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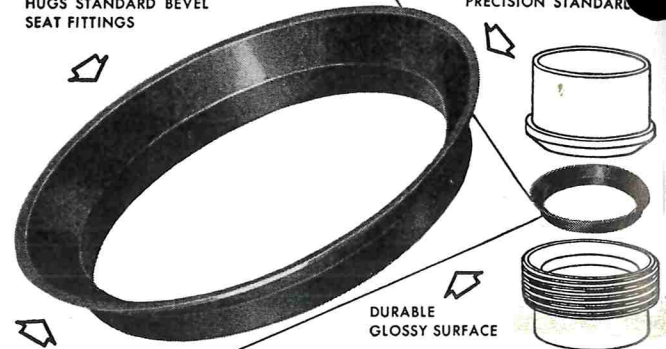
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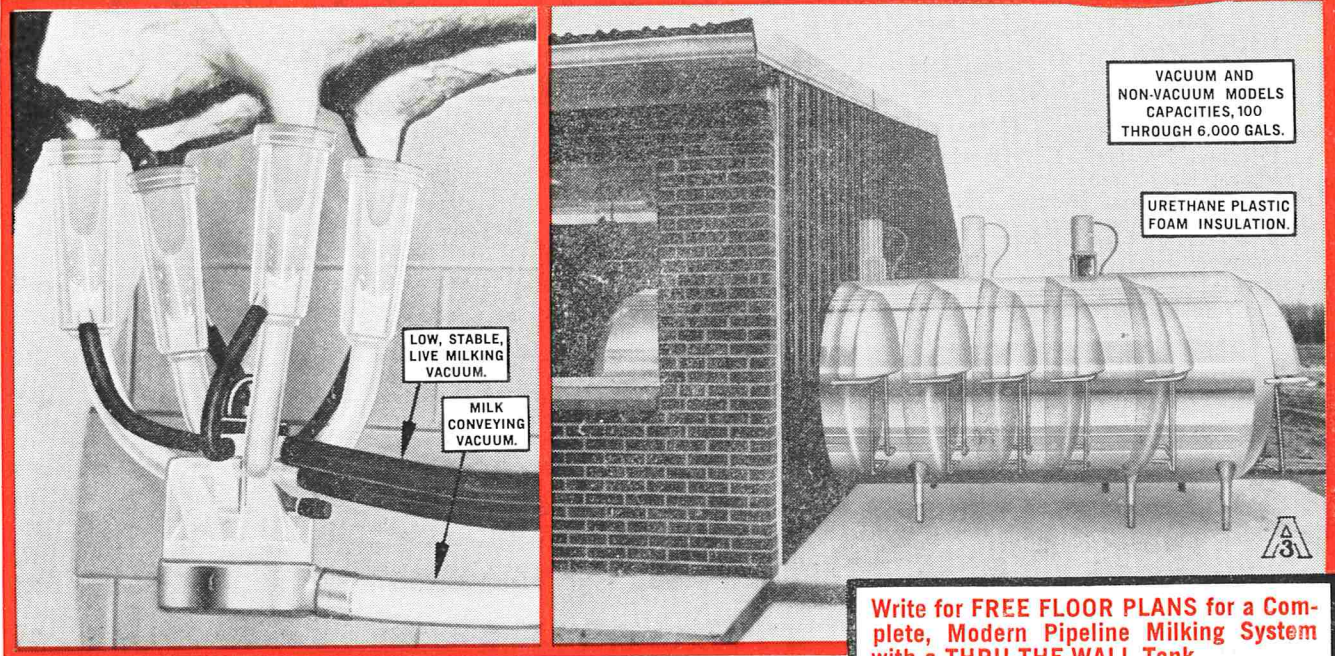
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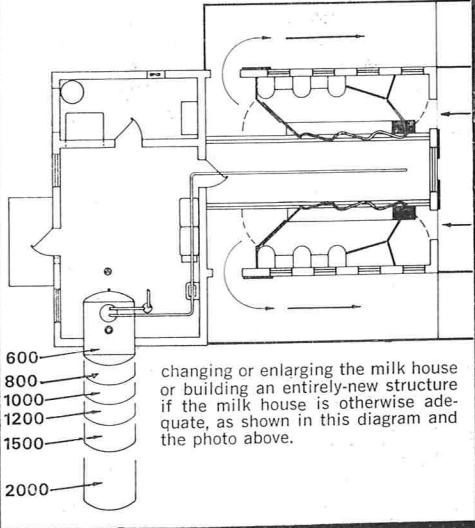
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Vol. 35

September, 1972

No. 9

The Effect of the Teats Dip "Bovadine" on Some Characteristics of Staphylococci Isolated from Bovine Udder Lana Sue Weckbach and B. E. Langlois .....	501
Letters to the Editor .....	503
Report of the Committee on Dairy Farm Methods 1970-1971 .....	508
Control of Clostridium Botulinum and Staphylococcus Aureus in Semi-Preserved Meat Products Hans Riemann, W. H. Lee and C. Genigeorgis .....	514
Environmental Aspects of Housing Irving Bell .....	524
Effects of Aerosolization on Salmonella Newbrunswick Attila K. Stersky, D. R. Heldman, and T. I. Hedrick .....	528
Inactivation of Salmonella Typhimurium by Sorbic Acid H. S. Park and E. H. Marth .....	532
Bacteriological Quality and Occurrence of Vibrios in Dungeness Crabmeat in Oregon Processing Plants and Markets Evelyn C. Allen and Margy Woodburn .....	540
Effect of Milking Machines Equipped with Automatic Quarter-Take Off Devices on Milk Quality and Health of the Udder W. N. Philpot .....	544
Loss of Viability by Staphylococcus Aureus in Acidified Media II. Inactivation by Acids in Combination with Sodium Chloride, Freezing, and Heat T. E. Minor and E. H. Marth .....	548
IAMFES, Inc., Committees and Representative Appointments 1971-1972, 1972-1973 O. M. Osten and Walter F. Wilson .....	556
Holders of 3-A Symbol Council Authorizations on August 20, 1972 .....	562
Affiliates of IAMFES, Inc. ....	566
News and Events .....	567
Index to Advertisers .....	569

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## A Research Note

THE EFFECT OF THE TEAT DIP "BOVADINE" ON SOME CHARACTERISTICS OF STAPHYLOCOCCI ISOLATED FROM THE BOVINE UDDER<sup>1</sup>

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(Received for publication February 14, 1972)

## ABSTRACT

The effect of "Bovadine," a post milking teat dip, on characteristics associated with staphylococci isolated from cows was determined. Comparisons were made between various characteristics of isolates obtained from foremilk samples from teats treated with Bovadine and those obtained from untreated teats. A significantly ( $P < .05$ ) greater number of isolates obtained from untreated teats produced coagulase, lysozyme, and phosphatase than did isolates from Bovadine treated teats. In contrast, more isolates from treated teats produced gelatinase and protease and utilized glucose and mannitol anaerobically than did those from untreated teats. These results suggest that Bovadine, when used as teat dip, may alter some of the characteristics used to identify staphylococci.

During a study on classification by numerical taxonomy of staphylococci isolated from the foremilk of cows, we noted a difference between some characteristics of isolates obtained from teats treated with Bovadine and those isolated from non-treated teats. Since most of the characteristics involved were those commonly associated with determining pathogenicity or proper classification of staphylococci, an analysis was made to determine if the use of the teat dip Bovadine under field conditions was responsible for the observed differences. Results of the work are presented in this paper.

## MATERIALS AND METHODS

Organisms used in this study were isolated from foremilk from Jersey and Holstein cows in the University of Kentucky herd. After each milking, diagonally opposite teats of each cow were treated with Bovadine, a commercial teat dip containing 10,000 ppm of non-irritating titratable iodine. The other two teats were not treated and served as controls.

Milk samples were streaked (0.01 ml) onto Baird-Parker medium and plates were incubated at 37 C for 48 hr. All colonies on the plates were picked into Trypticase Soy Broth (TSB) and were incubated at 37 C for 24 hr. Stock cultures of each isolate were maintained on Trypticase Soy Agar slants at 4 C. Before analysis, each isolate was subcultured several times in TSB.

Characteristics listed in Table I were determined for each isolate, with procedures similar to those described by other investigators: DNase (8), phosphatase (3), lecithinase (6), protease (6), lipase (10), gelatinase (4), lysozyme (9), and

mannitol and glucose fermentation (5). Coagulase production was determined by the tube method, using reconstituted rabbit coagulase plasma with EDTA (BBL) according to the manufacturer's directions. Catalase production was measured by adding 3% H<sub>2</sub>O<sub>2</sub> to an actively growing culture on an agar slant. Any evolution of gas was considered positive for catalase.

Data were punched onto IBM cards and analyzed by an IBM Fortran IV 360 Model 50 computer. The percentage of isolates possessing a particular characteristic was determined for each group. The chi-square test was used to determine if the differences observed between the isolates from Bovadine-treated teats and the untreated teats for each characteristic tested resulted from use of the teat dip.

## RESULTS AND DISCUSSION

A total of 287 organisms were isolated and used in this study; 113 isolates were obtained from Bovadine-treated teats and 174 isolates from untreated teats. Only those isolates which were catalase-positive, gram-positive cocci were studied.

Results obtained from this study are shown in Table I. Of all the isolates studied 91.3% utilized glucose anaerobically, whereas 93.0% were coagulase-positive. Since glucose fermentation is a characteristic of the genus *Staphylococcus* (2), and coagulase production is a characteristic of the species *Staphylococcus aureus* (2), the percentage of coagulase producers could be equal to but not greater than the percentage of glucose fermenters. Thus, based solely on glucose fermentation, 1.7% of the coagulase-

Table I. PERCENT OF ISOLATES OBTAINED FROM BOVADINE TREATED AND UNTREATED TEATS FOUND POSITIVE FOR SOME OF THE CHARACTERISTICS USED TO IDENTIFY STAPHYLOCOCCI

Characteristics	All isolates (%)	Bovadine treated teats isolates (%)	Untreated teats isolates (%)
Catalase	100	100	100
Coagulase	93.0	88.5	96.0
DNase	95.1	92.9	96.5
Phosphatase	88.5	81.4	93.1
Lecithinase	95.5	95.6	95.4
Protease	29.6	38.9	23.6
Lipase	89.9	86.7	92.0
Gelatinase	42.5	50.4	37.4
Lysozyme	87.9	80.5	92.5
Mannitol (anaerobic)	70.3	77.9	63.8
Glucose (anaerobic)	91.3	95.6	88.5

<sup>1</sup>Published with the approval of the Director of the Kentucky Agricultural Experiment Station as Journal Article 71-5-145.

positive isolates would have been placed in the genus *Micrococcus*, since members of this genus are unable to utilize glucose anaerobically (2). The largest discrepancy occurred with the untreated group where over 7% of the coagulase-positive organisms did not ferment glucose, and, therefore, could be placed in the wrong genus if classical methods of classification are used.

Baird-Parker (1) has reported that phosphatase production is a stable characteristic of coagulase-positive staphylococci and that the percentage of phosphatase producers should at least be equal to the coagulase producers. However, in this study almost 5% fewer isolates were phosphatase producers than were coagulase producers. These results are in agreement with those of Morton and Cohn (7) who found that not all coagulase-positive organisms were necessarily phosphatase-positive. A greater number of phosphatase-positive and coagulase-positive isolates was found in the untreated than in the treated group.

Mannitol fermentation and coagulase production have been used to separate species of the genus *Staphylococcus* (2, 11). Therefore, the percentage of isolates fermenting mannitol and producing coagulase should be similar. In this study, only 70.3% of the isolates fermented mannitol, while 93.0% produced coagulase. The greatest difference was in the untreated group where approximately 33% of the coagulase producers did not ferment mannitol. Several investigators have reported that staphylococci may lose the ability to produce coagulase or to ferment mannitol (1). Further, when compared to staphylococci from human sources, a lower percentage of isolates from bovine sources have been reported as able to ferment mannitol (12). The low percentage of isolates producing gelatinase (42.5%) and protease (29.6%) was not foreseen. A greater percentage of isolates from the treated group produced these two enzymes.

Differences observed between the treated and untreated group were significant ( $P < .05$ ) for coagulase, lysozyme, and phosphatase. These results would indicate that the post-milking teat dip either reduced the number of pathogenic staphylococci in the foremilk, or affected some of the characteristics without affecting pathogenicity. The ability of the isolates in the treated group to produce gelatinase and protease and to utilize glucose and mannitol anaerobically was significantly greater ( $P < .05$ ) than those in the untreated group. Since anaerobic utilization of glucose is used to separate the genus *Staphylococcus* from the genus *Micrococcus* (2, 11), a change in this character could affect the classification of the organism.

Results of this study seem to indicate that Bo-

vadine may have an effect on certain characteristics generally associated with staphylococci since a significant difference in various characteristics was found between the treated and untreated groups. One possible explanation for the observed differences may be that Bovadine acted as a mutagen. It also is possible, however, that the differences between the two groups resulted from the selection of more iodine resistant strains in the treated group. This selection may have resulted in a population that differed biochemically from the untreated isolates. Whatever the cause of these differences, data obtained in this study show that characteristics normally associated with staphylococci and generally used in their identification were affected by Bovadine. Since the purpose of this study was to determine if the use of Bovadine under field conditions had an effect on characteristics of staphylococci isolated from the udder, the exact cause of these differences was not explored further. Based on these results, it does seem that in some instances, the treatment received by a host before isolation of an organism may have to be considered to properly identify or classify the isolate.

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LETTERS TO THE EDITOR

What about the reported accuracy of cell counting methods?

DEAR SIR:

A paper entitled, "Collaborative Study of Confirmatory Testing Procedures for Somatic Cells in Milk" by Read et al. appeared on page 285 of volume 34 (1971) of the *Journal of Milk and Food Technology*. This article reporting the results of a collaborative study of cell counting contains a number of serious weaknesses which add confusion to the difficult task of evaluating laboratory procedures for use in regulatory programs. In this article the authors implied that all of the cell counting methods have been adequately tested and concluded that there are no important differences in accuracy and precision among the methods. Since the article

is being used to justify the acceptance and inclusion of the cell counting procedures in the 13th edition of *Standard Methods for the Examination of Dairy Products*, the validity of the work deserves serious examination.

The following analysis of Dr. Read's article derives from a review of the paper and an independent analysis of the data from the collaborative study as distributed in a mimeograph report by Dr. Read dated January 2, 1970 (4).

First, it should be pointed out that the study was poorly conducted in that it was not designed to test the use of procedures as published and available to laboratories by technicians trained in their use. Rather it was only an evaluation of the difference in cell counts obtained when the same slides were examined by different procedures. Thus, differences in cell count which may result from differences in the prescribed

TABLE I. EXAMPLE OF CALCULATIONS FOR MEAN, VARIANCE, AND COEFFICIENT OF VARIATION BY TWO METHODS

1. Sample data

	X Count/ml	Z = log <sub>10</sub> X Log <sub>10</sub> count/ml
	96	1.98227
	85	1.92942
	92	1.96379
	100	2.00000
	89	1.94939
Sum	462.0	9.82487

Sum of units

squared 42,826.0

Mean  $\bar{X} = 462.0/5 = 92.4$

19.30865

$\bar{Z} = 9.82487/5 = 1.96497$

$$\text{Sample variance } S_x^2 = \left[ \frac{42826.0 - \frac{(462.0)^2}{5}}{4} \right] = 34.3$$

$$S_z^2 = \left[ \frac{19.30865 - \frac{(9.82487)^2}{5}}{4} \right] = 0.00076$$

2. Mean, variance, and percent coefficient of variation (CV) using count/ml

$$\bar{X} = 92.4 \text{ (from 1 above)}$$

$$S_x^2 = 34.3$$

$$\% \text{ CV} = \frac{S_x}{\bar{X}} 100 = \frac{34.3^{1/2}}{92.4} 100 = 6.34$$

3. Arithmetic mean, variance, and percent coefficient of variation using log<sub>10</sub> units

a. Convert  $\bar{Z}$  and  $S_z^2$  to log<sub>e</sub> units

$$\log_{10} = 2.30259$$

$$M = 1.96497 (2.30259) = 4.52452$$

$$S^2 = 0.00076 (2.30259)^2 = 0.00403$$

b. Calculations

$$\text{mean} = \exp (M + \frac{1}{2} S^2)$$

$$= \exp [4.52452 + \frac{1}{2} (0.00403)] = 92.4$$

$$\text{variance} = [\exp (2M + S^2)] [\exp (S^2) - 1]$$

$$= [\exp 2 (4.52452) + 0.00403] (\exp 0.00403 - 1) = 34.4$$

$$\% \text{ CV} = 100 [\exp (S^2) - 1]^{1/2}$$

$$= 100 [\exp (0.00403) - 1]^{1/2} = 6.35$$

4. Comparison of estimates<sup>a</sup>

	Arithmetic results	Estimates from log <sub>10</sub> counts
Mean	92.4	92.4
Variance	34.3	34.4
Percent coefficient of variation	6.34	6.35

<sup>a</sup>Differ in third digit due to rounding errors

procedures for preparing milk films were ignored. The DMSCC (National Mastitis Council Method) (1) includes as an essential part of the method the use of especially-designed milk film slides, which permits greater accuracy and uniformity of film making. Since these were not used, the DMSCC (NMC method) was not performed as implied in their descriptive title "Strip Counting Procedure with Reticle" or in citation of the published method. The directions sent to the laboratories (3) for performing the microscopic field counting procedures deviate from those specified in USPHS's own publication (2) of the prescribed methodology, and represent an improved version of this method. Thus, the collaborative study actually compared an improved version of the field count with an incorrect modification of the DMSCC (National Mastitis Council). In addition there was apparently no training in slide examination methodology to acquaint analysts with the new or unfamiliar procedures before they participated in the study.

The most serious criticisms, however, relate to the analysis and presentation of the data. The stated objective was to obtain estimates of two variance components. While variance components are useful in evaluating the relative importance of various sources of variation, variance components alone are inadequate for evaluating the accuracy or precision of laboratory procedures. The authors transformed the cell counts to  $\log_{10}$  for statistical analysis. Thus the tabulated presentation contains the variances of the log of cell counts rather than variances of cell counts. These have very little meaning. The authors gave no indication of how the coefficients of variation were computed. It is inappropriate to reconvert the variance or standard deviation of the  $\log_{10}$  of cell count to variance or standard deviation of cell count. For example, in Table 1, the experimental error for the field count is re-

ported to be 0.00527. The square root of this error variance is 0.0726 and is the standard deviation of the logarithms of the cell counts. The antilog of this value (1.1) does not reflect the standard deviation of cell count. The comparison of mean cell counts obtained by the different procedures should be a major consideration in evaluating different procedures. Neither the means nor statistics required for computing means were included.

As indicated, the actual cell count data from this collaborative study were included in a preliminary report by Dr. Read (4). To check the validity of Dr. Read's conclusions I analyzed the data presented in his preliminary report. The results of my analysis are shown in Tables 1-3. Table 1 contains an analysis of the cell counts. There are important differences in the means: those by the field counting procedure were highest, those by strip counting without a reticle were the lowest, while the counts obtained with the reticle were intermediate. These results are in accord with published literature (5, 6) and theoretical expectations. The coefficients of variation 19.03, 14.71, and 12.70 for field counting, strip counting without reticle, and strip counting with a reticle indicate differences in the precision of methods under consideration. Table 2 shows the partitioning of the count variance into the components: between samples, between analysts, and within analyst error. Here the field counting at 73.7% and strip counting with reticle at 76.19% of the total variance attributable to between sample differences appear to be superior to strip counting without the reticle in which only 62.29% of the variance was attributed to sample differences. Table 3 gives the geometric mean and partition of the variance into the three major components using the  $\log_{10}$  transformation. The similarity of results presented in Tables 2 and 3 suggest that the transformation was not necessary for partitioning of variances. We have indicated above that it led to the computation of spurious standard deviations.

For an in depth comparison of counting procedures consideration should be given to the number of particles counted and the microscope factors required to convert the counts to cells per milliliter. Neither these data nor the values of the individual strip reticle counts required to compute the data in Table 2 of Dr. Read's article were available to me for analysis. Thus I can only point out that there is an inconsistency in the units indicated in the variance and coefficient of variation. Also the ranges given indicate considerably larger errors below the mean than above the mean. This is the opposite of the expectation for data normalized by a  $\log_{10}$  transformation. If in fact the data were not normally distributed the larger margin of error should have been above the mean.

The data presented for the Electronic Somatic Cell Count Procedure are totally inadequate for evaluation. All variances are in terms of the  $\log_{10}$  of something, presumably the instrument read out. There is no indication of the relation between the counts obtained by this procedure and a reference standard. In fact the author could have presented an analysis of the fat content and the reader would have no basis to detect the error.

The strip counting method with a reticle referred to in Dr. Read's study was adapted from a procedure, "Direct Microscopic Somatic Cell Count in Milk," developed by a subcommittee of the National Mastitis Council. This committee was formed to develop and evaluate procedures to be used in the control of abnormal milk. Dr. Brazis of the USPHS was chairman of the committee and guided the development of the written protocol to tailor it to needs of the abnormal milk control program. The method has been field tested and reported. Dr. Read's own collaborative test data

TABLE 1. COMPARISON OF MEAN, STANDARD DEVIATION AND COEFFICIENT FOR THREE METHODS OF EXAMINING MILK FILMS

	Method		
	Field counting	Strip counting	Strip counting with reticle
Mean	1.703	1.356	1.570
sd	.324	.199	.199
c.v. (%)	19.03	14.71	12.70
Cell count (given in count $\times 10^6$ )			

TABLE 2. DISTRIBUTION OF CELL COUNT VARIANCE

	Method		
	Field counting	Strip counting	Strip counting with reticle
<i>Percentage of variance attributed to:</i>			
Sample	73.70	62.29	76.19
Analyst in sample	11.39	30.79	17.41
Error	14.91	6.92	6.41

TABLE 3. DISTRIBUTION OF VARIANCE WHEN CELL COUNTS ARE TRANSFORMED TO  $\log_{10}$

	Method		
	Field counting	Strip counting	Strip counting with reticle
Geometric mean	1.510	1.135	1.379
<i>Percentage of variance attributed to:</i>			
Sample	77.22	59.36	77.21
Analyst in sample	11.05	33.52	16.92
Error	11.73	7.11	5.87



shows it to be more precise, even in the hands of inexperienced analysts, yet PHS has not acknowledged its superiority.

It is unfortunate that the Division of Microbiology, Bureau of Foods, FDA, would prepare a publication claiming to show acceptable levels of performance of laboratory procedures without proper design and conduct of the research and without accurately analyzing, interpreting, and presenting the assembled data.

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*Authors say data and conclusions are valid*

DEAR SIR:

We are pleased to hear of Dr. Smith's interest in our collaborative study on methods for counting milk somatic cells and welcome the opportunity to comment on the points which cause him so much concern. For easy reference, specific statements made by Dr. Smith are first quoted and then commented upon.

1. "The DMSCC (National Mastitis Council Method) includes as an essential part of the method the use of specially designed milk film slides which permits (sic) great accuracy and uniformity of film making. Since these were not used, the DMSCC (NMC method) was not performed as implied in their descriptive title."

The specially designed slides have clear circular areas of 1 cm<sup>2</sup> marked on the surface. The slides used in this study had clear circular areas of 1 cm<sup>2</sup> surrounded by a frosted surface. The method for making milk films is identical for the field counting, strip counting, and strip-reticle counting modifications of the DMSCC—in fact, the new edition of *Standard Methods for the Examination of Dairy Products* contains a single set of directions for making slides for all modifications of the DMSCC procedure. Since the methods for film preparation are identical, we believe that use of a single set of slides for counting by all three modifications is valid.

2. "The directions sent to the laboratories for performing the microscopic field counting procedures deviate from those specified in USPH's own publication of the prescribed methodology, and represent an improved version of this method."

The directions that were sent out referred the analysts to the 12th edition of *Standard Methods for the Examination of Dairy Products* for the details of the microscopic field counting

technique. These details were not spelled out in the instruction sheet since this publication was readily available to each analyst.

3. "In addition there was apparently no training in slide examination methodology to acquaint analysts with new or unfamiliar procedures before they participated in the study."

This Division conducts a national program for the approval of milk testing laboratories and for the certification of State Milk Laboratory Survey Officers. The latter people were asked for names of analysts who were certified to perform the DMSCC procedure and who were doing this work routinely. The analysts who participated in the study came from this group. We believe that the training and knowledge necessary to obtain certification together with the experience these analysts had certainly qualifies them for a collaborative study of the DMSCC procedure as practiced by people engaged in routine analyses—which was the objective of the study.

4. "While variance components are useful in evaluating the relative importance of various sources of variation, variance components alone are inadequate for evaluating the accuracy or precision of laboratory procedures."

Collaborative studies are routinely employed by chemists and microbiologists to evaluate methods to be included as official AOAC methods. The present series of collaborative studies was designed to compute components of variance as suggested by Youden (14). While other techniques may also aid in the interpretation of collaborative study results, the variance components approach is the usually accepted way of assessing accuracy/precision. The adequacy of variance components is in part dependent on the underlying assumptions of normality and homogeneity of variance among collaborators. This dependence was specifically considered in the statistical analysis of the study (6) and is discussed subsequently.

Youden suggests the use of blind duplicate samples and the estimation of replicate and systematic (among analysts) error. Aside from being part of the standard procedure, these errors are useful in further evaluation of a test method. Methods that are precise in a research lab sometimes prove difficult to standardize among analysts. The among-analyst component of variation and the graphs suggested by Youden (14) are quite helpful in analyzing the usefulness of a method. After a method is accepted, it may then be desired to employ the information gained about the variation among analysts to detect analysts who make errors in using the method. The components of variance technique is thus not only necessary in testing a method, but also provides a means of testing the proficiency of analysts, if the method is accepted. Since Dr. Smith does not suggest any specific alternative or addition to variance components, no further evaluation can be made of this comment.

5. "The authors transformed the cell counts to log (base 10) for statistical analysis. Thus, the tabulated presentation contains the variances of the log of cell counts rather than variances of cell counts. These have very little meaning."

Two sensitive assumptions in the components of variance model are normality and homogeneity of variances among sub-populations (collaborators). It is quite common (and often-times necessary) to transform the data to achieve a better fit to a normal distribution. Quite often the log transformation will improve the fit. In addition, the log transformation will assist in improving homogeneity of variances especially when the sub-population means and variances are proportional. When log transformations are utilized for two or more sets of data, comparisons can readily be made among the results.

The analysis of variance models (4) used in the collaborative

study require several assumptions in order to be valid. One of the assumptions, as stated above, is that the variate or its transformation is normally distributed. A brief history of the current procedures is summarized below. In 1907, Student (11) wrote a paper on counting with a hemacytometer. He suggested that the Poisson distribution was adequate to analyze the error using the hemacytometer. Deviations from this theory were soon noted. Whitaker (13) in 1914 and Student (12), himself, in 1919, gave applications where the distribution and the underlying assumptions were not even approximately met. This problem of the distributional assumption was still present in 1957 when Turner (9) made a review of the literature and offered suggestions for modifications of the hemacytometer method to meet the Poisson assumptions. The field procedure, as quoted from Smith (10), also deviates from a Poisson distribution.

In instances where the results show that the variance is less than the arithmetic mean or greater than the arithmetic mean, the positive or negative binomial may be more applicable. Student (12) discusses the negative binomial distribution of cells in prespecified divisions. He notes that "... the presence of an individual in a division influences the chance of other individuals falling in that division" when the variance is larger than the mean. This situation would be expected on a slide when the film drying from the outer edges forces the cells closer together in some divisions near the center. Thus, a transformation was used on data in the study. This transform was tested and determined to be satisfactory.

The counts of the somatic cells per milliliter were converted to logarithms to base 10 in order to use normal probability theory in analysis of this study. This assumption can be routinely tested. Pearson and Hartley (5) give a test based on the sample moments of the data. The test results show that there was insufficient evidence to reject the transformation. All tests were performed at the 0.01 level.

Dr. Smith (10) has demonstrated (unintentionally) the need for such a transformation. In discussing the results of his analysis he states, "The fact that the error variance averaged almost 130% of the mean caused some concern initially, since we expected the mean and variance to be equal." This was after eliminating some data. "These two samples were deleted prior to the analysis but other equally suspicious values in this and other groups of data were included in the analysis since the cause of the excessive variation was not determined." Apparently no attempt was made by Dr. Smith to study the distribution of the data when it did not fit his theory.

6. "It is inappropriate to reconvert the variance or standard deviation of the log of the cell count to variance or standard deviation of cell count."

Comments on the meaning of the variance of  $\log_{10}$  counts/ml and the calculation of the coefficient of variation from the variance can be discussed together. Dr. Smith states that the variance of  $\log_{10}$  counts/ml and the coefficient of variation computed from it have no meaning. As it stands, this is an incorrect statement. What Dr. Smith probably meant to say is that the antilog of the variance of log counts is not equal to the variance of counts. However, the variance of the log counts can be converted to the variance of the counts. It is also noted that the coefficient of variation (CV) can be estimated in at least two ways. The CV can be computed directly from the counts or alternatively by estimation from the estimate of the variance of  $\log_{10}$  counts/ml. Aitchison and Brown (1) give a simple relation for arithmetic results in terms of  $\log_e$  units. A constant will convert the results to  $\log_{10}$  units.

Table 1 shows a sample set of calculations in the simple case where the degrees of freedom are  $(n-1)$ . The equations are as follows:

$$\begin{aligned} \text{mean} &= \exp(M + 1/2 S^2) & (1) \\ \text{variance} &= [\exp(2M + S^2) - 1] / [\exp(S^2) - 1] & (2) \\ \text{C.V.} &= [\exp(S^2) - 1]^{1/2} & (3) \end{aligned}$$

where,

$M$  = mean of  $\log_e$  counts

$S^2$  = variance of  $\log_e$  counts

When this transformation is used, the coefficient of variation depends only on the variance. Hoyle (2) and Land (3) give a discussion of more complex situations and some comments on confidence intervals. Thus, it can be easily seen that the variance in terms of logarithms to a base  $e$  or base 10 do have meaning and can be related to the arithmetic mean and to the coefficient of variation.

7. "To check the validity of Dr. Read's conclusions, I analyzed the data presented in his preliminary report."

Dr. Smith performed several calculations different from ours which is certainly within his prerogative, although it is really not clear what they add. Although his calculations have not been checked, one result appears puzzling: he obtained a standard deviation for strip counting lower than that for field counting and equal to that of strip counting with reticle. Even casual examination of the data shows the greater overall variability of the strip count data due to the uniformly low counts of 4 analysts (as pointed out in our paper). Accordingly, it is not clear how Dr. Smith is making his calculations and it is impossible to comment on their validity.

8. "The data presented for the Electronic Somatic Cell Procedure are totally inadequate for evaluation. . . . There is no indication of the relation between the counts obtained by this procedure and a reference standard. In fact the author (sic) could have presented an analysis of the fat content and the reader would have no basis to detect the error."

It is unfortunate that Dr. Smith has not had an opportunity to read the literature on the use of the Coulter Counter for counting somatic cells in milk. The relationship between the Electronic Somatic Cell Count and the Direct Microscopic Somatic Cell Count has been published by several investigators using similar techniques, including two studies from our laboratories (7, 8). The primary purpose of a collaborative study as opposed to a comparative study is to establish the precision of a technique—not how well the results compare with another procedure. The latter is done typically before the collaborative study as it was in this instance. In our studies the correlation coefficient between the electronic and the DMSCC procedure (field counting modification) ranged between 0.97 and 0.997 for milks with somatic cell counts of  $>300,000$  per milliliter. From these data, together with similar results from other laboratories, we believe that there is a correlation between the Electronic Somatic Cell Count and the Direct Microscopic Somatic Cell Count.

9. "Dr. Read's own collaborative test data shows it (DMS-CC-strip reticle counting modification) to be more precise, even in the hands of inexperienced analysts, yet PHS has not acknowledged its superiority."

As was pointed out in our paper, the precision of a method is dependent upon two sources of variance: (a) the experimental error or the ability of a given analyst to repeat results from the same sample, and (b) the among-analyst variance which, as it implies, is the variation obtained when different people perform the same test. In the study the experimental error gave a coefficient of variation of 17% and 14%, respectively, for the field counting and strip-reticle counting modifications of the DMSCC. However, the lower experimental error of the latter procedure was not low enough to offset

the higher among-analyst variance. As a result, the total variance of the strip-reticle procedure was almost 1/3 greater than the field counting procedure or, simply, the strip-reticle procedure was not quite as precise as the field counting procedure in this collaborative study. We stated, "An evaluation of the relative precision of the three modifications of the Direct Somatic Cell Count . . . indicates that there is little difference with the exception of the among-analyst variance in the strip-counting modification without reticle." Our review of the data reinforces the validity of this conclusion.

In conclusion, this is the only collaborative study that has been done on the various modifications of the Direct Microscopic Somatic Cell Count when performed in routine testing laboratories. We believe that a collaborative study on procedures proposed for use in routine testing should be done by the analysts who do this work routinely for the study to be meaningful. We believe the study is sound and the results valid. As Dr. Smith stated, the results of this study were considered when decisions were made on methods to be included in the next edition of *Standard Methods for the Examination of Dairy Products*. It would seem that if anyone has serious reservations about whether the results of this study are misleading it would be appropriate for him to conduct another collaborative study so that people who must make decisions as to such things as what to include in *Standard Methods for the Examination of Dairy Products* would have the benefit of the broader data base.

