

Cut-off controls. The members of AMHIC received reports on some of the technical problems encountered by industry and public health in obtaining effective and reliable automatic controls to prevent the storage and dispensing of potentially hazardous foods at unsafe temperatures, between 45 F and 140 F, and in obtaining a workable field test to determine the accuracy and reliability of these controls. To overcome these problems and to accomplish the objectives of the cut-off controls, several major changes in machine design are presently being considered by the manufacturers and the members of the Committee on Cut-Off Controls; and it is anticipated that within the next year appropriate and essential changes will be made in the Evaluation Manual to more adequately protect the consumer and to aid the evaluator and regulatory officials in carrying out their responsibilities.

Remanufactured machines

The program for Evaluation of Remanufactured Vending Machines seemed to have tremendous appeal, during the early planning stage, to members of the public health agencies and vending machines operating companies; and several of the firms rebuilding vending machines requested this program for evaluation of other-than-new machines. However, to date, only three remanufacturers have submitted machines for evaluation and only two have received Letters of Compliance to manufacture machines under this NAMA program even though several written invitations to participate in the program have been sent by NAMA to all known remanufacturers.

Coin-operated special devices

The NAMA staff reported at the 1970 meeting that several requests had been receivd during the past year from members of the public health agencies and the vending industry to develop criteria for the evaluation of the small coin-operated office coffee service machines. After thoroughly discussing these requests and recognizing a possible conflict with the current edition of the PHS recommended ordinance and code, AMHIC appointed a Committee to make an in-depth study of the definition and sanitation requirements pertinent to coin-operated special food and beverage devices and present same to the membership by at least the next AMHIC meeting and, subsequently, to the appropriate Federal agency. Only a few of these special coffee service devices on the market today could be manufactured to comply with the current Evaluation Manual without some major changes in design.

Commissary guide

The second draft of the proposed *NAMA Vending Commissary Guide,* which is intended to advise vending machine operators of basic sanitation requirements as to site, layout, design, construction, and equipment installation for food preparation commissaries, has been reviewed by this Committee. The third draft of this proposal is probably being delayed until questions posed by some state regulatory officials and by the NAMA staff have been adequately resolved concerning the applicability of the Federal meat inspection laws and regulations to the typical vending-catering-food management commissaries.

RECOMMENDATIONS

1. The Association reaffirm its support of the National Sanitation Foundation and the National Automatic Merchandising Association and continue to work with these two organizations in developing acceptable standards and educational materials for the food industry and public health.

2. The Association urge all sanitarians to obtain a complete set of the National Sanitation Foundation's Food Equipment Standards and Criteria and a copy of the National Automatic Merchandising Association—Automatic Merchandising Health-Industry Council's Vending Machine Evaluation Manual and related materials; to evaluate each piece of food equipment and vending machine in the field to determine compliance with the applicable sanitation guidelines; and to let this Committee and the appropriate evaluation agency know of any manufacturer, installer, or operator failing to comply with these guidelines.

3. The Association urge all sanitarians and regulatory agencies to support the work of the Association's Committee, submit suggestions for developing new guidelines and for amending same, and subscribe, by law or administrative policy, to the principles represented by the Standards, Criteria, and Evaluation Manual for Food Equipment and Vending Machines.

This report of the Committee on Food Equipment Sanitary standards respectfully submitted by: Karl K. Jones, *Chairman,* Purdue University, West Lafayette, Indiana; Irving L. Bell, State Department of Health, Frankfort, Kentucky; Glenn Brauner, National Canners Association, Washington, D.C.; Robert R. Dalton, State Department of Public Health, Lansing, Michigan; Carl Henderson, State Department of Health and Social Services, Santa Fe, New Mexico; Howard Hutchings, State Department of Health, Pierre, South Dakota; O. Donald Moore, Food & Drug Administration, Atlanta, Georgia; W. Joel Simpson, State Department of Environmental Resources, Harrisburg, Pennsylvania; and Harold Wainess, Harold Wainess and Associates, Northfield, Illinois.

ASSOCIATION AFFAIRS

NOTICE TO MEMBERSHIP

Deadline for submission of nominations for 1972 Annual Sanitarians Award is June 1, 1972. The award is to a local sanitarian this year (City or County). See January 1972 issue of the Journal for details.

