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Journal of

MILK and FOOD TECHNOLOGY

59TH ANNUAL MEETING
August 21, 22, 23, 24, 1972
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NOTICE
Page I and II
National Mastitis Council Meeting

Official Publication

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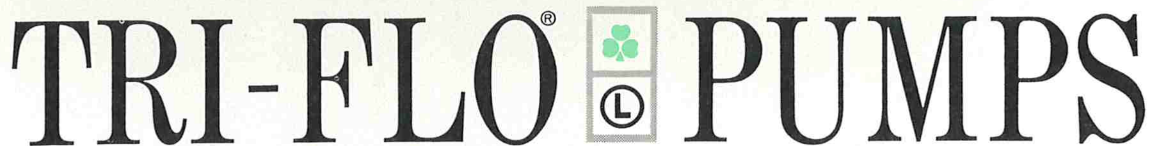

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NATIONAL MASTITIS COUNCIL ANNUAL MEETING

LA SALLE HOTEL—CHICAGO, ILLINOIS
FEBRUARY 8-9, 1972

Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the eleventh annual meeting of the National Mastitis Council.

The program will concentrate on subjects and speakers recommended by popular request through the 1971 postmeeting questionnaire. The general theme will be "Looking Ahead" as the speakers discuss current progress and look to the future.

"Evaluation of Teat Dips and Teat Dipping Procedures" will be followed by a panel discussion of "Newer Milking Machine Concepts and their Relationship to Mastitis Control" presented by the Milking Machine Manufacturers Council.

The pharmaceutical industry and the Food and Drug Administration will be represented in discussions of mastitis remedies, problems at hand, and products that may be used in the future.

A director of a quality control laboratory will review his experience with the direct microscopic somatic cell count, and the results of a critical examination of herds whose market is in jeopardy will be presented.

An outstanding dairyman and a well-known veterinary practioner from California will appear by request of NMC members who heard them at the NMC meeting in San Diego.

Of major significance will be the address by Dr. E. E. Saulmon, Agricultural Research Service, USDA, who will consider the merits of possible regulatory measures for control of mastitis.

And to give perspective to our thinking a prominent dairyman will bring his reflections on the events now taking place and those he sees in the future.

Informal evening discussions will consider: (1) Milking machine installation, operation, maintenance. (2) Mastitis therapy. (3) Regulation of abnormal milk.

Plan now to attend this meeting. It will start at 8:45 a.m. on February 8 and will adjourn at noon on February 9. Fill in the registration form and return it today to the National Mastitis Council. It will save time for you and NMC if you send the registration fee with the form.

Send request for room reservation directly to the LaSalle Hotel.

William L. Arledge
President

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La Salle Hotel—Chicago, Illinois

February 7-9, 1972

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Journal of

MILK and FOOD TECHNOLOGY

INCLUDING MILK AND FOOD SANITATION

Official Publication

International Association of Milk, Food and Environmental Sanitarians, Inc.

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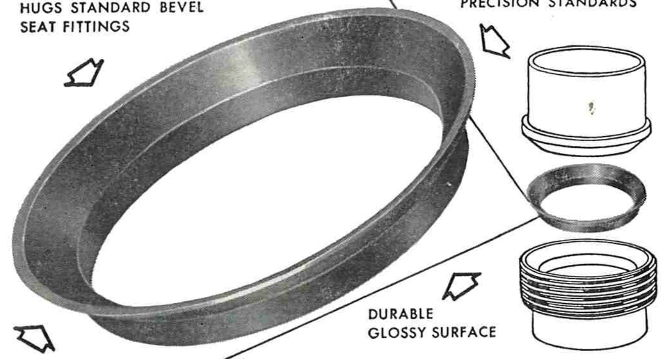

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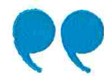

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FACTORS IN THE ECOSYSTEM OF FOOD PROCESSING EQUIPMENT CONTRIBUTING TO OUTGROWTH OF MICROORGANISMS ON STAINLESS STEEL SURFACES[1]

R. B. MAXCY

Department of Food Science and Technology
University of Nebraska, Lincoln 68503

(Received for publication July 6, 1971)

ABSTRACT

A laboratory system using 1 cm^2 pieces of stainless steel with controlled temperature, humidity, and inocula was used to study the fate of the microflora of dairy origin on various films. Metal squares were treated to simulate incomplete cleaning, then inoculated with raw milk. After incubation for 16 hr at 80, 86, 93, or 100% RH, the microflora was recovered in sterile phosphate buffer and evaluated using the standard plate count method. Isolates from countable plates were observed for general characterization.

Humidity of the ecosystem was a major factor in rate of water loss from a film. Available water, and indirectly the solute concentration, influenced the microenvironment and the resulting population density of microorganisms. Likewise, the microenvironment had a selective effect on outgrowth of bacteria, because of varying sensitivity of bacteria to drying and solute concentration. The gram-negative bacteria were inhibited at a lower humidity (80% RH), while the higher humidities (93 or 100% RH) provided sufficient water for abundant outgrowth. Reduction of area of a film exposed to air reduced water loss and provided protection for harborage of microorganisms.

Pre-soiling prior to addition of a film of milk decreased the rate of water loss from the film and increased bacterial growth. Induced lipolysis within a film of milk influenced outgrowth of bacteria with an inhibitory effect on gram-positive types. Results indicate that modern closed systems and extensive mechanical handling of milk may be favoring growth of undesirable gram-negative bacteria, e.g., coliforms and psychrotrophs.

Modern food processing equipment provides a complex system for protection and growth of microorganisms. Microenvironment includes chemical and physical factors that affect the individual cells; ecosystem includes secondary and interacting factors, e.g., temperature, humidity, and physical enclosures *(2)*. Though modern cleaning procedures, e.g., "cleaned-in-place" (CIP) operations are quite effective, soil residues and microorganisms remain *(5, 7)*. Soil consisting of food residues, cleaning compounds, and constituents of water provides nutrients for microorganisms. Thus, microorganisms may grow and contribute contaminants to subsequent food processing operations. The microflora of milk handling equipment varies qualitatively and quantitatively *(8)* depending on the many complex factors of the ecosystem. The most commonly recognized factors influencing the microflora are soil residue, detergent residue, available moisture, temperature, incubation time, and population density of microorganisms.

Most of the above factors are influenced by conditions beyond the microenvironment. For example, a major factor in the ecosystem appears to be the humidity of the environment, because humidity determines the rate and extent of drying and has a secondary effect on many other factors in the microenvironment *(2)*. Available water directly influences metabolism and indirectly influences the transport system for assimilation of food and voiding of waste materials. Microorganisms have strict limits of water activity in which growth occurs *(6)*, and water activity in thin films of food is dependent on the relative humidity (RH) of the environment *(3)*. At water activity below an equilibrium of 95% RH many bacteria are inhibited *(6)*. This humidity is rather uncommon in arid areas. Yet there is a universal problem with bacterial contamination from equipment. Barnhart et al. *(2)* showed factors other than equilibrium humidity to be involved in determining the outgrowth of microorganisms on soiled surfaces, and more recently, they *(3)* showed the solute concentration to be a critical factor in inhibiting microorganisms in a film. Varying sensitivity of microorganisms might be expected to produce a selective effect on the microflora. Major factors in the ecosystem were investigated to determine their importance in controlling microorganisms.

MATERIALS AND METHODS

Sources of raw materials

Whole Grade "A" mixed raw milk, collected from the dairy plant of the Department of Food Science and Technology, was used for inoculating metal squares. The inherent microorganisms constituted the inoculum.

Surface material

Pieces of 1 cm^2 stainless steel were cut from commercial plate (#4 finish). The squares were washed by hand brushing using an alkaline solution and warm water, rinsed in distilled water, dipped in ethyl alcohol, placed in petri dishes, and sterilized in hot air *(1)*.

[1]Published as paper No. 3195, Journal Series, Nebraska Agricultural Experiment Station, Lincoln.

TABLE 1. EFFECT OF HUMIDITY ON MICROORGANISMS INCUBATED FOR 16 HR AT 25 C IN A FILM OF MILK ON STAINLESS STEEL.[1]

Initial inoculum	Humidity level			
	80%	86%	93%	100%
9.2×10^4	1.8×10^2	7.3×10^7	2.2×10^9	2.7×10^9

[1]Numbers of microorganisms per milliliter are based on the initial inoculum. After incubation the counts were based on the original weight of the sample then expressed as numbers per gram.

Control of humidity

Desired humidity was obtained using saturated chemical solutions in 1-2 liter desiccators *(9)*. The following chemicals were used at 25 C: ammonium sulfate - 80% RH, potassium-sodium-tartrate - 86% RH, and sodium tartrate - 93% RH. To obtain approximately 100% RH, the bottom of the desiccator was covered with distilled water and allowed to equilibrate. These humidity levels were checked by the use of a precision hygrometer.

Determination of water loss from milk films

For determining immediate loss of moisture, approximately 0.01 ml of whole milk was pipetted onto each test square on the balance pan of a direct reading analytical balance. Sample preparations and weighings were made in a room which had been equilibrated to the desired humidity. Weighings were made every 30 sec for the first 5 min and every 1 min for the next 5 min.

When 16 hr incubation was used, the squares were weighed and immediately placed in the desired humidity chamber for equilibration.

Evaluation of growth

The microbial content of milk samples for inoculation was determined by standard plate count *(1)*. An inoculum of 0.01 ml of milk was added to each previously sterilized metal square to cover approximately 80 to 90% of the upper surface and incubated in the appropriate humidity chamber. Fate of the microflora was evaluated after recovery by shaking the individual squares in a tube of sterile phosphate buffer *(1)* based on the method of Barnhart et al. *(2)*. Plating and counting procedures were those described in *Standard Methods for the Examination of Dairy Products (1)*.

From plates chosen for counting, 5 colonies were selected by random design from each of duplicate plates. Colonies were picked, transferred to litmus milk, and streaked on milk agar plates. Nature of the isolates was determined by gram staining, proteolysis on milk agar plates, catalase production, characteristic growth in litmus milk, gas production in brilliant green lactose bile broth, and spore formation. Combination of results of these tests was used to group the bacteria according to their physiological significance in the dairy industry *(4)*.

Presoiling of stainless steel surfaces

Pre-treatment of the stainless steel surface involved spreading a thin layer of milkfat on the test strip prior to adding raw milk. Following incubation for 16 hr in the 100% RH chamber at 25 C, counts and identification of isolates were made to determine the fate of the microflora.

Induced rancidity in a milk film

Approximately 0.01 ml of milk (combinations of raw and homogenized milk) was added to each test square and incubated at 25 C. Incubation was for 16 hr in a 100% RH chamber, after which the fate of the microflora was determined.

Simulated soil harborages

Stacking of squares, to simulate loose metal to metal fittings, was accomplished by drilling holes in two opposite corners of each square and then bending stainless steel wire so as to support the squares when placed one on top of the other.

The desired number of stainless steel squares and their support were tared prior to adding whole raw milk. Approximately 5 mg was applied between each square, avoiding spillage of sample over the edges of the square. Squares were manipulated using sterile forceps. Test assemblies were placed into open sterile petri dishes and incubated in the desired humidity chamber for 16 hr.

To simulate pressure fittings of metal to metal joints, in some experiments a sterile C-clamp was used to provide pressure on the above assembly. Other conditions were the same as those described for simple stacking.

RESULTS

Effect of relative humidity on water loss from a film of raw milk

The relationship between relative humidity and water loss from a film of milk was determined in 12, 48, and 67% RH at 25 C. Figure 1 shows the results from an average of 2 trials. Approximately 33% of the water was lost from the film at 12% RH in 10 min. Water loss from higher humidities was only

TABLE 2. EFFECT OF HUMIDITY ON THE FATE OF THE MICROFLORA IN A FILM OF RAW MILK AT 25 C.

Microorganisms	Initial inoculum		Distribution of isolates after incubation							
			80% RH		86% RH		93% RH		100% RH	
	No.	%	No.	%	No.	%	No.	%	No.	%
Streptococci	21	28	3	18	9	8	4	7	0	0
Micrococci	8	11	8	50	18	15	5	8	5	6
Bacillus sp.	0	0	0	0	0	0	0	0	0	0
Gram-positive-non-sporeforming rods	6	8	4	25	5	28	4	7	6	8
Coliform	18	24	0	0	14	12	13	22	17	21
Gram-negative proteolytic rods	13	17	0	0	55	47	26	43	24	30
Gram-negative non-proteolytic rods (Excluding coliforms)	10	13	1	6	17	14	8	13	28	35
Total number isolates	76		16		118		60		80	

TABLE 3. SELECTIVE EFFECT ON THE MICROFLORA OF A SINGLE LAYER OF RANCID MILK EXPOSED TO 100% RH FOR 16 HR.

Microorganisms	Distribution of isolates					
	Initial inoculum		80% RH		100% RH	
	No.	%	No.	%	No.	%
Streptococci	5	13	17	31	10	3
Micrococci	7	18	5	9	33	11
Bacillus sp.	1	3	1	2	2	1
Gram-positive non-sporeforming rods	5	13	12	22	17	6
Coliform	1	3	8	15	39	13
Gram-negative proteolytic rods	5	13	3	6	64	21
Gram-negative non-proteolytic rods (Excluding coliforms)	15	39	9	16	144	47
Total number isolates	39		55		309	

TABLE 4. EFFECT OF A PRESOIL OF FAT (WITH A SUBSEQUENT RAW MILK FILM) ON THE MICROFLORA WHEN EXPOSED TO 100% RH FOR 16 HR.

Microorganisms	Distribution of isolates					
	Initial inoculum		With fat presoil		Without fat presoil	
	No.	%	No.	%	No.	%
Streptococci	13	43	21	27	18	20
Micrococci	5	17	10	13	12	13
Bacillus sp.	0	0	0	0	0	0
Gram-positive non-sporeforming rods	2	7	4	5	11	12
Coliform	7	23	24	30	31	34
Gram-negative proteolytic rods	3	10	7	9	11	12
Gram-negative non-proteolytic rods (Excluding coliforms)	0	0	13	17	7	8
Total number isolates	30		79		90	

TABLE 5. EFFECT OF STACKING AND/OR CLAMPING OF STAINLESS STEEL SURFACES ON THE NUMBERS OF MICROORGANISMS AFTER INCUBATION FOR 16 HR AT 25 C AT DIFFERENT HUMIDITY LEVELS.[1]

Initial inoculum	80% RH incubation		100% RH incubation	
	Stacking	Clamping	Stacking	Clamping
2.0×10^4	1.7×10^4	2.1×10^4	1.9×10^9	6.6×10^8

[1]Numbers of microorganisms per milliliter are based on the initial inoculum. After incubation the counts were based on the original weight of sample then expressed as numbers per gram.

slightly less rapid. This rate of water loss produces an inhibitory level of solutes in a milk film before microorganisms could pass through the lag phase *(3)*.

Variations occurred in the quantity of water lost by duplicate squares due to different sample weights. Smaller samples consistently lost more water per unit weight, because relatively more surface was exposed to the atmosphere.

Equilibrium of a single layer of raw milk was determined by exposure to different humidities (80, 86, 93, and 100% RH) for a 16 hr period. Average results of 3 trials are given in Fig. 2. At 80% RH, there was an almost complete loss of water. Higher humidities allowed less loss thereby leaving adequate water for microbial growth. Some water was lost at the conditions used to simulate approximately 100% RH. This loss was attributed to a partial exchange of air at the time of adding the sample to the desiccator and exposure to air at the time of final weighing.

Effect of humidity on microorganisms in a film of milk on stainless steel

Milk samples of approximately 0.01 ml were incubated for 16 hr at 80, 86, 93, and 100% RH. The population was evaluated by total count and by observations on isolates. Results of an average of 3 trials are given in Table 1. Between 80 and 86% RH, there was a very critical humidity below which there was no bacterial outgrowth. Above 86% RH, there was an outgrowth of bacteria which increased with an increase in humidity.

Results from the observation of isolates obtained from total counts are given in Table 2. A selective effect can be seen for the two extremes (80 and 100%

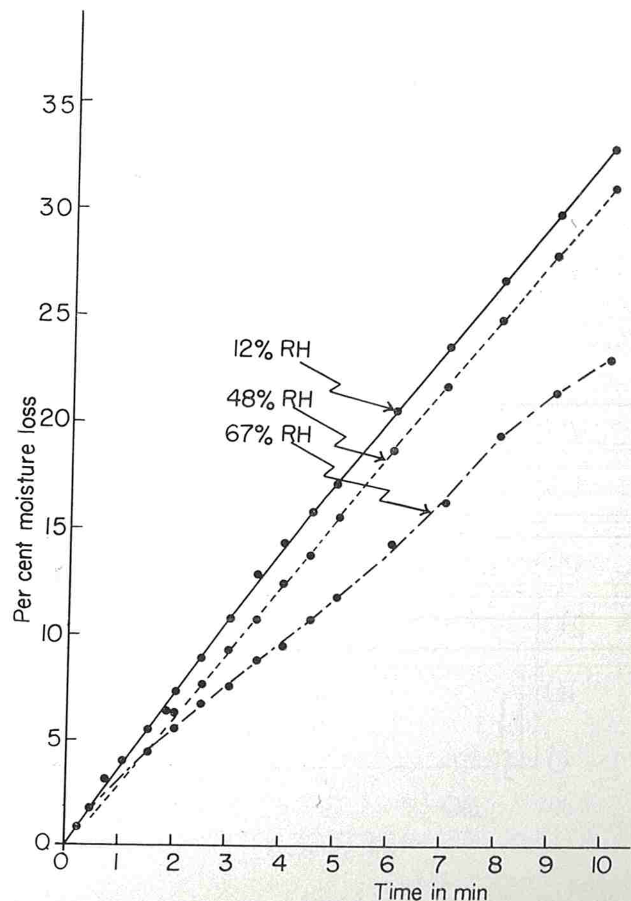

Figure 1. Effect of humidity on the desorption isotherm of a single layer of raw milk at 25 C on stainless steel surfaces.

RH). At 80% RH, the majority of bacteria surviving were gram-positive. At the other extreme (100% RH), approximately 50% of bacteria were gram-negative. Two intermediate levels (86 and 93% RH) had no apparent selective effect upon bacteria.

Lipolysis as a selective factor in milk soil

Raw and homogenized milk (a combination known to result in lipolysis) were mixed, applied to stainless steel squares, and incubated for 16 hr at 80 and 100% RH. An average of 3 trials (each trial consisting of 3 squares plated in duplicate) showed a total count of 2.2×10^9 microorganisms per gram when exposed to 100% RH, whereas exposure to 80% RH gave a total count of 5.6×10^4 per gram. The latter count was somewhat higher than when raw milk was exposed to comparable treatments.

Random isolates taken from the countable plates were observed and grouped into major classes (Table 3). Lipolytic activity and storage at the higher humidity suppressed the gram-positive bacteria to the benefit of the other organisms. On the other hand, there was no apparent difference in the nature of the microflora from the initial raw milk and that incubated at 80% RH.

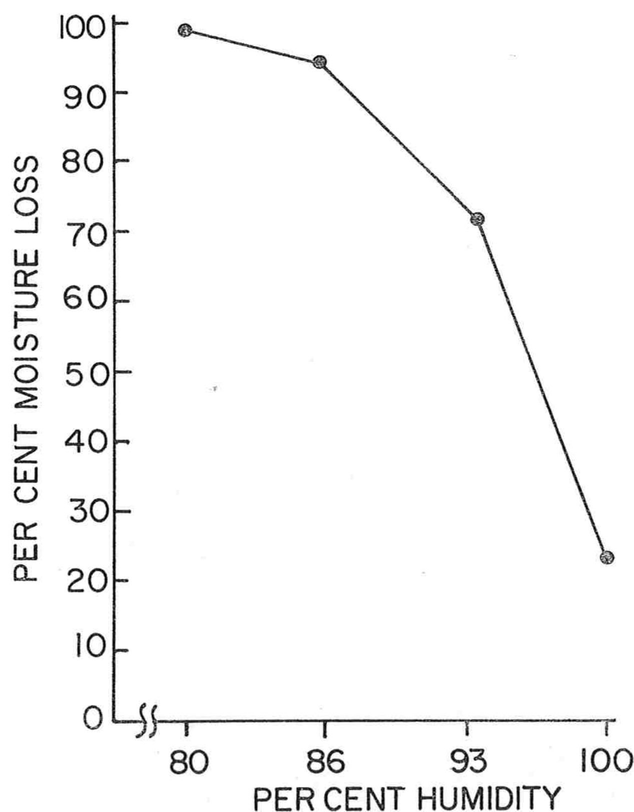

Figure 2. The effect of humidity on the extent of moisture loss during equilibration of a layer of raw milk for a period of 16 hr at 25 C.

Effect of presoiling with milkfat on the subsequent microflora

Presoiling stainless steel squares with milkfat was to determine if the microflora would be altered in a subsequent layer of raw milk. After 16 hr incubation at 100% RH, the microorganisms were recovered for a total count and further observations on the isolates.

An average of 3 trials showed the total count to be 45×10^8 bacteria per gram. Comparing counts when a presoil was involved to samples of a simple layer of raw milk, no difference could be seen. The results in Table 4 show that bacteria surviving on the squares presoiled with milkfat were essentially the same as those bacteria found in the initial raw milk. Therefore, it can be concluded that presoiling with milkfat has no selective effect on the fate of bacteria in a film of raw milk.

Effect of simulated harborages for the microflora.

Decreased surface exposure of soil to surrounding atmosphere was obtained by placing raw milk between stainless steel surfaces. Stacks of stainless steel squares were then incubated at 80 or 100% RH for 16 hr at 25 C. Results of an average of 3 trials at each humidity are given in Table 5. At 80% RH the protective effect was only adequate to maintain numbers equivalent to the original inoculum. At 100% RH, however, the outgrowth was similar to that attained with fully exposed films. Application of pressure to the metal to metal contact had no apparent effect on the results.

Observations to determine the nature of the microflora from harborages of simulated limited space indicated no marked selective effect from stacking or clamping at either of the two humidities (Table 6). There was a 5-log increase of bacteria at the 100% RH. Observation of the isolates indicated essentially the same microflora as compared to a fully exposed film. Likewise, at 80% RH fewer bacteria survived and the majority were gram-positive bacteria.

DISCUSSION

Humidity of the environment is an extremely influential factor in the ecosystem of food handling equipment. The system is dynamic because of evaporation and condensation which may occur simultaneously within an enclosed system because of temperature differentials within different parts of the equipment. Humidity influences the rate of water loss as well as the equilibrium water in a film. Rate of water loss and equilibrium level are also influenced by nature of interaction between a milk film and stainless steel. Both quantity of water and time available determine the fate of the microflora on dairy food processing equipment.

Table 6. Effect of limiting exposure (stacking and/or clamping) upon the microflora of raw milk which was exposed to different humidity levels for 16 hr at 25C.