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### OCTOBER NATIONAL RESTAURANT MONTH

The nation's 335,300 eating places and the millions of Americans who eat in them are being recognized during National Restaurant Month held annually in October.

Theme of this year's month-long program is "We're Glad You're Here!", said Robert D. Flickinger, National Restaurant Association President.

This year, National Restaurant Month has special meaning to everyone who operates a foodservice business and to their millions of customers as the NRA calls on the foodservice industry to boost the American tradition of hospitality by joining in the campaign to improve courtesy and warmth in dealing

with millions of customers.

The foodservice industry has over 3 1/2 million employees, most of whom are in daily contact with the public as waiters, waitresses, busboys, and bartenders. Nearly 30 per cent of all food consumed in this country is eaten in a restaurant or other food-serving establishment.

As the leadership organization of the foodservice industry, the NRA is urging restaurateurs and other foodservice people to renew their efforts during National Restaurant Month to train their personnel in the benefits of "good old fashioned hospitality in serving the American consumer."

## REPORT OF THE COMMITTEE ON DAIRY FARM METHODS 1970-1971

A. K. SAUNDERS—*Chairman*  
A. E. PARKER—*Western Asst. Chairman*  
J. B. SMATHERS—*Eastern Asst. Chairman*

The 1970-1971 Farm Methods Committee of the International Association of Milk, Food, and Environmental Sanitarians is made up of 53 individual members and 14 state affiliate members. In 1970-1971 we had nine task committees and one sub-committee. In Cedar Rapids last year our executive board asked that we start work on Animal Waste Disposal. Our water protection committee in some ways was closely related, so we added Animal Waste Disposal to this committee, chaired by Henry Atherton, as a sub-committee with Russel Lock as sub-committee chairman. We believe Mr. Atherton and Mr. Lock with their committee members have given you an excellent starting report in the very short period of time they had to work. We are sure that the members of the International Association of Milk, Food and Environmental Sanitarians will benefit greatly from the reports of the nine task committees.

### ANTIBIOTICS, PESTICIDES, AND OTHER ADULTERANTS

M. W. JEFFERSON—*Chairman*

Concern over adulteration of milk supplies by antibiotics has increased in many areas of the country since the I.M.S. Abnormal Milk Program went into effect on July 1, 1970. Control and use of antibiotics varies between areas of the country. Regulatory programs on control of milk supplies with antibiotic adulteration vary to some degree and should be strengthened in view of the abnormal milk control programs now made mandatory. Since emphasis is being placed on high leucocyte counts, and this raises the possibility of an increase in the use of antibiotics, the task committee recommends the following:

A. That educational programs be strengthened concerning the use of antibiotics. These educational programs should be directed not only to the milk producer, but to the practicing veterinarian, the sanitarian, and others working directly with the milk producer.

B. That all antibiotics used or prescribed by the practicing veterinarian, as well as those used by the dairy farmer and purchased through various channels, be properly labeled, giving clear and precise directions as to use. The label should also state specific time limits milk should be withheld from the market.

C. That controls be established on a nationwide basis to limit the dose rate of antibiotics, and, in particular, to control the different types of antibiotics and their use by the dairy farmer.

Pesticides continue to be a problem in certain areas of the country. The task committee has received several reports of pesticide contamination of milk supplies, the cause of which has not been determined. Evidence indicates that surveillance of milk supplies by regulatory agencies for pesticide adulteration should be strengthened. Failure to follow label directions is a problem in the use of pesticides. The task committee makes the following recommendations:

A. That the directions on the label of all pesticides be as

plain, simple, and distinct as possible in order to be understood.

B. That educational programs concerning the use of pesticides be strengthened.

C. That commercial applicators be licensed and controlled by each state.

D. That chlorinated hydrocarbon pesticides be prevented from being used if residues cannot be controlled.

The possibility of adulteration of milk supplies by sanitizers and detergents through automatic milking systems which use automatic cleaning and sanitizing systems, is of concern to regulatory agencies. Even though little testing of milk and milk products has been done for adulteration of this type, the task committee recommends that all agencies give consideration to this possibility. The task committee recommends that research be directed toward development of suitable and rapid test procedures that will detect the presence of cleaning and sanitizing chemicals in milk.

The problem of excess water in milk continues to exist in most areas of the country. The task committee recommends that positive programs be carried out by industry and regulatory agencies to eliminate this problem.

Little information is available concerning the testing of milk supplies for mercury and herbicide adulteration. Many agencies have incorporated these tests into their surveillance programs. Use of mercuric chloride to preserve milk samples for butterfat tests should be discontinued to eliminate this compound from dairy plants and laboratories and eliminate the possibility of pollution problems.

Some members of the task committee have expressed concern over the disposition of milk containing high leucocyte counts.

The task committee feels that limitations should be established for the sale of milk suspended from a Grade A market because of high leucocyte counts. Manufacturing milk standards vary from state to state. Some members feel that leucocytes are a normal constituent of milk and cannot be considered as adulteration. Whether or not this problem should be considered by this task committee is questionable, and it is recommended that the Farm Methods Committee give very serious consideration to this question at its next annual meeting.

### CLEANING AND SANITIZING OF FARM MILK EQUIPMENT

JAMES R. WELCH—*Chairman*

The following are the recommendations of the task committee:

A. *Cold water cleaning*: This type of cleaning is not feasible for the following reasons: (a) increased cost to producer, (b) poor solubility of compounds, (c) milkfat does not go into solution, and (d) soil suspension is reduced.

B. *Temperature recording device*: Temperature devices for cleaning solutions are advantageous on pipelines, coolers, and storage tanks that are cleaned automatically.

C. *Welded pipelines*: This type of installation is advantageous as it eliminates improperly fitted gaskets and replacement of gaskets and it offers more suitable cleaning of all surfaces. Welding of joints should meet all criteria established by regulatory agencies.

D. *Rinsing temperatures*: 1. Pre-rinse: For best results temperature should not exceed 110 F. 2. Post-rinse: Tap temperature will help eliminate mineral precipitation.

E. *Inking of rubber parts*: 1. Inking conditions in rubber parts can cause a detrimental effect on milk quality since: (a) cleaning is affected because of surface breakdown, and (b) minute amounts of carbon drop into the milk causing discoloring and sediment. 2. Inking of rubber can be controlled by proper use of an acidified rinse. The time and concentration of the acidified rinse depends upon the water hardness.

F. *Surveillance of equipment cleaning*: Complete cleanup of all milk equipment after use is essential to obtain high quality milk. Therefore, off-hour surveillance programs should be instituted. Milk pick-up times can also be programmed to give producers adequate time for complete clean up.

### EDUCATION

VERNON D. NICKEL—*Chairman*

The Educational Sub-Committee has the continual assignment of gathering new material for publication in the *Journal*. Material is forwarded to Dr. Elmer H. Marth, Editor of the *Journal of Milk and Food Technology*.

The Committee has been active and material continues to come in from many parts of the country. The membership of the International Association of Milk, Food, and Environmental Sanitarians, Inc. should be interested in the fine material that the committee has collected. Please note in the *Journal* the different published articles and where you may send for the articles you are interested in.

### PLASTICS

BERNARD M. SAFFIAN—*Chairman*

In recent years, the Plastics Task Committee has been concerned with making recommendations for proper cleaning of plastics. To make meaningful recommendations it was necessary to consider the minimum temperature at which plastics can be cleaned efficiently, the maximum temperature where the useful life of the plastic is not drastically shortened, a temperature at which metal and glass components which are in the same coupled system with plastic can be cleaned efficiently and types of cleaning chemicals which clean efficiently.

Recently, there has been considerable concern voiced about the effect of phosphates in detergents on water in streams, lakes, etc. The detergent industry claims that if the use of phosphates is blocked, the country will face severe health dangers from such things as improperly cleaned food processing equipment. It should be mentioned that it is probably not the dairy detergents used in cleaning of farm equipment which are causing this problem because the effluents are not dumped directly into sewage systems which empty into streams, rivers, or lakes. Therefore, use of phosphates in dairy farm detergents may continue to be permissible.

For the 1971 report, the Plastics Task Committee wished to determine what effect substitutes for chlorinated polyphosphates would have on dairy farm cleaning procedures, especially since high cleaning temperature and caustic soda reduce the useful life of many plastics. The following questions were asked of the major cleaner manufacturers: (a) Would the use of substitute materials for chlorinated poly-

phosphates require an increase in cleaning temperature or in cleaner concentration? (b) What significant effects on foaming and rinsing may be anticipated if phosphates are deleted or used in greatly reduced concentrations?

Eighty percent of the cleaner manufacturers stated that under the present state of technology there is no material available as a replacement for chlorinated polyphosphates which will maintain the same level of cleaning with economy. Since no replacement has been developed, the questions regarding increased cleaner concentration, cleaning temperature, and effects on foaming and rinsing cannot be answered at this time. Cleaning costs will increase unless a material is developed which has the same efficiency as chlorinated polyphosphates at the same price or lower.

A minority reply to the questions stated that substitute chemicals are available to replace chlorinated polyphosphates and that a detergent formulated with these materials will not have foaming or rinsing problems and that no change is needed, in solution temperature. No mention was made of increased concentration or cost of material.

### SEDIMENT

M. H. ROMAN—*Chairman*

The commonly known milk sediment test should be renamed as the "Cleanliness of Milk Production Test." Proper milking preparation procedures, and care must be exercised in the production and handling of milk on the farm to keep it clean and free of extraneous matter.

A universal milk sample obtained on each farm bulk milk collection lends itself well for monitoring milk cleanliness. A 4-oz. sample tested through a 0.2-inch diameter sediment test disc has merit in determining the degree of cleanliness of an individual producer's milk supply. This test is so simple to perform that milk producers should be encouraged to conduct their own tests at frequent intervals to assure that clean milk is being offered for sale.

The test area of the disc will be almost indiscernible if milk is normal, production is clean, and contaminants are not allowed to enter the milk. The liquid portion of contaminants cannot be filtered out nor can fragmented particles be effectively strained out by farm filtration, and such contamination will be revealed by stain-like appearance or deposits on the sediment test disc.

This task committee recommends the following for clean milk production: (a) keep cows clean and properly clipped; (b) use clean tepid sanitizing solution for thorough udder and teat cleaning and massaging with NO REUSE of the solution; (c) use individual paper towels for washing and drying of teats and udders; and (d) protect the milk from sediment and dust contamination.

The most common site of contamination of milk with sediment is at cowside generally because of incomplete cleaning and drying of teats and udders before milking. The single-service drying towel showing soil after use indicates that the original cleaning was incomplete and should be repeated. Faulty handling of machine inflations, dusty air, and unclean water used for cleaning may be other contributing sources.

A mixed sample sediment test of milk from a farm tank will reveal instances of faulty milking practices. Continued unclean tests of a milk supply should serve as a clue to the sanitarian that a milking time inspection is in order. The universal milk sample simplifies monitoring of milk supplies and provides for follow-up where needed.

Clean milk production is a prerequisite for high quality milk. APHA *Standard Methods* recognizes the sediment test as one of the milk quality tests. The USPHS *Pasteurized*

*Milk Ordinance* spells out procedures for clean milk production, and unclean milk is encompassed by the definition of adulterated milk. USDA, FDA, and APHA have cooperated in the preparation of sediment grading standards. USDA has proposed maximum sediment levels for State adoption as standards for cleanliness of milk used for manufacturing. Some state and local regulatory agencies require sediment testing of farm milk supplies and rejection of unclean milk, but many regulatory agencies do not require this testing and thus may be assuming that cleanliness of milk is not a problem. However, maintaining all producer milk supplies at an acceptable cleanliness level has been found to be a major milk quality problem and one in need of constant attention.

This task committee recommends that: (a) industry and regulatory agencies recognize the sediment test as a test of cleanliness of milk production, (b) a mixed milk sample of at least 4 oz. taken in a sterile container be procured on each farm bulk milk collection to serve for quality and chemical tests, (c) all receivers of milk institute a routine mixed sample sediment testing program using procedures outlined in APHA *Standard Methods*, (d) as a rapid screening test a 4-oz. mixed milk sample be tested through a 0.2-inch diameter area of a sediment test disc; thus it can be ascertained which producers should effect improvement in cleanliness of milk production, (e) milking time inspections be made in those instances where producers do not effect improvement after notification; tests made on milk before farm straining will show the degree of cleanliness of production, (f) an abnormal milk test be conducted on milk samples which foul the test disc and resist passage or which show the presence of yellow color on the back side of the disc, (g) to be better versed in clean milk production, each sanitarian should conduct at least two milking time inspections with sediment testing of each cow's milk prior to straining; one such milking time inspection should be made at a farm where sediment tests have been consistently clean and the other at a farm where tests have been found frequently unclean, and (h) producers be encouraged to conduct their own sediment tests and govern their production methods accordingly in order to assure that clean milk is being produced.

## PROPER MILKING PRACTICES

KENNETH HARRINGTON—*Chairman*

### Preparation

Proper preparation of teats and udders before milking serves a three fold purpose: (a) sediment control, (b) mastitis control, and (c) stimulates milk let down. Clipping of udders and flanks will greatly improve sanitary preparation of the cow. Observe teats and udders for cuts, cracks, bruises, and swelling while preparing the udder.

Thoroughly wash and massage teats and lower udder for approximately 30 sec with a single-service towel that has been soaked in an approved germicidal solution. A warm water (110-120 F) is recommended. This solution should be changed as frequently as necessary to maintain proper germicidal strength and temperature.

Whenever possible, a metering device should be installed in the water line to feed the proper amount of clean warm water and sanitizer to the hose being used. This will eliminate the problem of contaminated solutions. A single service towel should be used.

Drying the teats and lower udder with another single service towel will further stimulate let down and remove excess

water that could get into the milk.

Use strip cup or strip plate to check all cows for abnormalities. Discard all abnormal milk showing flakes, clots, discoloration, and unusually thick or thin milk. When abnormal milk is suspected, the cow should be milked last, the milk discarded, and affected equipment sterilized. Remove three to four streams of milk from each teat into the strip cup. This fore-milk is low in fat and high in bacteria. The strip cup should be used regularly to detect clinical mastitis on individual cows and quarters. Use of the strip cup also helps stimulate milk let down. A mastitis test should be used on a scheduled basis to determine sub-clinical mastitis.

### Milking procedure

Approximately 1 min after stimulation and preparation, gently apply milker unit. Use only the number of units per man that will not cause over milking. The number of units per operator will vary considerably due to agility of the operator, type of installation, milk flow per cow, and adequacy of stimulation. The task committee recommends the following number of units per operator:

Bucket type units	2
Stanchion pipeline units	3
Milking parlor (Tandem)	3
Herringbone parlor	4

Where highly automated systems are used, the number of pipeline units per operator can be increased.

Cows should be trained to milk out completely in three to five minutes. Operator should follow manufacturer's recommendations as to inches of vacuum and rate of pulsation. Operator should observe all milker units closely.

If stripping is necessary, machine stripping rather than hand stripping should be practiced. Machine stripping is accomplished by gently pulling down on teat cups. Prolonged machine stripping induces bad milking habits and may result in injury to the delicate tissue lining the teat, the udder cistern and the teat end. Do not allow teat cups to creep up on udder to shut off flow of milk.

As the quarter is milked out, the teat cup should be removed. Remove teat cups gently by shutting off the vacuum. Break the vacuum by pressing the thumb between the inflation and the top of the teat. This breaks the vacuum seal so that inflation can be easily and gently removed.

### Post milking procedure

Teat dipping with an approved non-irritating germicidal preparation is recommended. This germicidal solution will eliminate milk film on teat end which is an excellent medium for bacterial growth.

Since the muscle surrounding the teat canal opening is relaxed after milking, bacteria may gain entrance through the teat canal if teat dipping is not accomplished.

### Automatic stimulation

*Machine massage.* Stimulating milk let down by machine massage should be practiced only with machines designed for that purpose. When such machines are used, follow the procedures listed above under "Preparation" except delete the statement, "Stimulates Milk Let Down."

*Prep stalls.* Where milk let down is induced by automatic prep stalls, the paragraphs under "Preparation" beginning with the words "Thoroughly wash and massage" and "Whenever possible" should be eliminated.

*Spray nozzles in milking stalls.* Where this application of automatic stimulation is being practiced, the statements under "Preparation" beginning with: "A warm water", and "Whenever possible," should be deleted.

## CLEANING AND SANITIZING OF FARM MILK PICK-UP TANKERS

STEPHAN B. SPENCER, *Chairman*

The farm bulk pick-up tanker is a vital link in the collection and transportation of milk. The sanitation standards of these units must be of a high order to supply quality products to the consumer.

The available facilities to clean and sanitize farm pick-up tankers are important in accomplishing these necessary tasks in an efficient and effective manner. Previous reports stress the importance of facilities to wash the tanker immediately after use.

The task committee was surveyed to ascertain the necessary facilities in tank receiving rooms. We recommend the following: (a) concrete block with epoxy paint or tile for the wall surfaces, (b) concrete is the most satisfactory floor material, (c) overhead doors with automatic door lifters, (d) both drive-thru or back-in types of receiving room are acceptable, (e) a minimum of 10 ft candles; 20 ft candles is preferred in the receiving area; either incandescent or fluorescent light is satisfactory, (f) a drop light should be provided with an automatic retracting reel, (g) in areas where heat is necessary, forced air heaters are suggested, and (h) unloading facilities include adequately sloped floors and adequate drain systems with clean-out traps. Some items of equipment that task committee members consider important in addition to equipment for C.I.P. or hand cleaning of the tank are: (a) high pressure gun for cleaning the outside of the tanker, (b) an agitation system either mechanical or air, (c) a refrigerator for holding samples, (d) a hand wash sink, (e) sink for parts washing, and (f) non-corrosive storage cupboards.

The facilities for cleaning farm pick up tankers are important in the sanitation chain. The dairy industry can ill afford to neglect this important link.

## SAMPLING OF BULK TANK MILK

WILLIAM L. ARLEDGE, *Chairman*

The primary concern of the task committee this year was the sampling of milk on loaded over the road tankers at destination. From replies to questions throughout the country, there are many discrepancies in proper agitation procedures to collect a true representative sample.

Methods of agitation of over the road tankers at destination prior to sampling are as follows: (a) none, (b) agitation using old fashioned hand agitator designed for the ten gallon can, (c) thief sample — using a long stainless steel rod in an attempt to get a core sample, (d) air agitation: 1. sanitary air hose placed in tanker using air velocity to roll the milk for agitation; 2. air driven motor to actuate a small propeller or agitator placed into the tanker through the manhole; 3. air injected through a quick coupling in rear of tanker in an attempt to circulate milk; and 4. air line built into bottom of tanker where air is blown through stainless tube throughout the length of the bottom of tanker to roll milk for agitation. (Same device used for C.I.P. cleaning of tanker.) (e) mechanical agitation: 1. propeller or agitator driven by an electric motor; unit placed on manhole with propeller down in milk; and 2. mechanical agitator built into tanker driven by electric motor mounted on underside of tanker, (f) circulation of milk by use of unloading pump out of outlet valve and back into tanker through manhole; plastic hose being the most common hook up to accomplish, (g) pumping

of milk into storage tank in plant then sampling through storage tank pet cock, and (h) drip sampling as milk is unloaded.

Data received to date are inconclusive as to recommendation of a positive method of agitation to insure proper mixing whereby an accurate sample for bacteriological or chemical testing can be obtained routinely without contamination.

It has been shown through studies made by Brazis (*Journal of Milk and Food Technology*, Volume 25, June, 1962; August, 1962) if bulk tank milk is not properly agitated the bacteria count will increase almost logarithmically as the butterfat content of the sample increases point by point above the actual test of the load. Since the methods of distribution of raw milk have changed drastically during the past several years and the points of production are now further and further from the processing facility, the demand for proper means of agitation to get a representative sample is critical.

Comments received by this committee indicate many loads of milk per year are rejected at destination, not because of the true quality of the milk, but because of inferior or improper agitation and sampling techniques. It is now recommended that a butterfat test be conducted on any sample collected and if the butterfat result is not within 0.2 unit or less of the actual recorded test of the load of milk, the results of such other bacteriological or chemical tests should not be accepted as official. Such results will not be representative of the tanker load of milk.

The committee recommends a continuation of this study in an attempt to gather additional data for which sound recommendations may be made in proper agitation procedures to collect a true representative sample.

## WATER PROTECTION

HENRY ATHERTON, *Chairman*

Task committee activity this year has assumed a "watch-dog" status in the area of water protection. Many areas have held or are holding waste management conferences. It would appear that we are still in a fact-seeking situation to determine the extent, type, and implications of agricultural wastes in water supplies. There have been several proposed solutions to waste management on the one hand and water protection on the other, but the literature indicates a considerable lack of agreement. Some systems having a good potential in one area of the country fail to offer the same advantages in other areas because of differences in weather, seasonal temperatures, concentration of agricultural enterprises, etc. Some question the relative importance of some commonly accepted parameters of pollution (i.e., Are phosphates the controlling variable in algal growth or must carbon, nitrogen, etc. be considered? Should ammonia losses in the air be given consideration as well as nitrate-nitrite relationships in waste management?).

This task committee recommends further study of the complex movement of chemicals in soil and water as animal wastes or discharges from industrial operations are recycled in the traditional practices of the agricultural enterprise.

Several areas of potential concern have been brought to the attention of the task committee. Recycled water has assumed an important role in water management. Treated effluents are becoming important sources of water. There is ample evidence in the literature that water treating systems render supplies "potable," yet do not destroy certain pathogenic or spoilage organisms commonly associated with waste waters. For instance, certain psychrotrophic microorganisms with dangerous potential for food spoilage survive chemical treatments which render water coliform-free.

There are reports that chlorine doses at least ten times that necessary to destroy coliforms are needed to destroy polio virus. The resistance characteristics of protozoa, amoebic cysts, worms, and the numerous viral agents (hepatitis particularly) should be determined and publicized. It was reported at the winter meeting of the American Society of Agricultural Engineers that superchlorination-dechlorination systems, water pasteurization, or ultraviolet systems were able to safeguard the water supply. The former was preferred for practical reasons for farm or small plant operations. It was suggested the savings in labor cost might well pay the cost of chlorinating the system. Superchlorination of livestock waters has been shown to greatly decrease animal health problems resulting from past-contamination and cross-contamination from poultry and livestock droppings, nasal discharges, saliva, etc. in water cups and troughs.

Resistance characteristics of microorganisms surviving ordinary waste disposal treatment should be studied. New standards for potability should be determined to prevent contamination of water supplies by chlorine-resistant microorganisms in waste treatment effluents and in natural water supplies.

The National Academy of Sciences has appointed new task committees to review Water Quality Criteria Standards. Task committee Co-Chairman Atherton is a member of the Panel on Water Quality Criteria for Agricultural Purposes. New information will be made available to the membership as this panel review developments in this area.

### ANIMAL WASTE DISPOSAL

R. E. LOCK, *Sub-Chairman*

In the task committee study of animal waste disposal it was found that many states are now regulating or developing regulations governing the disposal of manure. At present, most such legislation has been directed toward beef cattle feed lots. A summary of state regulations can be secured by writing for Bulletin ARS42-189 from the U. S. Department of Agriculture, Agriculture Engineering Research Division, Beltsville, Maryland 20705.

It was the consensus of the task committee that a limit should be placed on the spreading of manure on frozen ground. During those months when ground is frozen, manure should be stored in tanks or stacked in an area constructed to prevent runoff.

It was felt that there should be an increase in control over location and design of livestock buildings and lots in relation to the local environment. This is not only to protect against the hazard to water quality but to minimize malodors.

More care should be exercised in the design and installation of manure flush systems to assure that the volume increase in disposable wastes is not in excess of what the soil in the disposal area can absorb. Topography, climate, and soil type should be carefully studied to ascertain the limits of each system. The limit thus established on the number of livestock units and total waste volume should not be exceeded.

Anaerobic and/or aerobic lagoon systems are becoming more commonly used for manure disposal. Specific criteria for the climatic and soil conditions of the area in which they are constructed should be established. The area of potential concern in use of lagoons is composition and disposition of digested wastes from lagoons.

Because of increasing concern of a gradually urban society for our environment, it is inevitable that there will be statutory control of all rivers and ground waters against pollu-

tion. Undoubtedly, this control will extend to control of malodors emanating from livestock operations especially when they are located in close proximity to populated areas. The most reasonable measure for the disposal of animal wastes at the present time is to recycle the wastes through plant growth by spreading the manure on the fields at reasonable intervals and in reasonable quantities.

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II. *Cleaning and Sanitizing of Farm Milk Equipment*: JAMES WELCH - *Chairman*, D. G. RAFFEL, GLENN CAVIN, C. C. GEHRMAN, KEITH A. HARVEY, JOHN W. RING, D. F. GOYER, ROY PERKINS, FLOYD M. COPENHAVER, and State of Connecticut - RUSSELL WEINHOLT

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## CONTROL OF CLOSTRIDIUM BOTULINUM AND STAPHYLOCOCCUS AUREUS IN SEMI-PRESERVED MEAT PRODUCTS

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### ABSTRACT

*Clostridium botulinum* and *Staphylococcus aureus* are naturally occurring contaminants in semi-preserved meat products. They can be inhibited by (a) storage below 3 C, (b) 10% sodium chloride (brine concentration), (c) pH values below 4.5, or (d) proper combinations of these factors. However, most meat products do not have the pH values and brine concentrations required to completely inhibit *C. botulinum* and *S. aureus* and there is always a risk of temperature abuse. Improved safety can be achieved by adding 1% or more glucose to the product. The glucose will, in the event of temperature abuse, generally be fermented to lactic acid by the indigenous microflora in the product. As a result, the pH value drops to a level at which the brine concentration is sufficient to inhibit *C. botulinum* and *S. aureus*. A better approach to safety is to add, together with glucose, a radiation-killed preparation of lactic acid bacteria, e.g., *Pediococcus cerevisiae*. Such preparations cause a rapid decline in pH only when the product is exposed to a high temperature, and they are stable during storage of meat products. Addition of irradiated lactic acid bacteria to meat products has not yet been officially approved.

Another way to improve the safety of semi-preserved meat is to add sufficient glucono- $\delta$ -lactone to reduce the initial pH of the product to a level at which the salt concentration is inhibitory. Use of larger amounts of glucono- $\delta$ -lactone may result in flavor and color problems even when the meat product is kept at refrigeration temperatures.

Mildly processed cured-meat products are not infrequently involved in food poisoning, the causative organism often being *Staphylococcus aureus* (13), which is well adapted to grow in a salt-containing environment. Dack (19) reported that of 137 outbreaks of staphylococcal food poisoning in the years 1956-1961, 57 were associated with meat products, 45 of which were contaminated hams. Data accumulated by the National Center for Disease Control (13) for the years 1961-1968 indicate that ham was responsible for 21.4% of the outbreaks in that period. Manufactured meat products such as ham and bacon are also the food items most commonly associated with staphylococcal food poisoning in England and Wales (38).

Other food poisoning bacteria, such as *Salmonella*, *Clostridium perfringens*, and *Clostridium botulinum*, are seldom involved in poisoning caused by semi-preserved meat. The only one which represents a considerable risk is *C. botulinum*, as it typically

grows in incompletely preserved products and produces a toxin that causes a high mortality in affected persons. Even small outbreaks of botulism cause great alarm, and food processing and distribution must be designed to exclude growth of *C. botulinum*.

When vacuum-packed, smoked, and cured (semi-preserved) fish was involved in outbreaks in the early 1960's, interest was renewed in potential botulinogenic foods, including semi-preserved meat products. A number of studies were initiated, and it is our purpose to summarize the results which have been obtained, both by other investigators and by ourselves.

### DEFINITION OF PRODUCTS

Semi-preserved meats, as the term is used in this article, means the group of "moist" meat products which have been cured with moderate amounts of salt (brine concentration<sup>2</sup> generally in the range 1% to 7%) and often subjected to a "pasteurization" process (maximum temperatures well below 100 C) after, or more generally, before packaging. Curing includes addition of chemicals such as nitrate, nitrite, and sometimes phosphates and compounds contained in wood smoke.

The products, often after being sliced, are generally packed in plastic pouches. These are flushed with inert gas (nitrogen or carbon dioxide) and sealed, or sealed under vacuum. Exclusion of oxygen from the packages serves to preserve color and other quality characteristics of the meats. Some products (canned hams of 3 lb. or above, canned bacon) are vacuum-packed in tin cans instead of in plastic pouches. Examples of "semi-preserved meats" are listed in Table 1.

Practically all the products, because of their perishable nature, are distributed and stored under refrigeration. They hardly present a public health hazard so long as they are kept at temperatures not exceeding 4 C (40 F), although large populations of saprophytic organisms may build up during prolonged storage. A few products which have a very high brine concentration, notably hard salami, are stored and distributed at ambient temperatures.

<sup>2</sup>Brine concentration is a measure of salt concentration in the water phase of foods and equals:

$$\frac{\text{gram salt}}{\text{gram salt} + \text{gram water}} \times 100.$$

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TABLE 1. COMPOSITION OF SEMI-PRESERVED MEATS PURCHASED IN SUPERMARKETS IN 1966, 1968, AND 1969<sup>1</sup>

Product	Brine concentration (%)	pH	Year
Canadian bacon	3.3	6.3	1966
Chopped ham	5.3	6.5	1966
Cooked ham	2.0	6.0	1966
Corned beef loaf	3.9	5.7	1966
Galantina	4.9	5.8	1966
Mortadella	3.6	5.4	1966
Pressed pork	3.4	6.2	1966
Salami	5.1	5.2	1966
Sliced turkey, white	1.5	6.1	1966
Sliced turkey, dark	3.0	6.4	1966
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Beef bacon	4.0	5.6	1968
Bologna	3.0	5.2	1968
Chopped ham	2.9	5.4	1968
Cooked bacon	4.1	—	1968
Cooked ham	3.2	6.3	1968
Cooked salami	2.8	4.8	1968
Jellied corned beef, brand 1	2.3	5.5	1968
Jellied corned beef, brand 2	1.9	5.1	1968
Sliced dark turkey	3.3	6.4	1968
Smoked sliced chicken, brand 1	2.1	6.3	1968
Smoked sliced chicken, brand 2	2.0	6.3	1968
Spiced luncheon loaf	1.6	6.0	1968
Spicy beef	3.9	6.0	1968
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Bologna	3.6-5.3	5.2-6.3	1969
Ham	4.5-4.8	6.0	1969
Pepper loaf	3.6-4.5	5.4	1969
Salami	4.5	4.9	1969
Sliced dark turkey	2.6-4.1	6.2-6.5	1969
Smoked sliced chicken	2.9-3.4	6.3	1969
Sliced beef	5.2	5.9	1969
Spiced luncheon loaf	3.2-3.6	6.2	1969
Spicy beef	4.6-6.5	6.0	1969

<sup>1</sup>References: Samples from 1966 and 1968, Riemann (79); samples from 1969, Akrabawi and Riemann (5).

Typical compositions of a number of semi-preserved meat products, purchased in California supermarkets, are indicated in Table 1. One sample of each product was analyzed for pH and brine concentration, except in the 1969 survey, in which 5 to 15 samples of each product were analyzed. The salt concentration and pH of many of the meat products analyzed were insufficient to inhibit the growth of *C. botulinum* and *S. aureus*, as will be discussed later.

#### PREVALENCE OF *Clostridium Botulinum* AND *Staphylococcus Aureus* IN MEAT

It is generally assumed that *C. botulinum* is a rare contaminant in meat. The excellent public health record of shelf-stable, canned, cured meat which has been given a heat process that permits *C. botulinum* spores to survive supports this assumption.

Direct information concerning occurrence of *C. botulinum* in meat and meat products is scarce. Greenberg et al. (36) conducted a survey of meat sampled shortly after slaughter and found *C. botulinum* (type C) in only one sample (chicken meat) out of more than 2000. Surveys of prepared foods, including semi-preserved meats, have indicated a higher prevalence. Insalata et al. (45) found one sample (vacuum-packed frankfurters) out of 400 contaminated with *C. botulinum* (type B). Taclindo et al. (91) found type B in one out of 73 samples of luncheon meats, and Abrahamsson and Riemann (2) found 6 out of 372 samples of semi-preserved meat and poultry products contaminated with types A or B. In earlier studies, Saleh and Ordal (80) demonstrated botulinum toxin in three of 12 packages of commercially frozen chicken a la king which had been defrosted at an elevated temperature.

The apparent discrepancy among reported findings is probably the result of differences in sample size. Greenberg et al. (36) inoculated only 3 g, whereas other authors cited used sample sizes of 24 g or more. The contamination with *C. botulinum* found in the surveys quoted above is therefore of about the same order of magnitude.

Without going into more detail, it seems safe to assume that *C. botulinum* is infrequently present in semi-preserved meats and when detected, it is present in very low numbers, probably less than one organism per pound of product. Contamination may occasionally be much higher, but even one cell per pound is enough to justify strict safety measures.