The membership is urged to send in nominations at once and to remember that previous nominees who have not received the award are eligible for re-nomination.

Melton E. Held,
Senior Past President
Chairman of Awards
Committee

KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC., 1972 EDUCATIONAL CONFERENCE AND BUSINESS MEETING

The 1972 Educational Conference for Fieldmen and Sanitarians was held February 22-23, 1972 at the Executive Inn Motor Hotel, Louisville, Kentucky. A total of 275 (county and state health department sanitarians, milk and food industry fieldmen, plant managers and related service companies and University officials) were registered. States other than Kentucky represented were as follows: Colorado, Georgia, Indiana, Michigan, Missouri, New York, Ohio, Pennsylvania, Tennessee, Texas, Washington, D.C., and Wisconsin. The program was broken into general sessions, food and environmental sanitarians sections and milk sanitarians sections. Fifteen papers were presented during the conference.

The following awards were given at the awards banquet by the Kentucky Association of Milk, Food, and Environmental Sanitarians, Inc.

Outstanding Sanitarian Award: (Co. awards were given due to a tie in voting on two of those nominated). William T. Vincent, Lexington-Fayette Co. Health Dept., Lexington, Kentucky A. R. Himes, Ohio Co. Health Dept., Hartford, Kentucky.

Outstanding Fieldman Award: Harold McCurry, Southern Bell Dairy, Somerset, Kentucky.

Oustanding Service Award: Charles F. Spears, Kraft Foods, Taylorsville, Kentucky.

A past presidents plaque was also presented to out going president, Irving Bell, Kentucky State Health Department, Frankfort, Kentucky.

New officers and directors elected at the business meeting were as follows: President–Donald L. Colgan, Fleming Co. Health Dept., Flemingsburg; President-elect–Dudley J. Conner, Kentucky State Health Dept., Frankfort; Vice President–R. T. Winbigler, Swift and Company, Glasgow; Secretary-Treasurer–Leon Townsend, Kentucky State Health Dept., Frankfort; Director Western Region–Max Weaver, Kentucky State Health Dept., Paducah; Director N. Central Region–Gayle Shrader, Jefferson Co. Health Dept., Louisville; Director Eastern Region–William L. Stephenson, K.I.O. Dairy Supply, Union.

The conference is sponsored by the Kentucky Association of Milk, Food and Environmental Sanitarians Inc., the Kentucky State Health Department, Division of Environmental Services, The Kentucky Dairy Products Association, and the American Dairy Association of Kentucky.

Officers and Directors elected at the 1972 annual meeting of the Kentucky Association of Milk, Food and Environmental Sanitarians Inc.

Back row L. to R. Donald L. Colgan, President, Irving Bell, Past President, R. T. Winbigler, Max Weaver, Director. Front row L. to R. Gayle Shrader, Director, William Stephenson, Director, Leon Townsend, Secretary-Treasurer. (absent from picture Dudley J. Conner, President elect).

Awards winners, presented by the Kentucky Association of Milk, Food and Environmental Sanitarians, Inc., at the 1972 Educational Conference of Fieldmen and Sanitarians, February 22-23, 1972, Executive Inn, Louisville, Kentucky.

L. to R. A. R. Himes, and W. T. Vincent, Outstanding Sanitarians Award, Charles F. Spears, Outstanding Service Award, Harold McCurry, Outstanding Fieldmen Award and J. L. Wallace and Louis Smith, Honorary Memberships.

THE SANITARIAN: HEALTH CAREER FACTS

Performing preventive medical services to an entire population is quite an extensive assignment. And it is not unrealistic. This responsibility is carried out daily by the professional sanitarian. If communities are clean and free of damaging hazards to overall health it reflects the sanitarian's awareness and ability to deal with environmental health problems.

Most people assume that the food they eat, the liquids they drink, the air they breathe, the public swimming pools they use are safe. The sanitarian cannot assume any of these, and his investigations and evaluations assure safety for the general public. The sanitarian finds and eliminates hazards in order to make the physical environment safe for everyone. He is primarily involved with improving man's environment by alleviating metropolitan and rural health problems. Promoting and securing water supply extension, providing safer recreational areas, and developing better hygienic conditions in nursing and convalescent homes are several of his many duties.