Microorganisms	Initial inoculum		Stacking 80% RH		Stacking 100% RH		Clamping 80% RH		Clamping 100% RH	
	No.	%	No.	%	No.	%	No.	%	No.	%
Streptococci	11	37	11	19	7	9	3	19	1	3
Micrococci	0	0	15	25	13	16	7	44	0	0
Bacillus sp.	0	0	5	9	1	1	2	13	0	0
Gram-positive non-sporeforming rods	3	10	7	12	11	14	2	13	5	13
Coliforms	0	0	2	3	4	5	0	0	5	13
Gram-negative proteolytic rods	2	7	6	10	19	24	0	0	11	28
Gram-negative non-proteolytic rods (Excluding coliforms)	14	47	13	22	25	31	2	13	18	45
Total number isolates	30		59		80		16		40	

The influence of water activity on the growth of microorganisms has been widely studied, and the particular importance in foods has been pointed out by Mossel *(6)*. Most of the work has been based on systems designed to avoid the problem of equilibration rate. Equilibration rate is a prime factor in films of food. Furthermore, most work has been based on a homogenous environment which did not necessarily apply to the microenvironment of a film of milk on stainless steel, where a factor of surface interaction would be involved. Our work involved surface interaction and showed an outgrowth of bacteria at 86% RH and above. By mechanical reduction of the amount of sample exposed to low humidity, the drying rate of the film, even at 80% RH, was decreased to a point that there was a major increase in the population density.

The wide variation in the requirement for water by different bacteria indicates humidity of the ecosystem has a selective effect in a developing microflora. The data showed that at 80% RH the majority of the microorganisms surviving were gram-positive (50% micrococci; 25% gram-positive non-sporeforming rods; 18% streptococci indicating a nonselective outgrowth). Isolates from the samples incubated at 100% RH were of the same general types as were in the inoculum of raw milk, and the frequency of occurrence of the various types at the two test times was similar. Reduction of area of a film exposed to air reduced water loss and provided protection for the harborage of microorganisms so that soil exposed to 80% RH produced a microflora similar to that developed in 100% RH. Other examples of the sensitivity of bacteria to changes in the microenvironment arise from a consideration of presoiling with raw and homogenized milk. Interaction may involve lipolytic activity. Increase in surface activity resulting from lipolysis is more inhibitory to gram-positive bacteria thus contributing to a swing in the microflora to gram-negative bacteria.

Many elements of modern dairy food processing equipment may contribute to a selective outgrowth of microflora. Closed equipment such as that designed for circulation cleaning maintains a high humidity. Modern systems of extensive mechanical handling may contribute to lipolysis thereby producing a selective factor in the microenvironment. These factors may be involved in the apparent growing problem of gram-negative bacteria; e.g., psychrotrophic and coliform bacteria.

Acknowledgment

This investigation was supported in part by Public Health Service Research Grant No. FD-00116. Appreciation is due Francis D. Lavicky for technical assistance.

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A Research Note

THE EFFECTS OF STORAGE TEMPERATURES ON THE KEEPING QUALITY OF HALF AND HALF[1]

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Abstract

Half and half containing 10% butterfat was pasteurized at 90.6 C for 25 sec and bottled on a Regent filler in 0.5-oz aluminum foil topped plastic containers. Samples were analyzed after storage at 1.7, 4.4, and 7.2 C for 0, 1, 2, 3, 4, 5, and 6 weeks. Product quality decreased with an increase in the storage temperature and the half and half was judged acceptable for coffee after 4, 3, and 2 weeks of storage at 1.7, 3.3, and 7.2 C, respectively.

Dairy processors are interested in extending the shelf life of dairy products. They are particularly interested in half and half for coffee supplied in small 0.5-oz packages (coffee creamers), since many milk plant operators with a small volume in coffee creamers process once a week or obtain supplies of creamers from large processors. In either event they desire a shelf life of 4 weeks.

Recent information has been reported *(4)* on the shelf life at various storage temperatures of high-temperature short-time pasteurized milk, but apparently there is a lack of information on half and half. Extending shelf life of half and half through pasteurization at 90.6 C should be of interest to dairy processors. The objective of this study was to determine the storage life of pasteurized half and half at various storage temperatures.

Experimental Procedure

In three replicate trials, half and half containing 10% butterfat was homogenized at 700 lb. pressure at 82.2 C, pasteurized at 90.6 C for 25 sec, cooled to 4.4 C, and bottled on a Regent Model M 450 filler-sealer in 0.5-oz aluminum foil topped plastic containers. The samples were immediately placed in styrofoam containers, covered with ice, and transported to the laboratory. Sufficient samples were collected for analysis after storage at 1.7, 4.4, and 7.2 C (± 1 C) for 0, 1, 2, 3, 4, 5, and 6 weeks in household type refrigerators.

The samples at the end of each storage period were analyzed *(1)* for Standard Plate Count (SPC). The SPC procedure was modified in that 1 ml of product was distributed among 3 plates to provide for improved visibility of the bacterial colonies. The samples were checked for flavor by three experienced judges. "Potentially" psychrotrophic counts were determined by the method of Hankin and Dillman *(2)*. Feathering values were determined as specified in the Sealtest Laboratory Manual *(3)* in which half and half, with a value of 4 or above, is considered stable in most coffee under average conditions.

The data were averaged arithmetically except for SPC which were averaged geometrically. The latter included actual values noted on the plates even if there were < 30 colonies so that an indication of the actual count could be recorded.

Results and Discussion

The effects of storage temperature on the keeping quality of pasteurized half and half may be noted in Table 1. The SPC and "potentially" psychrotrophic count increased with an increase in time and temperature of storage, whereas the flavor score and feathering value decreased. The relatively low flavor scores of the samples even at 0 weeks of storage resulted from the pronounced cooked flavor caused by the pasteurization treatment. The comparatively low SPC's require some explanation in view of the low flavor scores and feathering values. This is particularly true at the 7.2 C storage temperature. One of the replicate trials had pasteurized half and half in which the SPC did not increase appreciably with age. Consequently, the values for this replicate, when averaged geometrically with the values for the other 2 replicates, markedly affected the average. Thus, comparison of the bacterial data with the remaining data which were averaged arithmetically appears misleading. The differences among replicates indicate that there was probably a variation in the thoroughness of cleaning and sanitizing the equipment. Such variations may be experienced in a commercial operation and will affect the length of time half and half may be stored.

The results indicate that half and half pasteurized at 90.6 C for 25 sec may maintain a desirable quality for up to 4, 3, and 2 weeks of storage at 1.7, 4.4, and 7.2 C, respectively. However, only minimal further loss of quality occurred for up to 6 weeks of storage at 1.7 C. These statements are based primarily on flavor scores of 36 or above and feathering values

[1]Scientific Contribution No. 475. Agricultural Experiment Station, University of Connecticut, Storrs.

TABLE 1. THE EFFECTS OF THE TEMPERATURE OF STORAGE ON KEEPING QUALITY OF HALF AND HALF

Weeks of storage	SPC/ml	"Potentially" psychrotrophic/ml	Flavor		Feathering value
			Score	Criticism	
			1.7 C		
0	2.3	<1	36.7	Cooked & feed	5.0
1	2.3	1.4	36.4	Cooked & feed	5.0
2	2.4	<1	36.3	Cooked & feed	5.0
3	1.0	<1	36.1	Cooked & lacks freshness	4.7
4	<1.	<1	36.1	" " "	4.3
5	2.0	1.0	35.8	" " "	4.3
6	1.8	<1	35.8	" " "	4.3
			4.4 C		
0	1.6	1.0	36.8	Cooked & feed	5.0
1	1.4	1.4	36.4	Cooked & feed	5.0
2	1.8	<1	36.3	Cooked & feed	4.3
3	1.8	<1	36.0	Cooked & feed & lacks freshness	4.7
4	24	10	35.9	Cooked & lacks freshness	5.0
5	33	18	35.4	" " "	4.7
6	77	53	34.9	" " "	4.3
			7.2 C		
0	3.2	1.6	36.9	Cooked & feed	5.0
1	27	15	36.6	Cooked & feed	4.7
2	3700	1300	36.2	Cooked, feed & lacks freshness	4.0
3	170,000	9700	35.4	Cooked, feed & lacks freshness	2.7
4	420,000	110,000	30.6	Slight sour & bitter	1.0

of 4 or above. Mention should be made that the utmost in sanitation must be maintained during filling operations. Furthermore, the sales force handling a product such as coffee creamers must be made aware of the importance of maintaining storage temperatures at 1.7 C or under. Low storage temperatures must be maintained from the filler to the consumer. Milk plant operators may well be advised to consider the newer sterile product that is aseptically packaged in place of 90.6 C-pasteurized half and half because of the improved shelf life that can be expected when properly processed and packaged.

ACKNOWLEDGMENTS

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USE OF TIME-TEMPERATURE EVALUATIONS IN DETECTING THE RESPONSIBLE VEHICLE AND CONTRIBUTING FACTORS OF FOODBORNE DISEASE OUTBREAKS[1]

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ABSTRACT

An investigation of an outbreak of *Clostridium perfringens* foodborne illness indicated that turkey or dressing prepared in a school kitchen was responsible. When turkey was again prepared in the kitchen, a bacteriological survey and a time-temperature evaluation were made of the thawing, cooking, chilling, and reheating to which the turkey, stock, or dressing were subjected. During thawing of 22-lb. turkeys in plastic wrappers and in paper bags at room temperature for 18 hr, neither internal nor surface temperatures reached a level at which *C. perfringens* could grow. Cooking the turkeys in a steamer or in a pot of boiling water raised internal temperatures to a level lethal to vegetative cells. The stock (in gallon jars and a large rectangular pan) and deboned meat (in similar pan) were stored overnight in a reach-in refrigerator. During storage the temperature of both were within a range so that *C. perfringens* spores could germinate and its vegetative cells multiply for 7-9 hr. The stock was later used in dressing, which when baked, reached internal temperatures known to destroy vegetative cells of *C. perfringens*. Meat and gravy, when reheated, did not reach such levels.

Clostridium perfringens, Staphylococcus aureus, and *Salmonella* were isolated from raw turkey; *C. perfringens* was isolated from cooked meat, stock, and kitchen equipment. Nine recommendations for heat destruction of vegetative cells, inhibition of bacterial growth during storage, and cleaning and sanitizing equipment are made. These recommendations will help prevent foodborne illness whenever turkey and dressing are prepared.

On numerous occasions foodborne disease outbreaks have followed the serving of turkey meat in school lunchrooms. In one instance, 8-24 hr after a school lunch, approximately 80% of the students and teachers of an elementary school in Georgia experienced a mild gastroenteritis characterized by abdominal cramps and diarrhea. *Clostridium perfringens* Hobbs type 11 was identified from 13 of 14 isolates that were cultured from stool specimens from five of the victims[3]. This finding, together with the clinical symptoms, suggested that *C. perfringens* caused the outbreak. No food was available for laboratory examination; however, the attack rates calculated from food histories incriminated either turkey or dressing as the vehicle.

Two days before the outbreak, frozen turkeys were thawed at room temperature at the school kitchen. The following day some of the turkeys were cooked in a steamer, others in a large stockpot on a range. The cooked turkey and stock were cooled on table tops, then transferred to refrigerators in which they were chilled overnight. The next morning, heated stock was mixed with bread crumbs and the resulting dressing was baked in ovens. Cold turkey meat was diced, gravy that was made from stock was poured over the meat, and the mixture was warmed in ovens.

The foregoing information, derived from the epidemiologic investigation, was inadequate to confirm either turkey or dressing as the responsible vehicle. Neither did it suggest which stage of preparation (thawing, cooking, chilling, dicing, reheating, serving) created circumstances leading to contamination, survival, or multiplication of *C. perfringens* which caused the outbreak. Therefore, a further investigation of the methods used, the product temperatures, and the type of contamination on the product and on equipment surfaces at each stage of the operation was conducted the next time turkey and dressing were prepared at the school. Data was interpreted

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[1]Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service of the Georgia Department of Public Health.

[2]Present Address: Carr-Scarborough Microbiologics, Inc., Forest Park, Georgia.

[3]Typing done by the Anaerobic Laboratory Unit, Bacterial Disease Branch, Laboratory Division, Center for Disease Control, Atlanta, Georgia.

in reference to the growth and survival of *C. perfringens* (3).

METHODS

Time-temperature measures

A continuous time-temperature recording was made of all stages of preparation—thawing, cooking, chilling, and reheating[4]. Frozen turkeys, weighing approximately 22 lb. each, were thawed in their original plastic wrappers on work tables at room temperature for 18 hr. Temperature recordings were made of three such turkeys. Another plastic wrapped, frozen turkey was put into a double kraft paper bag; the open end of the bag was then folded and taped shut. Thermocouples with stainless steel bayonet (type T)[5] sensors 4 inches in length were inserted into the breasts and legs of frozen turkeys. Holes for the sensors were made by first pushing a heated ice pick about 3 inches into the meat and then extending the holes with an unheated ice pick to a depth to accommodate the bayonet. The thermocouples were then pushed into the holes until the tips of the sensors were near the geometric center of each piece of meat. Flat, button-type (type T) thermocouples were attached to the surfaces of the frozen turkeys. Two wires were wrapped around each thermocouple lead near the sensor, and the sensor was held to the turkey surface by rubber bands passed around the turkey and hooked to the free ends of each wire. The room temperatures and the temperature inside the bag were measured with plain, welded-end (type T) thermocouples; the sensor ends were located approximately 2 inches from a turkey.

After the turkeys were thawed, they were cut into quarters and put into pans or a large stockpot. Bayonet-type thermocouples were inserted into the center of the thickest part of a piece of meat so that most of the metal shaft was buried in the meat. Surface temperatures were measured with flat, button-type thermocouples. These were held to the meat surface by attaching wires around the thermocouple lead near the sensor and threading and pulling these wires through the meat to produce tension. A plain welded-end thermocouple was placed a few inches above the meat to measure ambient temperature in the steamer. Water temperature in the stockpot was measured with a welded-end thermocouple at a depth midway between the bottom of the pot and the water surface. Turkeys were cooked in a pressure-type steamer or in a pot of boiling water for 4.5 to 5 hr. At the completion of cooking, hot stock was poured into several 1 gal glass (mayonnaise type) jars. Stock was also poured into a 5-gal (8-inch deep) rectangular metal pan. Bayonet-type thermocouples were inserted through the lids and into the geometric center of the stock in some jars and fastened in that position. The same type of thermocouple was fastened in the geometric center of the stock in the rectangular pan.

Thermocouples were left in each piece of the meat until that particular piece was deboned by workers wearing disposable plastic gloves. After the meat was deboned, thermocouples were reinserted so that the bayonet tip was in the thickest part of a large piece of meat. The meat was put into 6-inch deep rectangular pans to form a layer about 3 inches deep.

[4]Temperatures were recorded on Chart No. 5270 (type T), 0 to 500 F range, in an Electronic 16 multipoint recorder (Potentiometer), Honeywell, Fort Washington, Pa.

[5]Type T refers to a copper-constantan junction. Premium-grade wires were used.

The jars and pan of stock and cross-stacked pans of meat were put into a previously empty 2-door, forced-air, reach-in refrigerator. They completely filled this refrigerator. Initially the refrigerator air temperature was 34 F. Subsequent temperatures were measured a few inches away from the stored food, at two locations: one close to pans of meat, the other close to several jars of stock.

After being refrigerated overnight, the stock was put into large pots and heated on a gas range. Normally, the stock would have been heated only until congealed fat melted, but at the suggestion of the investigators, stock was heated until it reached a rolling boil. The temperature was recorded with a blunt-end thermocouple (type T) having its tip located in the center of the pot, midway between pot bottom and stock surface.

Some of the heated stock was added to refrigerated cornbread crumbs. Mixing was done by hand by workers who wore disposable plastic gloves. The dressing was then put into 2-inch deep baking pans. A plain welded-end thermocouple was inserted into the dressing, and the wire was secured with tape, so that the end of the thermocouple was in the middle of the pan halfway down in the dressing. Pans of dressing were baked in an oven set at 375 F. Oven air temperatures were taken with plain welded-end thermocouples about an inch above the dressing. Temperatures were recorded until the dressing was served.

Chunks of chilled meat were diced with a knife on a cutting board and put into a pan to which hot gravy (made from stock, flour and seasoning) was subsequently added. Pans were then put directly on an electrically heated serving table, into an oven (set at 300 F) to be held until required for serving, or on a range and heated until the mixture reached 165 F. The first two procedures were used during the outbreak. The last procedure was suggested by the investigators as an alternative method. In all situations, a 4-inch bayonet-type thermocouple sensor was inserted into one of the larger pieces (slightly less than 1 inch in thickness) of diced, cooked turkey. Temperatures were recorded until the last portion of turkey was served.

Microbiological examinations[6]

Swabs were taken from raw turkeys and from equipment at various stages of the preparation of the turkeys. Surfaces of the turkeys or equipment were swabbed simultaneously with four swabs. Each of two swabs was then put into cooked meat-dextrose broth. The third swab was put into a tube of tetrathionate broth, and the fourth swab was put into a tube of brain-heart infusion broth.

One of the tubes of cooked meat-dextrose broth was heated to 80 C (176 F) for 10 min, the other was not heated. Both tubes were then incubated at 37 C (98.6 F) for 24 hr. The incubated broth was streaked to modified McClung-Toabe egg yolk agar which was incubated anaerobically at 37 C for 24 hr. Suspicious colonies were picked. Some of the picked material was inoculated into cooked meat-dextrose broth and some used for gram staining. After overnight incubation at 37 C, the broth culture was subcultured to indol-nitrite medium and to motility medium to be confirmed or rejected as *C. perfringens*. Twenty grams of shredded cooked turkey meat were added to 100 ml of cooked meat-dextrose broth. Stock was shaken in the sample bags and then 1 ml was added to each of two tubes of cooked meat-dextrose

[6]Laboratory tests for *Salmonella* and *Staphylococcus* were performed by the Bacteriology Section, Laboratory Branch, Georgia Department of Public Health.

TABLE 1. FOODBORNE PATHOGENS ISOLATED DURING THE PREPARATION OF TURKEYS IN A SCHOOL LUNCH KITCHEN

Samples	Number of samples	Number positive		
		Clostridium prefringens	*Staphylococcus aureus*	*Salmonella*
Raw turkey (surface or cavity)	20	3	2[a]	4
Sink and drainboard	2	0	0	0
Knife	2	0	0	0
Glass jars	3	1	0	0
Meat block	1	1	0	0
Pan (after cooking)	1	0	0	0
Cooked turkey	2	1	0	0
Boned meat	2	2	0	0
Refrigerated meat	1	1	0	0
Stock, cooked	1	0	0	0
Stock after refrigeration	1	0	0	0
Stock after dissolving congealed fat by heat	1	1	0	0

[a]One isolate produced enterotoxin D.

broth. These were then examined in the same way as swab samples.

Each tetrathionate broth tube was incubated at 37 C overnight and streaked to brilliant green agar plates. After 18-24 hr incubation at 37 C, suspicious colonies were picked and stabbed in and streaked on triple-sugar-iron agar slants, incubated overnight at 37 C, and confirmed or rejected as *Salmonella* by urease testing and slide agglutination. Samples of stock were examined for *Salmonella* by adding 20 ml of stock to 200 ml of tetrathionate brilliant green tergitol broth. Fifty grams of turkey meat were added to 450 ml of sterile phosphate buffered diluent and homogenized in a blender for 2 min, 1 min at low speed and 1 min at high speed. Twenty milliliters of the homogenate were then added to 200 ml of tetrathionate brilliant green tergitol broth. The stock and meat cultures were tested for *Salmonella* in the same way as swab samples.

Each brain-heart infusion broth tube was incubated at 37 C overnight. The incubated broth was streaked to mannitol salt agar and incubated at 37 C overnight. Colonies that were suspected of being staphylococci were picked for gram staining and coagulase testing. (Coagulase production was tested by the tube method.) Selected coagulase-positive staphylococcal colonies were tested for enterotoxin production according to the method of Casman *(4)*. Samples of stock and homogenized turkey were streaked directly onto mannitol salt agar plates and examined in the same way as the swabs.

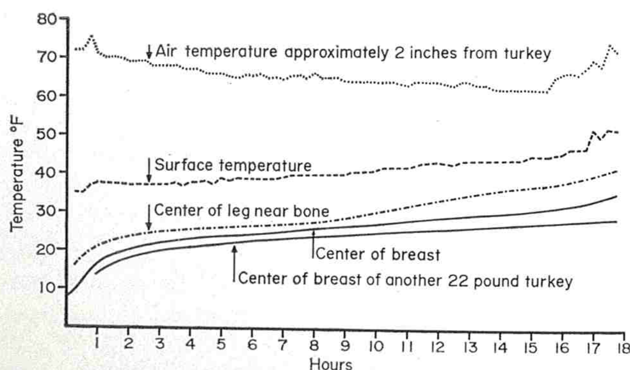

Figure 1. Surface and internal temperatures of 22 lb. turkeys (in packaging film) thawed at room temperature.

RESULTS AND DISCUSSION

Thawing

During overnight thawing at room temperature, the interiors and surfaces of turkeys weighing approximately 22 lb. each did not reach temperatures that were high enough to support multiplication of *C. perfringens* (Fig. 1). Only toward the end of the thawing period (after 17 hr) did surface temperatures exceed 50 F. After 18 hr at room temperature in a double kraft paper bag, a turkey was still frozen inside and had a surface temperature below 40 F (Fig. 2). Thawing turkeys in paper bags at room temperature appears to be satisfactory if the thawing time does not exceed 1 hr per pound *(5)*.

Raw turkeys

Of 20 microbiological swab samples from raw turkeys, three yielded *C. perfringens,* two yielded *Staphylococcus aureus* (a culture from one turkey produced enterotoxin D), and four yielded *Salmonella* (Table 1). Thus, raw turkeys can serve as vehicles for bringing foodborne pathogens into kitchens. If these organisms are not killed during cooking, and if they have an opportunity to multiply during subsequent storage, a foodborne disease outbreak could result.

Cooking

The surface temperature of turkeys that were cooked in a steamer rose rapidly and leveled off near the vapor temperature in the steamer. The geometric center of turkey breasts reached 165 F in 1 to 1.5

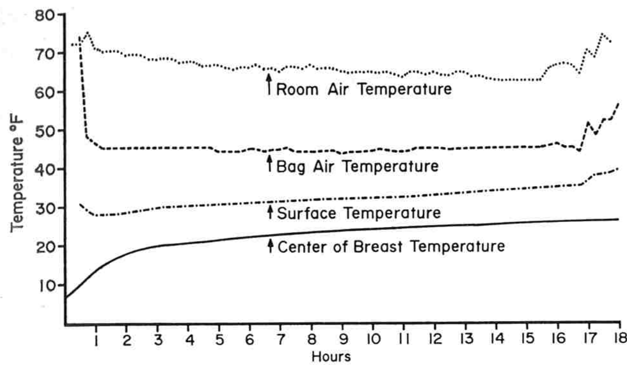

Figure 2. Surface and internal temperatures of 21.9 lb. turkey thawed in double kraft paper bag.