*Staphylococcus aureus*, which is more ubiquitous than *C. botulinum*, is often associated with infections in man and animals. Epidemiologic information indicated that the main reservoir of *S. aureus* is man (13), with the nose being the principal site of multiplication. Colonization of *S. aureus* in the nose begins in infancy and, within the first few days of life, as many as 90% of children may become nasal carriers (13). About 30 to 50% of healthy adult individuals are nasal carriers, in contrast to 60 to 80% for patients and hospital personnel (106). Hands are the second most common source of staphylococci (62), and have been found contaminated in 4 to 44% of individuals examined (106). One study revealed staphylococci present on the skin of 30% of food handlers (50). Untermann (102) found 37% of 268 food handlers were carriers (nose and throat) of coagulase-positive staphylococci, and 8.2% carried enterotoxigenic staphylococcal strains (types A, B, and C). Staphylococci are also quite common on the skin of healthy animals (61).

In view of these facts it is not surprising that *S.*

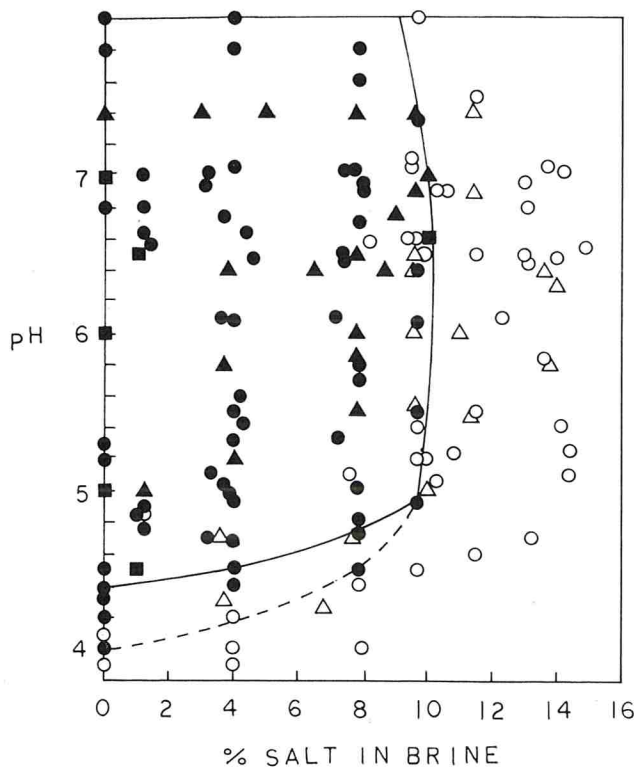


Figure 4. Combination of pH values and brine concentrations which supported (closed symbols) or did not support (open symbols) production of enterotoxin A (squares), B (triangles), and C (circles) aerobically in laboratory media and foods at inoculum levels up to  $1 \times 10^7$  cells/ml or g (solid line) or  $1 \times 10^8$  cells/ml or g (broken line). Based on references 9, 25, 27, 29, 30, 40, 51, 56, 60, 76, and 94.

and this compound, together with salt, a low pH, and perhaps smoking, might cause complete inhibition of the two pathogens. However, many of the products indicated in Fig. 1-4 contained nitrite in commercially used concentrations, and some had also been exposed to wood smoke. Still, the effect of nitrite is not well established. It is an unstable compound, and, when used in foods in low concentrations, it may mainly delay toxin formation as far as *C. botulinum* is concerned (75).

It might also be argued that the levels of acidity and brine concentration required to inhibit a moderate number of *S. aureus*, and especially *C. botulinum*, organisms which occur naturally in meat, would not be as high as indicated by the experiments represented in Fig. 1-4, in which cell numbers of a magnitude up to  $1 \times 10^8$  were used. Many observations indicate that this argument is valid to the extent that toxin production occurs earlier when many cells are present in the food. However, studies in which a broad range of *C. botulinum* cells were used in laboratory media have indicated that even very low numbers of cells may initiate growth at brine concentrations and acidities which are only slightly below the levels required to inhibit a very large number of cells (77).

It has been established that the levels of brine concentration and acidity required to inhibit *S. aureus* depend to a large extent on the number of cells present (26, 28, 29). However, only a few of the products represented in Table 1, namely those with pH values below 5.4, are inhibitory to even low numbers of staphylococcal cells.

#### General effects of wood smoke

Some semi-preserved meat products are treated with wood smoke as a part of the processing. Smoke causes a drop in surface pH and has a noticeable influence on the bacterial flora in meat products, causing a shift in the composition of the flora from catalase-positive (micrococci and staphylococci) to catalase-negative (mainly lactic acid bacteria) (37). *Staphylococcus aureus* injected into ham has little chance to survive the smoking process (52), but there is always a risk of recontamination during slicing and packaging. However, the shift in microflora in the direction of a more acid-producing bacterial population tends to inhibit staphylococci especially if fermentable substances are present. Thus smoking has some inhibitory effect on *S. aureus*. The effect of lactic acid bacteria will be discussed in more detail later.

The situation is similar with respect to *C. botulinum*. Fish which have been intensively exposed to wood smoke will not support growth of *C. botulinum* (1, 63); but mildly smoked fish will, as was demonstrated in the fish-borne outbreak of botulism in the early 1960's.

#### Effect of packaging

Packaging can have a definite influence on the safety of semi-preserved meats. If meat is vacuum packed, the gaseous phase in the package will change because of consumption of oxygen and accumulation of carbon dioxide (43). This change is much more favorable for the facultative anaerobic and carbon-dioxide-resistant lactobocilli than for staphylococci and micrococci. One result of this is a change in the spoilage pattern, with the meats often turning sour and developing, upon prolonged storage, a flavor somewhat similar to that of sour milk or buttermilk. Another consequence is that the growth of *S. aureus* is limited by competition with other bacteria in the meat (18, 22, 27, 44). It must be emphasized that staphylococci in the absence of competition can grow and produce enterotoxins A and B in meats under anaerobic conditions (26, 27, 97). However, neither the extent of growth nor the amount of enterotoxin produced is as prolific as in the presence of oxygen (27).

The reaction of *C. botulinum* to anaerobic conditions differs from that of staphylococci. *Clostridium*

*botulinum* is an anaerobic, carbon-dioxide-tolerant microorganism and is not inhibited under conditions created by vacuum packaging. However, growth of *C. botulinum* may not be significantly promoted by vacuum packaging under practical conditions. It has been demonstrated that vacuum packaging is not a requirement for *C. botulinum* to grow and produce toxin on fish (49, 97). However, vacuum packaging may delay spoilage and change the spoilage pattern of food, with the result that *C. botulinum* gets an opportunity to grow and produce toxin without typical spoilage signs in the food. It is believed that this, in part, explains the outbreak of botulism caused by smoked fish in the 1960's. Prevention of drying imparted by plastic packaging may be a factor of importance in *C. botulinum* growth. It has been observed (90) that evaporation of water through a non-barrier material (natural casing) can cause a decrease in water activity in sausage to a level where *C. botulinum* fails to grow.

#### Effect of glucose in semi-preserved meats

The information discussed above indicates that the salt and pH levels in most semi-preserved meat products are insufficient to prevent growth of, and toxin formation by *S. aureus* and *C. botulinum* at elevated storage temperatures. Thus the safety of semi-preserved meat products seems to rest mainly on maintenance of a low storage temperature. There is little doubt that storage temperature requirements are generally fulfilled in packing plants and in the distribution chain, but there is also much opportunity for temperature abuse. Therefore, it seems desirable to explore other ways in which the safety of these products can be maintained even when the products are exposed to increased storage temperatures.

One possible procedure is to add glucose to the product so inhibitory concentrations of lactic acid are formed in case of temperature mishandling. A number of samples of semi-preserved meat products obtained from supermarkets were inoculated with *C. botulinum* spores and tested for toxin after 2 and 4 days at 20-30 C (5). Out of 9 product categories, all with salt and initial pH levels that permit growth, only 3 became toxic. A common characteristic of these 3 types of products was that the pH values after storage were in the range of 5.6-6.7. In contrast, products which did not become toxic all had pH values in the range of 5.4 to 4.4. These observations indicated that naturally occurring microorganisms in semi-preserved meat products can produce acid in amounts that inhibit *C. botulinum* and probably also *S. aureus*. Experiments in which 1% glucose was added to commercially produced semi-preserved meat products confirmed this. In most instances, but

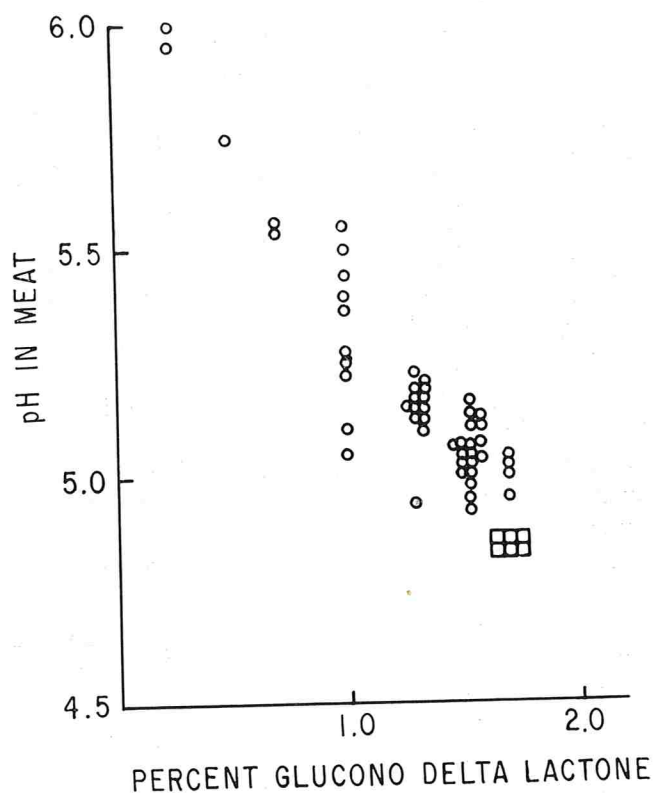


Figure 5. Effect of adding various concentrations of glucono-delta-lactone to meat during curing. pH of the meat was measured after pasteurization or autoclaving. Circles, pork; squares, beef.

not in all, glucose addition resulted in a pH drop to below 5.3 during 4 days of incubation at 20-30 C, and no toxin was produced by *S. aureus* or *C. botulinum*. Strains of *Streptococcus faecalis* and micrococci were isolated from glucose-containing "souring" meat products, and proved to be active acid producers in the presence of normal concentrations of curing salts. The inhibitory effect of these strains on *C. botulinum* in glucose-containing cured meat was tested and proven. It has been observed before that natural contaminants in foods may inhibit *C. botulinum* and *S. aureus* growth and toxin production (15, 18, 20, 22, 34, 43, 44, 47, 48, 58, 80, 81, 103) and that the inhibition generally is due to acid formation.

Examples of inhibition of *S. aureus* without involvement of acid formation are known (87, 100, 101), and one inhibitory factor, hydrogen peroxide, has been identified (21). However, inhibition does not always occur, even when other organisms outnumber staphylococci by a factor of 44 (89) and is probably of minor importance compared to the inhibition caused by acid-producing bacteria.

The inhibitory effect of competing organisms on *S. aureus* depends on storage temperature and salt concentration (32, 58, 70, 71, 72). Higher tempera-

tures and brine concentrations tend to favor the mesophilic, salt-tolerant staphylococci.

Some other nonacid-producing microorganisms may actually promote the growth of *C. botulinum* by consuming oxygen (reducing the oxidation-reduction potential) or neutralizing acid (10, 14, 103). Spoilage bacteria that do not produce acid have not been found inhibitory to *C. botulinum* (39, 55). In our experiments with bacteria isolated from semi-preserved meat products, inhibition occurred only when acid was formed.

Addition of 1-2% glucose to semi-preserved meat products undoubtedly results in increased safety, but the procedure has some drawbacks: (a) it requires presence in the meat of fermenting (lactic-acid-producing) bacteria; (b) the safety depends on the ability of the acid-producing bacteria to initiate growth faster than *C. botulinum* and *S. aureus* in the event of temperature abuse; and (c) some acid-producing bacteria will grow at refrigeration temperature and thus reduce the shelf-life of the product by producing sour flavor, discoloration, or both.

Because of these drawbacks, other methods to control food poisoning bacteria in semi-preserved meats seem desirable. We have considered three other possibilities: (a) inoculation of meat products with a pure culture of lactic acid bacteria as, for example, *Pediococcus cerevisiae*, which is used in fermented sausages; (b) addition of an irradiated lactic acid bacterial preparation that is inactive at refrigeration temperature but is able to rapidly produce lactic acid if the product is exposed to higher temperatures; (c) direct addition of acid, or of compounds such as glucono- $\delta$ -lactone which will form acid by hydrolysis and decrease the pH of the product.

These methods were considered to have the following advantages and disadvantages. Inoculation of food with lactic acid bacteria has the advantage that rather few organisms need be added, as multiplication and formation of acid will take place in the event of temperature abuse. However, precautions must be taken to ensure that the lactic acid bacteria will remain viable as long as the food is stored, and that they will be able to multiply faster than the food-poisoning organisms, if the need arises. Addition of irradiated lactic acid bacteria which can rapidly convert glucose to lactic acid could overcome the difficulties mentioned above. Such a preparation is easier to standardize and control than is a viable bacterial culture. Use of an irradiated cell preparation will also ensure rapid fermentation and a decrease in pH if there is temperature abuse, as no cell multiplication is required. Addition of a substance that will form acid after having been dissolved in the food product, such as glucono- $\delta$ -lactone, is a

simple and effective solution. The main drawback is that treatment with this compound results in a drop in pH even without exposure of the food to hazardous temperature. Thus, glucono- $\delta$ -lactone treatment will result in foods being more acid, and this may not be acceptable. Another drawback is that a low pH induced at the beginning of storage may cause color problems in cured products. Also, there may be a gradual drift toward higher pH values, with concomitant loss of safety, during prolonged storage. These problems deserve more study.

On the basis of considerations discussed above, we decided to attempt to develop a nonviable lactic acid bacterial preparation that would rapidly produce lactic acid from glucose and be stable enough to be used in products with a refrigerated shelf-life of many months. *Pediococcus cerevisiae* was selected for this as it is an active lactic acid producer, and has already been approved as an additive to meat products. Methods were developed to produce and harvest cells with a high content of acid-forming enzymes (Lee, Riemann, and Al-Mashat; Patent application, 1971). The cells were concentrated to a density of about  $10^{12}$  per milliliter and then killed by exposure in the frozen state to 0.75 Mrad from a 60-cobalt source. The resulting nonviable cell preparation diluted a thousand times with 1% glucose solution and incubated at room temperature caused a rapid pH drop. A series of inoculated-pack studies yielded similar results; in semi-preserved meats pH dropped to below 5.0 in a shorter time (a few hours) than *C. botulinum*, *C. perfringens*, and *Salmonella* required to initiate growth. The pH drop occurred at all temperatures from 10 to 37 (50-99 F) C, and was slowed down, but not prevented, by brine concentration up to 9%. At a 10 C toxin formation by *C. botulinum* type E may be faster than acid production by irradiated *P. cerevisiae*.

The irradiated preparation of *P. cerevisiae* is stable for months in meat kept at refrigeration temperatures, and it does not produce acid or change the flavor, color, or texture of the meat, so long as the meat is kept cold. Only if there is temperature abuse will fermentation take place and cause a rapid pH drop. The fermentation gives the meat a flavor that resembles that of buttermilk. This flavor may be offensive to some people, but consumption of meat fermented with *Pediococcus cerevisiae* involves hardly any risk.

The use of glucono- $\delta$ -lactone to extend shelf-life of semi-preserved meat products has been described by Mol and Timmers in 1970. Concentrations of 0.2 to 0.6% reduce the pH in meat to such a degree that stability can be achieved with less salt than is normally used. The decrease in pH caused by the

compound may also be selective for naturally occurring, acid-producing microorganisms, and thus result in a further drop in pH. The amount of gluconolactone required to inhibit staphylococci and *C. botulinum* depends on the salt content of the meat product. Figure 5 illustrates the amounts of gluconolactone required to achieve different pH values in meat. These pH values remained practically constant in products with very low bacterial contamination for 2 months or longer refrigerated storage.

## ACKNOWLEDGMENTS

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## ENVIRONMENTAL ASPECTS OF HOUSING

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### ABSTRACT

Housing is a part of man's total environment and hence it is to some extent responsible for the status of his health and well-being. The residential environment may affect man's physical health by influencing the transmission of communicable diseases, by not fulfilling his physiological needs, or by causing injury. Although approximately 60% of the U. S. population is currently covered by an adopted local or state housing code, it has been estimated that 16 to 20 million of the some 70 million dwelling units in the United States are so far below standard they should be replaced.

To cope with environmental problems related to housing and to protect the health of the nation's citizens, health agencies must accept the role of either stimulating or carrying out the four kinds of governmental action set forth below: (a) adoption of minimum health standards in housing; (b) conduct a program to achieve and maintain such standards; (c) conduct periodic evaluation of the standards to ensure their current adequacy; and (d) monitor the standards enforcement effort to guarantee that public health values are provided.

There is no doubt that the *environmental aspects of housing* have a direct effect on the total health of people. To comprehend the far reaching effect that environmental conditions of housing can have toward the total health of people, we should define the modern concept of health. The World Health Organization defines health as a "state of complete physical, mental, and social well-being, not merely the absence of disease or infirmity." Thus we must readily recognize that health involves more than the absence of physical illness, therefore we must consider that housing has effects on people beyond that of just physical illness. Furthermore, it is a recognized and accepted fact that the total environment of man is a dominant force in determining the level of his physical and mental health and his social well-being. Housing is a part of this total environment and, being a part, it is to some extent responsible for the status of man's health and well-being. This fact is pointed out by Public Building Administration reports, which indicate that slums occupy approximately 20% of the average cities area; however, they produce 60% of all tuberculosis and 50% of all disease.

With the fact established that quality of housing has a direct effect on human health, let's look at the scope of the environmental aspects of housing. Man has always concerned himself with the provision of shelter and protection against the elements and cre-

ation of an environment which is safe and secure and provides privacy for himself and his family. We commonly refer to this shelter as housing. Perhaps a more descriptive term would be "residential environment," which is defined as the physical structure that man uses for shelter and the environs of that structure including all necessary services, facilities, equipment, and devices needed or desired for the physical and mental health and social well-being of the family and individual.

How may this residential environment affect man's physical health? It may affect his physical health in one of the following three ways: (a) it may concern the transmission of communicable diseases, (b) it may not fulfill his physiological needs and/or it may place undue stress upon physiological responses, and (c) it may cause injury (6).

### FUNDAMENTALS OF HEALTHFUL RESIDENCE

Now let's look at what the fundamentals of healthful residential environment should consist of to prevent one or more of the three affects listed.

1. A safe and structurally sound, adequately maintained, separate, self-contained dwelling-unit for each household, if so desired, with each dwelling-unit providing at least:
  - a. A sufficient number of rooms, usable floor area, and volume of enclosed space to satisfy human requirements for health and for family life consistent with the prevailing cultural and social pattern of that region and so utilized that there is neither overcrowding of living or sleeping rooms;
  - b. At least a minimum degree of desired privacy, both;
    - (i) As between individual persons within the household; and
    - (ii) For the members of the household against undue disturbance by external factors;
  - c. Suitable separation of rooms as used for:
    - (i) Sleeping by adolescent and adult members of the opposite sex except husband and wife; and
    - (ii) Housing of domestic animals apart from the living area of the dwelling unit;
  - d. A potable and palatable water supply, piped by sanitary plumbing into the dwelling-unit or in the courtyard, in quantities ample enough to provide for all the personal and household uses essential for sanitation, comfort, and cleanliness;
  - e. A safe and sanitary means for the disposal of sewage, garbage, and other wastes;

- f. Sufficient facilities for washing and bathing;
  - g. Appropriate facilities for cooking, dining, and the storage of food, household goods, and personal belongings;
  - h. Appropriate protection against excess heat, cold, noise, and dampness;
  - i. Adequate ventilation and internal air free of toxic or noxious agents;
2. A neighborhood or micro-district setting for the dwelling which conforms with sound town, country, and regional planning practice and consists of:
- a. When economically feasible, a community water supply, sewage collection and treatment, collection and disposal of garbage and other wastes, and storm-water drainage;
  - b. An atmosphere which is free of toxic or noxious gases, odors, fumes, or dusts;
  - c. Protective facilities of police and fire services;
  - d. Industrial, commercial, cultural, social, religious, educational, recreational, and health and welfare facilities connected to the residential structures by a network of roads and public transportation and a system of footpaths;
  - e. Freedom from hazards to health, welfare, and public morals (6).

### HOUSING CODES

To provide the above fundamentals, governmental agencies are attempting to control the residential environment through enforcement of building and housing codes. We must keep in mind that a building code and a housing code are different. "A building code is a performance code. It regulates the kinds of materials that go into dwellings and kinds of construction that are acceptable to provide structural safety. A housing code deals with the facilities, maintenance and occupancy of existing buildings and the rehabilitation of such a building" (3).

It is primarily with the aspects of a housing code that health departments have concerned themselves. Approximately 60% of the U.S. population is currently covered by an adopted local or State housing code. But even with 60% of the U. S. covered by a housing code, it has been estimated that 16 to 20 million of the some 70 million dwelling units in the U. S. are so far below standard that they should be replaced (2). Space will not permit us to discuss all the various aspects of a housing code, but I would like to make a few comments regarding some of the basic requirements if we are to have acceptable housing. I have used the APHA-PHS Recommended Housing Maintenance and Occupancy Ordinance (1) as a reference for the following comments.

#### *Responsibilities of owners and occupants*

Owners and occupants have a responsibility to maintain the premises in a clean and sanitary condition, to store and dispose of garbage and rubbish in a sanitary manner, to prevent the accumulation of food and rubbish which will attract and harbor rodents and insects, and to maintain construction of the dwelling unit in such a manner as to prevent the

entry of insects and rodents.

#### *Minimum standards for basic equipment and facilities*

Every dwelling unit should have a room in which food may be prepared. Such a room should have a kitchen sink, stove, refrigerator, and cabinets and/or shelves for the storage of food and cooking and eating utensils. Every dwelling unit should have a nonhabitable room equipped with a flush water closet, lavatory, and a bathtub or shower. Every dwelling unit should have dual means of egress, with minimum head room of 6 ft, 6 inches without having to pass through any other dwelling or dwelling unit.

#### *Minimum standards for light and ventilation*

Every habitable room should have at least one window or skylight facing the outdoors and capable of being opened. Non-habitable rooms may have a ventilation system in lieu of a window. Every room should be supplied with electric service and outlets.

#### *Minimum thermal standards*

Every dwelling should have heating facilities which will maintain a temperature of at least 68 F at a distance of 18 inches above floor level under ordinary winter conditions.

#### *General requirements relating to the safe and sanitary maintenance of parts of dwellings and dwelling units*

These are obviously too numerous to mention in this discussion, however, in general the requirements pertain to adequate construction to provide protection from the elements, rodents and insects, and to permit sanitary maintenance.

#### *Maximum density, minimum space, use, and location requirements*

Every dwelling unit should have 150 ft<sup>2</sup> of floor space for the first occupant and at least 100 ft<sup>2</sup> of floor space for each additional occupant. The ceiling height of all habitable rooms should be at least 7 ft.

### PROBLEMS

Let's turn now to some of the problem areas. "Some factors contributing to housing problems are land, natural resources, building materials, production methods; building, zoning, and housing codes; industrialization, economics, distribution, population mobility, marketing; demand and supply; politics, government, industry, labor; obsolescence, conversions, progress, American standard of living and the American standard of wastefulness; income, education, climate, geography, activities both of omission and commission of officials, agencies of federal, state, and local government, private and unofficial organizations . . . and the public-people and their health (4).

I want to comment on four environment problem areas that are probably of most concern from the public health standpoints relative to housing construction and maintenance.

#### *Water supply*

If the proposed housing does not have access to a public water supply the local health department should be contacted for information regarding ap-

proved type of construction and treatment of private water supplies whether the supply is from a well, cistern, or spring. In all instances the supply should be subjected to continuous chlorination.

#### *Sewage disposal*

If proposed housing does not have access to a public sewage disposal system the local health department should be contacted for information regarding construction of an approved waste disposal system. Some local boards of health have subdivision and lot size regulations which govern installation of septic tanks and drainfields. The State Plumbing Code also specifies requirements for installation of septic tanks. In all instances it is necessary to make a percolation test and/or a soil study to determine if the soil will absorb the waste water and to determine the number of feet of drainfield that will be required. If a private sewage treatment system is to be installed that will discharge its effluent into a stream, a permit for such installation must be obtained from the State Water Pollution Control Commission.

#### *Plumbing*

The State Plumbing Code regulates installation of plumbing by statute in all public buildings and in homes in all counties having a city of 4th class or higher. In addition some local boards of health and cities have adopted the state plumbing code by regulation or ordinance in those counties not covered by statute, making a total of 109 counties in Kentucky covered by the code. The State Plumbing Code does exempt farmsteads that are not connected to a public water supply from the requirements of the code. State Plumbing Inspectors can be contacted at the local health department.

#### *Solid waste*

Storage of solid wastes is generally neglected; however, in many respects it is one of the most important aspects of solid waste management. Since storage is generally the responsibility of the waste generator, storage practices are frequently varied and difficult to control, especially around the home. Storage practices are important in two respects. First, good storage practices contribute to the day-to-day maintenance of a healthful, clean, attractive, and nuisance-free environment. In addition, storage practices have a direct influence on the cost of collection. Poor storage practices generally result in an unattractive, unsanitary community and excessive collection costs.

Although storage practices are important, there has not been a great deal of innovation in this area, particularly in regard to residential waste containers. The fundamental requirements for waste containers are as follows: (a) to prevent the escape and scatter-

ing of waste before collection; (b) to prevent rodents, flies, and other pests or animals from using wastes as food or breeding grounds; (c) to protect the community from the health hazards or nuisance associated with decomposing wastes; (d) to provide a relatively attractive covering for unattractive contents; and (e) to aid in the collection of wastes, and to allow economical collection practices.

To fulfill these requirements a container should, in general, be weather-resistant and leak-proof, durable, easy to empty and clean, attractive, of the proper size, and should be equipped with a durable tightfitting lid and convenient handles.

Some building codes are requiring either a refuse grinder or a home incinerator in each new residential structure. In most instances the grinders are no problem; however, the incinerator causes an air pollution problem as it is not feasible to install a home-type incinerator that will meet air pollution emission standards. It would seem reasonable and justifiable to require plans for handling and storage areas to be submitted as part of building permit application for each new business, industry, institution or multi family residential development.

Mobile home parks are becoming more popular as a source of housing in Kentucky and elsewhere. Therefore, I want to mention that the State Health Department administers the Kentucky Trailer Park Law and Regulations which requires submission of plans for approval and a construction permit before constructing or altering a park and also requires a permit to operate. Routine inspections are made by local health department sanitarians to assure compliance with established requirements. Our main problem with mobile home parks is created by installations that do not submit plans for approval before construction.

#### CONTROL PROGRAMS

Let's turn now to environmental control programs that should be carried out by state and local health departments to alleviate some of the problems associated with housing and to prevent future problems. Looking from the health viewpoint there are four goals which could be set for a housing program: (a) prevention of premature death, (b) prevention of disease, illness and injury, (c) attainment of efficiency of living, and (d) provision of comfort (6).

To accomplish these goals there are four kinds of governmental actions needed. They are: (a) adoption of minimum health standards in housing, (b) conduct of a program to achieve and maintain such standards, (c) periodic evaluation of the standards to ensure their current adequacy, and (d) monitoring of the stan-

dards enforcement effort to guarantee that public health values are provided (5).

Health agencies, to meet their responsibility, must accept the role of either stimulating or carrying out, as the case may be, these four required kinds of governmental action (5).

It is with regret that I must report that the Kentucky State Department of Health and, with few exceptions, the local health departments are not meeting this responsibility. Although many of the environmental programs at the State Health Department have an affect on housing there is not an established program that is giving attention to the entire environmental aspect of housing. The Division of Environmental Services has requested funds for establishing such a program; however, as of this date they have not been forthcoming. Hopefully they will be provided in the near future.

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### EXPO '72 TO SPORT THE 'NEWEST AND LATEST'

Exhibitors will feature an array of new or redesigned processing and handling equipment and components at Food & Dairy Expo '72, perhaps more than at any previous Show.

As booth plans are received, the tally of the "newest" or the "latest" products continues to mount—horizontal sanitary blender, a case stack pusher, a ribbon blender, closed-headed polyethylene container, solids mixer, freeze drier, computerized blenders, chemical meters, control panels, osmosis equipment, cheese grinders, colloid mill—the list goes on.

Far and away the largest of the eight exhibitor categories, the 78 companies in the processing and handling grouping will use a total of 64,000 square feet in the mammoth Atlantic City Convention Hall.

"We believe this early indication of exhibitor plans for Expo '72 points to what may be the most outstanding Show ever," said G. F. Barnum, commodity

director of processing and handling equipment and components of Dairy and Food Industries Supply Association.

Nearly half the 78 processing exhibitors have listed new or redesigned equipment as basic features of their Expo booths.

Food & Dairy Expo '72 will be held Oct. 1 to 5, 1972, the 28th biennial exposition.

Additional new or modified wares expected to be shown will be a programmed ice cream freezer; batch-type scraped surface cooker-cooler; super-duty "sanitizer" agitator, featuring instant "dial-a-speed"; a vertical swept surface heat exchanger; a "whipper-chiller"; rotary screw compressor; puree incorporator; spiral flow heat exchanger; tray pac caser, and glide line inventory system.

An estimated 15,000 to 20,000 processors are expected to attend.

## EFFECTS OF AEROSOLIZATION ON SALMONELLA NEWBRUNSWICK<sup>1</sup>

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### ABSTRACT

*Salmonella newbrunswick* was aerosolized with distilled water into a duct with temperature and relative humidity control of the air. Airborne bacteria were collected on a membrane filter and held in the airstream for ½ hr. Cells were rehydrated and held in distilled water for ½ hr; then they were centrifuged and the supernatant fluids tested for various inorganic chemicals and organic materials that are essential for normal functions. Spot tests revealed potassium, magnesium, phosphates, simple carbohydrates, and amino acids in the supernatant fluids from the bacteria. Sodium was definitely identified in < 10% of the tests on the fluids. An increase over the unaerosolized control in absorbance of the supernatant fluids at 265 nm was noticed. The increase may be attributed to partial destruction of ribosomes observed by electron microscopy.

Technical difficulties with studying biochemical changes in airborne bacteria are numerous and involve production and collection of bacterial aerosols in quantities sufficient to measure biochemical changes in airborne bacteria. By utilizing a special technique of recovering aerosolized bacteria on membrane filters this study was undertaken to determine the various leakage materials of these bacteria which might be associated with the airborne state and subsequent aerosol collection. Another objective of this study was to ascertain if the leakage of ultraviolet absorbing material was related to intracellular morphological changes in airborne *Salmonella newbrunswick*.

Anderson and Dark (1) found potassium and phosphate the most suitable to investigate the relationship between ion movement and survival of airborne *Escherichia coli* strain B. Anderson et al. (2) showed that after aerosolization, strains of *Escherichia coli*, *Aerobacter aerogenes*, *Serratia marcescens*, and *Staphylococcus epidermidis* rapidly lost their ability to retain intracellular potassium. Maltman (7) demonstrated that desiccation and rehydration of airborne staphylococci produced leakage of amino acids, phosphates, protein and ribonucleic acid components from cells. Cox (3) reported that airborne *E.*

*coli* K12 showed the greatest loss of viability at 80% relative humidity and inactivation was associated with almost complete breakdown of RNA and drastic inhibition of energy production.

### MATERIALS AND METHODS

Leakage materials resulting from aerosolization and rehydration of *S. newbrunswick* were determined as follows: (a) *S. newbrunswick* was grown at 37 C in chemically defined media (3), (b) cells were harvested by centrifuging at 12,000 × g and room temperature before washing in deionized sterile water three times, (c) the final suspension was atomized into an air stream produced by an Aminco unit (Fig. 1) with the temperature and relative humidity controlled, (d) bacteria in the air were collected for 15 min on a membrane filter and then bacteria on the filter were exposed to the air stream for an additional ½ hr (Fig. 1, A and B), and (e) one filter was removed and the bacteria were rehydrated rapidly by submerging the filter into distilled water, whereas the second filter was exposed to an air stream with gradually increasing relative humidity to nearly 100%. The gradual increase of relative humidity was accomplished during 4 hr by raising the temperature of the water bath of the Aminco unit to the dry-bulb temperature of the air thus giving a 100% saturation at the operating temperature of 26 C. When the relative humidity of the air stream reached 95%, the filter was transferred to a beaker containing water. Bacteria were suspended in the same quantity of water as the rapidly rehydrated sample.

Cells were resuspended in the distilled water and the absorbance of the samples was determined at 600 nm by the Spectronic 20 (Bausch and Lomb) spectrophotometer. The absorbances of all samples were adjusted to a 0.3 value (corresponding to  $4.6 \times 10^8$  cells/ml) by diluting the samples with distilled water to provide similar osmotic conditions for: (a) control (unaerosolized cells), (b) aerosolized with rapidly rehydrated cells, and (c) aerosolized with gradually rehydrated cells.