To meet the demands of many diverse roles the sanitarian must incorporate the knowledge of medical, engineering, scientific and legal areas into his occupation. Sanitarians work closely with other health specialists in the community to prevent outbreaks of disease, to plan for civil defense and emergency disaster aid, to make public health surveys, and to conduct health education seminars. Bacteriologist, chemist, physicist, engineer, teacher and lawyer—the sanitarian must be familiar with all these fields to effectively promote environmental health.

Currently sanitarians are fighting to eliminate many problems of crowded metropolitan areas. Industrial expansion has aggravated the problem of air pollution. The sanitarian assumes the responsibility of inspecting and attempting to alleviate many of the mobile and stationary sources of air pollution. He provides services for protection of air resources and assists in the planning of new industrial areas.

Proper housing for the American family is an integral part of environmental health. The sanitarian improves the neighborhood by protecting water resources and waste disposal, by promoting home safety and accident prevention, and by coordinating activities with urban renewal authorities, building inspection agencies, and public housing agencies.

No one can eliminate human immunity to accidents, but since the sanitarian is acutely aware of potential hazards he is needed to provide an accident control program. Professional training enables the sanitarian to recognize safety hazards and to develop a sense of diplomacy in working with other professionals to clear up the problem.

To protect our food supplies the sanitarian closely checks production and processing techniques. Inspections are made in food and dairy industries to see that clean, healthful, safe working conditions exist.

The sanitarian works with the problem areas that affect our health. But a constant watch must be kept over the potential hazards and developments of tomorrow. Sanitarians have been vital contributors to the space program by resolving sanitation problems of space flights. The projected necessity of a controlled environment for moon dwellers has also fallen within the sanitarian's area of concern.

EDUCATIONAL REQUIREMENTS: The desirable minimum qualification for the professional sanitarian is a college degree with a major in physical, biological, or sanitary science. A graduate degree in some specialty of public health is usually required for higher level positions. In Wisconsin, a person can become a registered sanitarian by examination after six years or practical experience following high school graduation.

Many sanitarians supplement their college background by enrolling in specialized short-term training courses in such subjects as occupational health, water supply and pollution control, air pollution, radiological health, milk and food protection, and metropolitan planning.

Wisconsin State University, Eau Claire, offers a B.S. degree in Environmental and Public Health. Universities having Schools of Public Health such as Minnesota, Michigan, California, North Carolina and Johns Hopkins offer postgradute degrees in public health sanitation. The Milwaukee Technical College offers a 2-year concentrated program in environmental sanitation. A certificate is issued upon completion.

OPPORTUNITIES: In recent years environmental health has expanded rapidly. And it is currently on the threshold of more extensive development. The problems of radiological health, occupational health, food protection, water and air pollution will expand as a result of population increases, migration to the cities and adoption of a highly technological civilization. To offset these problems more highly trained sanitarians will be needed.

Health organizations exist on city, state, national levels. International organizations—World Health Organization, Agency for International Development, the Peace Corps—have a need for sanitarians to deal with problems of environmental health—problems that may be far different and far greater than ours. In local, state and federal health departments the

sanitarian may specialize in a particular area of work, such as milk and other dairy products, food sanitation, waste control, air pollution, housing, or insect control. In rural areas and small cities they may be responsible for a wide range of environmental health activities.

The added significance of environmental health can be seen in the increasing number of sanitarians being employed by industries. Many sanitarians are employed by large businesses to control proper maintenance of buildings, equipment and employee facilities, to eliminate safety hazards, to assure the proper cleaning of plant equipment. The food and dairy industries, have a chronic need for qualified sanitarians to make sure that food ingredients and production procedures meet government specifications. Colleges and universities also have positions available to the professional sanitarian who may serve as a faculty member, conduct research, or direct the campus environmental health program.

SALARIES: The demand for more qualified individuals and the technological advancements within the field have resulted in improved salary scales for the sanitarian. Annual salaries with state and local governments usually range between $7,000 and $12,000. Industrial salary levels will vary according to educational background and the individual sanitarians knowledge of a particular specialty.