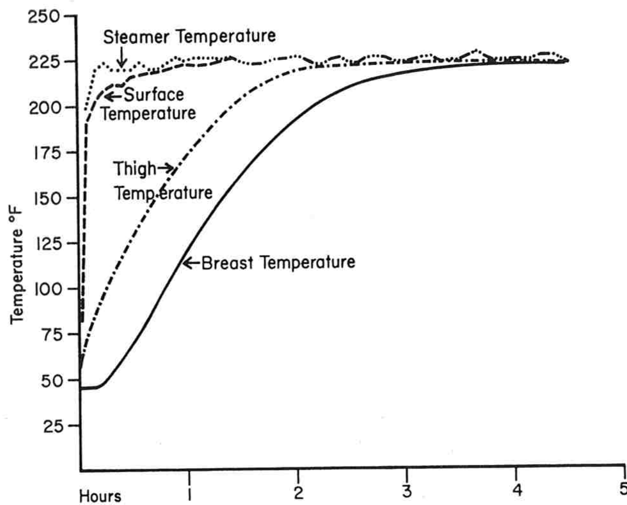

Figure 3. Internal temperature of turkey (breast and parts) cooked in steamer.

hr, and had almost reached the temperature (220 F) in the steamer during the last hour of cooking (Fig. 3).

The water around turkeys that were cooked in a stockpot began boiling about 25 min after the pot was put on the range. The geometric center of turkey breasts took almost 2 hr to reach 165 F and about 4 hr to reach the temperature of boiling water (Fig. 4).

Vegetative bacterial cells would be killed by these cooking procedures (Fig. 10), but heat-resistant *C. perfringens* spores could survive *(6, 7)*. Cooking meat establishes anaerobic conditions which are needed by *C. perfringens* to initiate growth. Cooking also activates spores which germinate when temperatures become favorable for bacterial growth *(2)*.

Clostridium perfringens — apparently having survived cooking in the spore state — was found in freshly cooked meat that had not yet been boned. This organism also was found in samples of meat from which the bones had been removed; its presence can be attributed either to survival of spores or to contamination during removal of bones, cutting meat or contact with utensils or pans.

Cooling

After cooking, the turkeys and stock sat on tables for as much as an hour. The turkeys were then deboned and the still-warm meat and stock were put into the reach-in refrigerator. The refrigerator air temperature rose from 34 F to more than 90 F and remained above 50 F for 10 hr (Fig. 5, 6).

Stock in gallon jars remained within the growth range for *C. perfringens* for 8.25 hr (Fig. 5). Stock in the large rectangular pan cooled somewhat more rapidly, but was still in the growth range for *C. perfringens* for almost 7 hr.

Turkey stock is an ideal growth medium for *C. perfringens.* Fat congealed on the surface of the stock in both the rectangular pan and the gallon jars. The jars had lids. These surface coverings, plus the fact that cooking establishes anaerobic conditions, tended to maintain anaerobiosis in the freshly cooked stock.

Turkey meat that was stored in layers approximately 3 inches deep in 6-inch deep pans also cooled slowly, remaining within a temperature range that would support multiplication of *C. perfringens* for 7.25 to 9 hr (Fig. 6). Turkey meat that was stored nearest the refrigerator fan in the fan's draft cooled faster than meat stored in other locations.

Clostridium perfringens will, after a lag period, multiply in the temperature range of 60 F to 122 F *(1, 3)*. If the bacterial lag period were 1 hr, for instance, these bacteria would have multiplied (rapidly from 122 to 86 F, and slowly at lower temperatures) over a period of 6 to 8 hr. During the 7 to

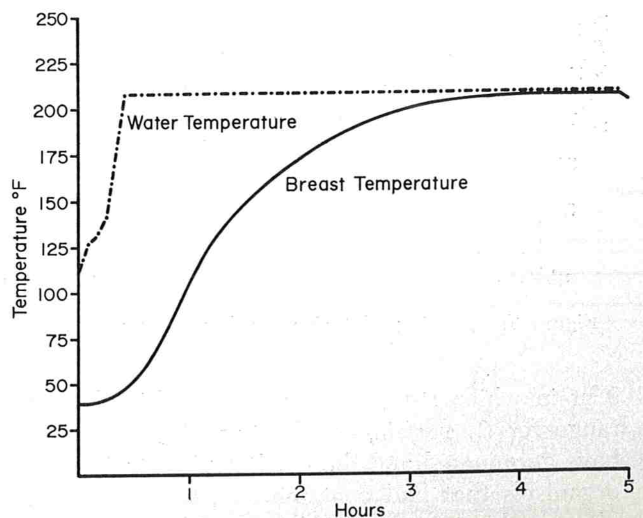

Figure 4. Internal temperature of turkey breast cooked in a stock pot on a gas range.

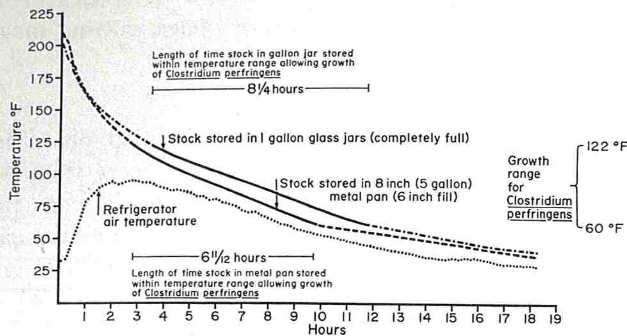

Figure 5. Cooling of turkey stock in a commercial-type reach-in refrigerator with the entire unit filled with hot turkey and stock.

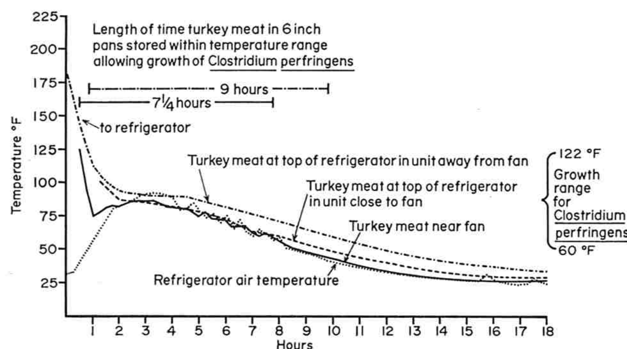

Figure 6. Cooling of boned turkey meat stored in 6-inch pans (3 inches deep) in commercial-type reach-in refrigerator that was filled to capacity with hot turkey and stock.

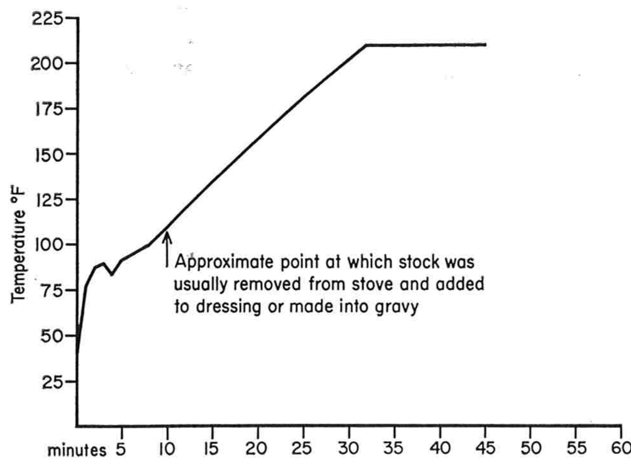

Figure 7. Heating of turkey stock on gas range.

9 hr in which stock or meat was within the growth range for *C. perfringens*, heat-resistant spores could have germinated and the resulting vegetative cells, or organisms that had contaminated the meat or stock after cooking, would multiply without competition from microflora that would normally be found on raw turkeys (Fig. 5, 6, 10).

Swab samples taken from a glass jar which was ready to receive the hot stock and from a meat block yielded *C. perfringens* (Table 1). This finding emphasizes the potential for contamination after cooking. This organism was also recovered from meat which had been refrigerated overnight.

Preparation and reheating

According to the kitchen supervisor, stock was routinely heated until congealed fat on the surface melted (at about 110 F) (Fig. 7). Because of the potential for bacterial growth during overnight storage in the refrigerator, the investigators suggested that stock be brought to a boil before it was used to make gravy or dressing. This practice would kill any vegetative cells that may have multiplied during storage and would prevent an outbreak of *C. perfringens* foodborne illness from this source.

When meat and gravy were mixed and put on an electrically heated serving table, the mixture never reached a temperature that could kill foodborne pathogens (Fig. 8). When meat and gravy were mixed and put into an oven for warm holding, the mixture reached a temperature of about 150 F in 1 hr, and at the time of serving, the temperature had risen to about 160 F (Fig. 8). The time-temperature relationship that resulted could kill vegetative bacteria. Some pans of meat, however, were not kept in the oven for an hour. If stock had not boiled prior to pouring it over the meat, the final temperature would have been several degrees lower, perhaps no more than 100 F in many instances. This temperature, of course, would not kill *C. perfringens* and, given time, would permit multiplication. Other than long periods in ovens, another safe procedure for handling meat and gravy would be to heat the two together on a range, stirring occasionally until the meat reaches 165 F, or, if a bayonet- type meat

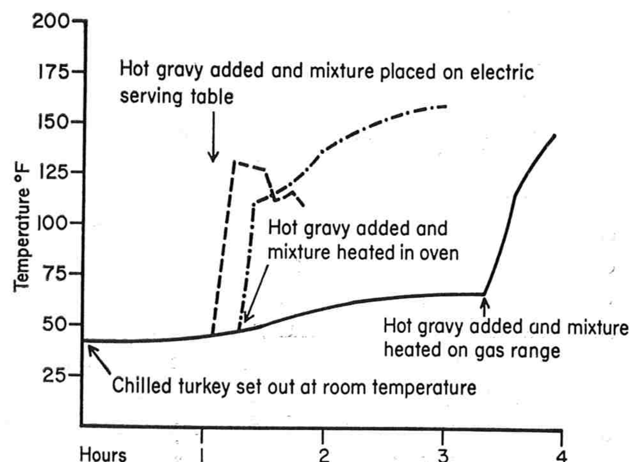

Figure 8. Preparation and cooking of chilled turkey meat.

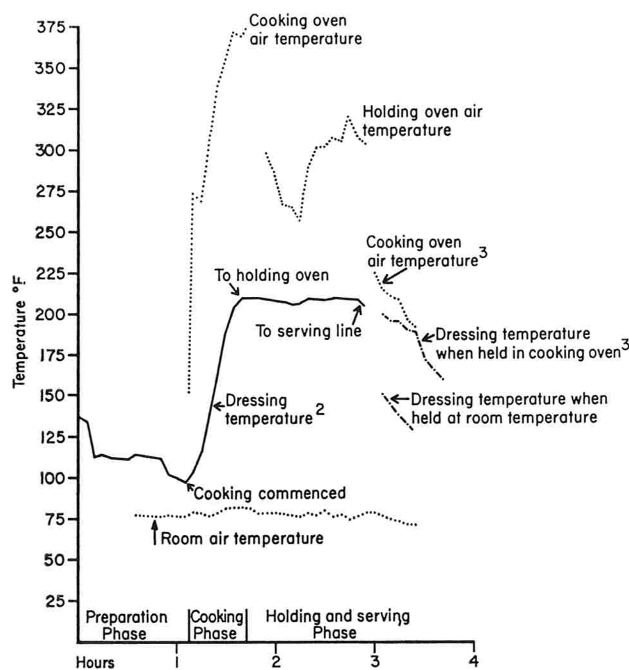

Figure 9. Preparation, cooking, holding, and serving of turkey dressing made from bread crumbs, corn bread crumbs, and turkey stock. [2]Two inches deep in 4-inch pans. [3]Temperature turned off.

thermometer is not available, until the gravy boils (Fig. 8).

During preparation of dressing, bacteria could possibly grow for more than an hour. The temperatures reached during baking, however, would kill vegetative cells, and even if spores germinated or if the baked dressing were recontaminated, there would not be time for multiplication before serving (Fig. 9, 10). If dressing were prepared under conditions observed in this investigation (Fig. 9), it could not cause an outbreak of *C. perfringens* foodborne illness.

Outbreak situation

The time-temperature relationships which probably occurred during the outbreak are illustrated in Fig. 10. These relationships are based on the observed time-temperature recordings (Fig. 1-9) and information provided by the school lunch supervisor during the epidemic investigation and the subsequent survey. Fig. 10 indicates that meat and gravy, but not dressing, were responsible for the illness. There is evidence that spores survived cooking, and it is possible that contamination was transferred to the meat or stock from containers and utensils after cooking. Ample opportunity followed for bacterial growth in both turkey meat and stock during storage, and the heat treatment prior to serving was, for the most part, inadequate to kill vegetative cells of *C. perfringens*. However, vegetative cells would have been destroyed by the temperature reached in the dressing during baking.

Recommendations

The most critical aspect of preventing foodborne illness from turkeys is adequate time-temperature control. Ideally, turkeys should be served immediately after cooking, but in school lunch or food service operation this is seldom practicable. The next best procedure would be to cool the turkey and stock to 50 C or below within 2 to 3 hr after cooking and to reheat both to at least 165 F just before

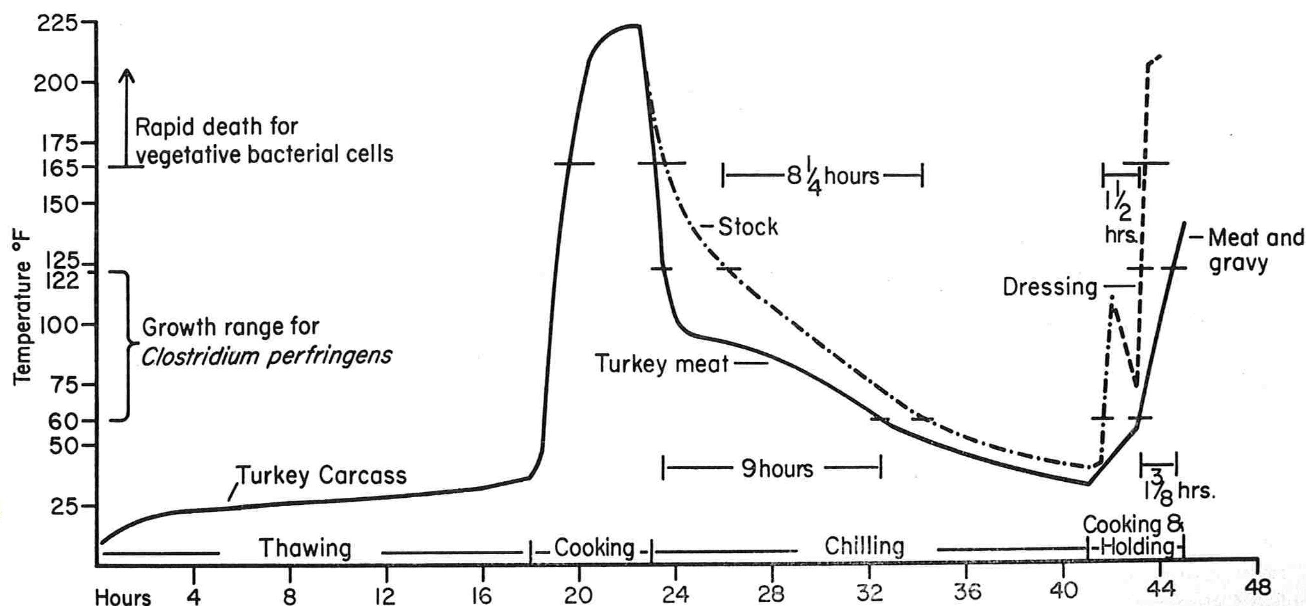

Figure 10. Illustration of possible time-temperature relationships during turkey preparation in a school lunch kitchen.

serving. Because of equipment limitations, schools are seldom able to accomplish rapid cooling of 10 to 12 large turkeys unless careful attention is given to the process. Therefore, the following recommendations should prove helpful in the prevention of foodborne illness from *C. perfringens*, as well as other agents, whenever turkey and dressing are prepared:

(*a*) Cook turkeys until the internal breast temperature reaches at least 165 F, preferably higher.[7]

(*b*) Wash hands and use disposable plastic gloves when deboning, dicing, or otherwise handling cooked turkey.[7]

(*c*) Separate turkey meat and stock before chilling.[7]

(*d*) Chill the turkey and stock as rapidly as possible after cooking. *Don't assume that simply getting them into the refrigerator will be satisfactory!* If the refrigerator is too small or the temperature too high or if the pans used to store meat or stock are too large, other procedures for rapid chilling should be employed prior to refrigeration. Procedures such as a running water bath, an ice or slush bath, continuous agitation in a mixer, or placing in a freezer for an hour or so increases the rate of cooling. Don't allow foods to simply cool to room temperature before refrigerating them.

(*e*) Use shallow pans (4 inches deep or less) for storing stock and deboned turkey in refrigerators. These pans should be cleaned and sanitized prior to use. The pans should be filled only to about one-half the depth.

(*f*) Thoroughly wash and sanitize all containers and equipment which previously had contact with raw turkeys before using for cooked meat or stock.[7]

(*g*) Bring stock to a rolling boil before making gravy or dressing. (If giblet gravy is made, it should also be boiled for about 10 min after giblets are added.)

(*h*) Bake dressing until all portions reach a temperature of 165 F or higher.[7]

(*i*) Just prior to serving, heat turkey pieces submerged in gravy on a range until the largest portions of meat reach 165 F. If a thermometer is not available to check temperatures, bring the mixture to a boil, stirring occasionally to ensure uniform heating. Covering the pan with a lid can be expected to shorten the heating time required.

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[7]Procedure already practiced in the kitchen where the study was conducted.

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A Research Note

A CASE STUDY: EFFECT OF MOLD ON GROWTH OF COAGULASE-POSITIVE STAPHYLOCOCCI IN CHEDDAR CHEESE

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ABSTRACT

A large lot of Cheddar cheese was refused by a food processing plant on the basis of extensive mold growth. The mold belonged to the genus *Penicillium*. There was a gradual increase in pH from the center of the cheese (pH 5.48) towards the moldy surface (pH 7.80). All portions of the cheese yielded staphylococci, but those isolated (100-1000/g) from the subsurface moldy areas were all coagulase-positive. Coliforms and salmonellae were absent. Aflatoxins could not be detected but the isolated coagulase-positive staphylococci were able to produce enterotoxin D.

A large quantity of Cheddar cheese, made in a modern Canadian cheese factory was refused by a food processing company because of extensive surface mold growth and consequent possible health hazards. Review of vat records disclosed that the milk for production of this cheese had been given a sub-pasteurization treatment of 140 F for 16 sec, which is common practice in Ontario cheese factories for the production of raw milk Cheddar cheese. The whole manufacturing process was normal and the milling acidity was 0.81%. The 3,000 lb. vat lot of curd had been pressed into 240 lb. blocks, which were later subdivided into 40 lb. portions and wrapped in "Parakote." This cheese had received a first grade (40/93) by the graders of the Canada Department of Agriculture *(1)*. During curing over a year period, abundant mold growth, of the genus *Penicillium*, had developed on some surfaces. For this reason, the prospective buyer felt that the cheese was unacceptable. Analysis of representative samples of the cheese by this laboratory showed a normal fat (33%) and moisture content (35.16%). Tests for the presence of aflatoxins were negative. Organoleptic evaluation confirmed the earlier score of 40/93. Microbiological tests were negative for coliforms and salmonellae, but 630 staphylococci per gram were detected of which 30% were coagulase-positive. Because of possible effect of mold growth on the pH of the cheese, which in turn would influence localized growth and toxin production by contaminating staphylococci, samples were taken from different portions of the cheese, both from the center and moldy surface areas. All portions yielded staphylococci, but coagulase-positive staphylococci could only be found near the moldy surface. Also, the total number of isolates was always higher in the subsurface samples (center portions 10-100/g, subsurface portions 100-1000/g).

Successive slices of about 1 mm thick, starting from the outside of the cheese had a pH of 7.8; 7.21; 7.47; 6.98, and 6.7, respectively. The pH at the center of the cheese block was 5.48. Thus, there seemed to be a correlation between pH and growth of coagulase-positive staphylococci. Isolates of coagulase-positive staphylococci were further tested *(2)* for their ability to produce enterotoxins A, B, C, and D. Small amounts of enterotoxin D were produced by all isolates.

This case study shows that when mold is allowed to develop on the surface of cheese during prolonged storage, it can cause a gradual increase in pH favoring localized growth of contaminating staphylococci and therefore possible production of toxin. These limited data seem to amplify the findings by Zehren and Zehren *(3)* who reported that insufficient acid development in cheese favors growth of staphylococci and toxin production.

It was also surprising that the organoleptic quality of this cheese did not deteriorate with the marked increase in pH. Removal of the surface mold growth prior to shipment would have never aroused any suspicion by the buyer about the real quality of the cheese.

ACKNOWLEDGEMENT

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A Research Note

SURFACE TENSION OF COMMON AQUEOUS AND ORGANIC PHASES IN FOOD EMULSIONS[1]

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ABSTRACT

Surface tensions of food grade oils, sodium chloride, sucrose, and dioctyl sodium sulfosuccinate were studied in a temperature range of 20 C to 50 C. Solutes in aqueous solution were varied in concentration. Regression equations were determined for each oil and solution except dioctyl sodium sulfosuccinate which exhibited a Type III surfactant response.

"All emulsions are thermodynamically unstable systems since the globule size progressively increases" *(4)*. J. Willard Gibbs is reported to have said that the only time an emulsion is stable is when it is broken *(2)*. Food emulsions constitute extremely complex systems of immiscible phases. To predict the "practical" stability of these systems, an evaluation of their properties is indicated. One of the first properties to be evaluated is the surface tension of the phases involved in the emulsion. Since relatively few systematic studies have been reported, the authors have investigated the effect of solute concentration and temperature on aqueous solutions of sodium chloride, sucrose, and dioctyl sodium sulfosuccinate.

MATERIALS AND METHODS

Fresh samples of corn, cottonseed, mustard, MCT (medium chain triglycerides), olive, peanut, safflower, "high oleic acid" safflower, and soya oils were studied. Reagent grade sodium chloride, sucrose, and dioctyl sodium sulfosuccinate (Complemix 50, American Cyanimid) were used for preparation of the aqueous phases studied.

Surface tensions were measured by the capillary rise method *(1, 3)* using glass capillaries with a mean radius of 0.0325 ± 0.0015 cm. The radius of each capillary was determined by measuring the capillary rise of liquids of known surface tension. Water, benzene, and N-hexane were employed at 20 C for this purpose. Their surface tensions are 72.75, 28.88, and 18.43 dynes/cm, respectively *(7)*.

The equation for the calculation of capillary radii and surface tensions is:

$$Y = 1/2 \text{ hdrg} = 1/2 \, (h_2 - h_1) \text{ drg}$$

where Y = surface tension, dynes/cm
h_2 = height of liquid in capillary, cm
h_1 = height of main menicus, cm
d = density of liquid, grams/cm^3
r = capillary radius, cm
g = gravity constant, 981 cm/sec^2

The temperature was maintained by a constant circulating water bath at ± 0.1 C at each temperature. The contact angle at the meniscus was assumed to be equal to zero. Densities were measured by weighing 50 ml aliquots in tared volumetric flasks. All statistical analyses were performed according to Ostle *(5)*.

TABLE 1. REGRESSION EQUATION, LACK OF FIT TEST, AND STANDARD DEVIATION OF THE SLOPE FOR THE RELATIONSHIP BETWEEN (20-50 C) AND THE SURFACE TENSION OF THE VEGETABLE OILS STUDIED.