After adjustment of absorbance, cell suspensions were incubated in distilled water at 35 C for ½ hr and then centrifuged. The supernatant fluid from each of the cell treatments was passed through a membrane filter of 0.45 μ pore size. Absorbance of the supernatant fluid was determined at 265 nm by the DBG Beckman spectrophotometer. The remainder of the supernatant fluid was frozen at -40 C for subsequent chemical analyses. For electron microscopy pellets of the culture were freeze-dried within 2 hr in screw cap tubes used for centrifugation. Atmospheric pressure in the tubes was restored with nitrogen gas of 99.99+% purity.

Inorganic ions of potassium, sodium, and magnesium plus

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TABLE 1. LEAKAGE OF MATERIALS FROM AIRBORNE *Salmonella newbrunswick* REHYDRATED AT VARIOUS RELATIVE HUMIDITIES

Leakage materials	Trial	% relative humidity																				
		30			40			50			60			70			80			90		
		C	S	R	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R
Potassium	A	?	+	+	?	+	+	?	?	?	-	-	-	?	-	?	?	+	+	-	-	-
	B	?	+	+	?	+	+	?	?	?	-	-	-	?	?	?	-	-	-	-	-	-
Magnesium	A	-	?	+	?	+	+	?	+	+	?	+	+	-	+	+	?	+	+	-	-	-
	B	?	+	+	?	+	+	?	+	+	?	+	+	-	+	+	?	+	+	?	+	+
Phosphate	A	-	-	-	-	+	+	-	-	-	-	-	-	?	+	+	?	+	+	-	-	-
	B	-	-	-	-	-	+	-	+	+	-	-	-	?	?	?	-	-	+	-	-	-
Simple carbohydrates	A	-	-	-	?	+	+	-	-	-	?	+	+	-	?	?	-	+	+	-	?	?
	B	-	-	-	?	+	+	-	?	?	+	+	+	-	?	?	-	+	+	-	?	?
Amino acids	A	-	?	?	-	-	-	?	+	+	?	+	+	-	+	+	?	+	+	-	-	-
	B	-	-	-	?	+	+	-	+	+	?	+	+	-	+	+	?	+	+	-	+	+

C = control; S = slowly rehydrated; R = rapidly rehydrated  
 - = no reaction; ? = doubtful reaction; + = definite reaction

the phosphates and organic materials which included simple carbohydrates, amino acids, and protein-like substances in the defrosted (20 to 24 C) supernatant fluids were determined by spot tests of Feigl (4, 5).

The appearance of UV light absorbing materials in the supernatant fluids was considered an indication of leakage of nucleic acid materials. Separation of the 265 nm absorbing material was carried out by column chromatography on dextran Biogel 2. The elution was done by 7 M urea in 0.02 M Tris/HCl buffer at pH 7.6. Void volume of the column was determined by blue dextran in 7 M urea and the Tris buffer. The position of a peak for mono-nucleotides was established by using yeast adenosine monophosphate eluted on the same column.

In electron microscopy studies, fixation of aerosolized *S. newbrunswick* cells was performed by a method of Stoeckenius and Rowen (9). Cells in the freeze-dried pellets were fixed with 4% formaldehyde in a stock salt solution (1% NaCl and 0.2% CaCl<sub>2</sub>) at 4 C for 6 to 7 hr. Then the cells were centrifuged at 3020 × g on Sorvall SS 1 centrifuge. The pellet was resuspended in the salt solution and held over-night at 4 C. After centrifugation and the removal of the supernatant fluid, a drop of 1.5% melted agar in the salt solution was added to the pellet and cooled to 4 C during ½ hr. Post-fixation of the agar pre-embedded pellet was accomplished in 1% osmium tetroxide in the stock salt solution for 6 hr at 4 C. The agar block was washed in deionized water at room temperature for 30 min.

The agar embedded pellet was cut into small pieces and stained with 2% aqueous uranyl nitrate for 30 min at room temperature. The agar pieces were dehydrated in graded acetone according to method by Reyter and Kellenberger (8). The agar pieces were transferred to a 1:1 of Epon-acetone mixture (6) and held overnight. The Epon-acetone mixture was replaced by 100% Epon for 12 hr and followed by another change of 100% Epon for 12 hr. Then the Epon impregnated agar blocks were transferred to the final 100% Epon in gelatin capsules. The Epon was hardened for 48 hr at 60 C and a week at room temperature. Sectioning was accomplished with a Sorvall MT 2 microtome using freshly broken glass knives. Only very thin sections were used (50-60 nm). Electron micrographs were obtained by the Philips EM-100 Electron Microscope.

## RESULTS AND DISCUSSION

Release of leakage materials from airborne *S. newbrunswick* after gradual and rapid rehydration

TABLE 2. RELEASE OF 265 NM LIGHT ABSORBING MATERIAL FROM AIRBORNE *Salmonella newbrunswick* REHYDRATED FROM VARIOUS RELATIVE HUMIDITIES OF AIR

RH %	Absorbance at 265 nm			Times increase compared to control	
	R	S	C	R	S
30	0.1475	0.111	0.015	9.83	7.40
40	0.140	0.147	0.0145	9.70	10.10
50	0.130	0.127	0.011	11.82	11.55
60	0.100	0.107	0.013	7.69	8.23
70	0.100	0.142	0.015	6.67	9.47
80	0.148	0.174	0.014	10.57	12.43
90	0.142	0.160	0.021	6.76	7.62
	Average			9.00	9.55

R = rapidly rehydrated; S = slowly rehydrated; C = un-aerosolized (control)

was investigated to observe if aerosolization caused permeability damage. Supernatant fluids of the rapidly and gradually rehydrated samples of *S. newbrunswick* were analyzed for the presence of various materials. Results in Table 1 indicate that the leakage materials tested were present in varying amounts. Since the tests were qualitative, arbitrary units (-, ?, +) were assigned according to the strength of the reaction.

### Leakage of inorganic materials

Potassium (Table 1) was present in at least one of the two trials at most of the relative humidities from 30 to 80%. Results of tests for phosphates were variable with approximately half of the rapidly and slowly rehydrated samples showing a doubtful to a very definite reaction. Magnesium was present in both rapidly and slowly rehydrated samples at the various humidities. The sodium leakage, if any, was too small for definite detection in more than 90% of the tests.

### Leakage of organic materials

Table 1 shows organic materials in the supernatant fluids of aerosolized *S. newbrunswick*. Protein-like substances were too weak to show a definite

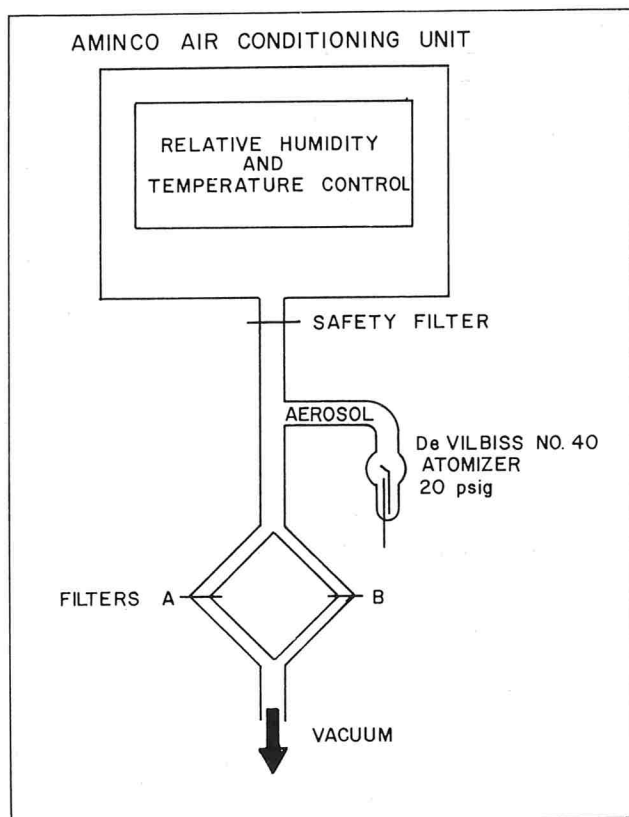


Figure 1. Equipment for aerosolizing, equilibrating, and rehydrating airborne *Salmonella newbrunswick*.

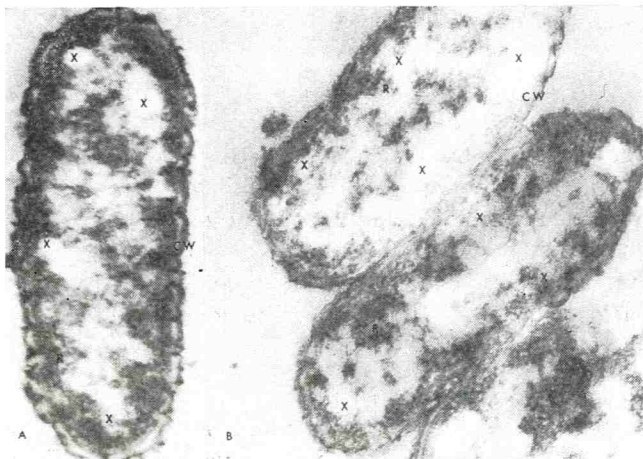


Figure 2. Electron micrograph ( $100,000\times$  magnification) of aerosolized and rapidly (A) or slowly (B) rehydrated and subsequently freeze dried cells of *Salmonella newbrunswick*. (CW) cell wall, (R) ribosomes, (X) areas of diminished ribosome content.

reaction in all trials. The significance of this observation is probably that the plasma membrane was not sufficiently damaged to permit release of large molecules.

Simple carbohydrates were detected by periodate oxidation without heating. They appeared to be present at various relative humidity levels in both

treatments. These substances may be derived from an internal pool of carbohydrate metabolic products, or may be associated with the nucleotides as are ribose or deoxyribose. Leakage of amino acids also was observed at various relative humidity levels (Table 1). Loss of some amino acids during rehydration may affect recovery of the organism after aerosolization.

Control bacteria did not have a definite positive test for the seven leakage materials at the seven relative humidities. There was one exception in 98 tests. The number of definite positive results for leakage indicated practically no difference between the slowly or rapidly rehydrated, aerosolized bacteria. Rehydration at 90% relative humidity seemed to result in less positive leakage tests than at relative humidities of 40 to 80%.

#### *Leakage materials absorbing ultraviolet light at 265 nm*

The release of 265 nm absorbing material from airborne *S. newbrunswick* showed considerable increase over the unaerosolized control at various relative humidity levels (Table 2). The significance of the 265 nm absorbing material is that it may be associated with nucleic acids or may result from their breakdown. Supernatant fluids of both slowly and rapidly rehydrated samples contained significant amounts of 265 nm absorbing materials when compared to the unaerosolized control. Release of these materials was slightly greater at five of the seven relative humidities when cells were rehydrated slowly than when rapid rehydration occurred.

#### *Electron microscope studies*

The aerosolized, rapidly or slowly rehydrated, and subsequently freeze-dried cells, showed some clear areas (Fig. 2, A and B, marked X) which may represent depletion of ribosomes. These clear areas and leakage of UV light absorbing materials (Table 2) suggest that partial destruction of ribosomes took place as the result of aerosolization. Subsequent treatments during preparation for analysis may have contributed to a limited extent to their disappearance.

#### CONCLUSIONS

Aerosolization of *S. newbrunswick* resulted in leakage of both inorganic and organic materials. Leakage of these materials probably signifies an increase in permeability damage to the cell membrane. Release of UV light absorbing material apparently is related to partial depletion of ribosomes.

#### ACKNOWLEDGEMENTS

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**DR. H. C. OLSON, OKLAHOMA STATE  
UNIVERSITY, WINNER OF PFIZER AWARD  
FOR CHEESE RESEARCH**

Dr. Harold C. Olson, Professor of Dairy Science at Oklahoma State University and a member of the American Dairy Science Association for 42 years, is the recipient of the 1972 Pfizer Award for Cheese Research. The presentation was made during the 67th annual ADSA meeting July 28 at Virginia Polytechnic Institute and State University, Blacksburg.

Dr. Olson, a native of Brookings, South Dakota, was honored for major research contributions in dairy microbiology and studies related to the performance of lactic cultures. "He has demonstrated an ability to identify problems of significance to the dairy industry and to obtain solutions that have won wide acclaim," the citation stated.

Author or co-author of more than 47 scientific papers on lactic cultures for the cheese industry, Dr. Olson was cited for developing a starter culture activity test which approximates the manufacture of cottage cheese by the short-set method.

Other noteworthy contributions were made in bacteriophage control; compatibility of multiple-strain starter cultures; symbiosis among starter culture bacteria; storage ability of starters, and on the effect of various factors on starter culture activity.

The Pfizer Award, consisting of a bronze plaque and \$1,000, was established in 1958 to recognize "meritorious scientific contribution to the cheese industry" and to serve as a stimulus to fundamental research. It was presented by John Shovers of Pfizer's Technical Development Department, Milwaukee Operations.

Dr. Olson, who helped establish the annual train-

ing school sponsored by the American Cultured Dairy Products Institute, is editor of the *Cultured Dairy Products Journal*. He received his B. S. degree from South Dakota State College in 1928, an M.S. degree from West Virginia University in 1930 and his Ph.D. in dairy microbiology from Iowa State College in 1932, when he was appointed Assistant Professor of Dairy Microbiology at Iowa State. He served in that post until his appointment in 1940 as Professor of Dairy Science at Oklahoma State.

Dr. Olson served as President of the Southern Division of the ADSA in 1957. A member of the International Association of Milk and Food Sanitarians, Phi Sigma and Sigma Xi, Dr. Olson served as an officer of the Oklahoma Dairy Technology Society, which he helped organize. He has held memberships in the Payne County Creamery Board of Directors and the Stillwater Chamber of Commerce.

Among other research achievements mentioned in the citation were the development by Dr. Olson of improved methods for the analysis of cottage cheese, including the use of an antibiotic to prevent bacterial growth on potato dextrose agar in the enumeration of yeasts and molds. His research on the effect of certain manufacturing variables on consumer preferences helped establish quality standards for cottage cheese. A test for detecting the agglutinating tendency of cultures also is credited to the award winner.

The Pfizer Award for Cheese Research is administered by ADSA and its selection committee. Pfizer is a leading producer of enzymes and other ingredients for the dairy and food processing industries.

## INACTIVATION OF SALMONELLA TYPHIMURIUM BY SORBIC ACID<sup>1</sup>

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### ABSTRACT

Nutrient broth, skim milk, and evaporated milk at pH 5.0, 5.5, or without pH adjustment and with and without 2,000 and 3,000 ppm sorbic acid were evaluated at 7, 13, and 37 C for their effects on *Salmonella typhimurium*. The combination of 3,000 ppm sorbic acid and acetic acid at pH 5.0 most effectively inactivated *S. typhimurium* in all media and at all temperatures. Complete inactivation by this treatment required from 12 hr or less in nutrient broth at 37 C to 55 days in evaporated milk at 7 C. In some instances, treatment with 3,000 ppm sorbic acid combined with lactic acid at pH 5.0 was equally effective. Reduction of sorbic acid concentration to 2,000 ppm or raising the pH of the substrate to 5.5 increased the time needed for inactivation of *S. typhimurium*. Inactivation of *S. typhimurium* was most rapid in nutrient broth at 37 C and required progressively more time either as the temperature was reduced or as more complex foods were substituted for the broth. Growth of *S. typhimurium* occurred at 37 and 13 C in plain nutrient broth, in nutrient broth at pH 5.0 or 5.5, and in nutrient broth with 2,000 or 3,000 ppm sorbic acid (pH not adjusted). Growth in skim milk occurred under similar conditions except when the pH was reduced to 5.0 with acetic acid. In evaporated milk, growth at both temperatures was possible only in untreated samples and in those acidified to pH 5.5. In some instances, a lag period of 25-29 days occurred at 13 C before growth was evident.

It has long been recognized that straight-chain monocarboxylic acids have fungistatic properties. In 1913 Kiesel (6) found that the antifungal activity of saturated fatty acids increases with the number of carbon atoms (up to 11) in the molecule. During the 1930's, according to Wyss et al. (12), Japanese workers observed that unsaturated fatty acids were more active antimycotic agents than were saturated acids with a similar number of carbon atoms. Gooding (4), in 1945, discovered that the  $\alpha$ - $\beta$ -unsaturated fatty acid, sorbic acid, was fungistatic. Since then sorbic acid (or its salts) has been widely used to protect a variety of foods from deterioration by mold. Included are: cheese, orange juice, fruit and fruit syrups, margarine, pickles, yogurt, and others. Although generally effective at a concentration of 3,000 ppm (the maximum permitted in some foods by

Federal regulations) or less, some molds can grow in the presence of at least 5,300 ppm of sorbic acid (7) and then can degrade the chemical to 1,3-pentadiene which imparts a hydrocarbon-like odor to the substrate.

Since sorbic acid has been used largely as an antifungal agent, only limited attention has been given to its effects on bacteria. Emard and Vaughn (3) reported that growth of salmonellae, staphylococci, and some other catalase-positive bacteria was inhibited during a 7-day incubation at 34 C when the yeast extract medium contained 77-1,200 ppm sorbic acid. Catalase-negative bacteria that were tested grew without noticeable inhibition by sorbic acid. In contrast, Hamdan et al. (5) found that both growth of and acid production by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (both catalase-negative) was reduced by 500 to 1,000 ppm sorbic acid. According to York and Vaughn (11), up to 30,000 ppm sorbic acid failed to inhibit growth or sporulation by *Clostridium botulinum* in liver infusion broth at pH 6.7. The pH of the medium was clearly too high for antimicrobial activity by sorbic acid.

Doell (2) found that approximately 7,500 ppm sorbic acid at pH 5.0 inactivated salmonellae and *Escherichia coli* in 48 hr at 37 C. Concentrations as low as approximately 750 ppm were bacteriostatic under similar conditions. Recently Park et al. (8) observed that *Salmonella typhimurium* was inactivated more rapidly in refrigerated cold-pack cheese food that contained sorbic acid rather than sodium propionate or no antimycotic agent. Since the effects of sorbic acid on salmonellae are poorly defined, especially at refrigerator temperatures, experiments were conducted to: (a) verify the observations made with cold-pack cheese food, and (b) establish the conditions under which sorbic acid will inactivate salmonellae in foods. Results of the experiments appear in this paper.

### MATERIALS AND METHODS

Eight-ounce prescription bottles were filled with sufficient broth, skim milk, or evaporated milk so that 150 ml remained in the bottles after autoclaving for 15 min at 121 C. Both

<sup>1</sup>Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

TABLE 1. TREATMENT OF SAMPLES REPRESENTED BY DIFFERENT CURVES IN FIGURES 1-9.

No. in figures	pH	Acid used	Ppm sorbic acid added <sup>2</sup>
1	NA <sup>1</sup>	None	None
2	5.5	Lactic	None
3	5.0	Lactic	None
4	5.5	Acetic	None
5	5.0	Acetic	None
6	NA	None	2,000
7	NA	None	3,000
8	5.5	Lactic	2,000
9	5.5	Lactic	3,000
10	5.0	Lactic	2,000
11	5.0	Lactic	3,000
12	5.5	Acetic	2,000
13	5.5	Acetic	3,000
14	5.0	Acetic	2,000
15	5.0	Acetic	3,000

<sup>1</sup>NA = Not acidified

<sup>2</sup>Added as potassium sorbate in quantities to provide the equivalent of 2,000 or 3,000 ppm sorbic acid.

a laboratory medium and foods which differed in complexity were represented by the substrates. After cooling, sufficient of a 40% solution of potassium sorbate was added to the substrates to provide 2,000 or 3,000 ppm of sorbic acid. These concentrations were selected because they are the maximum amounts permitted in several types of foods. The potassium sorbate (Chas. Pfizer and Company) solution was filter-sterilized (Millipore Filter) before it was used. The pH of some substrates fortified with sorbate was then adjusted to 5.0 or 5.5 with sterile 10 or 50% solutions of lactic or acetic acid. This was done to optimize effects of sorbic acid and to simulate conditions which exist in some foods. Substrates just described and appropriate controls were then held overnight at 7, 13, or 37 C. Treatment of samples is detailed in Table 1.

Temperature-equilibrated samples were inoculated with sufficient of a 24-hr old culture of *S. typhimurium* (8) to provide approximately 10<sup>3</sup> cells per milliliter of test substrate. Duplicate sets of test samples were then incubated at the temperatures (7, 13, or 37 C) at which they were previously stored. Samples held for 72 hr at 37 C were tested at 12-hr intervals for numbers of viable salmonellae, whereas those stored at 7 or 13 C were examined at 48-hr intervals for up to 97 days. Numbers of *S. typhimurium* were determined with plate count agar (Difco) and incubation of plates at 37 C. Data from duplicate samples were averaged and average values are reported.

RESULTS AND DISCUSSION

Inactivation of *S. typhimurium* was affected by both incubation temperature and kind of substrate in addition to pH and concentration of sorbic acid. The bacterium generally was most rapidly inactivated at 37 C and progressively slower at 13 and 7 C. Furthermore, inactivation was most rapid in nutrient broth and slower in skimmilk and evaporated milk. Data are arranged by temperature of incubation for ease of discussion.

Inactivation of *S. typhimurium* at 37 C

Data in Fig. 1, 2, and 3 indicate both growth and inactivation of *S. typhimurium* at 37 C when nutrient broth, skimmilk, and evaporated milk served as substrates. Growth by the bacterium in nutrient broth was possible at pH values of 5.5 (curves 2 and 4) and 5.0 (curves 3 and 5) when either lactic or acetic acid was used to achieve the desired pH value. Use of acetic acid at pH 5.0 was accompanied by a lag phase of at least 12 hr before growth was evident. This was not true when lactic acid served to provide the same pH value. Growth also occurred in nutrient broth which contained 2,000 or 3,000 ppm sorbic acid (curves 6 and 7). Since the pH of nutrient broth (6.7) approaches neutrality, sorbic acid probably was still present as the potassium salt and hence was without appreciable effect on growth of the bacterium.

Substitution of skimmilk (Fig. 2) for nutrient broth reduced the conditions under which growth of *S. typhimurium* was possible. Now acetic acid at pH

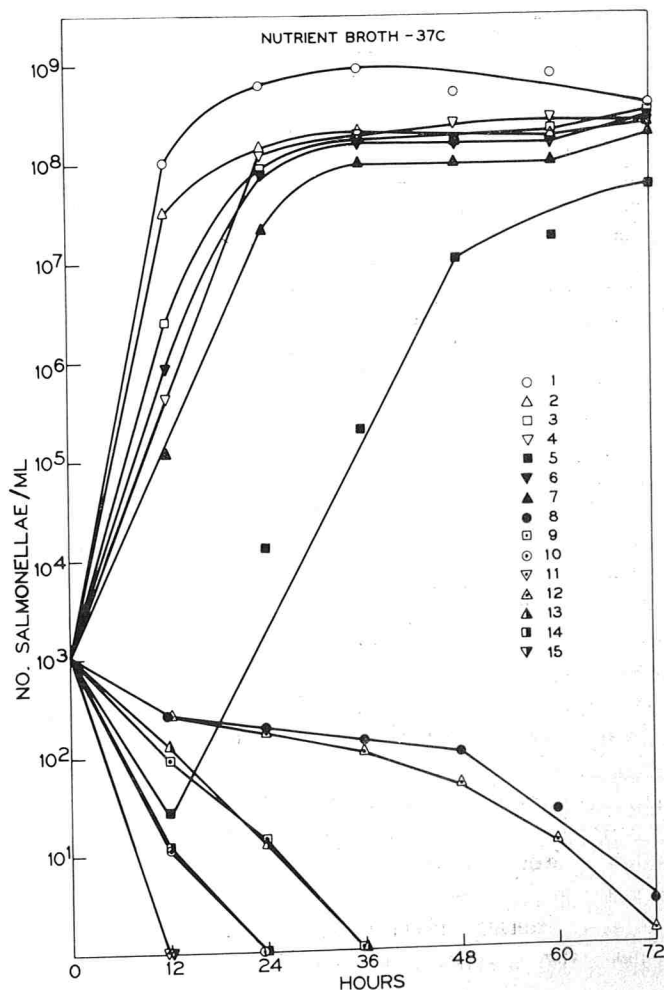


Figure 1. Behavior of *Salmonella typhimurium* at 37 C in plain or acidified nutrient broth with and without sorbic acid. Numbers of curves are identified in Table 1.

5.0 or presence of 3,000 ppm sorbic acid not only failed to permit growth but actually caused some inactivation of the bacterium during the 72-hr incubation period. Furthermore, presence of 2,000 ppm sorbic acid caused a lag of approximately 24 hr before growth of the *Salmonella* became evident. Perhaps the lower pH value of skim milk (6.4) (than of nutrient broth) contributed to the anti-salmonellae activity of sorbic acid in this substrate.

Growth of *S. typhimurium* was possible only in untreated evaporated milk (Fig. 3) and in evaporated milk at pH 5.5. Undoubtedly the naturally occurring low pH (5.70 untreated, 5.80 with 2,000 ppm and 5.86 with 3,000 ppm sorbic acid) of evaporated milk accounts for some of the detrimental effects of sorbic acid even in the otherwise untreated substrate.

Inactivation of *S. typhimurium* was most rapid in nutrient broth (Fig. 1) and was complete in 12 hr or less when the medium was at pH 5.0 and also contained 3,000 ppm of sorbic acid (curves 11 and 15). A reduction to 2,000 ppm sorbic acid (curves 10 and 14) at pH 5.0 resulted in complete inactivation after 24 hr. An additional 12 hr was required for complete inactivation when the pH was raised to 5.5 but the medium contained 3,000 ppm sorbic acid (curves 9 and 13). Presence of 2,000 ppm sorbic acid at pH 5.5 also caused inactivation of *S. typhimurium* but it was not complete in 72 hr (curves 8 and 12).

When skim milk served as the substrate (Fig. 2), complete inactivation of *S. typhimurium* required 24 hr and then occurred only when acetic acid served to reduce the pH to 5.0 and when 3,000 ppm sorbic acid were present. This is in contrast to nutrient broth when only 12 hr (or less) were required and when both lactic and acetic acid were equally effective. Complete inactivation also was achieved in 36 hr by 3,000 ppm sorbic acid at pH 5.0 when lactic acid was used and by 2,000 ppm at pH 5.0 when acetic acid was used. Furthermore, complete inactivation was evident after 48 hr at pH 5.0 (lactic acid) with 2,000 ppm sorbic acid. Treatments other than those just described, except those which allowed growth, caused partial inactivation of *S. typhimurium* during the 72-hr incubation at 37 C.

Whereas only four treatments achieved complete inactivation of *S. typhimurium* in skim milk, this was accomplished in evaporated milk by eight treatments, two more than did so in nutrient broth (Fig. 1 and 3). Included were 2,000 and 3,000 ppm sorbic acid at pH 5.0 (acetic and lactic acid), 3,000 ppm sorbic acid at pH 5.5 (acetic and lactic acid) and evaporated milk at pH 5.0 (acetic and lactic acid). To be sure, presence of sorbic acid hastened inactivation (24 to 48 hr versus 60 to 72 hr) over that observed

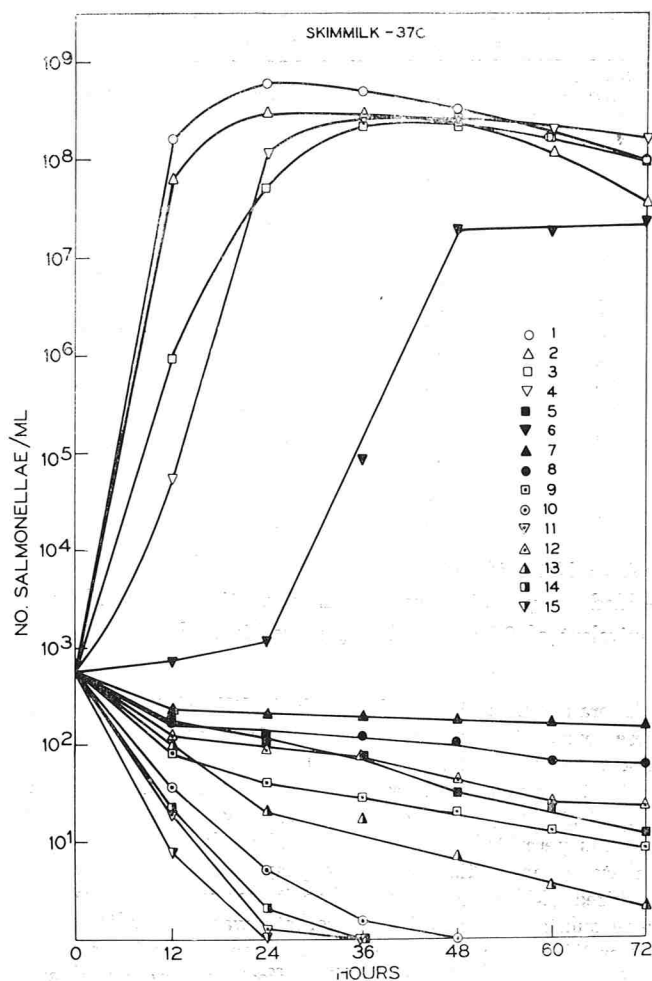


Figure 2. Behavior of *Salmonella typhimurium* at 37 C in plain or acidified skim milk with and without sorbic acid. Numbers of curves are identified in Table 1.

in its absence. Incomplete inactivation was accomplished by four other treatments, 2,000 and 3,000 ppm sorbic acid alone, and 2,000 ppm sorbic acid at pH 5.5 (acetic and lactic acid).

#### Inactivation of *S. typhimurium* at 13 C

Growth of *S. typhimurium* at 13 C occurred essentially in the same samples that permitted growth at 37 C, albeit in some instances an extended incubation was necessary before an increase in numbers was evident (Fig. 4, 5, and 6). This was particularly true in skim milk and evaporated milk (Fig. 5 and 6) where 27-29 days of incubation were required before growth occurred in samples adjusted to pH 5.5 with acetic acid. Incubation of up to 11 days also was required before *S. typhimurium* grew in skim milk which contained 3,000 ppm sorbic acid. The extended lag periods observed in these experiments suggest that a food is not necessarily protected against growth of salmonellae (or other bacteria) if that growth is not evident in a week or even several weeks.

It is also worthy to mention that after an initial

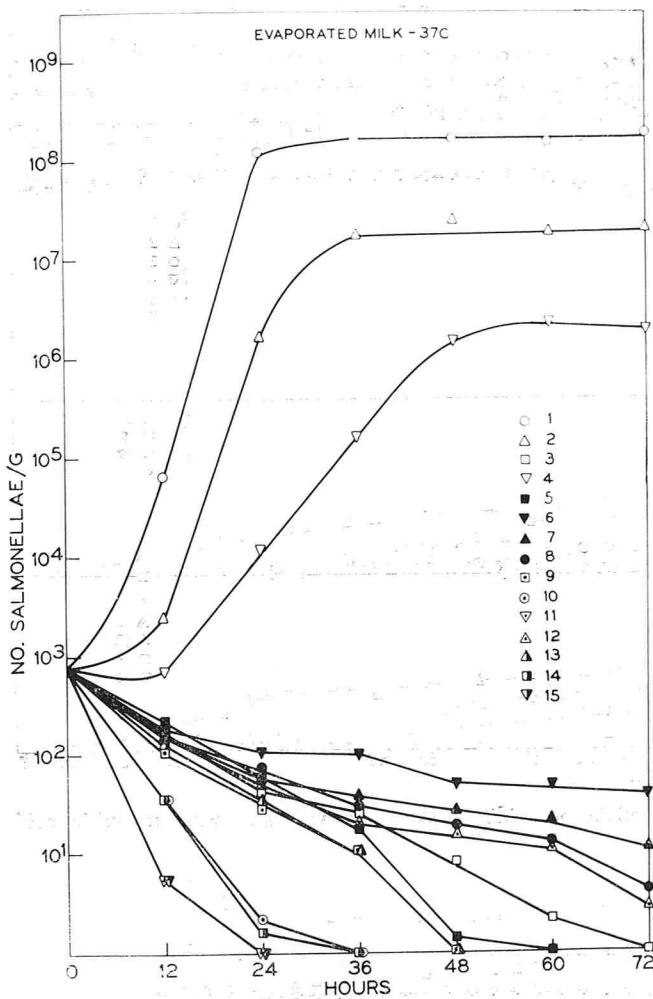


Figure 3. Behavior of *Salmonella typhimurium* at 37 C in plain or acidified evaporated milk with and without sorbic acid. Numbers of curves are identified in Table 1.

increase in numbers, *S. typhimurium* proved to be amazingly hardy and survived at 13 C without appreciable loss of numbers for 90 or more days in skimmilk (Fig. 5) and evaporated milk (Fig. 6) and for 35 or more days in nutrient broth (Fig. 4).

Inactivation of *S. typhimurium* at 13 C again was more rapid in nutrient broth than in skimmilk or evaporated milk. At this temperature, complete inactivation in nutrient broth always required more than a week of storage. Most rapid (11 days) inactivation was achieved by 3,000 ppm sorbic acid at pH 5.0. Progressively longer storage resulted in complete inactivation by some other treatments; 35 days were required before viable salmonellae were eliminated from nutrient broth at pH 5.5 and with 2,000 ppm sorbic acid.