GEORGIA ENVIRONMENTAL HEALTH ASSOCIATION

Persons employed in the various areas of Environmental Health in Georgia have an active professional association in their home state. The Georgia Environmental Health Association (formerly the Georgia Society of Registered Professional Sanitarians) is an organization which has as its goal the betterment of society through control of the surroundings. The association brings together in one group all environmentalists who are employed in local and state health departments, the Georgia Department of Agriculture, and numerous industry representatives who are employed to protect the environment.

Since its beginning 19 years ago, the organization has been concerned with the competence of its members. In the mid-50's, the association was instrumental in devising an evaluation procedure and establishing a review and examining board at the state level to evaluate competence of sanitarians, and to certify them as "registered professional sanitarians" when certain professional qualifications were met. This registration carried with it advancements in pay and promotion to additional areas of responsibility, and provided an incentive for the environmentalist to keep abreast of advancements in his profession.

The annual meeting of the association is designed to provide continuing education for the members. Once each year, members of the Georgia Environmental Health Association assemble on Jekyll Island for an educational conference. The theme for the 1971 conference was "A Future in Our Environment." with such outstanding speakers as the Deputy Director, Georgia Department of Health, a Regional Vice-President of a National Pest Control Association, the Director of Environmental Control for the Georgia-Pacific Corporation, the President of Acoustic and Vibration Association, Inc., and the Assistant Secretary for Health Services, U. S. Department of Health, Education and Welfare.

Also, members of the association saw a need and played an active role in getting the degree program "B. S. in Environmental Health Science" initiated at the University of Georgia. Since its beginning in the Fall of 1970, this program as developed into a very successful venture with 42 students currently working toward the degree.

The officers for the Georgia Environmental Health Association for the current year are as follows: President, Harry Forehand, Long County Health Department, Jesup, Georgia; President Elect, Ray N. Bliss, Airwick Industries, Inc., Forest Park, Georgia; Vice-President, Charles D. Stewart, Jr., Georgia Department of Agriculture, Atlanta, Georgia; and Secretary-Treasurer, James H. Martin, University of Georgia, Athens, Georgia.

SURGE ANNOUNCES RESULTS OF FIELD TESTS ON NEW QTO MILKER

The Surge QTO or Quarter Take-Off milker, a new concept in milking, has been developed by Babson Bros. Co. Research. Built around the proven Surge principle of tug and pull and fast efficient milking, the new unit automatically removes the teat cup when each quarter is milked out.

Results from almost one million milkings to date show advantages in labor efficiency while maintaining top milk production and good overall udder health.

Babson Bros. Research and Development Department began testing their first automatic take-off milker in 1958. Continuing research on the concept led to the development of the current model. The milking unit is governed by a solid state electronic control. Individual electrodes sense the milk flow from each teat. When the milk flow ceases, the low voltage sensing circuit is broken which signals the re-

moval of the teat cup. Actual removal is accomplished when an air powered cylinder draws the teat cup downward. Vacuum is shut off at the stem of the inflation allowing the teat cup to drop off. The operator can check the status of the QTO with the help of indicator lights on the control unit.

FOOD ENGINEERING AWARD JURY SELECTED

Dairy and Food Industries Supply Assn. (DFISA) and American Society of Agricultural Engineers (ASAE) have announced names of a blue-ribbon jury which will select the recipient of the industry's first Food Engineering Award.

Jury members who will evaluate nominees for the food processing, science and technology honor are: R. H. Brown, chairman, Agricultural Engineering Dept., University of Georgia; D. F. Farkas, chemical engineer, Engineering and Development Laboratory, Western Regional Research Laboratory, Agricultural Research Service, U. S. Dept. of Agriculture; V. A. Jones, associate professor, Dept. of Food Science, North Carolina State University; D. E. Kirk, professor, Agricultural Engineering Dept., Oregon State University; K. H. Lewis, director, Office of Food Sanitation, Bureau of Foods, Dept. of Health, Education and Welfare; H. L. Mitten, Jr., director of international operations, CP Division, St. Regis; and B. S. Schweigert, chairman, Dept. of Food Science and Technology, University of California.

The Food Engineering Award is designed to honor those who have made outstanding original contributions in research, development, design or management of food processing equipment or processes which have been of significant economic value to the food industry and the consuming public.