Oil	Regression (Y = a + bT) Intercept	Slope	S_b	Lack of fit
Corn	33.845	−0.054**	0.009	ns
Cottonseed	33.001	−0.036**	0.009	ns
Mustard	34.354	−0.053*	0.021	ns
MCT	30.140	−0.047**	0.006	ns
Olive	33.377	−0.057**	0.016	ns
Peanut	33.312	−0.047*	0.017	ns
Safflower	33.980	−0.054**	0.015	ns
Safflower (high oleic acid)	34.397	−0.076**	0.015	ns
Soya	34.598	−0.062**	0.006	ns

T = °C
ns = Not significant at 95% level
* = Significant at 95% level
** = Significant at 99% level
S_b = Standard deviation of slope

TABLE 2. ANALYSIS OF VARIANCE FOR MULTIPLE REGRESSION OF CONCENTRATION (C) AND TEMPERATURE (T) ON THE SURFACE TENSION OF SODIUM CHLORIDE AND SUCROSE SOLUTIONS.

Source	d_f	Sucrose Mean square	Sodium chloride Mean square
X_0	1	302,703.08	306,858.25
C/X_0	1	5.93**	4.16**
T/C, X_0	1	73.05**	37.30**
Residual	57	0.46	0.80

* = Significant at 95 % level
** = Significant at 99% level

RESULTS AND DISCUSSION

The resulting regression equations for each of the oils studied is given in Table 1. Three replications were made for each oil at 20, 25, 35, and 50 C. Each replication was an average of three determinations. The temperature effect was found to be significant at 95 or 99% level of significance. All of the regressions were found to fit a linear model as evidenced by the non-significant lack of fit tests. The intercepts and

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TABLE 3. SURFACE TENSION[a] OF DIOCTYL SODIUM SULFOSUCCINATE SOLUTIONS AT VARIOUS CONCENTRATIONS AND TEMPERATURES.

Concentration % (w/w)	Temperature (C)			
	20	25	35	50
0.01	59.81 ± 4.06	59.88 ± 3.09	57.69 ± 1.86	56.84 ± 2.45
0.025	53.15 ± 2.79	53.15 ± 4.42	51.41 ± 1.91	53.18 ± 1.63
0.05	42.17 ± 4.02	44.87 ± 1.61	43.41 ± 3.10	39.72 ± 1.66
0.1	38.62 ± 2.01	39.63 ± 1.47	38.09 ± 1.14	37.65 ± 1.50
0.175	32.80 ± 2.14	32.84 ± 1.34	31.14 ± 0.41	31.24 ± 1.09
0.25	30.29 ± 1.26	30.96 ± 0.63	30.78 ± 0.45	29.92 ± 1.04
0.5	28.90 ± 0.44	29.01 ± 0.44	28.79 ± 0.15	28.71 ± 0.30
1.0	28.32 ± 0.35	27.74 ± 0.61	27.48 ± 0.46	27.42 ± 0.29

[a]Standard deviations presented for each value.

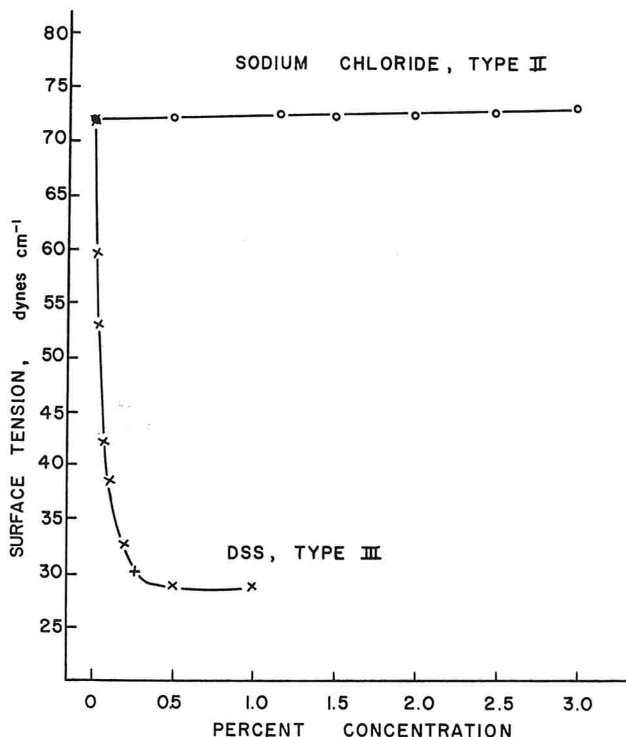

Figure 1. Relationship between the concentration of dioctyl sodium sulfosuccinate and the resulting surface tension at 20 C.

slopes were very similar with the exception of the MCT intercept. All of the slopes were negative, conforming to the fact that the surface tension of liquids decreases with increasing temperature *(1)*. At 20 C values of 32.24 and 32.28 dynes/cm were calculated for olive oil and cottonseed oil, respectively. According to Becher *(1)*, the surface tension of olive oil (20 C) is 33.0 dynes/cm and according to Swern *(6)*, the surface tension of cottonseed oil, at 20 C, is 35.4 dynes/cm. Neither author gives more than these figures in the 20-50 C range.

Sucrose solutions were studied at concentrations of 1.0, 3.0, 5.0, 7.5, and 10.0% (w/w) and at temperatures of 20, 25, 35, and 50 C. Sodium chloride solutions were evaluated at these same temperatures, but in concentrations of 0.5, 1.0, 1.5, 2.0, and 3.0% (w/w). Three replications (each consisting of the average of three determinations) were made for each concentration-temperature combination.

Table 2 gives the analyses of variance obtained for the regression of concentration and temperature on the surface tension of the sucrose and sodium chloride solutions tested. The resulting multiple regressions were:

$$\text{Sucrose: } Y = 73.636 + 0.099C - 0.096T$$
$$\text{Sodium Chloride: } Y = 73.261 + 0.306C - 0.069T$$

where Y = predicted surface tension, dynes/cm
C = percent solute (w/w)
T = °C

In both instances temperature was inversely related to surface tension while concentration was directly related. At a given temperature the electrolyte was approximately three times as effective as sucrose in raising the surface tension. All coefficients were found to be significant at 95 or 99% significance level. These linear relationships conform to the Type II curve *(1)* assigned to electrolytes and sugars.

Aqueous solutions of dioctyl sodium sulfosuccinate were evaluated at concentrations of 0.01, 0.025, 0.05, 0.1, 0.15, 0.25, 0.5, and 1.0% (w/w) at temperatures of 20, 25, 35, and 50 C. Once again, three replications (each representing the average of three determinations) were made for each concentration-temperature combination. The results are summarized in Table 3. The relationship between the concentration of dioctyl sodium sulfosuccinate and the resulting surface tension at 20 C is shown in Fig. 1. This curve compares well with the Type III curve *(1)* assigned to surfactants, i.e., compounds which drastically lower the surface tension of liquids when in extremely low concentration.

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BACTERIAL FLORA OF PREFROZEN DRY-CURED HAM AT THREE PROCESSING TIME PERIODS AND ITS RELATIONSHIP TO QUALITY

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Abstract

Hams frozen, stored, thawed, and dry-cured were sampled microbiologically after thawing, after curing, and after 30 days aging at about 34.5 C and about 62.5% relative humidity. Selective isolation techniques were made on samples from the surface of the butt, shank, and cushion surfaces and internally from the butt, shank, cushion, knuckle, *semitendinosus*, and *biceps femoris* of each ham. Surface samples and intramuscular shank samples contained the greatest total number of microorganisms. Coagulase-negative *Staphylococcus aureus* were present in highest numbers at each ham location and at each processing time. The number of microbial cells per gram increased after curing above that in the fresh hams and then decreased generally after aging. No adverse effects on quality were observed and quality appeared unaffected as bacterial numbers changed. Lancefield D group streptococci, *Staphylococcus epidermidis*, and species of *Micrococcus, Proteus*, and *Bacillus* were present in low numbers within the hams. Moisture content appeared to be the limiting factor of bacterial growth; however, salt tolerant microorganisms were the predominant flora. Variations in pH did not appear to influence the microbiology of the hams. Bacteria of public health significance were not isolated on or within the ham tissues.

Country style (CS) hams are produced commercially in sizeable volume primarily in southeastern United States. Production has increased rapidly in this area during the last 10 years and the trend has been to reduce the processing time to a minimum while attempting to improve and standardize quality. Few studies have been conducted on the microbiology of CS hams under conditions presently used for processing.

In this study thawed pork was used and it may be more permeable to curing ingredients and microorganisms than is fresh unfrozen pork. Frozen pork becomes more porous and ultimately more permeable to curing solutions than pork that has not been frozen *(1)*. However, salt diffusion rate in meat frozen at −20 C for 10 days and then thawed was compared with fresh meat and no significant difference in diffusion was observed at −2 C *(31)*. A decrease was shown in the number of *Staphylococcus aureus* in ground pork after 18 months of frozen storage *(21)*. Recovery of low numbers of *Salmonella* after freezing and storage for one month has been reported *(19)*. These low numbers may have been due to injury of cells, since it was reported that freezing injures *Salmonella, gallinarum (27)*. However, these workers found no relationship between injured and uninjured cells in relation to their pathogenicity.

A study with CS hams *(3)* showed that the fresh product contained species of *Sarcina, Micrococcus*, and *Bacillus* on the exterior surface. The interior tissues contained one yeast species, but no *Sarcina*. Control hams sampled after 2 weeks curing contained the same microorganisms as fresh indicating they would withstand high salt content.

The objectives of this study were to determine genera and the relative numbers of microorganisms present in hams that had been frozen and stored, then sampled after thawing, after curing, and after aging and to determine the relationships to quality.

Materials and Methods

Hams from 15 hogs grown on the University farm were allotted to an incomplete block design with the sampling times being treatments and the pairs of hams the blocks (Table 1). Three pairs of hams were required for one replication and a total of five replications for the experiment. The cutting procedure outlined *(10)* was modified in that the ham skin and subcutaneous fat were removed according to commercial procedure. Hams were then individually packaged, frozen, and stored for approximately 12 months at −17.8 C. Hams were removed from the freezer and allowed to thaw in the package for 7 days at 3 C.

Subjective evaluations of quality were made on representative samples at each processing time (Table 2). Hams allotted to the curing and aging treatments were rubbed with a mixture of 3.6 kg table grade granulated NaCl, 0.9 kg white cane sugar, and 85 g KNO_3 per 45.36 kg of ham. This mixture was applied at the rate of 101.1 g per kg of ham with one-half of the mixture applied on the first day and the remainder on the fifth day of cure. The hams were cured two days per 0.45 kg at 3-5 C. After curing, hams were soaked in free running cold water, placed in stockinette material, and hung shank down for 1-2 hr to dry. Hams were then weighed, evaluated for quality, and smoked at 30 C to a light mahogany color by burning moistened hardwood sawdust. Aging was accomplished with hams hung shank down at about 34.5 C and about 62.5% relative humidity for 30 days.

Hams in each treatment (Table 1) were sampled for micro-

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TABLE 1. ALLOTMENT OF HAM PAIRS TO TREATMENTS

Replication	Processing time		
	Thawed	Cured	Aged
	23-11-R[1]	23-11-L[2]	
1	24-1-R		24-1-L
		57-2-L	57-2-R
	57-4-L	57-4-R	
2	52-1-R		52-1-L
		22-1-L	22-1-R
	23-8-R	23-8-L	
3	24-4-L		24-4-R
		51-1-R	51-1-L
	51-7-R	51-7-L	
4	52-11-R		52-11-L
		23-3-L	23-3-R
	52-9-R	52-9-L	
5	51-5-R		51-5-L
		52-3-R	52-3-L

[1]R = right.
[2]L = left.

bial analyses. Surface samples cut about 2 mm thick were taken from the ham butt (BS), between the illium and the external fat layer or skin side; shank samples (SS) from cut surface of both sides of tibia; and cushion samples (CS) immediately ventral to the aitch bone (ischium). Intramuscular (internal) samples were taken from the butt (BI), shank (SI), and cushion (CI) areas. Muscles represented from the BI included the *gluteus medius, gluteus accessorius, gluteus profundus,* and *rectus femoris;* muscles of the SI included *deep digital flexor, extensor digiti quinti,* and *extensor digiti quarti;* and muscles of CI included the *semimembranosus, adductor,* and *gracilis.* The *semitendinosus* and *biceps femoris* muscles comprised the area designated SB. The knuckle sample internal area was designated KI and included the muscles *vastus lateralis, rectus femoris, vastus intermedius,* and *vastus medialis.*

All muscle areas were dipped in 95% ethyl alcohol, flamed, and trimmed twice; 11 g aliquots were aseptically removed and placed in sterile Waring blender cups. The samples were blended with 99 ml of 0.85% NaCl for 5 min (1:10 dilution) from which appropriate dilutions were prepared.

Isolation procedures and media used are shown (Table 3) and generally followed approved methods *(2).* Some additional information appears necessary to supplement that given in Table 3. APTA (APT + 0.03% NaN_3) medium used for *Streptococcus* selection was prepared by adding 1 ml of 3% sodium azide to 99 ml of APT agar (Difco). Commercially prepared media do not appear to be as effective in containing and isolating fungal colonies as acidified yeast nitrogen base agar (YNB) *(24).* After sterilization YNB was acidified with 1.6 ml of 5% tartaric acid per 100 ml of medium *(14).* For *Salmonella,* 1 ml and 0.1 ml of the 1:10 dilution were used to inoculate the lactose enrichment broth tubes *(23).* Growth from appropriate TSI slants was emulsified in a drop of saline on a glass slide and mixed with a drop of polyvalent antiserum *(29).*

Preplating treatment for spore-forming aerobes and anaerobes included the aseptic transfer of a 1:10 dilution from each area of the ham into sterile dilution bottles immediately after homogenizing and placing them in an 80 C water bath for 10 min. Inoculation into the appropriate media (Table 3) was then accomplished.

The liver broth and liver agar media were prepared as described elsewhere *(9)* except that the ground liver was soaked in 4.5 C water for 24 hr. Following autoclaving, the broth with the added compounds (10 g peptone and 1 g K_2HPO_4) was heated in a 100 C water bath for 45 min, adjusted to pH 7.0 with 1 N NaOH, and filtered. Two percent agar was added to the broth when this medium was needed. The media were used in the study of anaerobes.

Petri plates and culture tubes containing the inoculated liver media were placed in Trobal anaerobic jars (Model AJ-2) without inverting the plates but loosening the tube caps. The jar was evacuated, flushed with hydrogen, and incubated 72-96 hr at 30 C. The jar lid held a catalyst for activating formation of water. An externally attached oxidation-reduction indicator showed the state of anaerobiosis throughout the incubation period.

Plate counts were made on the incubated plates containing 30-300 colonies and the number present was expressed per gram of ham. Discrete colonies with morphology typical for the respective selective media were isolated for identification. Since the culture media APT and TGE are not selective, representatives of all colonies that appeared different were selected from each plate and duplicate selections were isolated from serially inoculated plates from the same sample.

Isolates were streaked on non-selective media to assure purity of cultures and then transferred to brain heart infusion agar, incubated 24 hr at 30 C and then stored at 4 C until confirmation procedures could be conducted (Table 3). Gram reactions and cell morphology were determined from 24-48 hr growth on brain heart infusion agar slants. Various differential media were inoculated from the stock cultures and results of differential tests were used to identify organisms as recommended in *Bergey's Manual (8).*

TABLE 2. CRITERIA FOR SUBJECTIVE EVALUATIONS OF QUALITY

Factor	1	2	3	4	5	6	7
Conformation[1]	Extremely poor	Moderately poor	Slightly poor	Fair	Slightly good	Moderately good	Extremely good
Marbling[2]	Devoid	Practically devoid	Traces	Slight	Small	Modest	Moderate
Firmness[3]	Extremely soft	Moderately soft	Slightly soft	Slightly firm	Moderately firm	Firm	Extremely firm
Color of lean[4]	Extremely pale	Moderately pale	Slightly pale	Ideal	Slightly dark	Moderately dark	Extremely dark

[1]Indicates muscling.
[2]Indicates intramuscular fat.
[3]Values of 4 and above are desirable.
[4]Pale grayish-white to dark red.

TABLE 3. METHODS OF ISOLATION AND IDENTIFICATION

Microorganisms	Isolation procedure	Incubation °C	Incubation Hrs.	Identification procedure
Lancefield group D streptococci	APTA agar	21	72	Cell morphology & reactions in: *(a)* Litmus milk; *(b)* APT broth at 10 C; *(c)* APT broth at 45 C; *(d)* APT broth at pH 9.6; *(e)* APT broth with 6.5% NaCl added and *(f)* milk containing 0.1% methylene blue
Yeast and molds	Acidified YNB agar	21	96-120	Cell morphology and growth on acidified YNB
Coliforms	VRB agar	30	48[1]	Cell morphology, type of growth on eosin methylene blue (EMB) agar and IMViC reactions
Enterobacteriaceae	MacConkey agar	30	48	Cell morphology, type of growth on EMB agar, growth in Urease medium and IMViC reactions
Staphylococcus aureus	TPEY agar	21[2]	72	Cell morphology, growth in mannitol broth, catalase and coagulase reactions
Salmonella	Lactose pre-enrichment broth ↓	30	48[1]	Growth on brilliant green agar, cell morphology, growth in triple sugar iron agar, and serotyping
	Selenite cystine broth	30	24	
Aerobic spore-formers	Heated at 80 C; trypticase soy agar	21	72	Heated at 80 C for 10 min, cell morphology and catalase
Halophilic aerobic spore-formers	Heated at 80 C; trypticase soy agar + 5% NaCl	21	72	Heated at 80 C for 10 min, cell morphology and catalase
Anaerobic spore-formers	Liver agar; liver broth; incubated in anaerobic jar	21	72-96	Cell morphology, aerobic growth, and biochemical reactions
All microorganisms on fresh, cured and aged hams	Colonies selected by morphology from APT and tryptone glucose agar	21	72	Selective and differential media, cell morphology, and biochemical reactions

[1]Longer incubation time allowed to permit recovery of cells.
[2]From APHA (1958).

RESULTS AND DISCUSSION

The total number of colonies isolated at three processing times at each ham location on the two media, APT and TGE, are shown in Table 4. Total counts per gram increased from fresh through curing both on the surface and within the hams at all locations. The total counts decreased slightly on the surface after aging one month at controlled temperature and humidity. Internal numbers decreased generally after aging compared to after curing when grown on APT medium, but increased on TGE medium. The reason for the difference between media was not discernable, but variation in average total counts between the two media resulted from the consistently higher counts obtained with TGE medium in replication 2.

The bacterial numbers increased in shank internal (SI) tissue on both media. The shank tissue

is the most difficult area to apply sufficient salt for adequate preservation; the percent NaCl ranged to a low of 2.2 in one ham. The average moisture content (60.1%) in shank tissue for all hams was nearly equal between the curing and aging periods. It appears logical therefore, that this tissue would contain a high number of bacteria. One reason for the high moisture content in the shank may be because the hams were hung with the shank-end down. Thus, the water in the ham would tend to migrate toward the shank-end.

The antimicrobial effects associated with the curing and aging periods were apparently not sufficient to suppress the growth of certain microorganisms. The decrease in surface growth after aging probably resulted from a reduction in water activity.

The most prevalent species of bacteria isolated was coagulase-negative *S. aureus* from TPEY (BBL) medium (Table 5). This species was present in appreciable numbers from each ham location and at each processing time. This species of bacteria was

TABLE 4. AVERAGE AND RANGE OF CELL FREQUENCY[1] ON APT AND TGE AT SEVERAL HAM LOCATIONS

Ham area[2]	Media[3]	Processing times: Thawed		Cured		Aged	
		Average[4]	Range	Average	Range	Average	Range
BS	APT	2.1[5]	(1-3)	6.5	(4-8)	3.8	(1-7)
	TGE	2.2[5]	(1-3)	7.3	(6-8)	5.1[6]	(1-8)
SS	APT	2.4[5]	(1-4)	7.3	(4-8)	6.6	(3-8)
	TGE	2.5[5]	(1-4)	7.5	(6-8)	7.1[6]	(5-8)
CS	APT	1.9[5]	(1-3)	6.4	(4-8)	5.1	(1-6)
	TGE	1.9[5]	(1-3)	6.5	(4-8)	5.8[6]	(4-8)
BI	APT	1.3	(1-2)	3.0	(1-4)	1.4	(1-3)
	TGE	1.3	(1-2)	2.9	(1-4)	2.6	(1-6)
SI	APT	2.0	(1-3)	3.9	(3-5)	2.7	(1-7)
	TGE	2.0	(1-3)	4.0	(3-5)	2.6	(1-6)
CI	APT	1.1	(1-2)	2.0	(1-3)	1.1	(1-2)
	TGE	1.3	(1-2)	2.5	(2-4)	1.7[6]	(1-6)
KI	APT	1.7	(1-3)	2.2	(1-4)	1.0	(1)
	TGE	1.7	(1-3)	2.4	(1-4)	1.5	(1-6)
SB	APT	1.1	(1-2)	2.1	(1-3)	1.0	(1)
	TGE	1.1[5]	(1-2)	2.1	(1-3)	1.7[6]	(1-6)

[1]Frequency expressed as range of cells per gram:
1 = 1-299 5 = 300,000-2,900,000
2 = 300-2900 6 = 3,000,000-29,000,000
3 = 3000-29,000 7 = 30,000,000-290,000,000
4 = 30,000-290,000 8 = 300,000,000
[2]BS (butt surface), SS (shank surface), CS (cushion surface), BI (butt internal), SI (shank internal), CI (cushion internal), KI (knuckle internal), and SB (*semitendinosus-biceps femoris*).
[3]Difco Laboratories, Detroit, Michigan.
[4]Average of ten observations.
[5]Average of eight observations.
[6]Average of nine observations.

TABLE 5. AVERAGE AND RANGE OF CELL FREQUENCY[1] ON TPEY[2] AT SEVERAL HAM LOCATIONS

Ham area[3]	Processing times: Thawed		Cured		Aged	
	Average[4]	Range	Average	Range	Average	Range
BS	2.1[5]	(1-3)	4.0	(4)	2.9[5]	(1-4)
SS	2.5[5]	(1-4)	4.0	(4)	3.8	(3-4)
CS	1.9	(1-4)	4.0	(4)	3.4[6]	(1-4)
BI	1.4	(1-2)	2.9	(1-4)	1.4[6]	(1-3)
SI	1.9[6]	(1-3)	3.5	(1-4)	1.9	(1-4)
CI	1.3	(1-2)	2.1	(1-3)	1.0	(1)
KI	1.6	(1-2)	2.2[6]	(1-4)	1.0	(1)
SB	1.3	(1-2)	2.1[6]	(1-4)	1.1	(1-2)

[1]Frequency = range of cells per gram:
1 = 1-299 3 = 3000-29,000
2 = 300-2900 4 = 30,000-290,000
[2]Baltimore Biological Laboratory, Inc., Baltimore, Maryland.
[3]BS (butt surface), SS (shank surface), CS (cushion surface), BI (butt internal), SI (shank internal), CI (cushion internal), KI (knuckle internal), and SB (*semitendinosus-biceps femoris*).
[4]Average of ten observations.
[5]Average of eight observations.
[6]Average of nine observations.