Replacement of nutrient broth with skimmilk (Fig. 5) served to lengthen the time at 13 C required for inactivation of *S. typhimurium*. In this instance, 35 days had to elapse before the most active combination of chemicals (3,000 ppm sorbic acid plus acetic acid

at pH 5.0) did the job. When lactic acid was substituted for acetic acid and other conditions remained the same, 59 days were required before inactivation of the bacterium was complete. Other combinations of sorbic acid with lactic or acetic acid required additional time (up to 97 days) before *S. typhimurium* was completely inactivated.

Although inactivation of *S. typhimurium* was slow in evaporated milk, it was somewhat faster than in skimmilk. For example, 31 rather than 35 days were required for 3,000 ppm sorbic acid plus acetic acid at pH 5.0 to inactivate the bacteria. Furthermore, more treatments (12 versus 9) caused complete inactivation of the bacterium in evaporated rather than skimmilk and inactivation by the least active of treatments was complete in 79 days (versus 97 days for skimmilk). Perhaps the heat treatments given evaporated milk may have produced compounds which were unfavorable for salmonellae and thus hastened their demise when other conditions became detrimen-

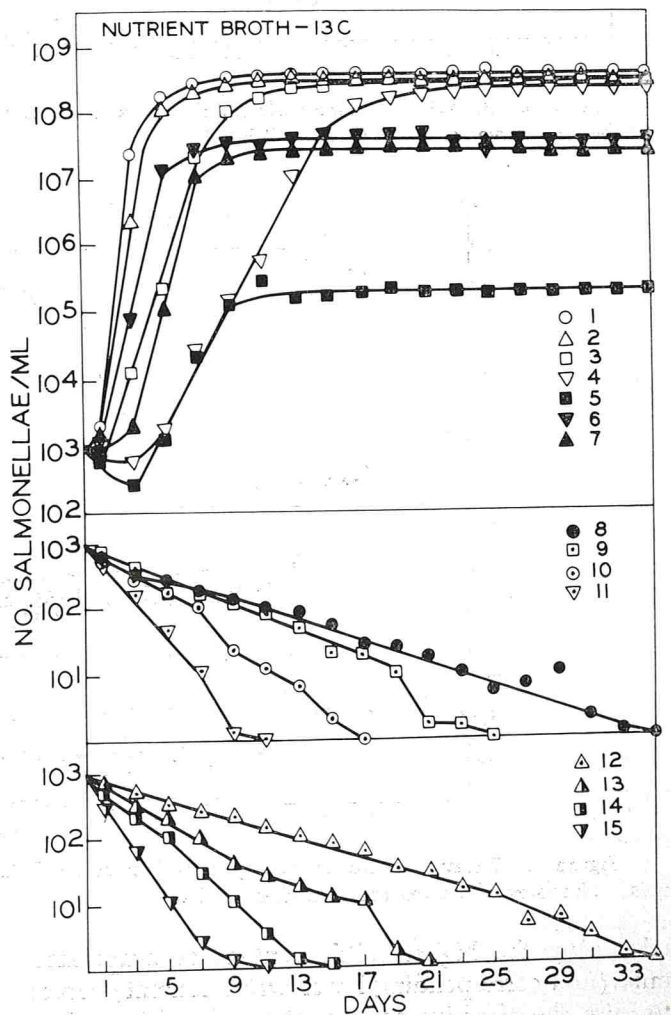


Figure 4. Behavior of *Salmonella typhimurium* at 13 C in plain or acidified nutrient broth with and without sorbic acid. Numbers of curves are identified in Table 1.

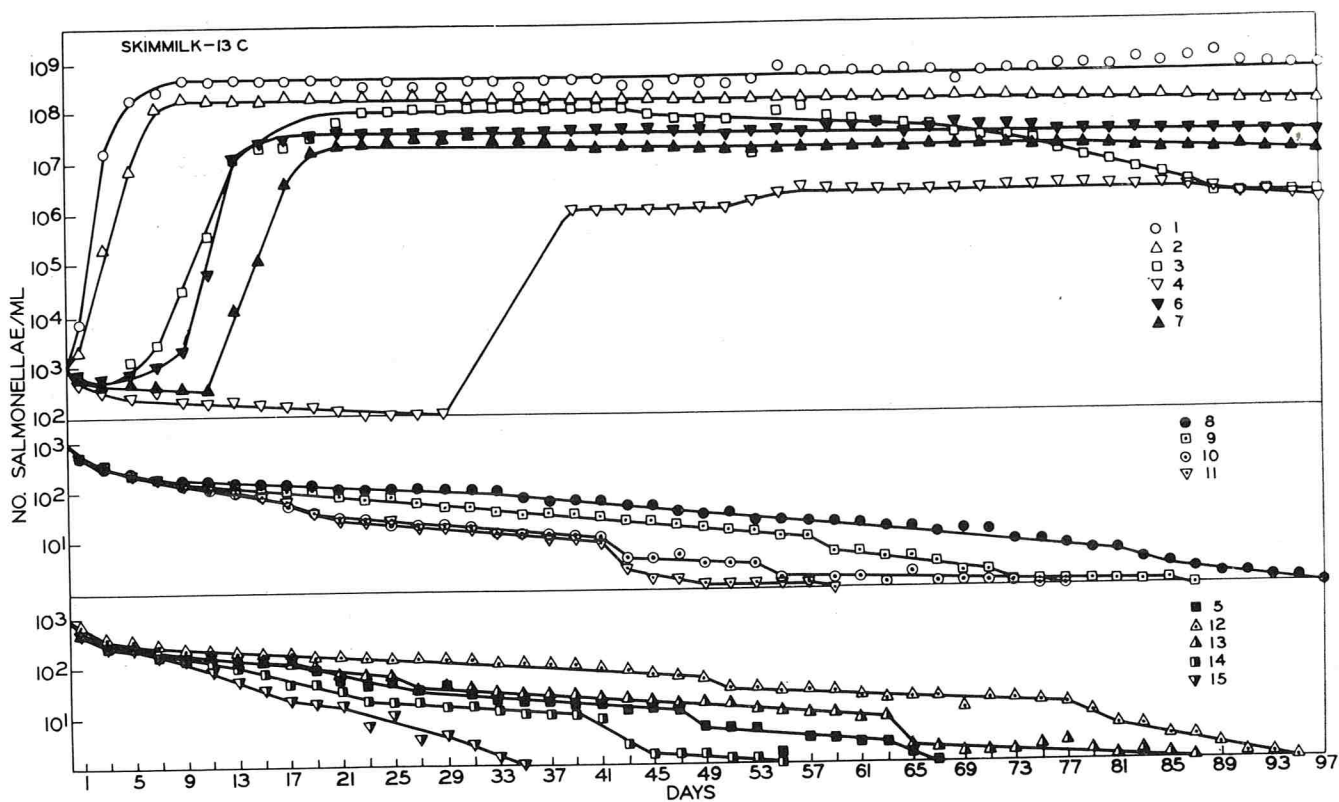


Figure 5. Behavior of *Salmonella typhimurium* at 13 C in plain or acidified skim milk with and without sorbic acid. Numbers of curves are identified in Table 1.

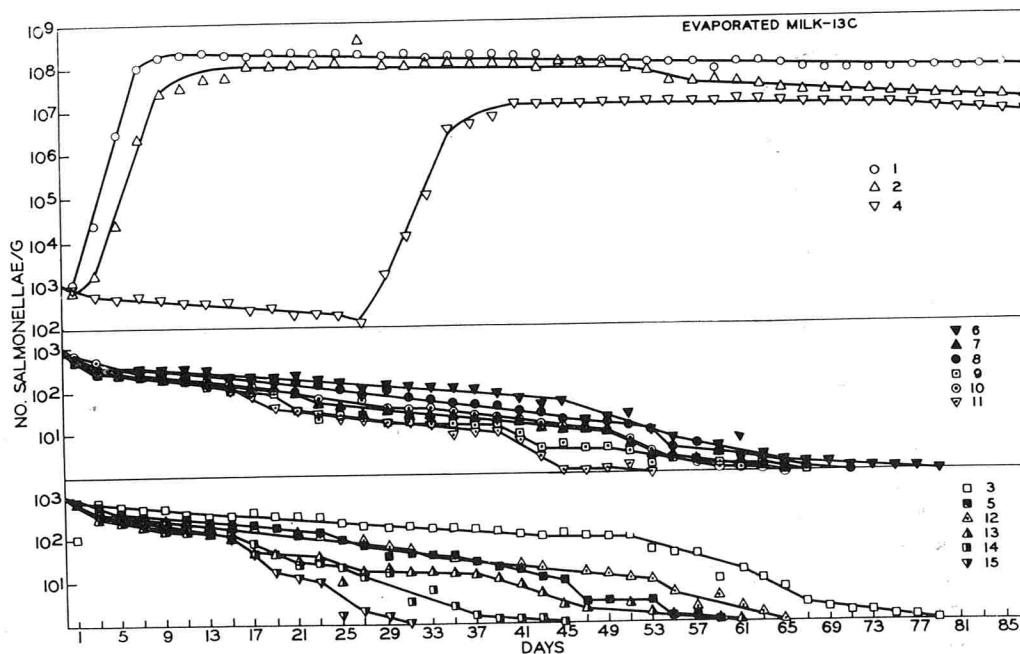


Figure 6. Behavior of *Salmonella typhimurium* at 13 C in plain or acidified evaporated milk with and without sorbic acid. Numbers of curves are identified in Table 1.

tal. Also, the higher solids content of evaporated milk (and corresponding lower water content) serves to raise the effective concentration of sorbic acid in the water phase. This, too, may have enhanced inactivation of salmonellae.

#### Inactivation of *S. typhimurium* at 7 C

At 7 C, growth of *S. typhimurium* was not evident in samples of nutrient broth (Fig. 7), skim milk (Fig. 8), or evaporated milk (Fig. 9), regardless of the treatment given the substrates. Instead, there was

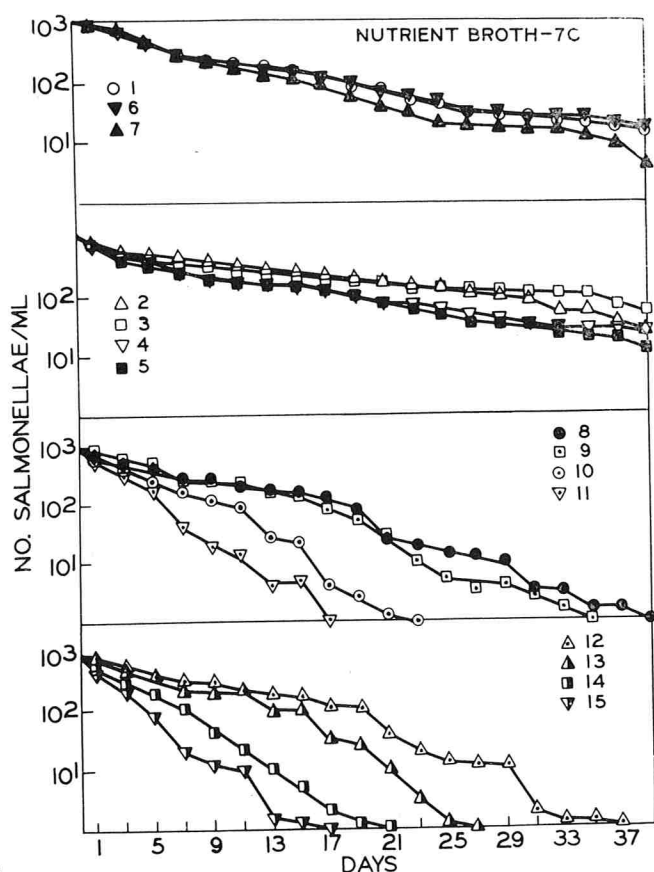


Figure 7. Behavior of *Salmonella typhimurium* at 7 C in plain or acidified nutrient broth with and without sorbic acid. Numbers of curves are identified in Table 1.

a gradual loss of viability by the bacterium in untreated or minimally treated samples and more rapid inactivation as the severity of the treatment increased.

A slow but steady decline in numbers of viable salmonellae occurred in all three untreated substrates as well as three substrates only acidified to pH 5.0 or 5.5 with lactic or acetic acid and in all three substrates that only contained 2,000 or 3,000 ppm sorbic acid. However, detectable salmonellae remained in the nutrient broth samples just described after 39 days and in milk samples after 87 to 97 days.

In contrast, when sorbic acid was combined with either lactic or acetic acid, complete demise of *S. typhimurium* was observed and this often occurred in less time. Use of the most active combination (3,000 ppm sorbic acid plus acetic acid to pH 5.0) caused complete inactivation after 17, 31, and 55 days in nutrient broth, skim milk, and evaporated milk, respectively. The combination of lactic acid at pH 5.0 plus 3,000 ppm sorbic acid was as effective in nutrient broth as the one described earlier but required 10 additional days in skim milk and 2 additional days in evaporated milk before inactivation was complete. Use of six additional treatments (2,000 ppm sorbic acid plus either lactic or acetic

acid at pH 5.0; 2,000 or 3,000 ppm sorbic acid plus either lactic or acetic acid at pH 5.5) caused complete inactivation of *S. typhimurium* in all three substrates during the test period. The time required for inactivation by these treatments ranged from 21 to 39 days in nutrient broth, from 39 to 85 days in skim milk, and from 71 to 87 days in evaporated milk.

Results of this investigation verify the observation made earlier by Park et al. (8) that sorbic acid hastened the demise of *S. typhimurium* from cold-pack cheese food stored at refrigerator temperatures. They are also somewhat in accord with a report by Shiflett et al. (10) who noted a loss of viability by *S. typhimurium* in sorbate-treated oysters stored at 7 C for 14 days. Unfortunately, these investigators used only 1,000 ppm sorbate, a maximum storage of 14 days, and failed to state the pH values for oysters. Hence, a direct comparison with their results is impossible.

It is evident that several factors govern inactivation of salmonellae by sorbic acid. These include temperature (inactivation was most rapid at 37 C and markedly slower at 7 and 13 C), substrate (inactivation was most rapid in nutrient broth and required substantially more time in food systems, particularly at 7 and 13 C), pH of the substrate (activity was greater at pH 5.0 than at 5.5), acid used to adjust pH (activity was often greater when acetic rather than lactic acid was used), and concentration of sorbic acid (at the same pH value, 3,000 ppm was more effective than 2,000 ppm).

The data again emphasize the need for conducting microbiological studies with actual food systems rather than only with laboratory media. Conclusions based only on data obtained with nutrient broth as the substrate would be substantially different from those based on observations made with food systems.

Although sorbic acid and its salts have long been used to control growth of mold on cheese and other foods, these data suggest that an additional benefit may accrue from use of these additives—inactivation of some bacteria, and in particular, food-poisoning bacteria such as salmonellae. Of course, the bacteria must be in contact with the chemical for this to happen. Hence, inactivation of bacteria below the surface of a food treated with sorbate on the surface only is unlikely unless the sorbate, at a sufficient concentration, can diffuse into the product. This possibility remains for a future investigation.

Recently enteropathogenic strains of *Escherichia coli* have been identified as the cause of illness in persons who consumed some French Camembert cheese (1). Occurrence of *Escherichia* - *Enterobacter* bacteria in cheese is not uncommon and was described by Russell (9) as early as 1895. Undoubtedly, these bacteria, including some enteropathogenic strains,

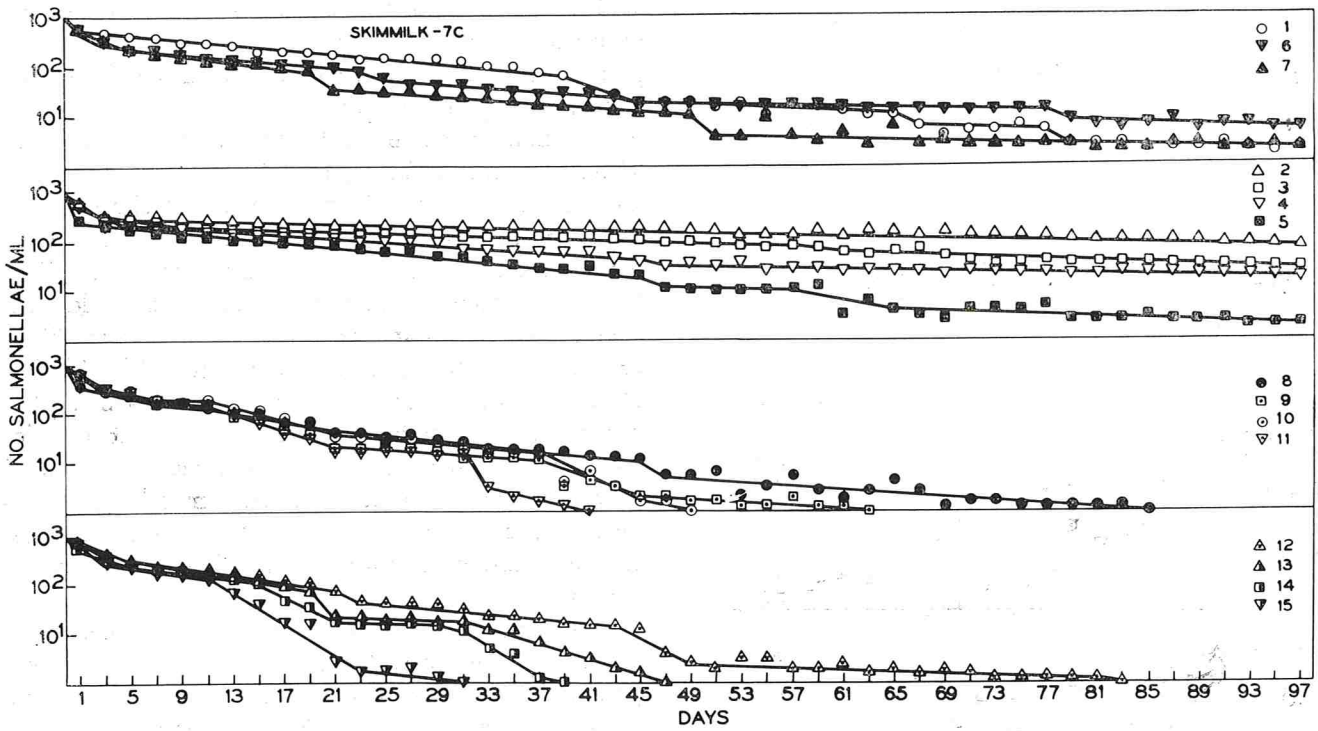


Figure 8. Behavior of *Salmonella typhimurium* at 7 C in plain or acidified skim milk with and without sorbic acid. Numbers of curves are identified in Table 1.

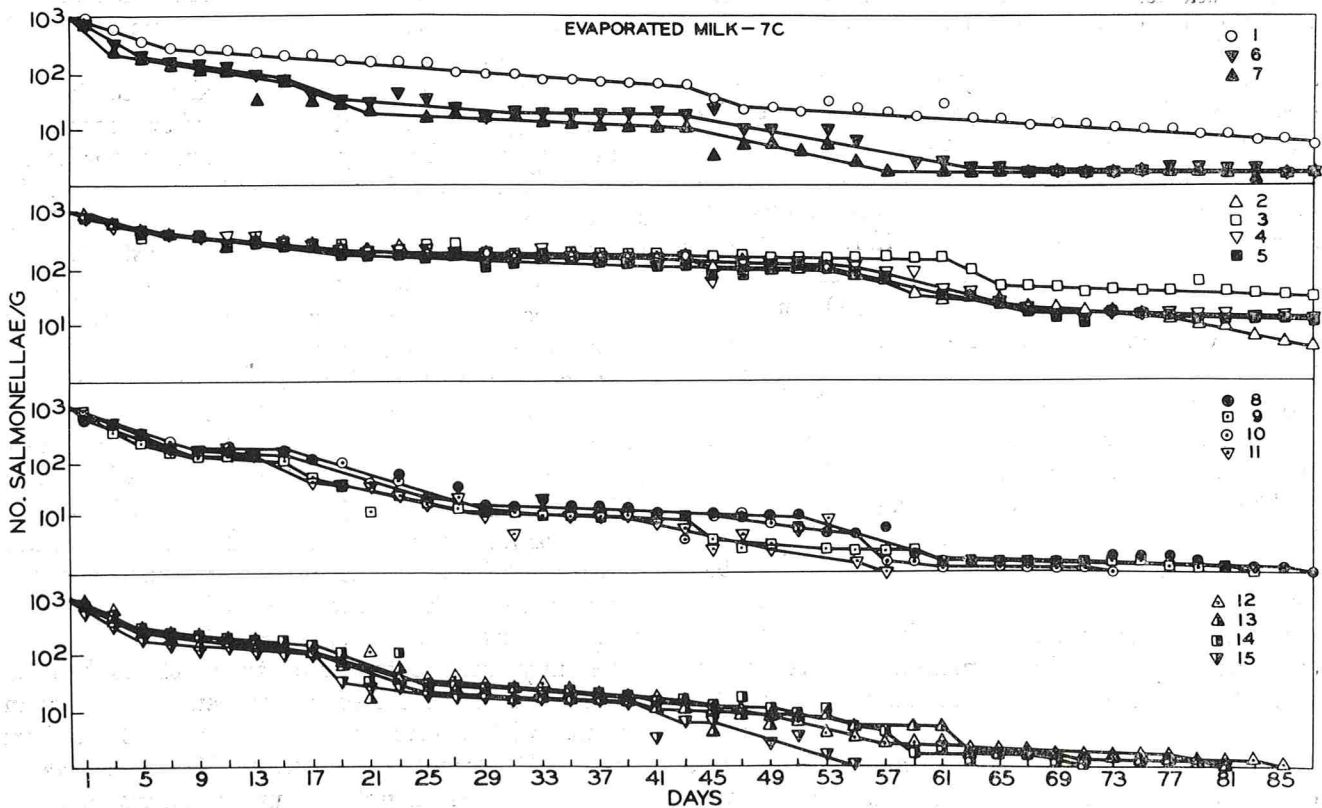


Figure 9. Behavior of *Salmonella typhimurium* at 7 C in plain or acidified evaporated milk with and without sorbic acid. Numbers of curves are identified in Table 1.



can be recovered from some present-day U. S. cheese which is not mold ripened. Since the genera *Escherichia* and *Salmonella* are closely related, it would seem reasonable to expect that sorbic acid could inactivate *E. coli*. Experiments need to be done to confirm this belief.

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## FOOD TECHNOLOGISTS TO MEET IN BOSTON

REPRESENTATIVE OF PEOPLE'S REPUBLIC  
OF CHINA INVITED

A Sections East meeting of some 500 members of U. S. Institute of Food Technology and the Canadian Institute of Food Science and Technology, will be held in Boston for three days beginning October 29. The theme of the Sections East Meeting is "Food Under Fire."

According to the General Chairman of the conference, Dr. Charles Pyne, Manager of Technical Research at H. P. Hood Inc., "The conference will provide for the exchange of thoughts and ideas on the wholesomeness of the food we eat among representatives from academic, industry, government, and consumer groups. The subject is vital to all consumers as they have the right to complete information about the foods they eat."

Mr. John M. Fox, President of H. P. Hood Inc., has been named Honorary Chairman of the conference. Mr. Fox disclosed today that for the past six months he and members of the IFT and various government officials have been working on the possibility of getting a food scientist or representative from the People's Republic of China to attend the conference and address the Banquet. Mr. Fox said that in his opinion, "One of the world's most exciting food stories is how the People's Republic of China has been able to feed its 800 million people. It is a story that has never been told and I am sure it is fascinating," said Mr.

Fox, "and it is a story that should be told to the food industry of America and the world. The forthcoming Sections East conference in Boston provides an ideal forum to tell the story."

Mr. Fox said that the conference planners have been in touch with many people and a formal invitation to the People's Republic of China has been extended, and the conference is now awaiting word from Peking. He expressed optimism that the invitation will be accepted because both the United States and the People's Republic of China seem anxious to establish contact between their respective scientific communities.

J. P. MacFarland, Chairman of General Mills and Vice Chairman of the Grocery Manufacturers Association, will address the group on the subject of the "Industry's Role in Protecting the Consumer." Other prominent names on the program include: Dr. Jean Mayer, Harvard School of Public Health; Dr. Mike Jacobson, Centre of Science in the Public Interest; Dr. Walter Smith, Department of National Health and Welfare in Canada.

Miss Pamela Low, Chairman of the Northeast Section, IFT, expressed her appreciation for the continuing interest and financial support given by H. P. Hood Inc. to Sections East.

## BACTERIOLOGICAL QUALITY AND OCCURRENCE OF VIBRIOS IN DUNGENESS CRABMEAT IN OREGON PROCESSING PLANTS AND MARKETS<sup>1</sup>

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### ABSTRACT

Dungeness crabmeat samples from Oregon processors and retail markets were analyzed for *Vibrio parahaemolyticus*, numbers of fecal streptococci, and aerobic total plate count. Samples were collected from three crab processors during one commercial season from the following points along the processing line: (a) raw, whole crabs; (b) cooked, cooled crabs; (c) picked crabmeat; (d) brined, or brined and inspected crabmeat; and (e) to-be-packed crabmeat.

Five of 31 samples of crabmeat from Oregon retail markets and 4 of 75 crabmeat samples from Oregon processors were positive for halophilic vibrios. Positive samples from processors were raw crabmeat and picked crabmeat. Biochemical characteristics of the isolates from only one of these, a raw sample, closely resembled the biochemical characteristics of known *V. parahaemolyticus* cultures. The remainder of the isolates were other halophilic vibrios.

The numbers of fecal streptococci in crabmeat from both processors and retail markets were generally within the guideline of < 1000 organisms per gram of crabmeat. The median aerobic total plate count of  $1.2 \times 10^6$  organisms per gram for the crabmeat samples from retail markets was above the limit of 100,000 organisms per gram established by New York City and used elsewhere. The median plate count of to-be-packed crabmeat from processors remained within this limit.

*Vibrio parahaemolyticus* has recently become of concern to food microbiologists and public health officials in the United States (7). It has been estimated that it causes 40-70% of all bacterial outbreaks of food poisoning in Japan (13). In the U. S. the extent of the problem is not known since it is not routinely sought in the detection of causative organisms in a food poisoning outbreak. However, it was identified as the cause in a Maryland outbreak in 1971 (7).

In Japan, the food which causes illness from *V. parahaemolyticus* is generally seafood, seafish, or a salted vegetable which is eaten raw or partially cooked (13). There is an indication that fish and shellfish in the United States may be naturally contaminated with *V. parahaemolyticus*. Market fresh-frozen Chesapeake Bay blue crab (6) were found to be contaminated, as well as oyster, clam, and mussel sam-

ples collected from retail markets on the Atlantic coast of Canada (2, 16). In Washington, the organism was most often isolated from oysters and other molluscan shellfish and from the intestines of crabs from a polluted area of Bellingham Bay (1).

In this study, crab samples from Oregon retail markets and from various points along crab processing lines of Oregon processors were analyzed to determine the occurrence of *V. parahaemolyticus* on Oregon crabmeat and whether any spots allowing large increases of *V. parahaemolyticus* existed along the processing line. Numbers of fecal streptococci and aerobic total plate counts were obtained to assess the bacteriological quality of the crabmeat.

### MATERIALS AND METHODS

Crab (*Cancer magister*) samples were collected during one commercial season (March to July, 1971) from three Oregon retail markets, each supplied by a different wholesaler, and from various points along the processing lines of three Oregon crab processors. The general outline of the processing procedure was described by Hipkins (10). All workers, except those at the inspection table, wore rubber gloves. The only wooden surface was some of the bins in which the raw crabs were transported. Samples included (a) whole crabs in bins; (b) butchered, cooked and cooled crabs; (c) picked crabmeat; (d) brined or brined and inspected crabmeat; and (e) rinsed and drained to-be-packed crabmeat. Thirty-one samples were collected from retail markets. Seventy-five duplicate samples were collected from crab processors. One sample of each set was transported at about 10 C to reduce loss of vibrios, the other was transported at about 0 C (2 to 4 hr).

The methods of Liston-Baross and Japanese workers as outlined by the Food and Drug Administration (8) were used to recover *V. parahaemolyticus*. In the Liston-Baross method, crabmeat dilutions are plated onto salt-water-starch agar (SWSA). These plates were incubated anaerobically at 37 C for 48 hr. Positive colonies were white and non-spreading with a clearing around each colony, indicative of starch hydrolysis. The Japanese method involved enrichment of crabmeat dilutions in glucose-salt-Teepol broth (GSTB) and salt-Colistin broth (SCB; Tanabe Seiyaku Co., Ltd., Osaka, Japan) for 18 hr incubation at 37 C followed by plating on thiosulfate-citrate-bile salts-sucrose agar (TCBS; Baltimore Biological Laboratories, Cockeysville, Maryland). The TCBS plates were incubated 24 hr at 37 C. Positive colonies were 2-3 mm in diameter with a green-blue center. Suspect colonies from both methods were

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TABLE 1. OCCURRENCE OF HALOPHILIC VIBRIOS, NUMBERS OF FECAL STREPTOCOCCI AND AEROBIC TOTAL PLATE COUNT (20 C) OF FRESH DUNGENESS CRABMEAT FROM OREGON PROCESSORS AND RETAIL MARKETS

Type of sample	Halophilic vibrios <sup>a</sup>	Fecal streptococci count/g		Plate Count (20 C) count/g	
		Median	Range	Median	Range
Crabmeat from retail markets	5/31	590	50 - 11,000	1.2×10 <sup>6</sup>	9.7×10 <sup>3</sup> -3.7×10 <sup>8</sup>
Crabmeat from processors:					
Raw	2/15	<10	<10 - 40	8.0×10 <sup>2</sup>	4.0×10 <sup>1</sup> -2.5×10 <sup>3</sup>
Cooked, cooled	0/15	<10	<10 - 100	4.4×10 <sup>3</sup>	2.0×10 <sup>1</sup> -9.7×10 <sup>4</sup>
Picked	2/15	530	200 - 1,500	7.2×10 <sup>4</sup>	2.0×10 <sup>2</sup> -9.1×10 <sup>5</sup>
Brined	0/15	250	<10 - 420	1.8×10 <sup>3</sup>	5.0×10 <sup>2</sup> -1.4×10 <sup>8</sup>
To-be-packed	0/15	100	30 - 180	8.0×10 <sup>3</sup>	3.7×10 <sup>2</sup> -5.0×10 <sup>4</sup>

<sup>a</sup>Number of positive samples/total number of samples.

TABLE 2. BIOCHEMICAL CHARACTERISTICS OF HALOPHILIC *Vibrio* ISOLATES FROM CRABMEAT SAMPLES

Sample	0% NaCl broth	7% NaCl broth	10% NaCl broth	TSI agar <sup>1</sup>	Kovac's oxidase	Nitrate reduction	Indole production	Voges-Proskauer reaction	Arabinose <sup>2</sup>	Cellobiose <sup>2</sup>	Inositol <sup>2</sup>	Lactose <sup>2</sup>	Sucrose <sup>2</sup>	Trehalose <sup>2</sup>	Malonate utilization	Citrate utilization
S-3	-	+	-	a	+	+	±	-	±	± <sup>3</sup>	-	-	+	+	-	-
S-1	-	+	-	a	+	+	-	+	-	± <sup>3</sup>	-	-	+	+	-	-
U-2	-	+	-	b	+	+	+	+	+	± <sup>3</sup>	-	-	+	±	+	±
U-3	-	+	-	b	+	+	+	+	+	± <sup>3</sup>	-	-	+	-	+	±
27	-	+	-	b	+	+	+	+	+	± <sup>3</sup>	-	-	+	+	±	+
5	-	+	±	a	± <sup>2</sup>	-	+	-	-	-	+	+	+	+	-	+
24	-	+	+	a	± <sup>2</sup>	-	-	-	-	± <sup>3</sup>	-	-	+	+	-	+
9	-	+	-	a	± <sup>2</sup>	-	±	-	-	-	-	-	+	+	-	+
13	-	+	-	b	± <sup>2</sup>	-	-	-	-	-	-	-	+	+	-	+
<i>V. parahaemolyticus</i> controls																
	-	+	-	b	+	+	+	-	+	± <sup>3</sup>	-	-	-	+	-	±

<sup>1</sup>a if acid; b if alkaline slant, acid butt

<sup>2</sup>Delayed positive reaction

<sup>3</sup>After 24 hr

tive *V. parahaemolyticus* were gram-negative, pleomorphic, motile rods which were hemolytic on sheep's blood agar. These presumptive *V. parahaemolyticus* cultures were carried through further biochemical tests (8). Oxidase production was also tested by Kovac's oxidase test (14). Cultures isolated on TCBS were tested for starch hydrolysis on SWSA. Sensitivity to penicillin was tested by growth at 37 C on SWSA with 2-2.5 units of penicillin per milliliter.

Numbers of fecal streptococci were determined by plating appropriate dilutions on KF-streptococcal agar (Difco, Detroit, Michigan) with incubation at 37 C for 48 hr. Aerobic total plate counts were determined after appropriate dilutions were plated on plate count agar (Difco) and incubated at 20 C for 48 hr. The pH of the 1:10 dilution of samples was determined.