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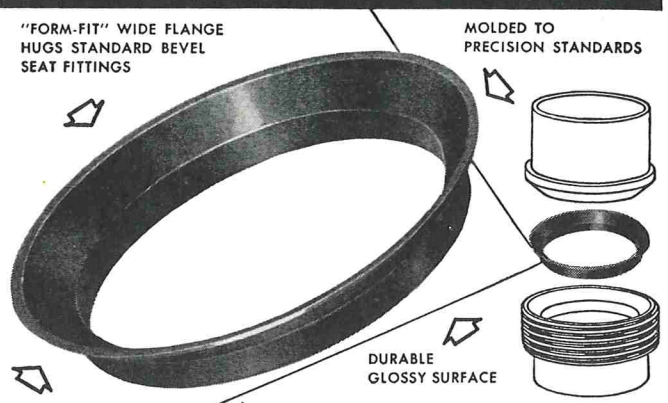

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Dairy authorities speak out on better cow milking.

Hazards of overmilking

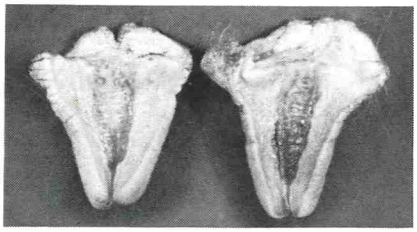
K. J. Peterson, D.V.M. / Oregon State University / Corvallis

Bovine mastitis is a common, costly and complex disease. Practically all cases are infectious in nature and result from the entrance into and multiplication of infective microorganisms within the mammary gland. Many types of infectious agents are capable of causing mastitis but natural teat barriers normally prevent their entrance. Factors which tend to break down these barriers play significant roles in the spread of the disease.

Of these many factors, one of the most important is improper machine milking. Our present dairy industry could not have evolved without the advent of the milking machine and considerable research has gone into its development. Most present day machines, if properly sanitized, maintained, and operated will perform satisfactorily. However, if any or all of these criteria are neglected, increased mastitis is likely to result.

No matter how good the machine, if it is improperly operated, teat injury is likely to occur. Overmilking, allowing the machine to remain on teats after milk flow has ceased, may cause teat injury. An overmilking trial was conducted on nineteen healthy dairy cows. These cows were subjected to varying periods of overmilking. Two teats on each cow were overmilked and two were not. At the conclusion of the trial, the cows were immediately slaughtered and comparisons made between overmilked teats and control teats of the same cow and between cows. This clearly demonstrated that overmilking was capable of causing severe injury to teats of some cows.

The changes consisted of hemorrhage and inflammation of the membrane lining the teat cavity. Injury to this lining results in lowered resistance to bacterial invasion and multiplication. Infection of this tissue is soon followed by infection of the mammary gland proper. In a number of cases injury also occurred in the deeper structures of the teat wall and in the lining of the streak canal. The streak canal has the very important function of preventing bacteria and other microorganisms from entering the mammary gland. An injured streak canal cannot properly perform this function and mastitis often results.



left front teat milked normally **right front teat extended overmilking**

In this trial, overmilking three minutes 20 times (twice a day for ten days) resulted in moderate injury to two cows, and none to one. Five minutes of overmilking 16 consecutive times resulted in severe injury to one cow, moderate injury to two cows and no injury to one cow. Ten minutes of overmilking twice a day for three days resulted in severe injury to all of three cows. As the overmilking time is increased, teat injury is also increased. The degree of teat injury varied with the number of times the cow was overmilked and the length of the overmilking period. However, it also varied between cows milked in identical manner. Certain cows for some undetermined reason are more susceptible to overmilking injury. This difference was also noted between teats on the same cow.

Cows vary in amount of milk produced and rate of milk flow. This variation exists not only between cows but between quarters of the same cow and at different stages of lactation. Some cows, often heavy producers, milk very rapidly and may be completely milked out in less than 3 minutes. Others giving less milk but with a slow flow rate, may require much more time. Unless the milker observes each cow and each quarter carefully, the rapidly milked cows and those late in lactation may be regularly overmilked. This may occur when milkers are careless, are operating too many machines or are following a routine that allows the same milking time for each cow and each quarter. Prolonged stripping may also result in excessive overmilking.

To maintain a dairy herd with a minimum of mastitis, the operator should carefully follow proven management practices and avoid the hazards of overmilking. Decreased mastitis means increased profits.