TABLE 6. BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF GRAM POSITIVE COCCI ISOLATES

	Biochemical test				Physiological characteristics		
	Aerobic		Anaerobic				
Identification	Glucose[1]	Mannitol	Glucose[1]	Mannitol	Pigmentation	Coagulase	Catalase
Control	−	−	−	−	−	−	−
Staphylococcus aureus	+	+	+	+	G	+	+
Staphylococcus aureus	+	+	+	+	GW	−	+
Staphylococcus epidermidis	+	−	+	−	W	−	+
Micrococcus	+	−	−	−	YO		+
Streptococcus	+	+	+	+	W		−

[1]From Hugh and Leifson, J. Bacteriol. 66:24, 1953.
\+ = Positive reaction.
− = Negative reaction.
G = Golden.
GW = Golden white.
W = White.
YO = Yellowish orange.

reported present in curing brines and sliced bacon *(13);* and in cured meats *(18);* and in fresh and cured hams *(5).* However, these studies were made on high moisture-low NaCl content products. The scheme of classification of the genus *Staphylococcus* and the genus *Micrococcus* is shown in Table 6 *(4).* A known coagulase-positive strain was used as a comparison for all identifying tests conducted on the staphylococci isolates. The genus *Streptococcus* was confirmed by determining the physiological pattern described in Bergey's Manual of Determinative Bacteriology *(8).*

Since most of the colonies recovered from the internal samples on TPEY medium were staphylococci as were those from APT medium (Table 7), the total number present on each medium tended to be nearly equal. However, there was poor agreement between counts on external surfaces on TPEY compared to both total plate count media, APT, and TGE. Other work has shown that TPEY may suppress the growth of organisms other than staphylococci *(11).* The reduction in number of cells per gram of surface sample after aging is most likely influenced by drying. It may be observed (Table 5) that this reduction also takes place intramuscularly between curing and aging, probably for the same reason. In addition, the increase in percentage of NaCl in the reduced water phase would tend to decrease the water available to the microorganisms. The increase in FFA during aging *(7, 17)* may also depress bacterial growth. A study of individual growth patterns on the various selective media when counts were considered significant (30-300) colonies revealed the same general trend of increased numbers after curing followed by a growth plateau or a decrease after aging.

Some groups of bacteria were isolated in very small numbers while others could not be isolated. Lancefield group D streptococci were isolated from the butt surface of an aged ham on APTA agar (APT + 0.03% NaN_3) plate. Also isolated from various media were *Staphylococcus epidermidis,* on APTA; and on APT medium, *Micrococcus* after curing, *Streptococcus* in fresh and cured, *Bacillus* at each processing period, *Escherichia coli* on fresh, *Enterobacteriaceae* on fresh, and yeasts on fresh and cured (Table 7). The lactose pre-enrichment technique did not yield *Salmonella* under the conditions of this study. All cultures with an acid butt and alkaline slant on triple sugar iron agar were tested directly with polyvalent "O" *Salmonella* antiserum. All cultures were negative by this test and were tested for urease activity. Cultures giving a rapid positive urease test were considered members of the genus *Proteus (30).* Yeasts and mold colonies were isolated

TABLE 7. PERCENTAGE INCIDENCE OF MICROORGANISMS SELECTED FROM APT[1] MEDIUM[2]

Identification	Processing times			
	Fresh	Cured	Aged	Total
I. Cocci; gram+				
Staphylococcus aureus	53.33	62.07	83.33	66.13
Staphylococcus epidermidis	0.00	0.00	5.55	1.61
Micrococcus	0.00	3.45	0.00	1.61
Streptococcus	13.33	6.89	0.00	6.45
II. Rods; gram +				
Bacillus	6.66	24.14	11.11	16.13
III. Rods; gram −				
Escherichia coli	6.66	0.00	0.00	1.61
Enterobacteriaceae	13.33	0.00	0.00	3.22
IV. Fungi				
Yeasts	6.66	3.45	0.00	3.22
				99.98

[1]Difco Laboratories, Detroit, Michigan.
[2]Total of 30 hams.

from the acidified YNB medium, but were not further characterized. One species of *Aerobacter* was isolated from VRBA and was probably a chance contaminant. MacConkey's agar did not yield any microorganisms from the family *Enterobacteriaceae*. However, *Micrococcus* species and coagulase-negative S. *aureus* grew on MacConkey's agar from cured and aged ham samples of the butt and shank areas but not until after 48 hr incubation at 30 C. It is well known that overgrowth may occur when incubation is extended beyond 24 hr at 37 C *(12)*.

Spore-forming anaerobes were not recovered with the isolation procedures used in this study. Isolates recovered from liver agar and liver broth were all characterized as facultative anaerobes and therefore not further identified. This result is in general agreement with the low incidence of anaerobic spore-forming bacteria which has been reported by several workers *(6, 15, 28)*. It is possible that vegetative forms of anaerobes were present in the fresh hams; however, no attempt was made to determine vegetative anaerobic growth since it was thought unlikely that they would survive the high NaCl content, reduced moisture, and the presence of nitrate during curing and aging.

Thus, under the conditions of this study, only *Staphylococcus* and *Bacillus* could be considered to have numbers large enough to produce significant change in the product. Species of these two genera grew in Trypticase Soy Broth plus NaCl after the pasteurization treatment (80 C for 10 min). Therefore, the combined effect of heat and NaCl did not render either genus incapable of growth. Subsequent monitoring of aliquots of the 1:10 dilution ham homogenate for 20 min at 80 C showed reduction of staphylococci but not complete growth inhibition. The final temperature of the homogenate was 77 C. The meat particles present apparently gave some protection to the bacteria during heating. A 10 ml sample of the 1:10 dilution of the homogenate placed in an 84 C water bath required 2.5 min to reach 80 C and this temperature could be maintained in the homogenate for the additional 10 min. No staphylococci were recovered after this treatment. Variation in heat resistance by staphylococci has been reported by several workers *(20, 26)* and a species of *Streptococcus* isolated from semi-perishable ham was able to withstand 40 min at 65.5 C.

Bacillus is considered to be the most common genus of conventionally processed meats because of its wide distribution and resistance to thermal processing *(25)*. The low numbers of *Bacillus* present in the hams from this study is probably not typical in the commercial production of CS hams because spores tend to increase with large scale production.

It is significant that these hams did not contain coagulase-positive S. *aureus*, particularly since the majority of food poisoning in meat comes from ham containing this species of bacteria *(22)*. Thus, the occurrence of staphylococcal food poisoning must result from contamination during packaging, preparation, and storage.

The good hygenic condition of this product is evidenced by the presence of only one *E. coli* and one *Aerobacter*, chance contaminants, and the failure to detect *Salmonella*. It must be realized, however, that the hams represented in this experiment were frozen and stored and that the sanitation was superior to that usually attainable in commercial establishments. It should also be noted, however, that these hams were placed directly at aging temperatures (about 34.5 C) after smoking without permitting the curing ingredients to equalize throughout the tissues. Thus, it would appear that microbial growth conditions were superior to those attained in usual commercial practice.

Since the ultimate judgment concerning the value of any food product lies with the consumer, it is imperative to relate all factors to the quality of the product. Individual sample measurements of the quality factors marbling, firmness, and color were not related to differences in number of microorganisms of the predominating genera or total number of microorganisms at any of the sampling times. While the quality factors evaluated here were not influenced by the number and kind of microorganisms present in this study, flavor may have been affected. A more complete discussion of the quality factors is presented in a subsequent paper.

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A NEW TEST TO DETECT LEAD POISONING IN CHILDREN–A SERIOUS ENVIRONMENTAL PROBLEM IN THE INNER CITY[1]

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ABSTRACT

Lead poisoning among children is a serious environmental and public health problem, especially in the inner city. Some children eat paint chips containing lead and thus become poisoned. A rapid screening test based on urinary delta-aminolevulinic acid and using a dipstick to collect samples was devised so that the mother could take the sample herself. The dipstick is composed of a piece of ion exchange paper stapled to a plastic handle. The research leading to the development of this test is discussed relative to overall environmental problems.

Problems of the environment, of pollution, and of ecology are gigantic in magnitude. These problems are so encompassing and concern so many of us that a personal commitment is needed to help solve them. Even as small a thing as picking up a piece of scrap paper blowing around is a start. We all work in different ways and there is no question that each of us can and should contribute in some way to the overall solution of the environmental problems of today.

To cite an example, I will relate how four of us worked towards solving a very serious environmental and public health problem. We are all in science and this is where we made our contribution. Of course, this is not the end for any of us. Hopefully, we also will contribute to helping solve ecological problems in our individual capacities as laymen and responsible citizens, as well as within our capabilities as scientists.

Working on the research I shall describe were Drs. William Ullmann and Joseph Kornfeld of the Laboratory Division of the Connecticut State Department of Health in Hartford and Dr. Kenneth Hanson and myself of the Connecticut Agricultural Experiment Station at New Haven. Incidentally, this research is a good example of how two state agencies cooperated to help solve an environmental problem.

The problem is lead poisoning among children which is a very serious environmental and public health problem; especially important in the inner city. Lead poisoning is, in a sense, a man-made disease. Man manufactured the paint containing lead and man used it both on the inside and outside of his home. Now after many years and many paintings, the lead paint, even though now covered with non-lead paint, can peel and chip off the walls, plaster, and woodwork. Some children pick up these chips of paint and eat them. Thus, they become poisoned by the lead. The eating of non-food items is called pica, but not all children indulge in this practice. High risk areas for lead poisoning are in the inner city where older housing is prevalent.

Lead poisoning is the third most important threat to children preceded only by auto accidents and the trauma of birth itself. Victims are usually between the ages of 1 and 6 years and unless lead intoxication is detected at an early stage, nausea, neurological disorders, mental retardation, and even death can result. The problem is to find the potential victims of lead intoxication and treat them before they become permanently impaired.

Fortunately, laboratory tests can detect lead poisoning before clinical symptoms are evident. It is important, therefore, to screen large numbers of children in order to find potential victims and to test them frequently. Children in high risk areas who have pica are in constant danger because of the possible presence of paint chips, both interior and exterior, and an initial negative laboratory test does not guarantee that the child may not be poisoned later on. It is precisely for this reason that detection methods should be easy to apply and be used as early and as frequently as possible.

LABORATORY TESTS

Two laboratory tests have been used to detect potential victims of lead poisoning, though neither was ideal for a mass screening program. One is the direct determination of lead in the blood and the other is the determination of delta-aminolevulinic acid in urine. Determination of lead in blood requires 5 to 10 ml of blood which must be taken by venipuncture. The sample must be taken by a phy-

[1]Presented at the mid-winter meeting of the Connecticut Association of Dairy and Food Sanitarians, January 20, 1971, Hartford, Connecticut

sician or highly trained technician and frequently a second person is needed to quiet or reassure the child. For such reasons as use of scarce medical personnel to collect samples, the obvious disadavantage of drawing blood, and the limit on the numbers of samples which can be tested, use of blood in a mass screening program is not feasible.

A second test in use when we started our research was determination of delta-aminolevulinic acid. (ALA) in urine. This test is based on the fact that ingested lead interferes with hemoglobin synthesis in which an enzyme, delta-aminolevulinate dehydrasase, is important. This enzyme converts ALA to the next material in the blood-making sequence and lead reduces activity of this essential enzyme. Conversion of ALA is hindered and thus, the ALA, with no place to go, begins to pile up and is excreted in abnormal amounts in the urine. The laboratory test previously used is called the column test. This method for determination of ALA requires collection of urine in bottles and sending or carrying them to the testing laboratory. There was the ever-present problem that ALA would deteriorate in transit. The collection and transport system involving bottles of urine was both clumsy and unwieldy. Further, the laboratory test required use of an ion exchange resin column and this cumbersome method severely limited the number of samples which could be processed in a day. In late 1969, the Connecticut State Department of Health started a program to test the urine of children for ALA by the column method. It was soon clear that a major gap existed between the number of children who should be tested for lead poisoning and the number that were examined. One estimate is that only about one-tenth of Connecticut children in high risk areas had been tested even once.

Need For New Test

It was clear to us that the sample collection and testing systems then used were not ideal for a mass screening program. The systems did not provide a means to test large numbers of children on a continuing basis. We felt that a mass screening test should meet the following criteria: *(a)* sample may be taken by an untrained person, *(b)* sampling should cause no pain or trauma to the patient, *(c)* sample must not be subject to deterioration, *(d)* method must not generate false negatives, and *(e)* laboratory and collection procedures must be so simple that frequent retesting is feasible.

Based on these criteria we agreed that an adaptation of the test for ALA in urine offered the most hope for success in finding and developing a good mass screening test to detect potential victims of lead poisoning.

The New Test

Let me describe briefly what we developed *(1, 2)*. It is a simple device called a dipstick and is a 6 × 4 cm piece of ion exchange paper (sulfonic acid cation exchange, Na form) stapled to a plastic handle. It is somewhat similar in construction to the familiar stick used to detect diabetes. The assembled dipstick is wrapped in a piece of aluminum foil and placed, together with instructions for use, in a mailing envelope. In use, the dipstick is first unwrapped from the foil. The mother has the child urinate into a cup, the paper part of the dipstick is then completely immersed into the urine, quickly withdrawn, and placed on the foil to dry. Two hours is sufficient. After drying the dipstick is rewrapped in the foil and mailed to the testing laboratory. The ion exchange paper binds the ALA and thus it is no longer subject to deterioration during transit.

In the laboratory, the ion exchange paper is cut from the holder. The paper is then placed in a tube with buffer solution and the ALA, if present, is washed by gentle shaking from the paper and goes into the buffer. The solution containing the ALA is treated with a chemical which forms a colored compound with ALA and the color is measured in a spectophotometer. Standards are tested concurrently. Results are reported to the physician or clinic, not to the parent.

Before dipsticks were sent out for trial, they were subjected to many tests in the laboratory. Each test was compared to the longer column test for ALA. Our data indicated that the dipsticks were as good as the column test for quantitatively determining the amount of ALA in the urine. In addition, by eliminating the need of a column we were able to shorten the laboratory time so that many more samples could be examined per day.

The Field Test

Satisfied that the dipsticks worked well in the laboratory, we embarked on a field test. During the summer of 1970 volunteers of the American Friends Service Committee came to Hartford to work in the inner city. They were directed by Mr. Mahlon Hale, a second year medical student who had arranged for them to come to Hartford. Their project was to go into the inner city and collect bottles of urine from children and have them examined for ALA in order to find potential victims of lead poisoning. We asked these volunteers, all between 16 and 18 years of age, to use a dipstick on the same sample when they col-

lected a bottle of urine. They agreed, and in this way we obtained excellent data correlating the two tests for ALA. The field tests substantiated the laboratory findings.

In late 1970, the State Health Department decided to abandon the use of the column method which required collection of urine in bottles and use the dipstick test. They are now preparing 60,000 dipsticks for distribution. These will be distributed to public health officials throughout the State of Connecticut who, in turn, will see that other groups such as visiting nurses, neighborhood groups, and others concerned with lead poisoning get them. In this way, they will eventually get to the mother so that she can collect the sample from her own child. Most mothers are able to do this and, in fact, we have even found 4-year olds who are able to use the dipsticks adequately.

An additional use for the test also has been suggested. That is, after a child is examined and if found to be poisoned, then a housing inspector visits the home to ascertain the condition relative to this environmental problem. In this way he can make recommendations if he finds flaking and peeling paint. I am told that there are insufficient housing inspectors to examine all homes which might have flaking paint. By use of diptick test results they can visit homes where children have been exposed and thus spend their inspection time more fruitfully.

What I have tried to do, through the example of the dipstick test, is to indicate how four of us worked together to help solve an environmental problem. I do not claim that lead poisoning is the most serious environmental problem, although it is very serious. However, my colleagues and I can only help with environmental problems where we feel we are qualified to make a contribution. All of us should think of some way where we can contribute. As professionals, I urge all of you to start now. For, if you do not participate in the solution to these environmental problems, then you become part of the problem itself.

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HIGH TEMPERATURE STORAGE AND MILK KEEPING QUALITY

The long shelf life of milk is remarkable, when one considers that milk is perishable food and that addition of preservatives is prohibited by law. To keep milk at peak flavor, texture and appearance, however, consumers would do well to give it proper care in the home by keeping it refrigerated at 40 F or lower.

Even the treatment milk receives from the time it is purchased until placed in a home refrigerator can affect its quality. For example, it is not uncommon for shoppers to have milk and other dairy foods in their automobiles for a sufficient time to affect product freshness and palatability.

A recent study conducted at the University of Florida Dairy Science Department, by Dr. C. Bronson Lane and Dr. Ken L. Smith, shows the effects of brief high-temperature storage conditions on milk keeping quality.

The scientists first placed recording thermometers in cars parked in direct autumn sunlight. Maximum temperatures recorded over a 24 hr time span were well above 120 F. Similar temperature checks on home refrigerators showed a minimum of 38 F with highs of 50 F (which is substantially higher than the 40 F recommended for milk storage).

Ten half-gallons of whole milk were purchased from supermarkets and convenience stores in the Gainesville area. Samples represented two half gallons each of five Florida brands.

Standard Plate Counts and Psychrotrophic Plate Counts were determined on all ten samples within an hour after they were brought into the laboratory.

The two milk samples from each plant were split—one was stored at 40 F immediately, as a control sample, and the other was divided and incubated at 120 F for 30, 60, and 120 min. (This incubation procedure was followed to simulate conditions the milk might be subjected to by a consumer who purchases the product and subsequently places it in a hot car for brief periods of time while continuing to shop.) The incubated samples were then refrigerated at 40 F.

Standard Plate Counts and psychrotrophic counts were obtained for both the incubated and control samples after 1, 4, 8, and 14 days storage. In addition, a panel of trained dairy product judges tasted both the controlled and heat exposed samples on the first, 7th, and 14th days.

Results from this preliminary experiment showed that in every instance bacterial numbers were higher in the samples exposed to "hot car" temperatures.

Even a half hour exposure to the 120 F temperature caused a measurable increase in the Standard Plate and psychrotrophic counts when compared to the control. Using a ranking test, judges were able to detect differences in taste between controlled and incubated samples after only one day of storage.

As the exposure time of 120 F temperature went up, taste deterioration in the incubated samples became more pronounced.

Dr. Lane and Dr. Smith point out that the shelf life of milk also depends on the number of organisms originally in the milk, and the handling procedures for the product at the retail level prior to purchase. However, they concluded, consumers can help significantly in protecting milk quality and retarding off-flavor development by minimizing or eliminating its storage in hot cars and properly refrigerating it at 40 F or lower in the home.

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CLOSTRIDIUM PERFRINGENS ENTEROTOXIN[1]

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Abstract

Clostridium perfringens type A food poisoning is caused by ingestion of food contaminated with large numbers of *C. perfringens* cells. The cells multiply and sporulate in the intestine and produce an enterotoxin *in situ*. The enterotoxin is released upon cell lysis and causes increased capillary permeability, vasodilation, and excess fluid movement into the intestinal lumen resulting in diarrhea.

Purified *C. perfringens* enterotoxin has a UV absorption spectrum characteristic for proteins, and is essentially free of nucleic acids, lipids, and reducing sugars. Its apparent molecular weight is 36,000 ± 4,000, its Stokes radius 2.6 nm, and its isoelectric point pH 4.3. Its specific toxicity is about 2,000 mouse MLD/mg N.

Clostridium perfringens type A enteritis

Food poisoning by *Clostridium perfringens* type A is characterized by diarrhea and abdominal pain, and results from ingestion of food contaminated with large numbers of *C. perfringens* cells *(21, 30)*. The enteric disease was reproduced in humans who had volunteered to ingest vegetative *C. perfringens* cells, free of the spent culture media *(3, 19)*. On the other hand, culture supernatant fluids had no effect in humans *(3, 22)*.

Experimental diarrhea also was produced in lambs *(14)* and rabbits *(7)* by administering into the small intestines live vegetative cells of *C. perfringens*, suspended in fresh medium. As will be described below, it is extremely important that in experiments designed to imitate food poisoning in humans, the *C. perfringens* cells are administered in the vegetative state.

A localized response can be obtained in lambs or rabbits by ligating the small intestine into segments, generally referred to as loops, injecting the loops with suspensions of vegetative *C. perfringens* cells in nutrient medium, and allowing the cells to grow for about 6 hr in the live animal *(9, 15)*. During this period, the bacteria multiply, sporulate, and cause excess movement of fluid from the blood stream into the intestinal lumen *(9, 18)*. As a result, the loops distend and take on a sausage-like appearance. Gas produced during growth of *C. perfringens* in the intestine contributes to the loop distention, but it does not appear to be a causative factor in the accumulation of fluid *(16, 17)*.

The loop distention resulting from excess fluid movement into the intestinal lumen is comparable to diarrhea, but the response of the animal is localized, and the normal removal of fluid is physically blocked. As in the production of diarrhea, a combination of live *C. perfringens cells* and nutrients also is required to cause loop distention; cells suspended in 0.85% NaCl, and nutrient medium without cells have no effect *(15)*.

The ligated intestinal loop technique has been used extensively as a convenient model to study *C. perfringens* food poisoning. The suitability of the loop technique for this purpose has been demonstrated *(6, 7, 15, 17)*, most convincingly in recent publications of Duncan et al. *(8, 19)* who correlated the ability of *C. perfringens* strains to cause loop distention in rabbits with the ability of the same strains to cause diarrhea in rhesus monkeys and human volunteers.

Production of C. perfringens enterotoxin in vitro

The inability of vegetative *C. perfringens* cells to cause loop distention or diarrhea in the absence of nutrient medium indicates that the causative factor in *C. perfringens* enteritis is not a component of the vegetative cells, but that it is produced *in vivo*. In an attempt to produce the causative factor *in vitro*, Duncan and Strong *(6)* prepared supernatant fluids and cell extracts from sporulated cultures of *C. perfringens (5)*. With both preparations they succeeded in producing loop distention and diarrhea in rabbits. Similar results were obtained with lambs *(16, 17)*. For reasons to be explained below, the causative agent has been termed "*C. perfringens* enterotoxin". In young, sporulated cultures of *C. perfringens*, the enterotoxin is located exclusively in the cells *(16, 17)*. It can be extracted from the cells by sonic lysis and be recovered in the soluble phase by centrifugation of the sonicate. The extracted and washed sediment containing cell debris, whole spores, and, usually, a few unbroken cells, is virtually free of enterotoxin. It appears, therefore, that the toxin is a component of the cytoplasm of the sporulated cell. The production of enterotoxin by sporulating cells

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suggested a possible relationship between sporulation and toxinogenesis *(6)*. This suggestion was supported by a direct correlation between the amount of enterotoxin produced by three individual strains of *C. perfringens*, and the degree of sporulation *(16, 17)*. Duncan *(Annual Meeting of the American Society for Microbiology, Minneapolis, 1971)* recently reported that, in contrast to the parent wild type strains, nonsporing mutants of *C. perfringens* type A lost the ability to produce enterotoxin. However, this ability was regained by mutants that reverted to sporulating forms.

Clostridium perfringens enterotoxin induces erythema without necrosis in the skins of guinea pigs and rabbits *(11)*. This reaction is distinctly different from the skin reactions to all of the other known toxins of *C. perfringens (29)*. The skin reaction is now being used for quantitative assay of the enterotoxin. The skin test is rapid, accurate, and about 1,000 times as sensitive as the ligated intestinal loop test *(11)*.