#### RESULTS AND DISCUSSION

Five of 31 samples of crabmeat from Oregon retail

markets were positive for halophilic vibrios. These samples had been collected throughout the testing period. All except one were from a processor in Washington. Four of 75 samples of crab from Oregon processors were positive for halophilic vibrios (Table 1). Two of these samples were meat of whole raw crabs and the other two were picked crabmeat. All of these isolates were gram-negative, pleomorphic, motile rods which were not sensitive to penicillin and would not grow in 0% NaCl but grew in 7% NaCl. All were hemolytic on sheep's blood agar. Biochemical characteristics are given in Table 2. Some isolates varied considerably from the schema of *V. parahaemolyticus* cultures. However, much variation in biochemical reactions has been reported in the literature: variable reactions in indole production

(16); growth in broth with 10% NaCl (4, 16); urease (4) and amylase (16) production; malonate (4, 18) and citrate (13, 18) utilization; and arabinose, cellobiose, trehalose (6) and sucrose (1, 18) fermentation.

The isolates from sample S-3 closely resembled *V. parahaemolyticus*. These isolates varied in that they produced acid from sucrose. Japanese workers question the pathogenicity of these strains which have been isolated from marine sources here (1, 13, 18). Moreover, they did not utilize citrate as the sole carbon source and the production of indole varied. On SWSA, 1600 organisms per gram were recovered. Isolates from the samples S-1, U-2, U-3, and 27 resembled *V. alginolyticus* in that they were Voges-Proskauer-positive and produced acid from sucrose. However, they did not grow in trypticase broth with 10% NaCl. The remainder of the *Vibrio* isolates varied considerably in biochemical characteristics.

A small number of samples (7 of 106) were positive for halophilic vibrios. There are several factors which could be related to this low level of contamination. The water temperature of areas in Oregon close to where the crabs were caught varied from about 8 C in March to 15 C in July. The growth temperature range of *V. parahaemolyticus* is 15-40 C (13). Consequently, the temperature of Oregon ocean waters was not conducive to growth of this organism. The one positive sample was taken in June.

The low level of pollution of Oregon coastal water, excluding estuaries (12), could also contribute to low numbers of *V. parahaemolyticus*. The crab processing procedure would also serve to reduce numbers considerably. Since there are several reports (11, 15) that *V. parahaemolyticus* is killed rapidly in fresh water, the many fresh water rinses during processing would serve to reduce the numbers also. The boiling in fresh water would greatly reduce the number of all vegetative bacteria (3, 15, 19).

The following changes occurred in the aerobic total plate count of crabmeat during processing: the numbers of bacteria, which were low on raw crabs, increased after cooking and cooling, increased further during picking, decreased after brining, then remained essentially the same. Samples were not taken directly from the cooling tank, but from racks after they had been cooked and cooled. In two of the five sampling periods, the crab had been cooked and cooled three days earlier and held on ice in the processing plant until the day of sampling.

The trends in total plate counts of samples among processors paralleled observations made during preliminary examination of the plants. The processor which took the most precautions to minimize contamination had products with the lowest plate counts. This processor added extra features to his line, such as flumes along the table to carry the shells out of

the shakers' work areas, hosing down the picking area with chlorinated water each time the shaker finished picking a batch of crab, and a fresh water rinse by means of a hose after the picked crab had been weighed and before it was brined. In all instances, however, the plate count on the to-be-packed crabmeat was below the 100,000 organisms per gram guideline (9). The median plate count was 8,000 organisms per gram. Data are reported for an incubation time of 48 hr at 20 C; incubation for 5 days gave slightly higher counts.

The plate counts of crabmeat from retail markets were quite high with a median count of 1,200,000 organisms per gram. This is well above the guideline. Crabmeat with counts in this range were considered to be spoiled by Tobin et al. (16). Each of the three markets had about the same range of counts ( $10^4$  to  $10^7$  or  $10^8$  cells per gram of crabmeat). Two markets prepackaged the meat, the third displayed it in a large pan on ice in an enclosed case. Display cases were -1 C at the coldest part in two stores and 5 to 6 C in the third. It appears that greater care in or supervision of the marketing of crabmeat is needed. Quality of crabmeat is important to the consumer who frequently serves the product without reheating and for its natural mild flavor.

The numbers of fecal streptococci on crabmeat from processors and retail markets were within the 1,000 organisms per gram guideline (9). Median count for to-be-packed crabmeat was 100 cells per gram and for crabmeat from retail markets, 590 per gram. The correlation coefficient for the relationship of aerobic total plate count and fecal streptococci was significant at the 1% level ( $r=0.73$ ) for retail samples but not for samples at the processor level.

The correlation coefficient of -0.14 for comparison of plate counts and pH of crabmeat from retail markets was not significant. This agrees with the report by Fieger and Novak (5) that pH is not a good indicator of bacterial numbers.

#### ACKNOWLEDGMENTS

Appreciation is expressed to the Oregon crab processors who cooperated in this study. A General Foods Fund fellowship held by the senior author partially supported the study.

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### LETTERS TO NATIONAL RESTAURANT ASSOCIATION MEMBERS

Sanitation and food protection are now receiving more and more attention from everyone. Knowing what is really important in order to protect your customers from the possibility of foodborne illness is not always clear, especially to your employees. However, a definite pattern of operational malpractices which contributed to foodborne illness outbreaks emerged from a check of a number of state, county and municipal health departments made recently by the NRA Public Health and Safety Committee.

We have published these practices on the enclosed chart, "Don't Serve Illness to Our Customers," which focuses employees' attention on the important points. You will surely want to emphasize these points as you instruct your employees and inspect and supervise your operation. The chart is provided as another membership service of the National Restaurant Association to assist you in assuring food safety for both your customers and employees.

Three other important points involving food pro-

tection were not included on the chart because they are primarily responsibilities of management. They are also very important to the prevention of foodborne illness outbreaks and should, thus, receive the same attention by management as you expect to give to the ten factors listed on the chart.

They are:

1. Food procured from unreliable or unsafe sources.
2. Unsafe water supply.
3. Equipment and equipment installation which do not permit necessary cleaning and sanitizing.

Please refer to your NRA Educational Materials Center catalog for aids to help you strengthen your foodservice sanitation and food protection program. Additional copies of this chart are available for purchase at fifty-cents each.

Sincerely,

NATIONAL RESTAURANT ASSOCIATION

Leslie W. Scott

President

# EFFECT OF MILKING MACHINES EQUIPPED WITH AUTOMATIC QUARTER-TAKE-OFF DEVICES ON MILK QUALITY AND HEALTH OF THE UDDER

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(Received for publication March 6, 1972)

## ABSTRACT

A commercial herd of approximately 550 lactating dairy cows was divided into two groups: one was designated as the Control Group and was milked in a routine fashion with conventional milking machines; the other was designated as the QTO Group and was milked with machines equipped with automatic quarter-take-off devices. Certain tests and observations were made on each animal in both groups at approximately 5-week intervals for 22 weeks. The QTO Group of animals was, by chance selection, at a slight disadvantage in terms of health of the udders at the outset of the study. The disadvantage was overcome quickly and, during the balance of the study, the QTO-milked animals maintained an advantage over the control animals. The advantages in favor of the animals milked with the QTO devices included fewer quarters infected with mastitis pathogens, reduced intramammary irritation as evidenced by lower scores for the California Mastitis Test, and fewer teats showing erosions or other abnormalities of the orifice. The differences were significant ( $P < 0.01$ ). Operators of the machines equipped with QTO devices walked 26.6% less during a routine milking than the operators of the conventional milking machines. The difference was significant ( $P < 0.05$ ).

The trend toward larger dairy herds and the shortage of qualified laborers for dairy work emphasizes the importance of further mechanizing the milking operation and increasing each operator's productivity. Hoglund et al. (2) reported that labor represents 76 to 82% of the cost of milking cows. In a similar study, Vos (3) estimated that as much as 18% of an operator's time is spent rechecking milking units to determine whether milk flow has ceased and in physically detaching the teat cups. It is also known that individual quarters of a cow's udder milk out at different rates and that the detachment of all four teat cups simultaneously often results in the over-milking of some quarters and the under-milking of others. It follows that improvements in both labor efficiency and health of the udder would result if mechanical devices were associated with milking machines to monitor milk flow and to automatically detach the teat cups individually as the flow of milk from respective quarters ceased. The use of such mechanical devices would also enable the operator to concentrate his judgement on matters in the parlor which are beyond the sphere of present technology.

Armstrong et al. (1) evaluated a prototype device

that automatically detached the milking unit from all quarters simultaneously when the completion of milking was signaled by a milk-flow monitoring device. Animals milked with the automatic units produced more milk on a daily basis than the animals milked with conventional machines. The differences were not significant. Milk Quality Test (MQT) ratings showed that the use of the automatic units did not have a detrimental effect upon the health of the udders.

## MATERIALS AND METHODS

A commercial dairy herd consisting of two groups of approximately 275 lactating cows each was used in the study. Animals were assigned to one of the two groups on an alternating basis following parturition and both groups were fed and managed in a similar manner. For reasons that were not apparent, animals in one of the groups had experienced previously an increased incidence of mastitis. That group was designated as the QTO Group and was milked with machines equipped with quarter-take-off (QTO)<sup>1</sup> devices that automatically removed the individual teat cups as the flow of milk from the respective quarters ceased. Animals in the second existing group were designated as the Control Group and were milked with conventional milking machines. The two groups were further divided into three lots of approximately 90 animals each according to level of daily milk production, i.e., those animals producing  $> 50$  lb., animals producing 35 to 50 lb., and animals producing  $< 35$  lb.

The milking facility consisted of two milking parlors placed back-to-back with cows exiting through separate corridors down the center between the parlors. Each parlor was equipped with four side opening, power operated, diagonal milking stalls set in a single row and two automatic prep stalls placed side-by-side between the entrance door and the first milking stall. Milk from animals in each parlor was conveyed to the milk receiving vessels via low-mounted 2-inch stainless steel milk lines. Each milking stall was equipped with a randle-mounted, low profile, siphon breaker cup that had an individual, unit-mounted, electrically controlled pulsator that operated on a 50:50 milk/massage ratio at 55 pulsations per minute. Vacuum was supplied by a two-pump Tonganoxie system that was common to both parlors. The vacuum for the milk side was supplied by a water cooled vacuum pump rated at 155 cfm<sup>2</sup> while vacuum for the pulsator side was supplied

<sup>1</sup>Trade name. Babson Bros. Company, 2100 South York Road, Oak Brook, Illinois 60521.

<sup>2</sup>The CFM for both pumps was based on the New Zealand method.

by an air cooled vacuum pump rated at 82 cfm. The vacuum lines were 2-inch stainless steel. The milking vacuum level was set at 12 1/2 inches Hg and the pulsator vacuum was set at 14 inches Hg, creating a 1 1/2 inch Hg vacuum differential.

The QTO system was comprised of two basic circuits, i.e., a milk sensing circuit and a solenoid valve operating circuit. The milking procedure was initiated by the operator pressing an auto-start button which caused the "milking-indicator" and "auto-start" lights on the milker control to go on and the four "quarter-status" lights to go off. Following attachment of the teat cups, there was a built-in delay of 70 to 80 sec which allowed time for milk flow to start.

Thereafter, the units functioned automatically. When milk stopped flowing into the respective electrode wells located on top of the breaker cup, the sensing circuit was broken and the solenoid valve operating circuit was activated. After a 2 to 3-sec delay, the solenoid was energized, allowing 55 psi air pressure to retract a cylinder piston located in the base of the QTO milker unit. This, in turn, pulled a lanyard cable downward to shut off vacuum at the end of the teat before the teat cup was pulled off. As each teat cup was removed, the corresponding "quarter-status" light on the milker control was energized, allowing the operator a visual check of the milker from a distance. Automatic QTO operation continued until each quarter was milked out and the individual teat cups were removed.

If a teat cup was accidentally removed before the quarter was milked out, the milker control was switched to manual operation, the teat cup was reattached, and the control was switched again to automatic operation when milk flow started.

Each QTO milker unit had a stainless steel base that provided protection for the air cylinder, the low voltage electrical connections, and the lanyard cables. The base was easily removed for checking of the electrical and air connections and for periodic cleaning of the air cylinders.

Certain tests and observations were made on each animal in both groups on the day before initiating the use of the QTO devices and, subsequently, at approximately 5-week intervals for 22 weeks. A sample of foremilk was collected from each quarter of 60 animals in each of the two highest production lots in both groups. Samples were collected in a similar manner from these particular animals at the beginning of the second, third, and fourth observational periods. A microbiological assay was conducted on each sample to identify any microorganisms that were present.

The California Mastitis Test (CMT) was conducted on foremilk from each quarter of the animals in both groups. The results were recorded as CMT 0, 1, 2, or 3. The teats of each cow were examined for erosions or other maladies of the orifice and milk samples collected from each quarter were examined for garget.

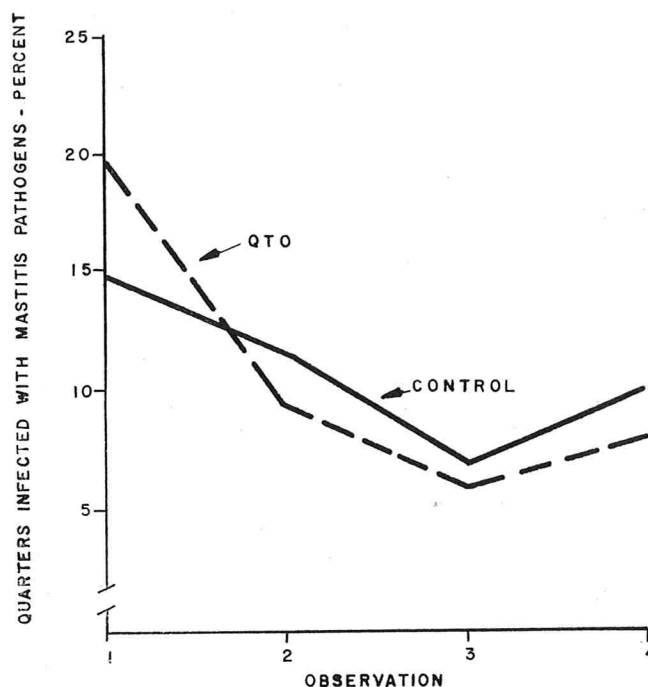


Figure 1. Results from the cultural examination of quarter milk samples.

## RESULTS AND DISCUSSION

### Microbiological content of quarter milk samples

As shown in Fig. 1, more of the animals in the QTO Group were infected with mastitis pathogens at the beginning of the study than in the Control Group. This situation had reversed, however, at the end of the first 5-week period and continued throughout the study in favor of the QTO devices. The differences between the two groups were tested using the following formula

$$t = \frac{P_1 - P_2}{\sqrt{\frac{P_1 Q_1}{N_1} + \frac{P_2 Q_2}{N_2}}}$$

where P = percentage of quarters with pathogens, Q = percentage of quarters with nonpathogens, and N = total number of quarters tested. The differences were significant ( $P < 0.01$ ) at observation 1 but were nonsignificant ( $P > 0.05$ ) at observations 2, 3, and 4. The decrease from observations 1 to 4 was significant

TABLE I. SUMMARY OF ANTIBIOTIC TREATMENTS ADMINISTERED INTRAMAMMARILY TO ANIMALS IN THE TWO EXPERIMENTAL GROUPS

During interval between observations	Group						Treatments administered
	Control			QTO			
	Cows	Quarters	Treatments administered (No.)	Cows	Quarters		
1 & 2	18	23	29	22	31	47	
2 & 3	37	61	142	33	57	153	
3 & 4	18	26	61	26	43	135	
4 & 5	41	76	157	52	75	181	
Total	114	186	389	133	206	516	

( $P < 0.01$ ) for both groups. Evidently this resulted from use of improved milking management methods with both groups of animals during the study period and from the extensive use of intramammary antibiotic infusions when abnormal milk was detected.

A record was kept of the antibiotic treatments administered to the udder of individual cows in both groups and the results are summarized in Table 1. Therapeutic agents were administered slightly more frequently to animals in the QTO Group than to animals in the Control Group. Seemingly, this was a consequence of the operators of the QTO devices having more time to observe the individual quarters of each cow during the milking process and they became more competent at detecting symptoms of the disease. This disparity in the number of quarters treated was disregarded in the statistical analyses conducted on the data because it was felt that the difference was a direct result of using the QTO devices.

The predominating mastitis pathogen observed was *Streptococcus agalactiae*. Infections caused by non-agalactiae streptococci as well as hemolytic staphylococci were extremely low. The incidence of non-pathogenic micrococci decreased steadily during the study, while a slight increase was observed in the incidence of saprophytic *Corynebacterium bovis* organisms. Coliform bacteria occurred only occasionally in the milk from either group.

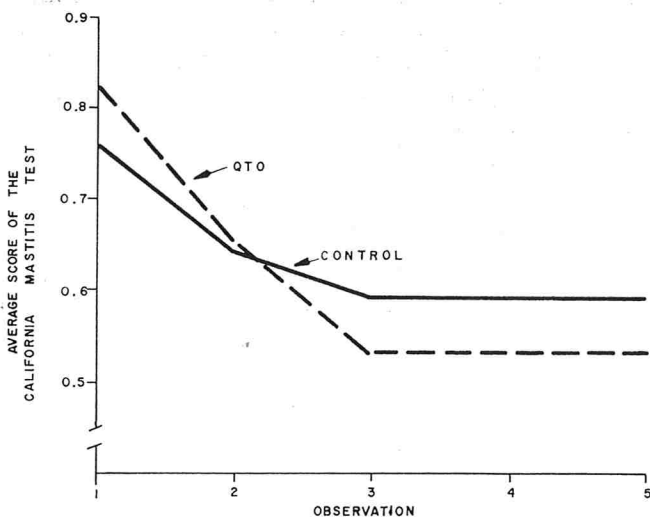


Figure 2. Results from conducting the California Mastitis Test on quarter milk samples.

#### California Mastitis Test results

The degree of intramammary inflammation present in specifically-affected quarters, as measured by the CMT, is summarized in Fig. 2. The average CMT score per quarter was determined by adding the CMT scores of 0, 1, 2, and 3 together and dividing by the total number of quarters tested. The CMT score was

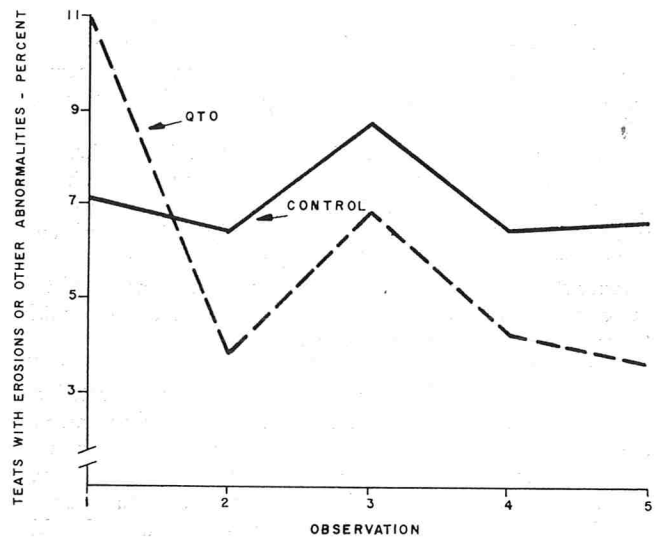


Figure 3. Results of evaluation of physical condition of teat ends.

higher in the milk from cows of the QTO Group at the beginning of the study, but decreased rapidly following installation of the QTO devices and leveled off during the last half of the study at a point below that observed for animals milked with conventional machines. An analysis of variance conducted on the data revealed a significant ( $P < 0.01$ ) difference for the individual observations and a significant ( $P < 0.05$ ) treatment  $\times$  observation interaction.

#### Physical condition of teat ends

As shown in Fig. 3, the number of teats showing some type of abnormality was highest in the QTO Group of animals at the beginning of the study. With the use of the QTO devices, the condition of the teats improved rapidly and, at the close of the study, teats of these cows were in better health than teats of cows that were milked with conventional machines. The differences between groups were significant at the 0.01 level of probability for observations 1, 2, 4, and 5 and at the 0.05 level for observation 3. The decrease from observation 1 to 5 was nonsignificant ( $P > 0.05$ ) for the Control Group but was significant ( $P < 0.01$ ) for the QTO Group.

#### Incidence of garget

A total of 10,091 samples of foremilk from individual quarters was examined. Differences between the two groups in the incidence of garget were small and statistically nonsignificant.

#### Miscellaneous observations

The number of blind or nonproductive quarters present in the QTO Group of animals increased from 23 to 44 during the first 5 weeks following installation of the QTO devices and became stabilized during the remainder of the study. It may be that the QTO de-



vices signaled the presence of nonproductive quarters more readily than operators normally recognized them when using conventional milking equipment. This observation is important in the production of high quality milk. The 21 quarters turned dry between the first and second observations had an average initial CMT score of 2.77. Drying off of these quarters contributed to the subsequent decrease in the average CMT score for the QTO Group. It did not, however, contribute markedly to the decrease in the percentage of quarters infected with mastitis pathogens because, by chance selection, only five of these cows were among the 120 animals from which individual quarter milk samples were collected for bacteriological culturing. Drying off of the specific quarters seemed to be a direct result of using the QTO devices. Thus, the original data collected from the quarters were included in the statistical analyses.

The numbers of animals that were removed from, or entered into, the two experimental groups between observations were recorded, and it was found that the turnover rate was 17% less for the QTO Group than for the Control Group.

Observations were made on the comparative number of cows milked per hour with the QTO-equipped machines and conventional machines. The rate of milking ranged from 35 cows per hour among the animals that produced the most milk to 55 cows per hour for the animals in the lowest production lot.

The number of animals milked per hour with the four QTO-equipped machines was approximately 10% higher than with the four conventional machines. It was estimated that an experienced operator could manage six units of QTO-equipped machines with the effort normally required to manage four conventional machines. This would result in a significant increase in the productivity of an operator.

The machine operators in the parlor of each group of animals were provided with pedometers adjusted to coincide with their individual stride length. It was found that the QTO operators walked an average distance of 3.64 miles while using the QTO devices to perform a routine program of milking that required an average of 4.61 miles of walking to perform without the use of the QTO devices, a reduction of 26.6% in the distance walked. The difference was significant ( $P < 0.05$ ) when tested by the paired "t" comparison.

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### REMOVAL OF MERCURY FROM FISH PROTEIN

A method of successfully removing at least part of the mercury in fish protein has been discovered by University of Wisconsin researchers.

S. Y. Lee and T. Richardson, College of Agricultural and Life Sciences Department of Food Science, have used insoluble thiolated aminoethyl cellulose (TAEC) at pH values from 6 to 11 to remove mercury. Eighty percent of the mercury from a 1 percent protein solution was removed with the TAEC suspension at pH 7. More concentrated protein solutions were too viscous to work with conveniently. The general

principle of the process is to use an insoluble reagent to capture mercury away from fish protein. The catch is finding a chemical with stronger affinity for mercury than the protein yet is easily removed.

The particular method being used does not seem to lend itself to practical application at this time but the general principle shows promise for removal of mercury, lead, cadmium and other poisonous heavy metals from fish protein.

## LOSS OF VIABILITY BY STAPHYLOCOCCUS AUREUS IN ACIDIFIED MEDIA

### II. INACTIVATION BY ACIDS IN COMBINATION WITH SODIUM CHLORIDE, FREEZING, AND HEAT<sup>1</sup>

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#### ABSTRACT

Determinations were made on the extent to which *Staphylococcus aureus* was inactivated [inability to grow on Trypticase Soy agar (TSA)] or injured [inability to grow on TSA + 7% added NaCl (TSAS)] in acidic artificial media and how inactivation was affected when some stresses common to food processing operations were inflicted on the organism. When cells were held in TS broth at  $>pH$  6 (24 hr, 37 C), presence of 7% salt added to the medium did not affect inactivation of staphylococci; but, at  $\leq pH$  5, salt enhanced inactivation. Cells that survived this treatment were not sensitive to salt present in the plating medium, whereas staphylococci from the stationary growth phase (TS broth, pH 7) were sensitive to salt. Cells exposed to low pH were only slightly more sensitive to salt when plated on TSAS than were cells held at pH 7. Exposure of organisms to salt did not render them appreciably more sensitive to subsequent acid treatment than were untreated organisms. Freezing (-30 C, 24 hr) did not substantially enhance inactivation of *S. aureus* at low pH but injury was greatly increased. A sublethal heat treatment (60 C, 15 min) failed to markedly enhance injury or death of cells at low pH values. Exposure to high and low temperatures failed to increase susceptibility of staphylococci to acid and acid-treated cells were no more sensitive to effects of these temperatures than were untreated cells. Staphylococci exposed to a low pH exhibited an extended lag phase when transferred to neutral nutrient media.

It is not uncommon for some foods to be contaminated with *Staphylococcus aureus* which under the right conditions can grow and produce enterotoxin, the causative agent of staphylococcal food poisoning (6, 7, 8, 9). Since most foods are more or less acidic, studies in this laboratory have been conducted to determine behavior of staphylococci in these environments. We have studied growth of the organism in acidified milks (5), survival in cultured dairy products (10), and inactivation in acidic artificial media (11). Evidence has been accumulated which indicates behavior of *S. aureus* varies with type of acid, degree of acidity, rate of acidification, medium, temperature, time, and other parameters.

Additional information is required to more fully understand how staphylococci might behave in acidic foods since factors other than acid alone are operative and together with acid they determine the fate of these organisms.

Staphylococci which survive food processing operations may have been exposed to a variety of environmental stresses. In the quality control laboratory, they are often enumerated by plating with selective media which impose additional stress. These accumulated stresses may destroy the ability of the cell to divide and, as we cannot detect non-dividing cells with the plate count, these organisms often are considered to be dead or non-existent. Some of these cells could just be injured and hence able to eventually undergo repair and recover the function of cell division. This investigation was conducted to determine the extent to which staphylococci might be injured or inactivated in acidic artificial media and how this might be affected by stresses common to food processing operations. Specifically, we studied, (a) injury and inactivation of *S. aureus* by acid during stress, (b) susceptibility of stressed cells to acids, (c) susceptibility of acid-treated cells to stress, and (d) recovery of acid-treated cells from the injured state in a nutrient medium.

#### MATERIALS AND METHODS

##### Media

**Basal medium.** Dehydrated Trypticase Soy (TS) broth (BBL, BioQuest) was reconstituted according to the manufacturer's directions and autoclaved (121 C, 15 min) in 30-ml quantities in 25 × 150 mm screw capped tubes. The pH of the sterilized medium was 7.2-7.3.

**Treated media.** Solutions (2 M) of the following acids were prepared in 10-ml quantities and autoclaved (121 C, 15 min): acetic, glacial, A.C.S. reagent (Allied); hydrochloric, reagent (Dupont); lactic, USP 85% (Mallinckrodt); and phosphoric (ortho), A.C.S. reagent (Allied). Appropriate quantities of these solutions were aseptically added to the previously prepared 30-ml quantities of TS broth. The volume of added solutions ranged between 0.15 and 1.35 ml per 30 ml of broth.

##### Culture

A culture of *S. aureus* strain 100 was obtained from K. F.

<sup>1</sup>Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by Public Health Service Grant No. FD00009-05 from the Food and Drug Administration.

Weiss (The Food Research Institute, University of Wisconsin). The organism was stocked on TS agar slants, refrigerated until used, and restocked every 2 to 3 months. Before each experiment, the organism was transferred to 16 × 125 mm screw capped tubes containing 10 ml of TS broth (two tubes were inoculated for each variable under study) and incubated 18 hr at 37 C (resulting in populations of approximately  $10^8$  cells per milliliter). Cells were then ready for treatment.

#### Treatment of cells

Before cells were treated, broth cultures were cooled to 10 C and centrifuged at about 4000 rpm for 5 min. The supernatant liquid was removed with a sterile Pasteur pipette, 10 ml cold (10 C) sodium phosphate buffer (pH 7.4) was added, and the cells resuspended by means of a Vortex mixer. This washing procedure was repeated once, then the buffer was removed.

**Exposure to acid.** A 10-ml volume of TS or acidified TS broth was added to cells, they were resuspended by means of a Vortex mixer, and tubes were incubated statically for 24 hr at 37 C. When staphylococci were suspended in untreated broth (control), their numbers remained fairly constant (approximately  $10^8$  per milliliter) during the 24-hr incubation period.

**Stressed cells.** Cells were subjected to three different types of stress: (a) exposure to 7.5% sodium chloride for 24 hr at 37 C, (b) storage at -30 C for 24 hr, and (c) heating at 60 C for 15 min. Cells were first washed, as described above, and then treated in either TS or acidified TS broth. Exposure to salt was accomplished by placing washed cells in 10 ml TS broth with 7% added NaCl. Cells were frozen in a domestic-type freezer or were heated in a water bath (timing was started when the temperature in a pilot tube reached 60 C and, after 15 min, tubes were plunged into ice water).

#### Recovery from the injured state

Studies were done to determine whether acid-treated staphylococci undergo an extended lag phase before they can divide after transfer to neutral media. Staphylococci were exposed to acid for 24 hr at 37 C, then washed (as above), and 10 ml buffer added. Untreated (control) cells were washed and diluted 1:1,000 in buffer. A 0.1 ml volume of control and treated cells were added to tubes which contained about 9.9 ml TS broth (two replicates were prepared). The organisms were resuspended by means of a Vortex mixer and then incubated at 37 C in a water bath.

#### Determination of numbers of staphylococci

A 1.0 ml aliquot was aseptically removed from each tube after the contents were mixed with a Vortex mixer, and pour plates were prepared according to recommendations of *Standard Methods for the Examination of Dairy Products* (17). Plates were poured with TS agar (TSA) and duplicate plates were prepared with TSA + 7% added salt (TSAS). Use of these media has been recommended for determining numbers of injured *S. aureus* (2, 12, 15). Reportedly, both "healthy" and injured organisms grow on TSA whereas the latter cannot grow on TSAS. Plates were incubated at 37 C for 48 hr, then the average number of organisms per milliliter was determined.

#### Experimental design

In the first series of experiments, hydrochloric acid was used to acidify media. Three variations in treatment were employed: (a) cells were stressed and then treated with acid, (b) cells were treated with acid and then stressed, and (c) cells were simultaneously stressed and treated with acid. In addition, a comparison was made between response of

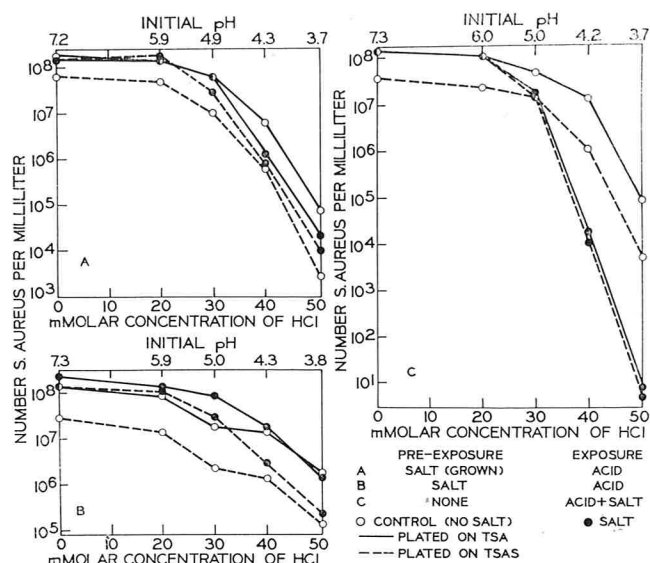


Figure 1. Inactivation of *S. aureus* by hydrochloric acid and 7.5% sodium chloride in Trypticase Soy (TS) broth (37 C, 24 hr) as determined by plating on TS agar (TSA) and TS + 7% added salt agar (TSAS).

non-dividing stressed cells to acid and behavior of cells grown in the presence of stress toward acid (the latter was accomplished by transfer of a loopful of culture to salt broth and incubating for 48 hr at 37 C).

A second series of experiments was done in which organisms were simultaneously stressed with either salt or at -30 C and treated with acetic, lactic, or phosphoric acid. Finally, staphylococci were treated with either acetic, hydrochloric, or lactic acid, and recovery from the injured state was determined by inoculating treated cells into TS broth and plating at 2-hr intervals for 12 hr.

## RESULTS

### Inactivation and injury of *S. aureus* by hydrochloric acid and stress

**Inactivation and injury by salt and acid.** Figure 1 shows the behavior of *S. aureus* when the stress and acid were 7.5% sodium chloride and hydrochloric, respectively. Fewer of the cells held in untreated or acidified TS broth were recovered when plated on TSAS medium rather than on TSA. If recovery of cells from untreated TS broth is compared for all experiments (Fig. 1, 2, 3, 4) and TSA counts are assigned a value of 100%, then recovery on TSAS ranged between 80 and 40% (avg. 54%). Average recovery on TSAS (TSA = 100%) when organisms were held in TS broth (37 C) containing 0, 20, 30, 40, and 50 mM hydrochloric acid (pH 7.3-3.7) and then plated was 56, 52, 39, 20, and 9%, respectively (Fig. 1, 2, 3). Salt sensitivity of cells, therefore, increased very little as pH decreased.