Antiserum against C. perfringens enterotoxin

Antisera prepared in rabbits against crude extracts from sporulated cells of *C. perfringens* completely neutralized the enterotoxin *in vitro (16)*. These sera could be converted to enterotoxin-specific antisera by absorption with cell extracts of either a nonsporulating strain of *C. perfringens* or, preferably, of the homologous strain grown in a medium in which all of the cells remained vegetative. Specificity of the absorbed antisera was indicated by the production of a single major precipitin band in agar gel immunodiffusion against crude extracts from sporulated cells *(18, 25)*.

Although *C. perfringens* enterotoxin was completely neutralized with antiserum *in vitro*, it appears that circulating antibody has little or no neutralizing effect on the enterotoxin in the intestinal lumen. Niilo et al. *(25)* reported that the enterotoxin caused essentially the same loop distention in lambs that had been immunized against the toxin as it did in nonimmunized control animals. Similar results *(Hauschild, unpublished data)* were obtained with rabbits, although the total amount of toxin administered per rabbit (about 300 erythema units) could be neutralized *in vitro* with 2-4 ml of blood serum from the immunized animals. On the other hand, large doses of enterotoxin (900 erythema units/rabbit) administered into ligated intestinal loops killed most of the control animals within 3-5 hr while the immunized animals survived. This indicates that, upon entering the blood stream, the toxin is neutralized by circulating antibody. Intravenous injection of the toxin has been shown to be lethal to several animal species *(12, 24)*.

Identity of C. perfringens enterotoxins produced in vitro and in vivo

When ligated intestinal loops of lambs are injected with enterotoxin-free, vegetative cells of *C. perfringens* in nutrient medium, the subsequent events in the loops, i.e., multiplication and sporulation of the cells and fluid accumulation, are associated with the production of an erythemal factor *(18)*. This factor was recently recovered both from supernatant fluids of the loop contents and from the *C. perfringens* cells in the sediments. The identity of the erythemal factor with the *in vitro*-produced enterotoxin was demonstrated by immunodiffusion. A single continuous precipitin line was obtained with both the *in vitro* and *in vivo* preparations against the enterotoxin-specific antiserum *(18)*.

Characteristics of C. perfringens enterotoxin

The induction of diarrhea, loop distention, and of erythema by the enterotoxin, and its lethality and antigenicity have been dealt with above. The toxin also is sensitive to heat *(6)* and to some proteolytic enzymes, i.e., pronase *(6)* and *Bacillus subtilis* protease *(12)*, but not to trypsin, chymotrypsin, or papain *(6, 12)*.

The toxin was recently purified by a combination of gel and ion exchange chromatography *(12)*. The purified toxin was essentially free of nucleic acids, fatty acids, lipid phosphorus, and reducing sugars, and had a UV absorption spectrum with a maximum at 278-280 nm and a minimum at 250 nm which is characteristic for proteins. It appears, therefore, that we are dealing with a protein.

The apparent molecular weight of the enterotoxin, calculated from its K_{av} value on Sephadex G-100, was 36,000 ± 4,000; its Stokes radius was 2.6 nm. Isoelectric focusing of the toxin resulted in a single peak with erythemal activity, and showed a relatively low isoelectric point of pH 4.3. The specific toxicity of the purified toxin in mice was 2000 MLD/mg N *(12)*.

Little is known about the mode of action of *C. perfringens* enterotoxin *in vivo*. Niilo *(24)* found that the erythemal response of guinea pigs to intradermal injection of the toxin *(11)* was preceded by an increase in the permeability of the blood capillaries around the injection site, and has suggested the following sequence of changes in the intestine that would lead to fluid accumulation and diarrhea: increased capillary permeability, increased vasodilation, and increased intestinal motility.

The actions of the enterotoxin are relatively fast. The permeability increase in the blood capillaries

of the guinea pig skin became apparent within 15-20 min after injection of the toxin *(24)*. Mice *(12)* and guinea pigs *(24)* could be killed within the same period by intravenous injection of the toxin. After 90 min, the fluid volumes of ligated intestinal loops in rabbits were significantly larger than the fluid volumes of the control loops *(13)*.

ROLE OF THE ENTEROTOXIN IN C. PERFRINGENS TYPE A ENTERITIS

The role of the enterotoxin in enteritis was tested by the following experimental sequence: *(a)* induction of loop distention in lambs by injection of enterotoxin-free vegetative cells of *C. perfringens* in nutrient medium; *(b)* preparation of supernatant fluids from the loop contents, and of extracts from the loop sediments, both of which contained enterotoxin; and *(c)* treatment of these preparations either with normal rabbit serum (controls) or with enterotoxin-specific rabbit antiserum, and transfer of the toxin-serum mixtures to new ligated intestinal loops of lambs. The preparations containing normal rabbit serum again caused significant accumulation of fluid in the recipient loops. However, treatment with the antiserum completely neutralized their enterotoxic actions, as well as their erythemal activities. These experiments leave little doubt that the *in vivo*-produced enterotoxin is significantly involved in enteropathogenicity.

The action of *C. perfringens* enterotoxin was recently confirmed in primates. Duncan and Strong *(8)* induced emesis and diarrhea in rhesus monkeys by administering either cells or supernatant fluids from sporulated *C. perfringens* cultures into the stomachs of these animals. The strains of *C. perfringens* capable of inducing the gastrointestinal responses had also been found to induce loop distention in rabbits. Strong et al. *(29)* obtained similar results in human volunteers. Emesis and diarrhea were recently also induced in cynomolgus monkeys by administering purified preparations of the enterotoxin by the same route *(20)*. On the other hand, no responses were obtained in monkeys when the preparations were first neutralized *in vitro* with enterotoxin-specific antiserum *(20)*.

Since emesis occurs very rarely in human food poisonings caused by *C. perfringens* type A *(2)*, the induction of emesis by the enterotoxin might appear to contradict the postulated role of the toxin in the enteric disease. However, we have to consider that human food poisoning is caused by live cells of *C. perfringens* that produce the enterotoxin *in vivo*, rather than by ingestion of a preformed toxin. In the natural disease, therefore, the toxin is unlikely to come into contact with the stomach.

The need to use enterotoxin-free, vegetative cells in the experimental production of *C. perfringens* type A enteritis is now apparent. Unfortunately, in routine investigations of foods incriminated in food poisoning outbreaks caused by *C. perfringens*, the state of the bacterial cells has usually been overlooked. However, direct staining of smears of incriminated foods, and heat sensitivity tests of the microorganisms in such foods *(B. C. Hobbs, personal communication)* have indicated that the *C. perfringens* cells responsible for food poisonings existed in the vegetative state. Barbecued chicken has been infected with *C. perfringens* and subsequently incubated by the author *(Hauschild, unpublished data)* who detected neither spores nor enterotoxin until after 24 hr when the meat was no longer palatable. It would be desirable to take note of the state of the *C. perfringens* cells in future investigations of food poisoning outbreaks.

SEQUENCE OF EVENTS LEADING TO C. PERFRINGENS FOOD POISONING

The foregoing discussions suggest the following sequence: *(a)* ingestion of food contaminated with large numbers of vegetative *C. perfringens* cells; *(b)* multiplication and sporulation of *C. perfringens* in the small intestine; *(c)* production of enterotoxin associated with sporulation and release of the toxin by cell lysis; *(d)* increased capillary permeability and vasodilation in the intestine, and possibly, increased intestinal motility; and *(e)* excess fluid accumulation in the intestinal lumen resulting in diarrhea.

NOMENCLATURE

In a recent publication, the term "*C. perfringens* enterotoxin" has been proposed to characterize the erythemal toxin of *C. perfringens*. However, it must be emphasized that this enterotoxin differs in two important aspects from staphylococcal enterotoxins: *(a)* in human food poisonings it is produced *in vivo*, whereas staphylococcal enterotoxins are preformed in the food; and *(b)* it acts directly upon the intestine as shown by the localized loop responses, whereas the action of the staphylococcal entertoxins is mediated by the central nervous system *(1, 30)*. In view of these differences, a brief discussion of the new term is warranted.

On the whole, definitions of the term "enterotoxin" are ambiguous, but most of them are in accord with the characteristics of *C. perfringens* enterotoxin. A few examples are: "a cytotoxin specific for the cells of the mucous membrane of the intestine" *(Stedman's Medical Dictionary, 21st ed., 1966)*; "a toxin arising in the intestine" *(Dorland's Illustrated Medical Dic-*

tionary, 24th ed., 1965); "a toxic substance produced by microorganisms that is responsible for the gastrointestinal symptoms of some forms of food poisoning" *(Webster's 3rd New International Dictionary, 1968).*

The enterotoxin of *C. perfringens* has two important characteristics in common with the enterotoxins of *Vibrio cholerae (4, 26), Escherichia coli (10, 27),* and *Shigella dysenteriae (23)*: they are all produced *in vivo,* and all have similar gross actions in ligated intestinal loops. The enterotoxins of both *V. cholerae* and *C. perfringens* are heat-labile, are destroyed by pronase but not by trypsin, and are essentially free of reducing sugars and lipids *(4, 26).*

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3-A SANITARY STANDARDS FOR STAINLESS STEEL AUTOMOTIVE MILK AND MILK PRODUCTS TRANSPORTATION TANKS FOR BULK DELIVERY AND/OR FARM PICK-UP SERVICE

Serial #0511

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. Milk transportation tank 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 the joint consideration of the IAMFES, USPHS, and DIC at any time.

A.

SCOPE

A.1

These standards cover the sanitary aspects of transportation tanks for milk and fluid milk products.

A.2

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

B.

DEFINITIONS

B.1

Bulk Milk Transportation Tank: Shall mean an over-the-road truck or trailer tank used to transport milk and milk products. It may have more than one compartment.

B.2

A Farm Pick-Up or Multiple Pick-Up and Delivery Tank: Shall mean a bulk milk transportation tank as defined above with milk transfer attachments and facilities, including a pump and/or hose cabinet, as specified herein.

B.3

Product: Shall mean the milk or fluid milk product transported in the tank.

B.4

Surfaces:

B.4.1

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

B.4.2

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

B.5

Milk Outlet: Shall mean the opening in the lining of a tank or a compartment and the outlet passage for milk to the exterior of the tank or compartment. The outlet passage starts at the opening in the lining and terminates at the connection for the outlet valve.

B.6

Pump and/or Hose Cabinet: Shall mean a cabinet on a farm pick-up or multiple pickup and delivery tank used to house the pump and/or transfer hose and may also house a compartment for product sample trays and samples.

B.7

Deck Plate: Shall mean the manhole dust cover seat or that part of the outer jacket on which the cover rests.

B.8

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

Product Contact Surfaces:

C.1.1

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 stainless steel that is non-toxic and non-absorbent and which under conditions of intended use is equally corrosion resistant as stainless steel of the AISI 300 series[1] or corresponding ACI[2] types, except that:

C.1.2

Rubber and rubber-like materials may be used for flexible product transfer tubing, 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," as amended.

C.1.3

Plastic materials may be used for flexible product transfer tubing, bearing, 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.1.4

Where functional properties are required for specific applications, such as agitator bearing surfaces and rotary seals, where dissimilar materials are necessary, carbon, and/or ceramics may be used. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring, and distortion by the temperature, chemicals, and methods to which they are normally subjected in operation, or cleaning and bactericidal treatment.

C.2

The gauge of material for the lining shall be not less than the following:

16 U.S. Standard Gauge for tanks of capacities of 1,000 gallons or less,

14 U.S. Standard Gauge for tanks of capacities of over 1,000 gallons and not exceeding 2,000 gallons,

12 U.S. Standard Gauge for tanks of over 2,000 gallons capacity,

except that lighter gauges of material shall be permitted if they are so supported that they will have equal resistance to denting, buckling and sagging, as provided by the three gauges specified above for the respective sizes of tanks.

C.3

Non-Product Contact Surfaces:

C.3.1

All non-product contact surfaces shall be of waterproof, corrosion-resistant material or waterproof material that is rendered corrosion-resistant. If coated, the coating used shall adhere.

C.3.2

All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

C.4

Pump and/or Hose Cabinet:

C.4.1

The lining of the pump and/or hose cabinet shall be stainless steel or equally corrosion-resistant durable material.

C.4.2

Gasket material for pump and/or hose cabinet doors shall be smooth, easily cleanable, non-absorbent and without crevices in the body.

C.4.3

Sample trays and sample compartments furnished by the tank manufacturers that will be in the pump and/or hose cabinet shall be made of stainless steel, plastic or other equally corrosion-resistant material.

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

All product contact surfaces shall be self-draining except for normal clingage. Tanks shall be so constructed that the lining will not sag, buckle or prevent complete drainage of water when the tank has a pitch of not more than 1 inch in 100 inches.

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[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 & Steel Institute, 633 3rd Avenue, New York, N. Y. 10017.

[2]Alloy Casting Institute, 300 Madison Avenue, New York, N. Y. 10017.

D.5

The height of the vertical axes of the lining of the tank shall not be less than the minimum heights shown in the following tables:

Tanks Having Uniform Vertical Axes	Minimum Height
Up to and including 500 gallons	36"
Over 500 gallons and up to and including 2,000 gallons	40"
Over 2,000 gallons and up to and including 2,800 gallons	42"
Over 2,800 gallons and up to and including 3,500 gallons	44"
Over 3,500 gallons	46"

Tanks Having Varying Vertical Axes	Minimum Height	
	Front	Rear
Up to and including 500 gallons	36"	36"
Over 500 gallons and up to and including 2,000 gallons	40"	40"
Over 2,000 gallons and up to and including 2,800 gallons	41"	51"
Over 2,800 gallons and up to and including 3,500 gallons	43"	55"
Over 3,500 gallons	43"	57"

D.6

The inside radii of all welded or permanent attachments shall be not less than 1/4 inch. Where the head(s) and the partition wall(s) join the lining of the tank the radius shall not be less than 3/4 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 supplemented.

D.9

The outer shell shall be smooth and effectively sealed except for a vent or weep hole in the outer shell of the tank. The vent or weep hole shall be located in a position that will provide drainage from the outer shell and shall be vermin proof. The outer jacket and doors of the pump and/or hose cabinet shall be smooth and effectively sealed. Outside welds need not be ground.

D.10

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.

D.11

The tank and the divider between the compartments of a multi-compartment tank shall be insulated with insulating material of a nature and amount sufficient that, in an 18 hour period, when the tank is full of water, the average change in the temperature of the water will not exceed 2 F when the average difference between the temperature of the water and that of the atmosphere surrounding the tank is 30 F. Insulation material shall be installed in such a manner as to prevent shifting or settling.

D.12

Outlet and Outlet Valve

D.12.1

Each tank or compartment shall have a separate outlet passage. The outlet shall be of all welded construction with the exception that a rolled-on flange may be used on the terminal end and shall have an inside diameter at least as large as that of a 2 inch 3-A Sanitary Fitting. The outlet(s) shall provide complete drainage of the tank(s) or compartment(s). In multi-compartment or multi-tank units, the top of the outlet passage(s) of the front compartment(s) or the front tank(s) shall be as low as the low point of the lining at the outlet at a point in the outlet passage. The horizontal distance from the opening in the lining to this point shall not be more than four times the diameter of the outlet passage. The outlet passage downstream of this point shall pitch towards the connection for the outlet valve. The terminal end shall have a welded or rolled-on flanged connection for the outlet valve. The terminal end of the outlet passage shall not extend more than 6 inches beyond the inside lining of the tank or compartment(s). The outlet passage may be increased in length provided that:

D.12.1.1

The outlet passage is straight or is straight downstream of the elbow(s) or bend(s) used either to change the direction of product flow from a bottom outlet or to comply with the requirement in D.12.1 that at a specified point the top of the outlet passage shall be as low as the lowpoint of the lining at the outlet.

D.12.1.2

The outlet and outlet passage may be adequately cleaned manually or the tank or compartment with the increased outlet passage is provided with a fixed spray device(s) so that the outlet and outlet passage may be mechanically cleaned.

D.12.1.3

The outlet passage is insulated sufficiently that the temperature rise of the water in the outlet passage does not exceed the allowable average temperature rise of the tank full of water (2 F) specified in D.11.

D.12.1.4

The outlet passage is protected against damage (denting) and is braced and sloped.

D.12.2

Outlet valves shall be either close-coupled sanitary plug type or compression type tank outlet valves. The valve body shall be designed so that it can be used without a gasket or with either a single-service or multiple-use gasket.

Note: Block tin gaskets are not considered to be multiple-use gaskets.

D.12.3

The tank outlet and valve bore shall be the same size and concentric.

D.12.4

A sanitary 3-A cap shall be furnished for the outlet opening of the outlet valve, except the outlet opening of a valve located in the pump and/or hose cabinet that is connected to the pump piping.

D.13

Unless the outlet valve is located in the pump and/or hose cabinet, it shall be provided with a dust cover which (1) encloses the entire valve assembly, (2) is dustproof and (3) has a smooth interior finish. Dust covers shall be provided with means of sealing to prevent opening or removing the cover without breaking the seal.

D.14

Manhole Opening and Cover:

D.14.1

The manhole(s) opening shall be not less than 16 inches by 20 inches oval or 18 inches in diameter.

D.14.2

The upper edge of a top manhole opening shall be not less than 3/8 inch higher than the surrounding area and if an exterior flange is incorporated in it, it shall slope and drain away from the opening.

D.14.3

Manholes shall be located so that the distance from either end of the tank shall not exceed 18 feet, 6 inches, except that this requirement shall not apply to tanks with a permanently installed mechanical cleaning device(s) so designed and installed that solutions are applied to all product contact surfaces except those areas requiring manual cleaning.

D.14.4

Manhole cover gaskets shall be readily removable and may have any one of the following cross-sections: flat, rectangular, square, oval, round, "L" or "Z" shape, or any other section which is equally cleanable.

D.14.5

Gasket grooves or gasket retaining grooves shall not exceed 1/4 inch in depth or be less than 1/4 inch wide. The minimum radius of any internal angle in a gasket groove or gasket retaining groove shall be not less than 1/16 inch.

D.14.6

A sanitary vent of sufficient free opening to prevent excess vacuum and/or internal pressure, shall be installed under the manhole dust cover. The air vent shall be designed so that parts are readily accessible, easily removable and readily cleanable. (See Appendix, Section G.)

D.15

Manhole Dust Cover:

D.15.1

Each manhole shall be provided with a dust cover.

D.15.2

The interior finish of the dust cover shall be smooth, readily cleanable and free from bolts and screws. Round or oval head rivets shall be deemed acceptable.

D.15.3

Welded interior attachments shall have minimum radii of 1/16 inch.

D.15.4

A suitable vent shall be provided to relieve vacuum and pressure when the dust cover is closed.

D.15.5

The dust cover when closed shall provide an effective seal to prevent entrance of dust.

D.15.6

If a rubber or rubber-like, or plastic gasket is used as a seal, it shall be smooth, either removable or firmly bonded to the dust cover to provide a smooth, easily cleanable surface without crevices.

D.15.7

Deck plate, if attached to the outer jacket, shall be effectively sealed and firmly bonded.

D.15.8

The dust cover shall be provided with means of sealing to prevent opening the dust cover without breaking the seal.

D.16
Agitation:

D.16.1
When specified, the tank or compartment thereof shall be provided with means for mechanical and/or air agitation that when operated 20 minutes in whole milk that has been stored 24 hours at 40 F will result in the milk fat content of the product throughout the tank or compartment being within a variation of plus or minus 0.1 per cent by the official AOAC[3] Babcock milk fat test.

D.16.2
The agitator, if not designed for mechanical cleaning, shall be located in such a manner that it shall be readily accessible for manual cleaning and inspection.

D.16.3
A mechanical agitator shall have a seal of the packless type, sanitary in design with all parts accessible for cleaning.

D.17
Air Under Pressure and/or Mechanical Cleaning:

D.17.1
Equipment and means for applying air under pressure or solutions for mechanical cleaning, when provided, shall conform to the applicable provisions of the "3-A Accepted Practices for Supplying Air Under Pressure in Contact with Milk, Milk Products and Product Contact Surfaces," Serial #60400, as amended, except that clamp type fittings shall not be used in the product zone.

D.17.2
Tubing and related fittings within the tank shall be readily and easily removable for cleaning outside the tank or be designed to be mechanically cleaned.

D.17.3
Openings for air agitation and/or mechanical cleaning applications shall be protected against contamination by means of a removable dust cover, except where such openings are within the pump and/or hose cabinet.

D.17.4
Permanently mounted air or solution tubing shall be constructed and installed so that it will not sag, buckle, vibrate or prevent complete drainage of the tank or tubing, and shall be located so that the distance from the outside of the tubing to the lining is at least two inches, except at point of entrance. If designed for mechanical cleaning, the tubing and all related fittings shall be self-draining.

D.17.5
Means for mechanically cleaning the tank or compartment when provided, shall clean the product contact surfaces and all non-removable appurtenances thereto except those areas that may be manually cleaned without entering the tank. (See suggestions Appendix, Section H.)

D.18
Baffles

D.18.1
Baffles, when provided, shall not interfere with the free drainage of the tank or compartment.

D.18.2
The area of any one baffle plate shall not exceed 40% of the cross-sectional area of the tank and the entire baffle shall be on one side of the longitudinal center line of the tank. If more than one baffle is installed, consecutive baffles shall be installed on opposite sides of the tank and shall be at least 48 inches apart. Baffles shall be so designed that walk-through accessibility will be provided to all areas for inspection, and if the tank is not provided with means for mechanically cleaning the tank or compartment, for cleaning purposes.

D.18.3
Baffles shall be permanently attached to the tank. The radius of inside corners formed where baffles are attached to the lining shall be at least 1/4 inch. There shall be no sharp edges on baffles.

D.19
Hose Cabinets:

D.19.1
Hose cabinets shall comply with the following as well as other applicable provisions of the Fabrication Section D:

D.19.2
The lining of cabinets, doors and fixed attachments shall be smooth.

D.19.3
All permanent joints in the lining shall be welded. All welded areas in the lining shall be at least as smooth as the adjoining surfaces.

D.19.4
The bottom shall be constructed so that it will not sag, buckle or prevent complete drainage when the truck is on a level surface.

D.19.5
All inside corners shall have minimum radii of 1/8 inch.

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[3]The method of making this test will be found in the following reference: Official Methods of Analysis; Available from the Association of Official Analytical Chemists, P. O. Box 540, Benjamin Franklin Station, Washington, D. C. 20004.

D.19.6

Cabinets shall be dust tight and doors shall be equipped with a compression type closing device. Gasket material for sealing cabinet doors may be installed on face of the cabinet or on the doors except along a drainage area where it shall be attached to the doors. Gasket material shall be removable or firmly bonded to provide smooth, easily cleanable surfaces without crevices.

D.19.7

A roof overhang or suitable drip molding shall be provided over the cabinet doors.

D.19.8

The cabinet and doors shall be insulated with an insulating material having an insulating value of not less than 1 inch of cork.

D.19.9

A carrier bracket shall be provided to support the flexible transfer tubing. Means shall be provided to support the loose end of the tubing above the cabinet floor.