Plating efficiency with TSAS was higher when cells were recovered from TS broth with 7% added salt rather than from TS (Fig. 1C). Recovery on TSAS (TSA = 100%) when staphylococci were held

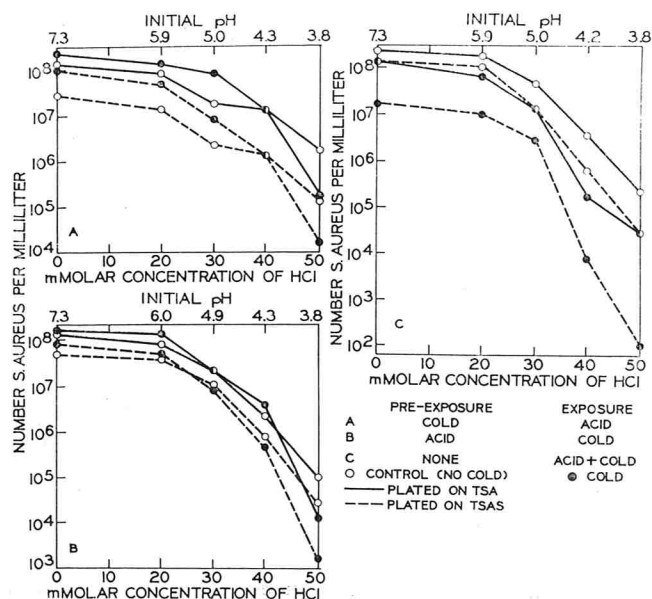


Figure 2. Inactivation of *S. aureus* by hydrochloric acid and freezing ( $-30^{\circ}\text{C}$ , 24 hr) in Trypticase Soy (TS) broth as determined by plating on TS agar (TSA) and TS + 7% salt agar (TSAS).

24 hr in TS + salt broth ( $37^{\circ}\text{C}$ ) containing 0, 20, 30, 40, and 50 mM hydrochloric acid (pH 7.3-3.7) and then plated was 100, 100, 90, 80, and 80%, respectively. Even when organisms treated with salt were held in untreated TS broth for 24 hr ( $37^{\circ}\text{C}$ ) before plating on TSAS, salt sensitivity was less than that of staphylococci with no previous salt exposure (Fig. 1A, 1B). Cells exposed to salt during the stationary phase of growth (Fig. 1B) were slightly more salt-sensitive on TSAS than cells which were grown in salt (Fig. 1A) (before transfer of both to broth containing no salt). Susceptibility of organisms to inactivation by acid (measured by plating survivors on TSA) was not appreciably affected by prior treatment with salt (Fig. 1A, 1B), regardless of type of salt treatment (growth versus exposure during stationary growth phase).

Inactivation of staphylococci by a decrease in pH was greater in TS broth with 7% added salt than in broth which contained no salt (Fig. 1C). Inactivation of organisms (measured by plating on TSA) held in broth which contained  $\leq 20$  mM hydrochloric acid ( $\geq \text{pH } 6.0$ ) was not affected by salt. When the concentration of acid was 30, 40, and 50 mM (pH 5.0-3.7), survival of cells in the presence of salt was decreased by 2.5-, 667-, and 10,000-fold, respectively.

**Inactivation and injury by freezing and acid.** Data obtained when the stress and acid were freezing and hydrochloric, respectively, are presented in Fig. 2. Freezing and storage at  $-30^{\circ}\text{C}$  for 24 hr had little effect on viability of *S. aureus* in untreated TS broth (Fig. 2C).

When the concentration of acid was 0, 20, 30, 40, and 50 mM (pH 7.3-3.8); freezing decreased survival by 2-, 3.8-, 2.5-, 20-, and 8-fold, respectively, according to TSA counts, and by 6.7-, 8-, 4-, 89-, and 500-fold, respectively, when TSAS was the plating medium (Fig. 2C). The organisms, therefore, were not appreciably susceptible to inactivation by freezing combined with a decrease in pH, but were substantially injured by this process.

Organisms exposed to 0-40 mM hydrochloric acid (pH 7.3-4.3) were not rendered more susceptible to inactivation or injury by subsequent freezing but cells exposed to 50 mM acid (pH 3.8) were inactivated 5-fold during the latter stage (Fig. 2B). Similarly, when this sequence was reversed (cells frozen, then treated with acid), staphylococci which were frozen were not appreciably more sensitive to the effects of acid than were organisms which were not previously frozen, provided that acid concentration was  $\leq 40$  mM ( $\geq \text{pH } 4.3$ ) (Fig. 2A). Survival of cells in TS + 50 mM acid (pH 3.8) was decreased 10-fold when pretreated by freezing.

**Inactivation and injury by heat and acid.** Behavior of staphylococci when the stress and acid were heat and hydrochloric, respectively, is displayed in Fig. 3. Numbers of *S. aureus* decreased from about  $10^8$  per milliliter to approximately  $10^3/\text{ml}$  after heating at  $60^{\circ}\text{C}$  for 15 min in untreated broth. As the acid concentration was increased to 20, 30, 40, and 50 mM

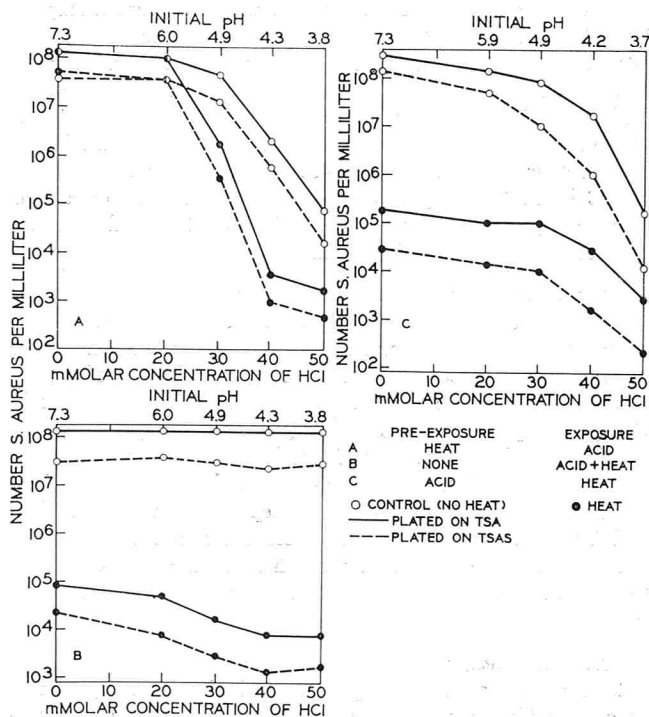


Figure 3. Inactivation of *S. aureus* by hydrochloric acid and heating ( $60^{\circ}\text{C}$ , 15 min) in Trypticase Soy (TS) broth as determined by plating on TS agar (TSA) and TS + 7% salt agar (TSAS).

(pH 5.9-3.7), survival of cells (plated on TSA) during heating decreased by 1.3-, 3-, 10-, and 10-fold, respectively (Fig. 3B). Thermal inactivation of the organisms, therefore, was affected moderately by a decrease in pH (7.3-3.8), but the 15-min period was not of sufficient duration for unheated cells to be affected by these acidities.

When cells of *S. aureus* were treated with 0.50 M hydrochloric acid (pH 7.3-3.7) in TS broth, washed, transferred to neutral TS broth for about 15 min, then plated on TSA; final inactivation increased less rapidly with a decrease in pH of acid treatment when cells were heated in the pH 7 broth at 60 C for 15 min than if these cells were held at 37 C during this period (Fig. 3C). Difference in cell survival with difference in pH (7.3-3.7) was 1667-fold when organisms received no thermal treatment and only 60-fold when heat was applied. Injury (TSA minus TSAS counts) roughly paralleled inactivation (TSA counts) during this process. These data suggest that either (a) some acid-inactivated cells recovered viability during heating, or (b) the acid treatment inactivated the more heat-labile cells in the population before heating (after the acid treatment, fewer than 0.1% of the original population was viable at the time of heating).

When staphylococci ( $10^8$ /ml) were heated at 60 C for 15 min [resulting in about  $10^5$  survivors per milliliter (see Fig. 3 C for the control)], washed, then transferred to TS broth containing 0.50 M acid (pH 7.3-3.7, 37 C, 24 hr); survivors of the thermal treatment either grew in the acidified broth or the pH was such that they were inactivated (Fig. 3A). After organisms were heated and held in broth containing  $\leq 20$  mM acid ( $\geq$  pH 6.0), populations of  $10^8$ /ml were observed. When the pH was 4.9 (30 mM acid), only about  $10^6$  *S. aureus* were detected (survivors after heating increased 10-fold in the acidic medium). Survivors of the thermal treatment were inactivated in broth with 40 and 50 mM acid (pH 4.3 and 3.7) by 50- and 100-fold, respectively. It is interesting to note that  $10^8$  cells per milliliter, which had no previous heat treatment, were inactivated in pH 4.9 broth by almost 3-fold, whereas  $10^5$  organisms per milliliter increased 10-fold in numbers at this pH after heating. Similarly,  $10^8$  *S. aureus* per milliliter, which had no previous heat treatment, were inactivated  $>2,000$ -fold in pH 3.8 broth whereas  $10^5$  organisms which survived heating at 60 C were only inactivated 100-fold at this pH. The more acid-labile cells in the population may have been inactivated during heating before treatment by acid.

*Comparison of staphylococcal behavior in the presence of different stresses.* Untreated stationary-phase staphylococci were not appreciably affected by freez-

ing at  $-30$  C for 24 hr and about 50% of organisms in a given population were inactivated in the presence of 7.5% salt for 24 hr at 37 C. On the other hand,  $<0.1\%$  of cells in staphylococcal populations survived heating at 60 C for 15 min.

When acid was combined with stress; inactivation (measured by plating survivors on TSA) increased most rapidly, with a decrease in pH, when the stress was salt. Inactivation increased very little with a decrease in pH when the stress was either freezing or heating. Survivors of a salt-plus-acid treatment were not salt-sensitive when plated on TSAS, whereas surviving organisms from frozen acidified media were markedly sensitive to salt. Salt sensitivity of organisms after heating did not increase with decrease in pH.

#### *Inactivation and injury of S. aureus by other acids and stress*

Trends observed when staphylococci were held in broth containing acetic, lactic, or phosphoric acid, and were simultaneously stressed with salt or by freezing, were similar to those obtained when hydrochloric acid was employed (Fig. 4). Cell inactivation and injury with these weak acids, however, was greater than that which occurred when hydrochloric acid was employed at an equivalent pH. The anti-staphylococcal activity of acids, as a function of pH, was acetic  $>$  lactic  $>$  phosphoric.

Staphylococci were inactivated (according to TSA counts) in the presence of 7.5% salt at pH 4.6 (37 C, 24 hr) by 1,000,000-, 400,000- and 50,000-fold when the acid was acetic, lactic, and phosphoric, respectively (Fig. 4). By comparison, when the acid was hydrochloric and the pH 4.2, inactivation by acid and salt was 6,667-fold (Fig. 1C). None of the cells in a population of  $10^8$  organisms per milliliter were able to divide in the presence of salt on TSAS after storage at  $-30$  C for 24 hr at pH 4.5 when the medium contained acetic or lactic acids (Fig. 4). When phosphoric and hydrochloric acids were employed at respective pH values of 4.1 (Fig. 4) and 3.8 (Fig. 2C), 100 and 50,000 *S. aureus* per milliliter, respectively, were able to grow on TSAS after freezing.

#### *Recovery of S. aureus from the injured state after exposure to acid*

Figure 5 shows how staphylococci in TS broth recovered from injury induced by exposure for 24 hr (37 C) to acetic, hydrochloric, and lactic acid. Type of acid used to treat cells had no apparent effect on eventual recovery. During the first 4 hr in TS broth, acid-treated cells showed little or no change in numbers (when plated on TSA), whereas untreated organisms increased about 8- to 9-fold during this period. Between 4 and 8 hr of incubation, the growth rate of acid-treated cells approached that of untreated

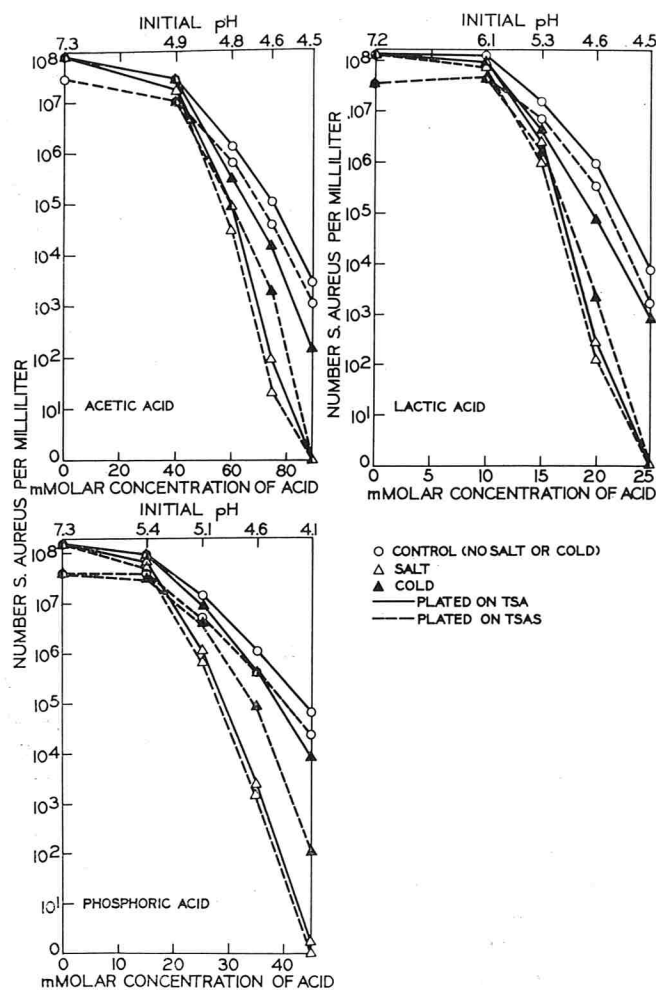


Figure 4. Inactivation of *S. aureus* by acetic, lactic, and phosphoric acid plus salt (7.5%, 24 hr) or freezing ( $-30^{\circ}\text{C}$ , 24 hr) in Trypticase Soy (TS) broth as determined by plating on TS agar (TSA) and TS + 7% salt agar (TSAS).

ed cells. After 8 hr, there was little difference in growth rate between the two types of treatments.

Immediately after cells were transferred to the recovery broth, the organisms showed characteristic sensitivity to salt when plated on TSAS medium, regardless of prior treatment. After 2-4 hr of incubation in the recovery broth, however, sensitivity to salt virtually disappeared.

#### DISCUSSION

##### Determination of acid-induced injury in staphylococci

Microbial cells exhibit tolerances to environmental stresses which are often consistent among members of a group such as a species or genus or between organisms which possess common morphological or physiological characteristics that affect response to the stress. When environmental conditions are held constant and inter-group differences are eliminated, then response to stress is determined by the internal

state of the cell. If cellular metabolism or physical integrity is altered so that characteristic tolerance to a given stress is impaired, then one might assume that some cellular injury may have occurred.

Authorities (2, 12, 15) agree that salt sensitivity is a useful tool to determine the extent of debilitation among cells in staphylococcal populations. Unlike a number of bacterial species; active, "healthy" staphylococci usually grow unhindered in nutrient media which contain 7.5% NaCl. Procedures which utilize this concept employ two media (which differ only in salt content) for enumerating *S. aureus*, e.g. TSA and TSAS. Those organisms which can grow on TSA but not on TSAS are believed to be injured. When staphylococci were exposed to a sublethal heat treatment and then tested with this procedure, the difference between counts obtained for the two media was reported in the literature to be 10- to 100-fold (2, 15) to 100,000-fold (12), depending on the severity of the thermal treatment and the laboratory reporting the results.

According to our data, metabolic activity is an important consideration in the cell's response to salt. Organisms transferred to TSAS medium during logarithmic growth were unaffected by salt, but cells plated during the stationary growth phase were salt-sensitive. On the other hand, stationary-phase staphylococci which were held in broth containing 7.5%

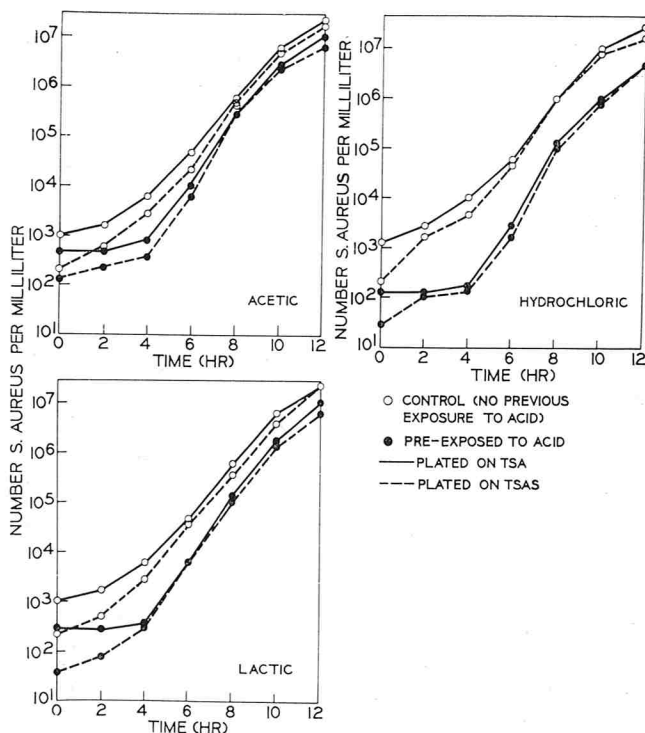


Figure 5. Recovery of *S. aureus* in Trypticase Soy (TS) broth ( $37^{\circ}\text{C}$ ) after exposure to low pH to acetic, hydrochloric, and lactic acids as determined by plating on TS agar (TSA) and TS + 7% salt agar (TSAS).

TSAS. Differences between cells in salt sensitivity after exposure to various concentrations of acid were small and hence are likely to be of only limited significance. If staphylococci which survived storage in acidic media were injured, this injury apparently did not make the organisms more vulnerable to salt. Conversely, cells stressed with salt were not rendered more susceptible to inactivation by acid.

Bacteria are also susceptible in varying degree to inactivation and injury by storage at low temperature. Types of injury which may occur at subfreezing temperatures include membrane damage and changes in metabolic or enzymatic activity (4). Metabolic changes may be manifested by a condition in which bacteria, normally able to grow on simple media, can only divide on rich, complex media after freezing (14, 16). Staphylococci, however, are rather resistant to inactivation by frozen storage, particularly when compared to gram-negative, rod-shaped, organisms such as *Escherichia coli* and salmonellae (1).

Freezing of stationary-phase cells in untreated TS broth did not result in appreciable inactivation or injury of *S. aureus*, according to results of this study. Storage at subfreezing temperatures, like exposure to salt, imposes sublethal stress on "healthy" staphylococci; but, theoretically, injured organisms should be less able to withstand this potentially-damaging environment. Again, if staphylococci were injured by storage in acidic environments, this state of injury did not make the organisms more susceptible to inactivation or injury by subsequent storage at subfreezing temperature except, perhaps, when the maximum exposure to acid used in these studies was tested. Conversely, frozen and thawed cells, for the most part, were not more vulnerable than control cells to inactivation or injury by acid treatment, but a difference was noted when the acid concentration was greatest.

Effects of sublethal heat treatment on bacteria have been described by Ordal (13). He believes lesions may be produced which include (a) damage or impairment of the cytoplasmic membrane (which permits leakage of pool material into the environment), (b) alteration of the cell's metabolic capabilities (with *S. aureus* there is a selective inactivation of cellular enzymes and partial denaturation of cellular protein), and (c) degradation of ribosomal ribonucleic acid.

Our data indicate that organisms given a sub-lethal acid treatment were not rendered more susceptible than untreated bacteria to inactivation or injury by heat. Conversely, heat did not increase acid-lability of staphylococci. On the contrary, as the severity of either treatment increased, surviving organisms appeared more resistant than untreated bacteria to

further stress.

When Jackson and Woodbine (3) subjected *S. aureus* to a sublethal heat treatment and subsequently transferred cells to nutrient broth (37 C), there was a slight drop in numbers followed by a 5.5-6.5-hr lag phase. Cells which were not heated exhibited a 2.5-hr lag in the recovery medium. After cells given both treatments reached the logarithmic phase of growth, they had similar doubling times and the same maximum populations were reached. Results of similar studies by Ordal (12) showed that staphylococci exposed to heat underwent a 4-hr lag phase during recovery, whereas unheated cells exhibited no lag. In addition, <10% of heated cells could grow on TSAS medium at 0-hr, but no differences were observed between counts on TSA and TSAS media after 3 hr.

Our results on recovery from acid-induced injury are consistent with findings reported for recovery from thermal-induced injury. Untreated cells underwent very little lag before initiating growth in the recovery broth, whereas acid-treated cells had lag phases which extended over 4 hr. After the initial 4 hr, growth rates of acid-treated and untreated staphylococci were roughly parallel.

The evidence suggests that at least some of the cells which survived exposure to acid at a low pH (<4.6) have been altered in some manner and so are likely to be injured. If one assumes that behavior of these survivors in salt, during frozen storage, or after exposure to a mild heat treatment, and extension of the lag phase during recovery (as employed in this study) is a valid indication of whether injury has occurred; then the extent of this injury is apparently small. Injury of staphylococci at very low pH (<4) can be detected by subjecting the cells to freezing or by studies of recovery in a nutrient broth (neutral pH) followed by plating on a non-selective agar medium.

#### *Inactivation and injury of S. aureus by acid in the presence of another stress*

Inactivation of *S. aureus* by acid was, in the presence of salt, greatly enhanced at pH values <5. Survivors of this dual treatment exhibited no apparent sensitivity to salt in the plating medium. Acid-induced inactivation of staphylococci was not markedly enhanced during frozen storage, but salt sensitivity of the organisms increased greatly at pH <5. A combination of heat (as used in this investigation) and acid did little to enhance cell inactivation or injury. The 15-min period, however, was much too short for acid to affect viability of *S. aureus* (11).

If inactivation and/or injury of a bacterium by two different environmental stresses occurs via similar mechanisms, then it is reasonable to expect that the combined effect of the two would at least be addi-

tive. Since, in our studies, salt had no lethal effect on cells in the absence of acid but inactivation (as defined by inability to grow on TSA medium) of cells by acid was accentuated by salt, salt and acid may affect closely related cell functions. This hypothesis is plausible in that both of these agents are low molecular weight ions. This phenomenon was not evident with the combination of freezing and acid, but injury (as defined by inability to grow on TSAS medium) induced by acid was greatly enhanced in the act of freezing and thawing.

Whereas low pH did not substantially enhance inactivation of *S. aureus* during heating at 60 C for 15 min; earlier work in our laboratory (11) demonstrated that lability of cells when held at 45 C for 24 hr was heightened about 10,000-fold at low pH. Results of this earlier study also showed that 12-24 hr must elapse before staphylococci held at 37 C are inactivated at low pH by acid.

#### Practical considerations

Research on behavior of microorganisms in foods often is directed toward studying effects produced by a single factor or a group of individual factors. Food systems, however, are extremely complex and organisms exist in this system within equally complex ecological niches. Many factors, therefore, interact with each other to affect the behavior of microorganisms within the system. Results of this study have demonstrated what happens when two relatively mild stresses are combined and allowed to act on microbial cells. Yet, the picture which we have presented is far from complete. In food systems, many antimicrobial factors are present along with growth stimulating agents, other microorganisms, complex food constituents, and intricate physical structures.

Governmental control agencies and the public are growing more concerned about chemical agents which are added to our foods. It is imperative that food microbiologists study more effective means of controlling microbial growth in foods by natural processes. Again, a single agent is often studied in the search for antimicrobial agents. Results of this study have shown that low pH and salt appear to be particularly effective against staphylococci. Other dual treatments and multiple combinations may be invaluable in control programs.

If enterotoxigenic staphylococci cannot be kept out of foods, then it is often essential to detect them before the food reaches a consumer. Because many foods contain a mixed microbial flora, methods to detect staphylococci in these foods employ a selective agent which allows *S. aureus* to grow and thus be detected while growth of other organisms is suppressed. One common selective agent is sodium

chloride (7.5%) added to the plating medium. Whereas actively growing staphylococci appear to be unaffected by this agent, organisms in the stationary growth phase may be appreciably inhibited. Moreover, our studies show that *S. aureus* cells which survived in media at low pH during storage at sub-freezing temperature were extremely sensitive to salt. This investigation affirms the caution which other researchers have issued to quality control personnel in the food industry, i.e. selective media are not reliable if one wishes to detect all potentially viable pathogens which may be present in processed foods.

The problem of debilitated bacteria in foods will continue to receive increasing concern in this decade. Many questions remain unanswered and are worthy of further investigation.

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### INSTITUTE OF SANITATION MANAGEMENT CHANGES NAME

Environmental Management Association, with its acronym "EMA," is the new name of the Institute of Sanitation Management, a professional membership society founded in 1957 to represent the environmental sanitation maintenance management personnel and its industry. The name change more accurately reflects the scope of the Association's interests, activities and the membership it serves, according to President James T. Maloney, who made the announcement. Maloney is manager of maintenance and building services for Xerox Corporation, Rochester, New York.

Balloting of the group's membership, completed last month, brought approval of the charter and By-Laws amendments necessary to effect the name change. Maloney pointed out that a survey of the membership brought forth the fact that they were involved in the complete elements of the environment and not just the work and product environment as was indicated by the former name.

This year's 15th Anniversary ISM/EMA National Environmental Sanitation & Maintenance Educational Conference and Exposition, being held October 15-19 in Philadelphia at the Sheraton Hotel, under the theme "Epilogue/Prologue - Out Of The Past Comes The Future," depicts the progressive history of the Association. For the Institute was formed in 1957 through the merger of the following organizations, The Association of Food Industrial Sanitarians, The National Association of Bakery Sanitarians, and The Industrial Sanitation Management Association.

In October, 1973, the Environmental Management Association will hold its National Educational Conference and Exposition in Clearwater, Florida, the site of its international headquarters.

Environmental Management Association is headquartered at 1710 Drew Street, Clearwater, Florida

33515, under the direction of Harold C. Rowe, Executive Director.

### N A M A ISSUES NEW VENDING MACHINE APPROVAL LISTING

A revised edition of the "Listing of Letters of Compliance" has been issued by N A M A, the national association of vending and food service management companies, according to David E. Hartley, N A M A public health and safety counsel.

The 24-page booklet lists all manufacturers and vending machine models which have been granted letters of compliance under the association's Vending Machine Evaluation Program since 1957 and which therefore comply with the U. S. Public Health Service Model Sanitation Code.

More than 600 different machine models from 23 currently active manufacturers and from 48 others who have either been merged or gone out of business are included in the listing. The booklet covers all approvals through July 1972.

Hartley said the publication is recognized nationally as the "approved vending machine list" by health and military officials who will receive more than half of the 7,500 copies which N A M A has printed.

The N A M A Machine Evaluation Program is carried out at the Department of Microbiology and Public Health of Michigan State University and at the Department of Public Health of Indiana University in Indianapolis.

They are available free to Health and Military Agencies from N A M A, 7 South Dearborn Street, Chicago, Illinois 60603.

Hartley urged vending service companies to keep the listings at hand for use with location managers, Occupational Safety and Health Act (OSHA) inspectors, and public health and military services inspection officials.

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(Expire 1974)

1971-72      1972-73

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## Committee Supervisor

A. E. Parker

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III

IV

V

VII

VI

IX

VIII

X

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(Expire 1973)

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NOMINATING COMMITTEE

(Expire Aug. 1972)

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## DAIRY HOUSING IS FOCUS OF NATIONAL MEETING

One of the most intensive looks at dairy farm facilities, systems, and equipment will be taken February 6, 7, and 8, 1973, at a National Dairy Housing Conference. This meeting, the first of its kind, will be held at the Kellogg Center on the Michigan State University campus, East Lansing.

More than 50 speakers from 20 states and Canada will report their latest observations and research on housing, feeding, and milking systems; environmental control; manure management; codes and regulations;

economics; and herd health. A down-to-earth approach to on-the-farm problems will be stressed.

Registration information can be obtained by contacting James Boyd, Agricultural Engineering Department, Michigan State University, East Lansing 48823.

The American Society of Agricultural Engineers is sponsoring the conference in cooperation with 14 industry, professional, and governmental groups from the United States, Canada, and Europe.

**HOLDERS OF 3-A SYMBOL COUNCIL  
AUTHORIZATIONS ON AUGUST 20, 1972**

**"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y-Treas., Dept. of Food Technology, Dairy Industry Bldg., Iowa State University, Ames, Iowa 50010."**

**0102 Storage Tanks for Milk and Milk Products  
As Amended**

- |     |  |            |
|-----|--|------------|
| 116 | Jacob Brenner Company, Inc.                            | (10/ 8/59) |
|     | 450 Arlington, Fond du Lac, Wisconsin 54935            |            |
| 28  | Cherry-Burrell Corporation                             | (10/ 3/56) |
|     | 575 E. Mill St., Little Falls, N. Y. 13365             |            |
| 102 | Chester-Jensen Company, Inc.                           | ( 6/ 6/58) |
|     | 5th & Tilgham Streets, Chester, Pennsylvania 19013     |            |
| 1   | Chicago Stainless Operations                           | ( 5/ 1/56) |
|     | 5001 No. Elston Avenue, Chicago, Illinois 60630        |            |
| 2   | CP Division, St. Regis                                 | ( 5/ 1/56) |
|     | 100 C. P. Ave., Lake Mills, Wisconsin 53551            |            |
| 117 | Dairy Craft, Inc.                                      | (10/28/59) |
|     | St. Cloud Industrial Park<br>St. Cloud, Minn. 56301    |            |
| 76  | Damrow Company   | (10/31/57) |
|     | 196 Western Avenue, Fond du Lac, Wisconsin 54935       |            |
| 115 | DeLaval Company, Ltd.                                  | ( 9/28/59) |
|     | 113 Park Street, So., Peterborough, Ont., Canada       |            |
| 109 | Girton Manufacturing Company                           | ( 9/30/58) |
|     | Millville, Pennsylvania 17846                          |            |
| 114 | C. E. Howard Corporation                               | ( 9/21/59) |
|     | 9001 Rayo Avenue, South Gate, California 90280         |            |
| 127 | Paul Mueller Company                                   | ( 6/29/60) |
|     | P. O. Box 828, Springfield, Missouri 65801             |            |
| 197 | Paul Mueller (Canada), Ltd.                            | ( 9/ 9/67) |
|     | 84 Wellington St., South, St. Marys, Ont., Canada      |            |
| 213 | Sanitary Processing Equipment Corp.                    | ( 2/18/72) |
|     | Butternut Drive, E. Syracuse, N. Y. 13057              |            |
| 233 | Stainless Steel Craft Corporation                      | ( 4/13/72) |
|     | 4503 Alger St., Los Angeles, Calif. 90039              |            |
| 21  | Technova, Inc. Gosselin Division                       | ( 9/20/56) |
|     | 1450 Hebert c. p. 758<br>Drummondville, Quebec, Canada |            |
| 31  | Walker Stainless Equipment Co.                         | (10/ 4/56) |
|     | Elroy, Wisconsin 53929                                 |            |

**0204 Pumps for Milk and Milk Products  
Revised, as Amended**

- |      |   |            |
|------|---|------------|
| 214R | Ben H. Anderson Manufacturers                   | ( 5/20/70) |
|      | Morrisonville, Wis. 53571                       |            |
| 212R | Babson Bros. Co.                                | ( 2/20/70) |
|      | 2100 S. York Rd., Oak Brook, Ill. 60621         |            |
| 29R  | Cherry-Burrell Corporation                      | (10/ 3/56) |
|      | 2400 Sixth St., S. W., Cedar Rapids, Iowa 52406 |            |
| 63R  | CP Division, St. Regis                          | ( 4/29/57) |
|      | 100 C. P. Ave., Lake Mills, Wisconsin 53551     |            |
| 205R | Dairy Equipment Co.                             | ( 5/22/69) |
|      | 1919 So. Stoughton Road, Madison, Wis. 53716    |            |
| 180R | The DeLaval Separator Co.                       | ( 5/ 5/66) |
|      | Duchess Turnpike, Poughkeepsie, N. Y. 12602     |            |
| 65R  | G & H Products, Inc.                            | ( 5/22/57) |
|      | 5718 52nd Street, Kenosha, Wisconsin 53140      |            |
| 145R | ITT Jabco, Incorporated                         | (11/20/63) |
|      | 1485 Dale Way, Costa Mesa, Calif. 92626         |            |

- |      |  |            |
|------|--|------------|
| 26R  | Ladish Co., Tri-Clover Division  | ( 9/29/56) |
|      | 9201 Wilmot Road, Kenosha, Wisconsin 53140   |            |
| 236  | Megator Corporation  | ( 5/ 2/72) |
|      | 125 Gamma Drive, Pittsburgh, Pa. 15238   |            |
| 148R | Robbins & Myers, Inc.  | ( 4/22/64) |
|      | Moyno Pump Division<br>1345 Lagonda Ave., Springfield, Ohio 45501                  |            |
| 163R | Sta-Rite Industries, Inc.  | ( 5/ 5/65) |
|      | P. O. Box 622, Delavan, Wisconsin 53115  |            |
| 72R  | L. C. Thomsen & Sons, Inc.   | ( 8/15/57) |
|      | 1303 53rd Street, Kenosha, Wisconsin 53140   |            |
| 219  | Tri-Canada Limited   | ( 2/15/71) |
|      | 6500 Northwest Drive, Mississauga, Ont., Canada                                    |            |
| 175R | Universal Milking Machine Div.   | (10/26/65) |
|      | National Cooperatives, Inc.<br>First Avenue at College, Albert Lea, Minn. 56007    |            |
| 52R  | Viking Pump Div.   |            |
|      | Houdaille Industries, Inc. (12/31/56)<br>406 State Street, Cedar Falls, Iowa 50613 |            |
| 5R   | Waukesha Foundry Company   | ( 7/ 6/56) |
|      | Waukesha, Wisconsin 53186  |            |