D.19.10

Fixed attachments such as pump support brackets, tubing carrier brackets and brackets for belt and pulley guards shall be easily accessible for cleaning. A pump having a base area of 1 square foot or less shall be installed so that there will be a minimum clearance of 2 inches between the base and the cabinet floor and 3 inches between the pump assembly and the cabinet walls. The minimum clearance between the base and the cabinet floor shall be increased to 3 inches if the base area of the pump exceeds 1 square foot. A pump assembly that is to be mounted on the floor of the cabinet shall have a solid base and be installed with a non-absorbent sealing gasket. It shall be installed in a position that (1) will not interfere with drainage and (2) will provide minimum clearance of 3″ between the pump assembly and the cabinet walls. Except when readily removable, a side wall mounted pump assembly shall be installed with a non-absorbent sealing gasket.

D.19.11

The size and location of the cabinet shall be such that will afford easy accessibility for assembly and disassembly of removable parts and provide ample clearance around permanently installed equipment and parts. (See Appendix, Section I, Facilities for Extra Fittings.)

D.20

Pumps:

D.20.1

Pumps, when furnished, shall conform to the "3-A Sanitary Standards for Pumps for Milk and Milk Products, Revised, Serial #0203," as amended. A sanitary closure shall be furnished for the outlet opening of the pump.

D.21

Motors for Pumps and/or Agitators:

D.12.1

A motor when located in the pump compartment, shall be totally enclosed, non-ventilated and the electric wiring shall be water-proof and shall be conducted through the wall of pump cabinet with water-tight connections.

D.21.2

Storage space for the pump motor electrical extension cord shall be located outside the pump compartment. It is suggested that a grounded cord reel be used to store the extension cord.

D.22

Transfer Tubing:

D.22.1

Single lengths of transfer tubing shall not exceed 8 feet except where adequate acceptable cleaning facilities are available at the place of cleaning. The minimum inside diameter of the tubing shall be 1-3/8 inches. A sanitary closure shall be furnished for the open end(s) of the tubing.

D.22.2

If two lengths of flexible tubing are used, they shall be connected either by the use of sanitary couplings or by a piece of rigid 3-A Sanitary Tubing.

D.22.3

A piece of flexible tubing may be used for the connection from the pump to the tank.

D.22.4

Flexible tubing shall be attached to rigid 3-A Sanitary Tubing, or the tank or pump in such a manner that the flexible tubing may easily be removed without tools. If clamps are used they shall be readily removable without tools.

D.23

Sample Trays and Sample Compartments:

D.23.1

Sample trays and sample compartments that are furnished by the tank manufacturer that are to be in the pump and/or hose cabinet shall be of sanitary design and readily cleanable.

D.23.2

Facilities shall be provided for keeping the samples cold.

D.23.3

Permanently installed sample compartments shall (1) be attached to the cabinets by welding or with bolted connections which have non-absorbent sealing gaskets in the joints, (2) have the supporting member(s) welded if supported from the floor of the cabinet and (3) be installed so there is a minimum clearance of 6 inches between the sample compartment and the cabinet floor.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series.

Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8 and CF-8M, respectively. These cast grades are covered by ASTM[4] specifications A 296-67 and A 351-65.

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.

G.

AIR VENTING

To insure adequate venting of the tank which will protect it from internal pressure or vacuum damage, the critical relationship between minimum vent size and maximum filling or emptying rates should be observed. A venting system of sufficient capacity to provide for venting during filling and emptying is not adequate during mechanical cleaning. During the cleaning cycle tanks when cleaned mechanically should be vented adequately by opening the manhole cover to prevent vacuum or pressure build-up due to sudden changes in temperature of very large volumes of air.[5] Means should be provided to prevent excess loss of cleaning solution through the manhole opening. The use of tempered water of about 95 F for both prerinsing and post-rinsing is recommended to reduce the effect of flash heating and cooling.

H.

MECHANICAL CLEANING

The mechanical cleaning system shall be so designed that solution is applied to all product contact surfaces except those areas requiring manual cleaning. When being cleaned, the tank should have sufficient pitch to accomplish draining and to have a fast flushing action across the bottom. The pitch should be at least 1/4 inch per foot. Means should be provided for manual cleaning of all surfaces not cleaned satisfactorily by mechanical cleaning procedures.

NOTE: Cleaning and/or sanitizing solutions should be made up in a separate tank—not in the transportation tank.

I.

FACILITIES FOR EXTRA FITTINGS

If extra sanitary fittings are supplied by the manufacturer of the farm pick-up tank, facilities should be provided in the pump compartment to adequately protect such items.

These standards are effective Feb. 1, 1972 at which time the 3-A "Sanitary Standards for Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-Up Service, Amended April 28, 1954," Serial #0501 and amendments thereto, are rescinded and become null and void.

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

[5]For example, when a 6,000 gallon tank (with 800 cu. ft. of 135 F hot air after cleaning) is suddenly flash cooled by 50 F water sprayed at 100 gpm the following takes place:

Within one second, the 800 cu. ft. of hot air shrinks approximately 51 cu. ft. in volume. This is the equivalent in occupied space of approximately 382 gallons of product. This shrinkage creates a vacuum sufficient to collapse the tank unless the vent, manhole, or other openings allow air to enter the tank at approximately the same rate as it shrinks. It is obvious, therefore, that a very large air vent such as the manhole opening is required to accommodate this air flow.

3-A ACCEPTED PRACTICES FOR MILK AND MILK PRODUCTS SPRAY DRYING SYSTEMS

Serial #60700

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United State 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. Milk and milk products spray drying systems heretofore or hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following practices, but which in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

A.
SCOPE

A.1
These 3-A Accepted Practices shall pertain to the sanitary aspects of equipment for spray drying milk and milk products, and includes all equipment necessary for spray drying milk and milk products beginning with the discharge of the pump which delivers the liquid product to the drying system and terminating at the point the final dried product enters either the packaging systems or storage for further processing. The drying system includes the equipment used for moving and cleaning the air, heating and/or cooling the air, atomizing the liquid, mixing the liquid in the hot air, removing the dry product from the air, additional drying of the product, cooling the product, pulverizing, sizing and conveying the product.

A.2
In order to comply to these 3-A Accepted Practices, equipment in spray drying systems shall comply with the following criteria for design, material, fabrication and air supply.

B.
DEFINITIONS

B.1
Product: Shall mean the milk or milk product and dry milk or dry milk product.

B.2
Air to be Heated: Shall mean processing air to be heated to at least 240°F.

B.3
Air not to be Heated: Shall mean processing air which either will not be heated or will be heated to a temperature less than 240°F.

B.4
Processing Air: Shall mean air prepared by filtration which is intended to be used in contact with the product for such purposes as heating, cooling, drying or conveying or will be used for sealing a bearing or similar purposes.

B.5
Product Contact Surfaces:

B.5.1
Shall mean all surfaces that are exposed to the product or from which liquids and/or solids may drain, drop or be drawn into the product.

B.5.2
Shall mean all surfaces in contact with air which is not to be heated prior to coming in contact with the product commencing at the discharge of the air inlet filter(s) and ending at the first downstream surface in contact with the product.

B.6
Air Contact Surfaces:

B.6.1
Inlet air contact surfaces shall mean all surfaces (1) in contact with air to be heated prior to coming in contact with the product and (2) in contact with heated air, commencing at the discharge of the air inlet filter(s) and ending at the first downstream product contact surface.

B.6.2
Exhaust air contact surfaces shall mean the surfaces of the air ducts, plenum chamber(s) (if provided) and appurtenances from the final product contact surface through the exhaust system.

B.7
Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.8
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

The materials of product contact surfaces of equipment included in the spray drying system for which there are 3-A Sanitary Standards or 3-A Accepted Practices shall comply with the material criteria of the applicable Standards or Accepted Practices.

C.2

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

C.2.1

Plastic materials may be used for scraper blades, sight and/or light glasses, bearings, bushings, short pieces of transparent tubing in dry product areas for observation purposes, short flexible connectors and sealing applications. These materials shall conform 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.2.2

Rubber and Rubber-like materials may be used for short flexible connectors, scraper blades and sealing applications. The materials shall conform 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.2.3

Cotton, wool, linen, silk, or synthetic fibers may be used for filtering and/or screening surfaces or entrainment separators, and for short flexible connectors used in dry product packaging areas. These materials shall be non-toxic, relatively insoluble in water, easily cleanable, and shall not impart a flavor to the product.

C.2.4

Aluminum alloys conforming to the Aluminum Association[3] designates 5052 and 6061 may be used as a product contact surface for dry product when used as a supporting or reinforcing member in light weight moving parts and other similar applications.

C.2.5

Welded areas and the deposited weld material shall be substantially as corrosion resistant as the parent materials.

C.2.6

Heat resistant glass[4] may be used in sight and/or light openings.

C.3

Air contact surfaces, except for those of flexible connectors, fans, burners and dampers, shall be of a corrosion resistant metal that maintains its original surface characteristics under the environment of intended use, or is rendered corrosion-resistant by a coating of corrosion-resistant material other than paint. If the portion of the plenum chamber at the inlet to the drying chamber is subject to washing, it shall be made of stainless steel.

C.4

Filter Media: Intake air filter media shall consist of one or more of the following: fiber glass with a downstream backing dense enough to prevent fiber glass break off from passing through, cotton flannel, wool flannel, spunmetal, activated carbon, activated alumina, non-woven fabric, absorbent cotton fibre, or other suitable materials which, under conditions of intended use, are non-toxic and non-shedding and which do not release toxic volatiles or other contaminants to the air, or volatiles which may impart any flavor or odor to the product. Chemical bonding materials contained in the media shall be non-toxic, non-volatile and insoluble under all conditions of use. Disposable media shall not be cleaned and re-used.

Note: Electronic air cleaners use electrostatic precipitation principles to collect particulate matter and therefore are not included in the preceding list of acceptable filter media. This does not preclude their use in spray drying systems.

C.5

Non-product contact surfaces, shall be of cor-

[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 & Steel Institute, 633 Third Ave., New York, N. Y. 10017.

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

[3]Aluminum Association, 420 Lexington Avenue, New York, N. Y. 10017.

[4]Glass of a borosilicate type with a coefficient of expansion between 30° C and 300° C of between 3.0 and 3.5 parts per million per degree centrigrade.

rosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. Non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.

FABRICATION

D.1

The fabrication criteria of equipment included in the spray drying system for which there are 3-A Sanitary Standards or 3-A Accepted Practices shall be those of the applicable Standards or Accepted Practices.

D.2

All other equipment shall conform to the following fabrication criteria.

D.2.1

The product contact surfaces of stainless steel sheets shall be as smooth as a No. 4 mill finish. Seam welds shall be smooth and pit free, and where grinding and polishing is required, such areas shall be at least as smooth as a finish obtained with 80 grit silicon carbide. Intricate fabricated and/or machined components shall be as smooth as a finish obtained with 80 grit silicon carbide, with welds pit free. If stainless steel sheets with a No. 2B mill finish are used, they shall be free of imperfections such as chips, flakes, or pits. Joints shall be smooth and flush. Permanent joints in metallic product contact surfaces shall be continuously welded.

D.2.2.

Product contact surfaces shall be easily accessible for thorough cleaning, either when in an assembled position or when removed. Parts that must be removed for cleaning shall be readily removable and easily dismantled, except that high pressure liquid product lines and such parts as fan wheels, air lock valves, fluidizer valves, conveying mechanisms, and similar parts need only be readily accessible for cleaning.

D.2.3

Product contact surfaces intended for regular wet cleaning shall be self-draining or self-purging except for normal clingage, except where self-draining is not feasible other drying methods including air drying may be used.

D.2.4

Internal angles of 135°F or less on product contact surfaces shall have minimum radii of 1/4 inch except where smaller radii are required for essential functional reasons such as those on internal parts of mechanical collectors, collector systems and air lock blades. In no case shall such radii be less than 1/32 inch, except those on atomizing devices and where external welding is necessary. In either case the internal product contact surface must be readily available for cleaning and inspection.

D.2.5

There shall be no exposed threads or crevices on product contact surfaces except where required for functional and safety reasons such as high pressure liquid product lines and atomizing devices, fan wheels, air lock valves, fluidizer valves and conveying mechanisms. The parts for which an exception is made that have exposed threads or crevices on product contact surfaces shall be designed to be mechanically cleaned or shall be readily accessible for cleaning.

D.2.6

Flexible connections having product contact surfaces shall have straight sides without corrugations.

D.3

Air contact surfaces shall be readily cleanable.

D.4

Sheet metal work constructed in accordance with conventional fabrication techniques[5] may be used for portions of the drier having air contact surfaces.

D.5

The construction of the portions of the spray drying system having air contact surfaces such as sheet metal work, air heating equipment, filtering equipment, pneumatic conveying equipment and exhaust systems shall be so constructed as to prevent the entrance of unfiltered air.

D.6

Non-product contact surfaces to be coated shall be effectively prepared for coating.

D.7

Sanitary tubing and fittings except those used (1) in high pressure liquid product lines and (2) in dry product conveying piping and equipment shall conform with the design and construction provisions of the "3-A Sanitary Standards for Fittings used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and

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[5]Information on sheet metal fabricating techniques will be found in: Paull, James H. *Industrial Sheet Metal Drawing.* 1938, Van Nostrand Co., Inc., New York, "Methods of Fastening," Ch. VII, p. 135.

Milk Products, Revised, Serial #0809", and Supplements thereto, as amended, and/or "3-A Accepted Practices for Permanently Installed Sanitary Product-Pipelines and Cleaning Systems, Serial #60500", as amended.

D.8

Non-product contact surfaces shall be free of pockets and crevices and shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable. Panels or doors shall be provided to allow easy access for cleaning of non-product areas of the equipment. They shall be constructed in a manner that will prevent air entrance. Use of hinges, wing nuts, latches, and similar easy-opening fastening devices are recommended to allow easy access without special tools.

D.9

Gaskets and Gasket Grooves on Product Contact Surfaces: Gaskets shall be removable or permanently bonded. Gasket retaining grooves for removable gaskets, if provided, shall be no deeper than their width. The minimum radius of any internal angle in a gasket retaining groove shall be not less than 1/8 inch, except that a 3/32 inch radius is permissible where a standard 1/4 inch O-Ring is to be used. Use of gasket positioning grooves or pins, premolded fitted gaskets or gaskets cut from sheet material are recommended.

D.10

When a fan is installed on the downstream side of the intake air filter, it shall be designed and installed in a manner to preclude entrance of air contaminants.

D.11

Fans of the air foil type shall be constructed with blade cavities sealed.

D.12

Any bearing having a product contact surface shall be of a non-lubricated type. Lubricated bearings shall be located outside the product contact surface with at least 1 inch clearance between the bearing and any product contact surface to assure (1) that the product does not contact the bearing or lubricant and (2) lubricants and/or product do not build up between the bearing and any product contact surface. When a shaft or cable passes through a product contact surface, the portion of the opening surrounding the shaft or cable shall be protected to prevent the entrance of contaminants.

D.13

When the exhausts of collectors are connected to the bottom of a plenum whose entire construction does not conform to the criteria for product contact surfaces, (1) the top of the plenum shall be constructed so as to conform to product contact surface criteria and (2) the collector exhaust connections shall extend upward into the plenum at least 6 inches. This provision does not apply to cloth collector bags.

D.14

A self-closing head shall be installed at the terminal end of all exhaust to atmosphere ducts.

E.

AIR SUPPLY FOR DRYING SYSTEMS

E.1

The location and nature of adjacent structures and the variations of wind and weather shall be considered in selecting the location of the air supply intake opening whether inside or outside a building. It shall be so located that it will reasonably insure that the character of the intake air will be suitable for its intended use.

E.2

Outside intake openings shall be suitably protected against the admission of all foreign objects. Openings should be provided with louvers which can be closed when processing equipment is not in use. Hoods should be used over these openings to minimize the intake of rain, snow, dust or other foreign material. Openings shall be equipped with sturdy screens having openings not larger than 3/4 inch in any dimension.

E.3

The air supply system and/or ducting shall be such that all of the air is caused to pass through air filters properly installed before coming in contact with product contact surfaces of the drying system.

E.3.1

Processing air which will be heated before product contact shall be passed through a properly installed and maintained filter(s), selected to have a minimum average efficiency of 90% when tested in accordance with the ASHRAE Synthetic Dust Arrestance Test[6] when operated at its design face velocity.

E.3.2

Processing air which will not be heated before product contact shall be passed through a prop-

[6]The method of making these tests will be found in the following reference: Method of Testing Air Cleaning Devices, ASHRAE Standard 52-68. Available from The American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 345 E. 47th Street, New York, New York 10017.

erly installed and maintained filter(s), selected to have a minimum average efficiency of 85% when tested in accordance with the ASHRAE Atmospheric Dust Spot Method[6] when operated at its design face velocity.

APPENDIX

F.

PRODUCT CONTACT SURFACE MATERIALS

F.1

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.2 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.2 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8 and CF-8M, respectively. These cast grades are covered by ASTM[7] Specifications A-296-68 and A351-70.

G.

CLEANING AND SANITIZING PROCEDURES

A cleaning and sanitizing regimen which is effective shall be employed. A description of this regimen shall be available at the drying plant. Because of the possibilities of corrosion, the recommendations of the cleaning compound manufacturer shall be followed with respect to the time, temperature, and the concentration of specific detergents and sanitizing agents. To insure proper strength of solution and to avoid corrosion, the detergent or sanitizer shall be completely dissolved or dispersed prior to use.

The following is adapted from "Special Sanitation Suggestions for Dry Milk Manufacturers" available from American Dry Milk Institute, 130 N. Franklin Street, Chicago, Ill. 60606, ADMI Handbook No. 917.

G.1

High Pressure Supply Pump, Milk Lines and Spray Devices

G.1.1

As soon as possible after the drier is shut down, remove the spray nozzles from chamber and place the nozzles in the solution tank. Direct the high pressure lines and/or nozzle pipes to the solution tank.

[7]Available from American Society for Testing & Materials, 1916 Race St., Philadelphia, Pa. 19103.

G.1.2

Remove and manually clean the line through which product is conducted from the high pressure regulating valve at the outlet of the high pressure pump to the inlet of the pump. Hook up the lines for the complete recirculating circuit including the preheater, high pressure or supply pump and high pressure lines. Do not put operating pressure on high pressure pump during rinsing and cleaning.

G.1.3

Using clear water at 110-115°F flush the entire circuit until rinse water is clear. Rinse water should go directly to the drain.

G.1.4

After rinsing, add enough water to the solution tank to avoid sucking air into lines during circulation. Slowly add an alkaline cleaning compound in the amount specified by the supplier. Circulate this solution for the length of time and at the temperature recommended by the cleaning compound manufacturer. To assure full flow through a multiple spray pipe system it may be necessary to circulate groups of pipes, valves and nozzle pipes alternately.

G.1.5

Rinse the alkaline cleaning solution thoroughly from the system and refill circuit with warm water.

G.1.6

Add an acid cleaning solution to the solution tank according to the cleaning compound manufacturer's direction. Circulate for the time and at the temperature recommended by the manufacturer of the cleaning compound. After completion of cleaning, completely rinse the acid cleaning solution from the system.

G.1.7

Disassemble the high pressure pump and check the entire system for effectiveness of cleaning. Allow the high pressure pump to dry before reassembly.

G.1.8

When paper gaskets are used in the preheater or anywhere in the circuit they must be changed daily.

G.1.9

Immediately prior to reuse of equipment, circulate a santitizing solution through system for 5 minutes and then discharge it to the drain.

G.1.10

Circulation time, temperature and strength of cleaning solutions may vary according to amount

Milk Products, Revised, Serial #0809", and Supplements thereto, as amended, and/or "3-A Accepted Practices for Permanently Installed Sanitary Product-Pipelines and Cleaning Systems, Serial #60500", as amended.

D.8
Non-product contact surfaces shall be free of pockets and crevices and shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable. Panels or doors shall be provided to allow easy access for cleaning of non-product areas of the equipment. They shall be constructed in a manner that will prevent air entrance. Use of hinges, wing nuts, latches, and similar easy-opening fastening devices are recommended to allow easy access without special tools.

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Outside intake openings shall be suitably protected against the admission of all foreign objects. Openings should be provided with louvers which can be closed when processing equipment is not in use. Hoods should be used over these openings to minimize the intake of rain, snow, dust or other foreign material. Openings shall be equipped with sturdy screens having openings not larger than 3/4 inch in any dimension.

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The air supply system and/or ducting shall be such that all of the air is caused to pass through air filters properly installed before coming in contact with product contact surfaces of the drying system.

E.3.1
Processing air which will be heated before product contact shall be passed through a properly installed and maintained filter(s), selected to have a minimum average efficiency of 90% when tested in accordance with the ASHRAE Synthetic Dust Arrestance Test[6] when operated at its design face velocity.

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Remove and manually clean the line through which product is conducted from the high pressure regulating valve at the outlet of the high pressure pump to the inlet of the pump. Hook up the lines for the complete recirculating circuit including the preheater, high pressure or supply pump and high pressure lines. Do not put operating pressure on high pressure pump during rinsing and cleaning.

G.1.3

Using clear water at 110-115°F flush the entire circuit until rinse water is clear. Rinse water should go directly to the drain.

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After rinsing, add enough water to the solution tank to avoid sucking air into lines during circulation. Slowly add an alkaline cleaning compound in the amount specified by the supplier. Circulate this solution for the length of time and at the temperature recommended by the cleaning compound manufacturer. To assure full flow through a multiple spray pipe system it may be necessary to circulate groups of pipes, valves and nozzle pipes alternately.

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Add an acid cleaning solution to the solution tank according to the cleaning compound manufacturer's direction. Circulate for the time and at the temperature recommended by the manufacturer of the cleaning compound. After completion of cleaning, completely rinse the acid cleaning solution from the system.

G.1.7

Disassemble the high pressure pump and check the entire system for effectiveness of cleaning. Allow the high pressure pump to dry before reassembly.

G.1.8

When paper gaskets are used in the preheater or anywhere in the circuit they must be changed daily.

G.1.9

Immediately prior to reuse of equipment, circulate a santitizing solution through system for 5 minutes and then discharge it to the drain.

G.1.10

Circulation time, temperature and strength of cleaning solutions may vary according to amount

[7]Available from American Society for Testing & Materials, 1916 Race St., Philadelphia, Pa. 19103.

of milk processed, temperatures used and water conditions in a particular plant.

G.1.11

Be sure to check spray nozzles daily for cleanliness and wear of cores, orifices, spinning devices, etc.

H.

GAS BURNER MAINTENANCE

It is essential that burners and their controls operate properly to produce a good quality product, and for the prevention of fires. It is suggested that burners be cleaned at least three times a year. If burners are extremely dirty, it is suggested that burners be removed and cleaned in the shop or other area away from the drier.

If in doubt about the operation of the burner, the drier manufacturer or a qualified service man recommended by him should be consulted.

At least once a year the burner and controls should be serviced by the manufacturer or a service man recommended by him.

I.

SANITARY ATTIRE AND CLEANING APPLIANCES

I.1

When it is necessary to enter the drier for cleaning:

I.1.1

The cleanup crew should be furnished with freshly laundered outer clothing and cleaned and sanitized multiple use or single service boots to wear while in the drier.

I.1.2

A suitable place should be provided for the storage of laundered outer clothing, cleaned and sanitized boots, unused single service items and cleaning tools and appliances.