**0403 Homogenizers and High Pressure Pumps of the  
Plunger Type, As Amended**

- |     |  |            |
|-----|--|------------|
| 87  | Cherry-Burrell Corporation                         | (12/20/57) |
|     | 2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404 |            |
| 37  | CP Division, St. Regis                             | (10/19/56) |
|     | Fort Atkinson, Wis. 53538                          |            |
| 75  | Gaulin, Inc.                                       | (9 /26/57) |
|     | 44 Garden Street, Everett, Massachusetts 02149     |            |
| 237 | Graco Inc.   | ( 6/ 3/72) |
|     | 60-Eleventh Ave., N.E., Minneapolis, Minn. 55413   |            |

**0506 Stainless Steel Automotive Milk Transportation  
Tanks for Bulk Delivery and/or Farm Pick-up Service,  
As Amended**

- |      |   |            |
|------|---|------------|
| 131R | Almont Welding Works, Inc.                          | ( 9/ 3/60) |
|      | 4091 Van Dyke Road, Almont, Michigan 48003          |            |
| 98R  | Beseler Steel Products, Inc.                        | ( 3/24/58) |
|      | 417 East 29th, Marshfield, Wisconsin 54449          |            |
| 70R  | Jacob Brenner Company                               | ( 8/ 5/57) |
|      | 450 Arlington, Fond du Lac, Wisconsin 54935         |            |
| 40   | Butler Manufacturing Co.                            | (10/20/56) |
|      | 900 Sixth Ave., S. E., Minneapolis, Minn. 55114     |            |
| 118  | Dairy Craft, Inc.                                   | (10/28/59) |
|      | St. Cloud Industrial Park<br>St. Cloud, Minn. 56301 |            |
| 66   | Dairy Equipment Company                             | ( 5/29/57) |
|      | 1818 So. Stoughton Road, Madison, Wisconsin 53716   |            |
| 123  | DeLaval Company, Ltd.                               | (12/31/59) |
|      | 113 Park Street, South Peterborough, Ont., Canada   |            |
| 45   | The Heil Company                                    | (10/26/56) |
|      | 3000 W. Montana Street, Milwaukee, Wisconsin 53235  |            |
| 201  | Paul Krohnert Mfg., Ltd.                            | ( 4/ 1/68) |
|      | West Hill, Ontario, Canada                          |            |
| 232  | Litewate Transport Equipment Corp.                  | ( 4/ 4/72) |
|      | 4220 South 13th Street, Milwaukee, Wis. 53221       |            |
| 80   | Paul Mueller (Canada), Ltd.                         | (11/24/57) |
|      | 84 Wellington Street, So., St. Marys, Ont., Canada  |            |
| 85   | Polar Manufacturing Company                         | (12/20/57) |
|      | Holdingford, Minn. 56340                            |            |



- 144 Portersville Equipment Company ( 5/16/63)  
Portersville, Pennsylvania 16051
- 71 Progress Industries, Inc. ( 8/ 8/57)  
400 E. Progress Street, Arthur, Illinois 61911
- 121 Technova Inc. Gosselin Division (12/ 9/59)  
1450 Hebert c. p. 758  
Drummondville, Quebec, Canada
- 47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)  
701 East 16th Ave., North Kansas City, Mo. 64116
- 189 A. & L. Tougas, Ltée (10/ 3/66)  
1 Tougas St., Iberville, Quebec, Canada
- 25 Walker Stainless Equipment Co. ( 9/28/56)  
New Lisbon, Wisconsin 53950

**0808 Fittings Used on Milk and Milk Products  
Equipment, and Used on Sanitary Lines Conducting  
Milk and Milk Products, Revised**

- 79R Alloy Products Corporation (11/23/57)  
1045 Perkins Avenue, Waukesha, Wisconsin 53186
- 138R A.P.V. (Canada) Equipment, Ltd. (12/17/62)  
103 Rivalda Rd., Weston, Ont., Canada
- 82R Cherry-Burrell Corporation (12/11/57)  
2400 Sixth Street, S.W. Cedar Rapids, Iowa 52406
- 124R DeLaval Company, Ltd. ( 2/18/60)  
113 Park Street, South Peterborough, Ont., Canada
- 184R The DeLaval Separator Co. ( 8/ 9/66)  
Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 67R G & H Products, Inc. ( 6/10/57)  
5718 52nd Street, Kenosha, Wisconsin 53140
- 199R Graco, Inc. (12/ 8/67)  
60 Eleventh Ave., N.E., Minneapolis, Minn. 55413
- 203R Grinnell Company (11/27/68)  
260 W. Exchange St., Providence, R. I. 02901
- 218 Highland Equipment Corporation ( 2/12/71)  
74-10 88th St., Glendale, N.Y. 11227
- 204R Hills McCanna Company ( 2/10/69)  
400 Maple Ave., Carpentersville, Ill. 60110
- 34R Ladish Co., Tri-Clover Division (10/15/56)  
2809 60th St., Kenosha, Wisconsin 53140  
LUMACO ( 6/30/72)  
Box 688, Teaneck, N. J. 07666
- 230 ITT Moreland Products, Inc. ( 3/27/72)  
1414 Tolland Turnpike, Manchester, Conn. 06040
- 200R Paul Mueller Co. ( 3/ 5/68)  
P. O. Box 828, Springfield, Mo. 65601
- 149R Q Controls ( 5/18/64)  
Occidental, California 95465
- 227 Stainless Steel Craft Corporation (1/11/72)  
4503 Alger Street, Los Angeles, California
- 89R Sta-Rite Industries, Inc. (12/23/68)  
P. O. Box 622, Delavan, Wis. 53115
- 73R L. C. Thomsen & Sons, Inc. ( 8/31/57)  
1303 43rd Street, Kenosha, Wisconsin 53140
- 191R Tri-Canada Fittings & Equipment, Ltd. (11/23/66)  
21 Newbridge Road, Toronto 18, Ontario, Canada
- 86R Waukesha Specialty Company, Inc. (12/20/57)  
Walworth, Wisconsin 53184

**0902 Thermometer Fittings and Connections Used  
on Milk and Milk Products Equipment and  
Supplement 1, As Amended**

- 32 Taylor Instrument Process Control, (10/ 4/56)  
Div. Sybron Corp.  
95 Ames Street, Rochester, New York 14611
- 206 The Foxboro Company ( 8/11/69)  
Neponset Ave., Foxboro, Mass. 02035

**1002 Milk and Milk Products Filters Using Disposable  
Filter Media, As Amended**

- 35 Ladish Co., Tri-Clover Division (10/15/56)  
2809 60th Street, Kenosha, Wisconsin 53140

**1102 Plate-Type Heat Exchangers for Milk and Milk  
Products, As Amended**

- 20 A.P.V. Company, Inc. ( 9/ 4/56)  
137 Arthur Street, Buffalo, New York 14207
- 30 Cherry-Burrell Corporation (10/ 1/56)  
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404
- 14 Chester-Jensen Co., Inc. ( 8/15/56)  
5th & Tilgham Streets, Chester, Pennsylvania 19013
- 38 CP Division, St. Regis (10/19/56)  
Fort Atkinson, Wisconsin 53538
- 120 DeLaval Company, Ltd. (12/ 3/59)  
113 Park Street, South Peterborough, Ont., Canada
- 17 The DeLaval Separator Company ( 8/30/56)  
Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 15 Kusel Dairy Equipment Company ( 8/15/56)  
100 W. Milwaukee Street, Watertown, Wisconsin 53094

**1202 Internal Return Tubular Heat Exchangers,  
for Milk and Milk Products, As Amended**

- 103 Chester-Jensen Company, Inc. ( 6/ 6/58)  
5th & Tilgham Street, Chester, Pennsylvania 19013
- 152 The DeLaval Separator Co. (11/18/69)  
350 Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 217 Girton Manufacturing Co. ( 1/23/71)  
Millville, Pa. 17846
- 238 Paul Mueller Company ( 6/28/72)  
P. O. Box 828, Springfield, Missouri 65801
- 96 C. E. Rogers Company ( 3/31/64)  
8731 Witt Street, Detroit, Michigan 48209
- 225 Sanitary Processing Equipment Corporation (11/24/71)  
Butternut Drive, East Syracuse, N.Y.

**1303 Farm Milk Cooling and Holding Tanks—  
Revised, As Amended**

- 11R CP Division, St. Regis ( 7/25/56)  
100 C. P. Ave.  
Lake Mills, Wisconsin 53551
- 119R Dairy Craft, Inc. (10/28/59)  
St. Cloud Industrial Park, St. Cloud, Minn. 56301
- 4R Dairy Equipment Company ( 6/15/56)  
1919 S. Stoughton Road, Madison, Wisconsin 53716
- 92R DeLaval Company, Ltd. (12/27/57)  
113 Park Street, South Peterborough, Ontario, Canada
- 49R The DeLaval Separator Company (12/ 5/56)  
Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 10R Girton Manufacturing Company ( 7/25/56)  
Millville, Pennsylvania 17846
- 95R Globe Fabricators, Inc. ( 3/14/58)  
3350 North Gilman Rd., El Monte, California 91732
- 179R Heavy Duty Products (Preston), Ltd. ( 3/ 8/66)  
1261 Industrial Road, Preston, Ont., Canada
- 12R Paul Mueller Company ( 7/31/56)  
P. O. Box 828, Springfield, Missouri 65801
- 58R Schweitzer's Metal Fabricators, Inc. ( 2/25/57)  
806 No. Todd Avenue, Azusa, California 91702
- 235 Stainless Steel Craft Corporation ( 4/13/72)  
4503 Alger St., Los Angeles, California 90039
- 216R Valco Manufacturing Company (10/22/70)  
3470 Randolph St., Huntington Pk., Calif. 90256

- 42R VanVetter, Inc. (10/22/56)  
2130 Harbor Avenue S.W., Seattle, Washington 98126
- 18R Whirlpool Corporation, St. Paul Division (9/20/56)  
850 Arcade Street, St. Paul, Minnesota 55106
- 55R Superior Metalware Industries,  
(Formerly John Wood Company) (1/23/57)  
509 Front Avenue, St. Paul, Minnesota 55117
- 170R The W. C. Wood Co., Ltd. (8/9/65)  
5 Arthur Street, South, Box 750, Guelph, Ont., Canada
- 16R Zero Manufacturing Company (8/27/56)  
Washington, Missouri 63090

**1400 Inlet and Outlet Leak Protector Plug Valves  
for Batch Pasteurizers, As Amended**

- 122R Cherry-Burrell Corporation (12/11/59)  
2400 Sixth St., S. W., Cedar Rapids, Iowa 52406
- 69 G & H Products Corporation (6/10/57)  
5718 52nd Street, Kenosha, Wisconsin 53140
- 27 Ladish Co. - Tri-Clover Division (9/29/56)  
2809 60th Street, Kenosha, Wisconsin 53140
- 78 L. C. Thomsen & Sons, Inc. (11/20/57)  
1303 43rd Street, Kenosha, Wisconsin 53140

**1603 Evaporators and Vacuum Pans for Milk and  
Milk Products, Revised**

- 132R A.P.V. Company, Inc. (10/26/60)  
137 Arthur Street, Buffalo, New York 14207
- 11R Blaw-Knox Food & Chemical Equip., Inc. (2/12/59)  
P. O. Box 1041  
Buffalo, N. Y. 14240
- 164R Mora Industries, Inc. (4/25/65)  
999 E. Maple Street, Mora, Minnesota 55051
- 107R C. E. Rogers Company (8/1/58)  
8731 Witt Street, Detroit, Michigan 48209
- 186R Marriott Walker Corporation (9/6/66)  
925 East Maple Road, Birmingham, Mich. 48010

**1702 Fillers and Sealers of Single Service Containers,  
For Milk and Milk Products, As Amended**

- 221 Bertopack Limited (5/25/71)  
75 Ardelt Place, Kitchener, Ontario, Canada
- 192 Cherry-Burrell Corporation (1/3/67)  
2400 Sixth St., S. W., Cedar Rapids, Iowa 52404
- 139 Exact Weight Scale Company (4/15/68)  
944 W. 5th Ave., Columbus, O. 43212
- 137 Ex-Cell-O Corporation (10/17/62)  
P. O. Box 386, Detroit, Michigan 48232
- 220 Haskon, Inc., Package Equipment Division (4/24/71)  
2285 University Ave., St. Paul, Minnesota 55114
- 142 Polygal Company (4/15/63)  
Div. of Inland Container Corp.  
P. O. Box 68074, Indianapolis, Indiana 46268
- 211 Twinpak, Inc. (2/4/70)  
2225 Hymus Blvd., Dorval 740, P.Q.

**1901 Batch and Continuous Freezers, For Ice Cream,  
Ices and Similarly Frozen Dairy Foods, As Amended**

- 141 CP Division, St. Regis (4/15/63)  
100 C. P. Avenue, Lake Mills, Wisconsin 53551
- 146 Cherry-Burrell Corporation (12/10/63)  
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

**2201 Silo-Type Storage Tanks for Milk and  
Milk Products**

- 168 Cherry-Burrell Corporation (6/16/65)  
575 E. Mill St., Little Falls, N. Y. 13365
- 154 CP Division, St. Regis (2/10/65)  
100 C. P. Ave., Lake Mills, Wisconsin 53551
- 160 Dairy Craft, Inc. (4/5/65)  
St. Cloud Industrial Park  
St. Cloud, Minn. 56301
- 181 Damrow Company (5/18/66)  
196 Western Ave., Fond du Lac, Wisconsin 54935
- 156 C. E. Howard Corporation (3/9/65)  
9001 Rayo Avenue, South Gate, California 90280
- 155 Paul Mueller Co. (2/10/65)  
P. O. Box 828, Springfield, Missouri 65801
- 195 Paul Mueller (Canada), Ltd. (7/6/67)  
84 Wellington St., So., St. Marys, Ont., Canada
- 234 Stainless Steel Craft Corporation (4/13/72)  
4503 Alger St., Los Angeles, California 90039
- 165 Walker Stainless Equipment Co. (4/26/65)  
Elroy, Wisconsin 53929

**2300 Equipment for Packaging Frozen Desserts,  
Cottage Cheese and Milk Products Similar to Cottage  
Cheese in Single Service Containers**

- 174 Anderson Bros. Mfg. Co. (9/28/35)  
1303 Samuelson Road, Rockford, Illinois 61109
- 209 Dobby Packaging Machinery (7/23/69)  
Domain Industries, Inc.  
869 S. Knowles Ave., New Richmond, Wis. 54017
- 222 Maryland Cup Corporation (11/15/71)  
Owings Mills, Maryland 21117
- 193 Triangle Package Machinery Co. (1/31/67)  
6655 West Diversey Ave., Chicago, Illinois 60635

**2400 Non-Coil Type Batch Pasteurizers**

- 161 Cherry-Burrell Corporation (4/5/65)  
575 E. Mill St., Little Falls, N. Y. 13365
- 158 CP Division, St. Regis (3/24/65)  
100 C. P. Avenue, Lake Mills, Wisconsin 53551
- 187 Dairy Craft, Inc. (9/26/66)  
St. Cloud Industrial Park  
St. Cloud, Minn. 56301
- 177 Girton Manufacturing Co. (2/18/66)  
Millville, Pennsylvania 17846
- 166 Paul Mueller Co. (4/26/65)  
P. O. Box 828, Springfield, Mo. 65601

**2500 Non-Coil Type Batch Processors for Milk and  
Milk Products**

- 162 Cherry-Burrell Corporation (4/5/65)  
575 E. Mill St., Little Falls, N. Y. 13365
- 159 CP Division, St. Regis (3/24/65)  
100 C. P. Avenue, Lake Mills, Wisconsin 53551
- 188 Dairy Craft, Inc. (9/26/66)  
St. Cloud Industrial Park  
St. Cloud, Minn. 56301
- 167 Paul Mueller Co. (4/26/65)  
Box 828, Springfield, Mo. 65801
- 196 Paul Mueller (Canada), Ltd. (7/6/67)  
84 Wellington St., So., St. Marys, Ont., Canada
- 202 Walker Stainless Equipment Co. (9/24/68)  
New Lisbon, Wis. 53950

**2600 Sifters for Dry Milk and Dry Milk Products**

- 228 J. H. Day Co. ( 2/28/72)  
4932 Beech Street, Cincinnati, Ohio 45202
- 171 Entoleter, Inc. ( 9/ 1/65)  
Subsidiary of American Mfg. Co.  
251 Welton St., Hamden, Connecticut 06511
- 229 Russell Finex Inc.  
156 W. Sandford Boulevard, Mt. Vernon, N. Y. 10550
- 173 B. F. Gump Division ( 9/20/65)  
Blaw-Knox Food & Chem. Equip. Inc.  
750 E. Ferry St., P. O. Box 1041  
Buffalo, New York 14240
- 185 The Orville-Simpson Co. ( 8/10/66)  
1230 Knowlton St., Cincinnati, Ohio 45223
- 176 Sprout, Waldron & Co., Inc. ( 1/ 4/66)  
Munsy, Pennsylvania 17756

- 172 SWECO, Inc. ( 9/ 1/65)  
6111 E. Bandini Blvd., Los Angeles, California 90022

**2800 Flow Meters for Milk and Liquid Milk Products**

- 223 C-E IN-VAL-CO, a division of Combustion  
Engineering, Inc. (11/15/71)  
P. O. Box 556, 3102 Charles Page Blvd., Tulsa,  
Oklahoma 74101
- 226 Fischer & Porter Company (12/ 9/71)  
County Line Road, Warminster, Pa. 18974
- 224 The Foxboro Company (11/16/71)  
Foxboro, Massachusetts 02035

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**ACCUMULATION OF NITRATE**

Nitrogen occupies a unique place in man's life. This element is both abundant and essential for all living things, yet supplies of the forms available to plants are inadequate in many parts of the world, thereby limiting the crop production of foods and fibers. Today, however, questions are being raised concerning the effects of the rapidly expanding use of nitrogen fertilizers on environmental quality and public health. Certain forms of nitrogen have been shown to be hazardous to man and animals; yet, it has also been found advantageous to add nitrate and nitrite to some processed foods. Now an important new report, **ACCUMULATION OF NITRATE**, examines the multifaceted issues associated directly and indirectly with the increased use and accumulation of nitrate nitrogen and related nitrogenous compounds in the environment, and recommends courses of action for the protection of man and the global environment.

Prepared by the Committee on Nitrate Accumulation of the National Research Council, this 106 page report specifically examines nitrogen compartments in the biosphere, sources of nitrogen, fertilizer and soil nitrogen, and the hazards of nitrate, nitrite, and nitrosamines to man and livestock. The report further evaluates the difficulties that arise from the un-

questioned need for large amounts of fertilizer nitrogen, the problems of concentrated sources of nitrogen from discharges of municipal and industrial wastes released directly into surface waterways, and the possible hazards of environmental deterioration associated with the appearance of eutrophic levels of nitrate in streams, rivers, lakes, and other waterways.

In discussing the hazards that may result from adding nitrate and nitrite to food products (such as those used in the meat industry as preservatives and color fixatives), **ACCUMULATION OF NITRATE** directs attention to the possible presence of nitrosamines in meats, vegetables, and canned goods, examines their carcinogenic, teratogenic, and mutagenic properties, and recommends extensive research in this area. Recommendations are also made for expanded, imaginative research on the ramifications of nitrogen as a fertilizer, as a waste component, and as a food constituent, additive, and preservative.

If you are concerned with the wide range of uses and the effects of such uses of nitrogen in any of its forms, you will find much of value in **ACCUMULATION OF NITRATE**. Copies of this paperbound book are available for \$4.25 each, from: National Academy of Sciences, Ptg. and Pub. Office, 2101 Constitution Ave., Washington, D. C. 20418.

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## NEWS & EVENTS

### DRINC AWARD GOES TO T. I. HEDRICK

Dr. T. I. Hedrick, the recipient of the Dairy Research Incorporated Award for 1972 has approximately 40 years experience in the dairy industry in positions of responsibility in research and production management. His originality, creativity, and foresight in research endeavors are illustrated by the wide variety of new products which have entered the market as a result of his research activity. The recipient has developed a process which has been patented for rapid curing of blue cheese curd which reduced the commercial production from 60-120 days to 7-10 days. He has developed two cheese confections which are fruit flavored and chocolate flavored cream-type cheeses. Under his supervision, a new cheese referred to as Dagano Cheese was developed and is now one of the popular varieties in his state.

Hedrick developed a frozen whipped cream and published recommended formulations several years before some of the commercial products were introduced to the market. He has also developed and published formulations for a whipped cultured milk dessert. His work has contributed to the improvement of natural smoke flavored cheese, flavored cultured buttermilk, and stabilized fruit flavored milks.

The recipient has also contributed materially to development of the polyethylene laminants on paper-board milk cartons. Because of his research on U.H.T. pasteurization, Hedrick has been able to contribute to improved engineering of such equipment. His work also has contributed to promulgation of state laws permitting direct steam injection in the pasteurization of liquid dairy products. Dr. Hedrick is well-known internationally for his research on air-

borne contamination and methods of control in dairy plants.

### A. L. RIPPEN HONORED WITH DELAVAL AWARD

The career of Professor A. L. Rippen, 1972 recipient of the American Dairy Science Association DeLaval Extension Award, is distinguished by more than 30 years of service in improving dairy products. The first half of this period was devoted to commercial activity in supervision of milk processing and packaging. In 1957 he became associated with the Dairy Department of Michigan State University as Extension Specialist in Dairy and Food Engineering and Manufacturing.

Rippen was selected by his peers as the outstanding extension specialist in his state in 1968. Among his many characteristics his co-workers and associates attest to his ability to get along with people; command the respect and good will of all; stimulate and motivate others; endow them with the spirit of service and action; and his superior ability to plan, organize, and develop projects.

Professor Rippen helped organize the Extension Section of the A.D.S.A. Manufacturing Division; helped prepare the by-laws for the Industry and Business Section and served as an officer for three years. He has conducted innumerable training conferences for industry-related executives and technicians in his state and participated in many for other states and nations. His versatile ability and understanding of the entire dairy industry has created a demand from governmental units, dairy and related

co-ops, other academic departments, and youth groups for his counsel, study, and help.

Born on a Nebraska farm in 1917 Rippen received degrees in dairy manufacturing and technology at the University of Nebraska in 1940, and at the Ohio State University in 1941. His wife, Leona, was born at West Unity, Ohio. He has two married daughters and a son who is a sophomore at the Community College of Lansing, Michigan.

---

### **STANLEY ARTHUR WITZEL, SR. RECIPIENT OF AWARD**

Stanley Arthur Witzel, Sr., now on assignment as professor and head of the agricultural engineering department at the University of Ife in Nigeria, has been selected by the American Society of Agricultural Engineers (ASAE) to receive the 1972 Cyrus Hall McCormick Medal Award for "exceptional and meritorious engineering achievement in agriculture."

The award was announced Friday evening, June 30, at the banquet which climaxed the 1972 annual meeting of the ASAE in the Arlington Hotel, Hot Springs, Arkansas.

The McCormick Medal first was donated by three of the then living children of the inventor of the self-rake reaper, as a memorial to their father and his achievements. The American Society of Agricultural Engineers is entrusted with the administration of the funds that support the award, as well as selection of the recipient.

During the past three years Witzel has been on assignment by the University of Wisconsin, Madison, to help establish, in collaboration with the U. S. Agency for International Development, and agricultural engineering department at the University of Ife in Nigeria. His work, with three of his Wisconsin colleagues who preceded him, has involved teaching, research, building a staff, developing curriculum constructing buildings, and collecting laboratory and shop equipment for their department. He has also served on all University Buildings Committee which has been working on a development program costing more than five million pounds when completed.

Nearing completion in Nigeria, under Witzel's direction, are the following research and development projects:

- a 100 percent retention bench terrace system with controlled but delayed provision for excess water removal — a soil saving arrangement for cultivated areas in the rain forest zones of Nigeria;

- the first concrete block and monolithic tower type silos;

- facility for 40 cows with individual experimental feeding shelter, milking parlor, and small dairy plant;

- a plant science and soils field laboratory of about 5,000 square feet for research;

- a farm office and assembly hall building of about 3,000 square feet.

From 1950 to 1967 the McCormick Medal Award winner was a consultant to his dean at the University of Wisconsin for the planning and development of campus and experimental farm lands, buildings, utilities, equipment, and building remodeling.

Witzel has been the editor of the Regional Bulletin on Dairy Cow Housing, a project of the Mid-West Plan Service, which started as an unofficial ASAE activity with the goal of establishing it nationally. This service, according to Witzel, has played a major role in the development of farm structures suitable for modern agriculture.

He was the principal investigator in 1963 for a farm animal waste research project supported by the National Institute of Health of the U. S. Department of Health, Education, and Welfare and the National Water Pollution Control Agency of the U. S. Department of the Interior. Witzel plans to prepare a report summarizing this project when he returns to the University of Wisconsin.

He was born on his family's farm in Rudd (Floyd County) Iowa in 1904. He received his bachelor's degree in civil engineering at Iowa State University and his master's degree at the University of Wisconsin in 1930.

---

### **NEW FOOD USES FOR ISOLATED WHEY PROTEINS OUTLINED BY USDA SCIENTIST:**

Protein concentrates isolated from cheese whey have potential uses in the nutritional fortification of soft drinks and bread, and in making foams for dessert toppings and meringues, a U. S. Department of Agriculture scientist reported today.

The use of whey proteins as food ingredients was described by Chemist Michael J. Pallansch, head of the Dairy Products Laboratory of USDA's Agricultural Research Service in Washington, D. C., in a symposium on agricultural and municipal wastes at the 164th national meeting of the American Chemical Society. Whey, a byproduct of cheesemaking that is sometimes viewed as a highly polluting waste, can become the source of nutritious food ingredients, the ARS scientist said.

Dr. Pallansch reviewed a number of experimental processes shown to be highly effective in removing lactose and salts from whey. The resulting liquid can be dried to a powder containing 30 to 90 percent protein. These processes, in which there is substantial commercial interest, include reverse osmosis, gel filtration, and ultra-filtration.

Proteins isolated by these processes from the acid whey produced in the manufacture of cottage cheese have been added to soft drinks with highly encouraging results, Dr. Pallansch reported. Both carbonated and noncarbonated beverages in a wide variety of flavors have been fortified with these protein concentrates. The protein had little effect on the flavor or color of the soft drinks, even after storage of the fortified beverages at room temperature for nearly a year.

The use of whey proteins in soft drinks is an attractive possibility for improving the nutrition of young people. These beverages are consumed in large quantities by children and teenagers.

Fortifying bread with whey proteins has posed some problems, because these additives generally lower the baking qualities of dough. Lactose in the whey inhibits yeast action. Also, the undenatured whey protein adversely affects baking quality.

Dr. Pallansch said that whey protein concentrates can be used in baking without undesirable side effects if most of the lactose is removed by the processes he described and the protein is then denatured with heat. Thus treated, the proteins retain most of their amino acids in a nutritionally available form and can be used in bread. Because these high-protein isolates are virtually lactose-free, only about a third to a half as much of them need be used as non-fat dry milk to provide equivalent protein fortification.

By selecting the processes or combination of processes used, denaturation of the protein can be controlled from 2 to 100 percent.

Dr. Pallansch also discussed the use of high-protein whey concentrates to make dessert toppings and meringues. He said there was considerable commercial interest in such products, and showed the results of experiments carried out in his laboratory on two commercially produced whey concentrate powders containing 75 per cent protein. When the powders were reconstituted with water and beaten in a high-speed mixer, they made a good amount (about 450 percent overrun) of stable foam at 30 percent solids. Some heat denaturation of the protein, however, appears to be necessary to produce the most stable foam, and the heating affects the flavor. The flavor can be improved by the addition of sucrose, which at the same time further improves the stability of the foam.

Babson Bros. after 20 years with Girton Manufacturing Company, where he was instrumental in the design, sales and service of milk cooling systems.

For many years, Evans has been active in dairy industry affairs. He is currently acting chairman of the 3A Task Committee on Farm Tanks for the Dairy Food Industry Supply Association, having served as chairman of that group for the preceding 10 years.

A recognized authority in the cooling systems field, Evans has written for both the Refrigeration Engineers Society and the Refrigeration Service Engineers on the subject of milk cooling and farm bulk tanks. The Evans family will be moving to the Chicago area from their present home in Bloomsburg, Pennsylvania.

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**INDEX TO ADVERTISERS**

Babson Bros., Co. ....	Back Cover
IAMFES, Inc. ....	570
Norton Plastics and Synthetics Division .....	Inside Back Cover
Sep-Ko Chemicals .....	569
The Haynes Mfg. Co. ....	Inside Front Cover
Zero Mfg. Co. ....	I

**DARL EVANS JOINS BABSON BROS. CO.  
TO HEAD COOLING SYSTEMS DIVISION**

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Dairy authorities speak out  
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## Understanding between man and cow can mean more milk

Some dairymen and milkers consistently get more milk out of cows because they understand and take advantage of the animals hormone functions. In fact, they get so much more milk that dairy experts suggest others follow their example.

At milking time, if a cow is not stimulated to let down her milk, her production will suffer. If fear, pain or disturbances occur, she will not produce as much. A creature of habit, she responds to procedures which are repeated day after day.

While milk let down must be fully stimulated, dairymen must remember that the stimulation lasts for only an average of six or seven minutes. The entire job of milking each cow must be completed within that time period. Good practice dictates that the milking unit should be placed on the cow very soon after milk is let down.

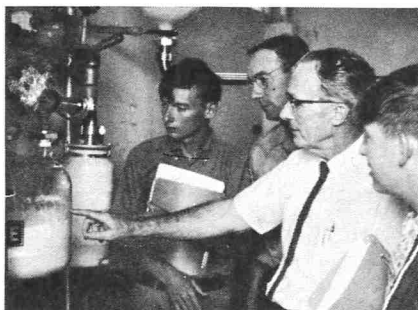
To get all the milk, the milker needs the cows full cooperation. High producing cows and those with tight sphincter muscles will take more time so the milking routine should take these animals into consideration.

Most cows are actually milked out in two to five minutes after the milk has been let down. Capable dairymen observe milk flow changes. When the flow slows down because most of the milk is removed, gently pull forward and down on the milking machine. This action along with gentle massaging of the individual quarters will help release trapped milk.

Overmilking causes irritation and stress. It creates conditions which could result in mastitis. Overmilking also tends to produce slow-milking cows. They begin to expect pain and at a certain point secrete the "interference" hormone, adrenalin, which prevents rapid milk let down. Many of

the hard-to-milk cows will even gradually change their habits if the milker will change his and encourage fast milking.

Best results occur when the milker limits the number of units he operates. His rule should be, no more than two units in a milking barn, and only three in a milking parlor. Only with such new aids as prep-stalls and automatic removal can one man successfully operate more machines.



Proper milking procedures are taught in Virginia Tech milking schools.

### EFFICIENT EQUIPMENT IMPORTANT

Perfect machine operation can not compensate for inadequate or improperly adjusted equipment. Inadequate air flow may result from a pump that is worn or too small, vacuum or milk lines which are too small, too long, not sloped enough, plugged, or having other restrictions including filters or flooding with milk, or from excessive leaks. The equipment representative can check these things. It is to your advantage to let him install and maintain a fully adequate system which will equal or exceed 3-A Accepted Practices recommendations.

Some simple checks can be

helpful when inadequate capacity is suspected. For example, when all components using air are attached, a petcock may be opened to reduce the vacuum level to 10" instead of 15". Then close the petcock and count 1000, 2000, etc. If it takes more than 2 seconds to recover to 15", the air flow capacity may be too low.

Vacuum (or air removal) actually operates the machine and takes milk from the cow. Air flow, measured in cubic feet per minute (C.F.M.), from both vacuum and milk pipelines is necessary to maintain the recommended vacuum level.

Air flow capacity of a pump and a system can be measured by an air flow meter. There should be no more than a 10% loss between the pump and the system air flow. Your equipment dealer should provide for and explain such things as the need for a reserve tank, traps, cleaning of vacuum lines, limiting vacuum fluctuations, keeping vacuum gauges, regulators, pulsators, pumps, etc. functioning properly.

Milking speed will tend to increase with increased vacuum level and pulsation ratio (milking-massage ratio). Possibility of irritation to the udder also increases accordingly. While milk is flowing it cushions irritations. A good milker will see that the machine is not attached to the cow when milk is not flowing. He will prepare the cow by using a strip cup to detect abnormal milk and remove bacteria from the teat end, then wash and dry the udder with a single service towel and attach the machine. He will keep his hands and the milking unit sanitized. When each quarter milks out, remove teat cups promptly and dip the teats in a specially prepared teat dip.

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