I.1.3

A clean place should be provided adjacent to the point of entry to the drier which provides (1) an area to which the clean outer clothing, can be carried, (2) an area in which, if required, outer clothing can be removed and stored, (3) an area in which the clean outer clothing and boots for use in the drier can be donned and (4) a clean floor (for example, a covering of clean paper) to maintain the cleanliness of the boots.

I.1.4

Garments and boots worn for interior drier cleaning should be worn only while cleaning the drier and not while performing other tasks. Boots that have been worn while walking outside the drier should be replaced with other suitable boots before re-entering the drier.

I.2

Cleaning tools and appliances that are used in the drier should be kept clean and used for no other purpose than cleaning the interior of the drier.

These Practices shall become effective March 14, 1972.

NUTRITION EDUCATION

The American Dietetic Association and National Dairy Council are cooperating in a nutrition education effort to inform American consumers about good eating habits.

A series of eight educational messages are available to all dairy processors and handlers nationwide for imprinting on side panels of milk cartons. Entitled "Nutrition Facts from National Dairy Council and the American Dietetic Association" the illustrated panel art and copy tell consumers about the importance of a well-balanced diet selected daily from the four food groups. Also, the functions of certain nutrients are explained and foods containing these nutrients are listed.

In a meeting between Mrs. Marjorie M. Donnelly, ADA President and Dr. M. F. Brink, NDC President, both agreed that this joint program could go a long way in "helping all Americans better understand the inter-relationships of all foods, and help them apply this knowledge to their daily living."

According to Dr. Brink, "If all of America's dairy processors cooperate by imprinting these educational messages on milk cartons there is a potential of more than 50 million impression daily with family members in their homes across the country."

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BACTERIOLOGICAL TESTING OF RAW MILK AND DAIRY PRODUCTS[1, 2]

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ABSTRACT

Bacteriological testing of raw milk and processed dairy products is not adequate for public health and quality control purposes. Bacteriological test results are not highly correlated with farm production conditions. Routine bacterial quality control tests are not always adequate for recovery of microorganisms injured during processing. Present systems and trends are discussed.

Sanitarians are more and more frequently asking the question, "Is bacteriological testing of raw milk and dairy products still adequate?" In reply a little allegory would be appropriate. According to Greek mythology, Pandora was the first woman on earth. She had or had found a jar, the so-called "Pandora's box" containing, according to a later version of the fable, an assortment of blessings for the human race. When the jar was opened through curiosity, all escaped except the blessing of hope, which has survived over the long years. This analogy is admittedly strained; many of us, however, will admit to approaching routine bacteriological tests like Pandora's box. The proffered blessings sometimes escape when the jar (or should I say, pipette canister) is opened. But, we still have hope. We perform our tests with the hope that they will be adequate for the purpose for which they are being used.

How often is this hope justified? And, is the implication of past adequacy in the question correct? Were our tests ever truly adequate? In a purely pedagogic approach, we should advance on this question with caution, pin it to a dissecting board, and carefully examine its separate parts. What is bacteriological testing today, and what was it yesterday? Why do we now perform these tests; has their use changed appreciably from that of yesteryear? Are they adequate now; were they really ever adequate?

If any among us are smart enough to answer the first half of each question, they may not be old enough to answer the last half. A sense and appreciation of history is required at this point.

Present these questions to a well-trained dairy and food technologist and, if his reflexes are intact, he will reach for his copy of *Standard Methods for the Examination of Dairy Products.* Why not? Throughout this century, this manual has served as *the* continuum of knowledge for generations of food technologists. What better source have we for answers to these questions?

DEVELOPMENT OF STANDARD METHODS

Sixty-five years ago, Professor S. C. Prescott reported on "The Need for Uniform Methods in the Sanitary Examination of Milk" at a meeting of the Laboratory Section of the American Public Health Association. He stated that differences in composition of culture media, variation in methods, dilution amount, time and temperature of incubation, and other factors made results from different laboratories valueless for comparison. As so frequently happens when someone speaks out, these comments got him appointed as chairman of a committee to study the existing methods and techniques used in bacteriological examination of milk *(3)*. Replies to a circular letter indicated, as Professor Prescott had already expressed, wide variations on most important procedural points. This, then, was the birth of *Standard Methods for the Examination of Dairy Products.*

After 2 years of committee work, a "Preliminary Statement by the Committee on Standard Methods of Bacterial Analysis" *(1)* was issued. Members of the committee were both modest and farsighted enough to state that "much work yet remains to be done on many important points." They also remarked that "There is no method known by which the exact number of bacteria in a sample of milk may be determined, and even when the best methods are used, the count is always less than the actual number of bacteria present . . . ." They justified this statement by offering the following reasons: the presence of adhesive membranes between clumps and chains of cells, various nutritional requirements between species and races, optimal temperature differences, oxygen requirements, growth rates, antagonism toward growth in close proximity to contiguous col-

[1]Journal Paper No. J-6716 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project 1839.
[2]Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Cedar Rapids, Iowa, August 17-20, 1970.

onies (200 colonies per plate was then considered the limit), the presence of "spreaders" and molds, and, finally, that storage of milk at 34 F for various periods or holding it in dilution water for several hours while plating would have a deterent effect upon colony development.

The conflict between the philosophy of total counts versus a Standard Plate count must have been of concern even then since the committee promised that "much work will be done on comparison of 37 C and 'room temperature' during the coming year." The committee was unwilling to recommend a numerical standard for leucocyte counts, but did regard high cell counts in milk with suspicion. As indicator organisms, long-chained streptococci (showing inflammatory reactions), gas-producers (careless handling), and *Bacterium coli* (dirty stables, cows, and vessels) were used.

In 1908, with only one member change in the committee of six, another progress report *(2)* was presented at the annual American Public Health Association meeting. The committee deemed it wisest not to make final recommendations. They were unable to decide between 48-hr incubation at 37 C or 5-day incubation at 21 C as the standard time-temperature combination. Consequently, use of either combination was deemed acceptable. We now know that the latter combination is most productive for colony formation. The microscopic estimate of bacteria in milk was categorized as useful as a preliminary test. Examination of milk for *B. coli* (*Escherichia coli*) had become a routine procedure in some laboratories; the number of *E. coli* present seeming to some degree to certain workers a more exact index to care of production than was the total bacterial count. The Wisconsin Curd test was no longer considered a sure test for the presence of fecal matter.

The first edition

In 1909, the same committee *(3)*, noting that its two previous preliminary reports had been favorably received, submitted a final report. This report, now considered the first edition of *Standard Methods,* was practically a restatement of the previous tentative proposals. In contrast to the preliminary reports, however, the 1909 report specifically stated that a count of 100,000 bacteria per cubic centimeter within a few hours after production indicated that the milk was seriously contaminated. After 24 to 36 hr, a count of this magnitude would indicate "ordinarily good care." They also stated that a leucocyte content of 500,000 or over per cubic centimeter, especially in mixed milk, should be regarded as suggestive of some inflammatory condition of the udder.

The second edition

The second edition *(4)*, published in report form in 1916, was greatly reduced in size, occupying only 10 journal pages compared with 30 pages in the preceding edition. This edition is noteworthy for its statement that bacteriological analysis of milk is required for routine and research purposes. This, of course, is so obvious to us now that our natural reaction is to wonder why it was not so evident then. The continuing demand for more rapid methods probably was responsible for the elimination of the total count determined by 5-day incubation at 21 C as a standard method. Although this edition pointed out that, in general, interpretation of the routine bacteriological analysis of market milk depends upon the history of the milk, it did give a few significant conclusions. For example, with properly cleaned and sterilized equipment and proper care in the farm and dairy, counts may easily be brought down to 5,000/cc. Bacterial counts over 50,000/cc within 5 to 7 hr after milking indicate that the milk has not been "properly guarded." Twenty-four hours from the cow, the bacterial number of the milk should not be over 100,000 in winter or 200,000 in summer; after 48 hr, the count should not exceed 1,000,000/cc. The microscopic count had not yet been recommended as a standard method, but it was recognized as an extremely useful method for rapid grading. The first hint of a supposed relationship between results of routine bacteriological analysis and public health appear in the concluding remark that, through microscopic examination of milk, the farmer could eliminate from his herds those cows that were discharging large numbers of streptococci. The milk supply thus could be protected "from this type of organisms that are today recognized as suspicious and decidedly undesirable."

The third edition

After an interval of 5 years, the 3rd edition *(5)* was published in 1921. There must have been a considerable amount of infighting by the entire field going on during this time because the committee offered a rather lengthy explanation in defense of shorter agar plate incubation periods. By this time, numerous investigations had shown that higher counts could be obtained by including various nutrients in the plating medium or by prolonged incubation. The Standard Plate count method was preferred, however, as furnishing an artificial means by which different laboratories could get reasonably accurate comparative results. Just why better media or longer incubation periods would not have yielded comparable counts in different laboratories was not explained. The authors correctly stated that neither the ordinary bacterial analysis nor the sediment test, which had been included in this edition, would reveal the presence of pathogenic bacteria. Through studies comparing di-

rect microscopic and plate counts, data had been gathered showing that agar plate counts were sufficiently accurate to justify their use in grading milk. By then, much of the uncertainty regarding the justification of their use had disappeared. Historically, however, workers had previously been uncertain of the value of the agar plate count in quality control work. Without fanfare or explanation, the agar plate incubation temperature was raised to 37.5 C.

At this time, reporting procedures were changed. No longer was it correct to report "number of bacteria per cc", but instead, the phrase "official plate count" followed by a number rounded to two significant left-hand digits was to be used. This, of course, was recognition of the estimate nature of the agar plate count.

The fourth edition

Only 2 years later in 1923, the 4th edition *(6)* of *Standard Methods* appeared. Rapid development in the use of laboratory methods for controlling milk quality made this repeat performance necessary. Since milk quality control was such a quickly expanding field, testing emphasis was still placed upon speed, reproducibility between laboratories, and reasonable accuracy or recovery. More refined techniques were not deemed necessary at this time. This still-early edition noted the coming of age of the reductase test (then referring specifically to the methylene blue reduction test). It was clearly recognized that exact agreement between reduction time and plate counts should not be expected, but that this method could rapidly detect poor-quality milk. The Frost "Little Plate" or microscopic colony count also was introduced as a provisional method with the emphasis again placed upon speed of gathering data. Once more, it was recognized that counts thus obtained would not agree with those of the standard method. There is no mention of the use of *E. coli* or other coliforms as indicator organisms in either the 3rd or 4th edition, although their use had been discussed previously.

The fifth edition

The 5th edition *(7)*, which did not appear until 1927, again stressed the necessity of moderate accuracy combined with a rapid and relatively inexpensive technique for obtaining bacterial estimates. The same phrases for justification of this approach as used in the preceding editions were repeated. Refinement of the direct microscopic count and the methylene blue reduction test had occurred to such an extent that they were now recognized as official procedures in this 5th edition. The agar-plate incubation temperature was lowered to 37 C with no comment. Reinstatement of coliforms as indicator organisms, however, did not yet occur.

The sixth edition

The next edition *(8)*, in 1934, did adopt the coliform count as a tentative procedure, stating that the coliform count and the total count provided essentially the same information when applied to fresh raw milk. Perhaps the most significant changes or shifts in emphasis in this edition were the inclusion of the coliform count and the formal recognition of "thermophilic, psychrophilic, saccharophilic, and other types of bacteria that do not develop readily on Standard agar at 37 C." Increasing attention to specific requirements for testing dairy products other than raw milk was indicated by inclusion of a section on the bacteriological study of ice cream.

The seventh edition

The 7th edition *(9)*, in 1939, plainly reflects the refinement induced by years of experience in quality control of dairy products. Testing for pathogenic and indicator organisms is discussed in considerable detail. Determination of yeasts and molds in butter appears for the first time. The agar plate counts, direct microscopic count, methylene blue reduction method, and the sediment test are described at great length. A standard agar of an improved formula containing dextrose, lactose and other milk solids, and a digested casein peptone was adopted.

Lest we seem to be straying from the subject, may I remind you that we are trying to decide if bacteriological testing of raw milk and dairy products ever was or is still adequate. Consider that, in 1910, it was estimated that about 50,000 samples of milk were examined annually by the agar plate method. In 1939, only about halfway through our historical review, millions of samples of milk were being analyzed by this method. Yet, this same year, the statement was made *(9)* that "Even with all of this work only a small part of the fluid milk supplies of the United States and Canada receives more than a cursory laboratory examination during each year." Bacteriological testing techniques and total application of these techniques were not adequate, yet the United States was rapidly making tremendous strides in advancing quality and safety of its dairy products. It must be evident that a test need not be complete or without fault to be helpful and that bacteriological testing alone is not the final answer to quality control, especially of raw milk. Good field work is imperative.

The eighth and ninth editions

In 1939, tolerances in incubation time and temperature were introduced; i.e., 48 hr $\pm$ 3 hr at 35 to 37 C. The 8th edition *(10)*, in 1941, was first to recognize optional agar-plate incubation at 32 C.

The number of pages in *Standard Methods* increased from 190 to 288, primarily by the inclusion of 70 pages of instructions for the microbiological examination of frozen desserts.

A glaring need for further analytical and standard methods was corrected in 1948 in the 9th edition *(11)* by addition of microbiological methods to study butter and cheese. The resazurin test and optional agar-plate incubation at 32 or 35 C also were introduced. After years of stating that total counts were not essential or necessary but that speed and reasonable accuracy were what mattered, the explanation for the reduction in incubation temperature seemed suddenly incongruous. "In order to minimize variations in plate counts due to unavoidable fluctuations in incubation temperatures and to make the count more uniformly indicative of the total bacterial content, plates should be incubated at 32 C, as recommended preferentially in *Standard Methods* since 1941. The counts on milks of good quality are not greatly affected by incubation at the lower temperature, but in the cases of milks having higher bacterial content, incubation at 32 C will more nearly reflect the actual bacterial densities in such products. The feasibility of using 32 C in a practical program for quality control of milk supplies has been shown repeatedly." How long will it take us to revert back to the progress report of 1908 in which a 5-day incubation period at 21 C was deemed acceptable? To further underline the evident inadequacy of the current tests, we note the introduction of the laboratory pasteurization test with its accompanying explanation. "None of the commonly used bacteriological tests is capable of detecting the presence of thermoduric bacteria in raw milk. . . . . it was concluded that laboratory pasteurization is the one test that determines compliance of the milk both before and after pasteurization." Up to a point, this is a true statement, but the laboratory pasteurization tests consists of two platings—one before and one after pasteurization of the milk.

The tenth edition

The primary aim of the committee responsible for publication of the 10th edition of *Standard Methods* in 1953 *(12)* was to promote uniformity of arrangement and style of presentation by using a simple cross-reference system and a more complete method of indexing. Some historical matter was sacrificed, and the trend toward ever-increasing size of the manual was reversed by elimination of 28 pages. Other changes included recognition of two milk-free media of different composition with total colony productivity indistinguishable from the previous standard medium and the introduction of improved staining procedures for the direct microscopic counting method.

The eleventh edition

The 11th edition *(13)*, in 1960, incorporated a great number of changes in bacteriological testing techniques, all of which may be interpreted, perhaps, as reflecting an ever-increasing awareness of the intricacies and inadequacies of testing methods. A test was provided for determination of toxicity of distilled water as well as a "three out of five" averaging method for bacterial counts. Revisions of material on the direct microscopic count, and enumeration methods for psychrophilic, thermophilic, thermoduric, pathogenic, and coliform bacteria were included. Allowances for optional incubation of coliform test media at 32 as well as at 35 C were made. In addition to this material, a sad commentary on a sort of progress may be read into the inclusion of discussions of radioactivity and tests for the detection of antibiotics in milk. Examination procedures for concentrated and cultured products were presented in this edition.

The twelfth edition

The basic philosophy underlying the development of the 12th edition *(14)* in 1967, was to refuse to allow introduction of any new method or modification unless it had undergone careful comparative testing with the data available to all interested parties. Several important changes emerged from these years of work and discussion, notably: the selection of 32 C as the incubation temperature for both the agar plate count and the coliform count (culminating, for at least a few years, the long downward trend in plate incubation temperatures), shortening the incubation period for Standard Plate counts on dry milk from 72 to 48 hr (a questionable decision), and determining psychrophilic bacteria counts at 7 C ± 1 C for 10 days instead of 5-7 C for 7-10 days *(15)* (thereby ending this use of a bacteriological blank check).

The thirteenth edition

Plans for publication of the 13th edition are now well under way, but it is too early to state with certainty the changes that may be introduced. Major changes in time and temperature of agar plate incubation, however, are not likely at this time.

By now it should be clear that our bacteriological testing procedures have never been considered completely adequate for the job of quality control. If they had been, field work could have been eliminated years ago, and quality control could have centered on routine laboratory analysis of gathered milk samples.

FUTURE NEEDS

We hear and, in turn, talk a lot about the change from handling milk in cans to bulk tanks invalidating the usefulness of our test procedures. Since we will

not decrease the efficiency of our refrigeration nor go back to collecting milk in 10-gallon cans, what should now be done?

It is unlikely that any test or series of tests will ever be devised that will tell all that should be known about the past history of a sample of milk. There is yet no substitute in sight for complete field service. For a complete discussion of this subject, read the papers by Hartley et al. *(16, 18)*, "Bacteriological methods for evaluation of raw milk quality. A review. I. Use of bacterial tests to evaluate production conditions, and II. Bacterial tests used to measure milk quality." These reviews reiterate the statement that no single test will accurately reflect farm production conditions. This, of course, will not and should not stop further investigation into the development of new tests and refinement of old tests for quality control purposes.

For those readers with a special interest in this subject, a series of four discussions on bacteriological testing of raw and pasteurized milk for regulatory purposes appeared in April, 1971 in the *Journal of Milk and Food Technology.* These papers are the result of a symposium, chaired by Dr. R. B. Reed, Jr. and given at the 1970 annual meeting of the American Dairy Science Association at Gainesville, Florida.

Today we have reviewed the history of bacteriological testing of milk and dairy products in the United States since 1905 through the changes made in *Standard Methods.* We have attempted to indicate that our methods probably never have been completely adequate, *but* we have made them work. Now, it may be possible to predict what will happen in the future from what we have seen in the past. Agar-plate incubation temperatures have edged cautiously downward from 37.5 C to 32 C. We have stopped stressing speed, partial recovery, and reasonable accuracy of plate counts to edge closer to a concept of as complete recovery of microorganisms as possible, a sort of *Total* Standard Plate count. Our interest in thermophilic bacteria waned as our processing procedures changed; we then became more concerned with the presence of thermoduric mesophiles. In due course, the psychrotolerant bacteria emerged as the most important type present. Now, our interest seems to be sensibly broadening to include concern over more complete recovery of injured microorganisms.

Two recent issues of the *Journal of Milk and Food Technology* contain excellent discussions of the present status of knowledge in this area. Reference is made to "Selective culture of spoilage and indicator organisms" by M. L. Speck *(21)* and "Current developments in detection of microorganisms in foods: Influence of environmental factors on detection methods" by Z. John Ordal *(20).*

We perform bacteriological tests to assure compliance with regulatory demands and to monitor attempts to prevent microbial spoilage. Both uses would seem to require as complete an enumeration of all viable organisms as could be achieved. Since, under favorable conditions, cellular injury is rapidly repaired, total counts of both injured and healthy cells are more useful than counts of only healthy cells. In other words, milk pasteurized and held at a low temperature (two sources of injury) might yield a deceptively low count, not indicating what could happen if the milk were inadvertently stored at a favorable growth temperature.

To keep our food safe, we try to limit initial bacterial contamination, then we eliminate as many of the contaminants possible by doing them physical violence (appropriate corrective heat or other treatment), and, finally, we try to prevent recontamination and suppress growth. These processes imply the development of specific flora and introduction of cellular stress and injury. These factors make it necessary to enhance colony productivity in both routine testing (as in the Standard Plate count and so called total counts) and special testing for indicator organisms with selective media (frequently inhibitory to even healthy cells).

Starting with raw milk, there does not seem to be any special, select group of microorganisms that can serve as an index of production conditions on dairy farms *(16, 18, 19).* Granted, some groups, such as: gram-negative; oxidase-positive; thermoduric; sodium desoxycholate, crystal violet, and psychrotolerant organisms have been used with some degree of success, but it still is probably better to rely on recovery of the maximum number of microorganisms as a partial measure of farm sanitation.

Much work has been done on the effects of media and incubation temperature on the recovery of microorganisms from both raw and pasteurized milk. One of the most recent of these studies was undertaken by Hartley et al. *(17).* They compared Standard Methods and Eugonagar at 7, 21, 28, and 32 C incubation temperatures for 10, 5, 4, and 2 days, respectively, using raw manufacturing grade, raw grade-A, and pasteurized milk. Incubation at 28 C for 4 days was the optimum time-temperature combination in their study. Differences between means of some of these counts may not seem great, but they were statistically significant. Data were presented as means; the individual count differences in some samples were much more dramatic. To some, the statement that a seemingly small difference of means carries statistical significance is like the proverbial waving of a red flag in front of a bull. It may be statistically significant, yes, but is it commercially signifi-

cant? If manipulation of agar plate pH and time and temperature of incubation enhance recovery of injured or fastidious or slow-growing microorganisms from a product, and if the media yielding the highest count generally produce the largest and most easily discernible colonies, then commercial significance is assured.

Studies by Thomas et al. *(22, 23, 24)* point out the insufficiency of the Standard Plate count for recovery of injured microorganisms. Of agar plate incubation temperatures of 35, 32, 28, and 21, incubation at 28 C for 4 days was found, on the average, to be the optimum for determining the maximum bacterial population of pasteurized milk. The mean thermoduric colony counts obtained after 2 days of incubation at 32 C were only 73.7% of the mean count obtained after 4 days at 28 C. The pH and type of bacteriological peptone used in the plating medium influenced colony size as well as count. Thermoduric cultures of *Arthrobacter, Micrococcus, and Streptococcus* species grew over a much wider cultural range before, than after, laboratory pasteurization. These genera may be missed by plating at 32 C on an unfavorable medium.

Plate counts of heated vegetative cells and spores of *Bacillus* species are higher in complex media than they are in media of nutritionally less desirable composition. Spores that survive heating will germinate only if they are given the most favorable conditions for growth and may, in fact, be even more demanding than the vegetative cells. This dormancy probably results from destruction of necessary enzymes that are reelaborated slowly in an unfavorable environment. With present-day processing and storage methods, the genera *Bacillus* and *Clostridium* are becoming increasingly important.

The same plating procedure is now recommended for enumeration of bacteria in raw and pasteurized milk, for dry milk, and for the laboratory pasteurization count. If the Standard Plate count is adequate for the enumeration of bacteria in some samples of raw milk, it may not serve as well with the flora in other samples. It certainly is suboptimal for the detection of the maxium viable population of much pasteurized milk and could be expected to be even less efficient with a product such as dry milk.

In conclusion, bacteriological testing of some dairy products is not completely adequate and may never be so. It would be desirable to develop methods that would give greater enumeration of all viable microorganisms in dairy products if possible. Until then, we could improve at least the Standard Plate count if we would lower the incubation temperature and increase incubation time. For plating pasteurized products, it would be helpful to raise the pH to 7.5.

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