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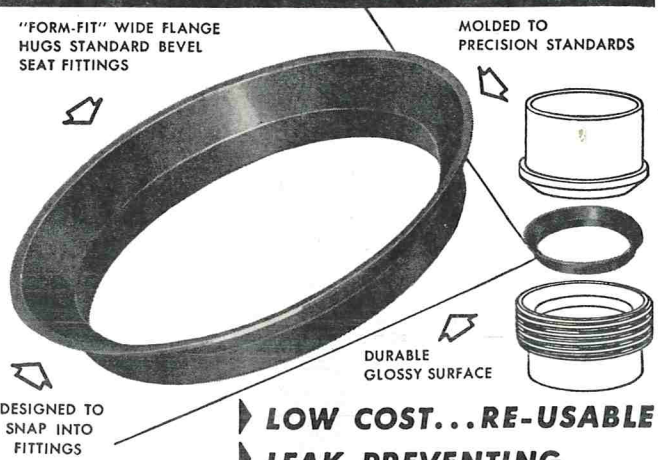

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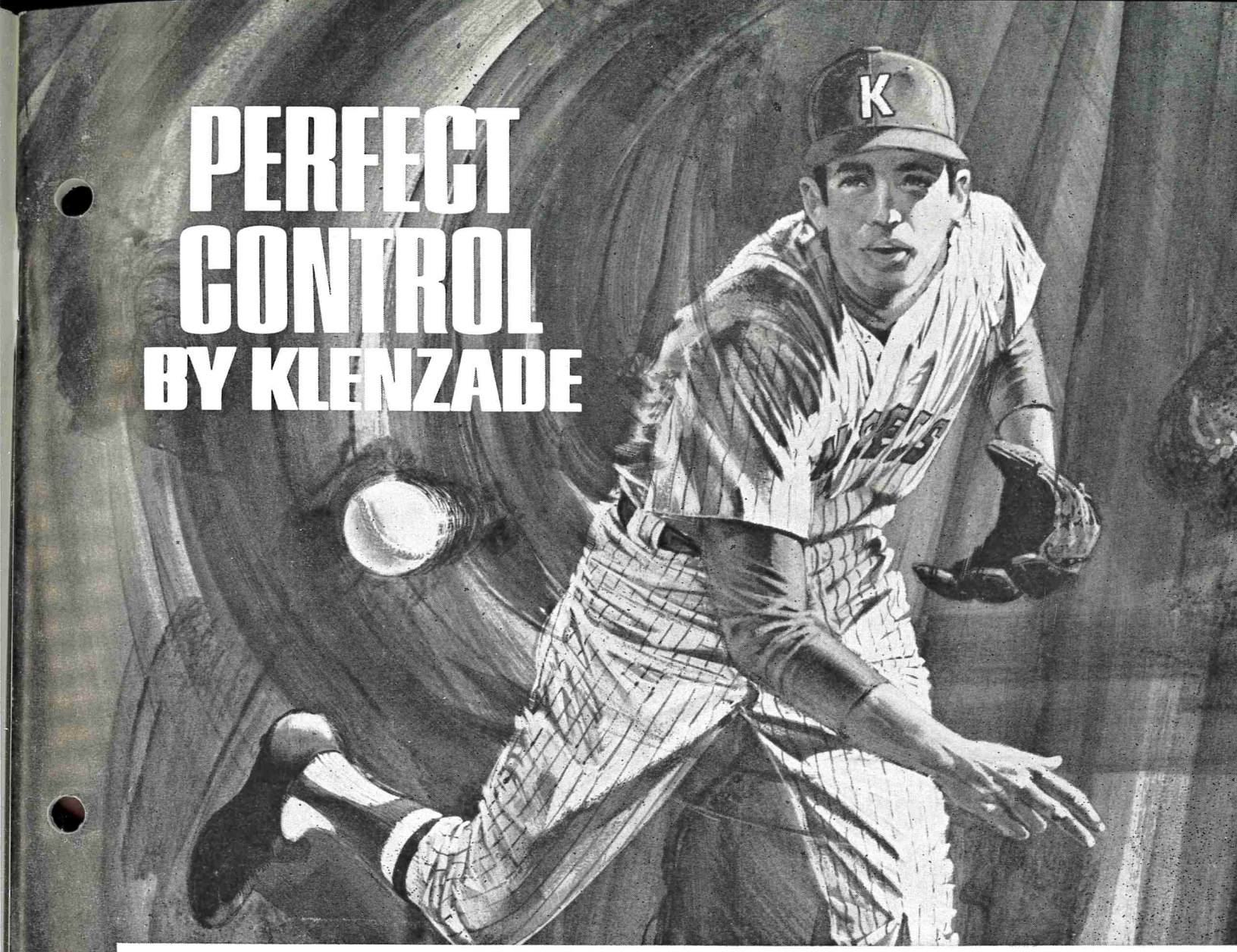

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RELATIONSHIPS BETWEEN LEUKOCYTE COUNTS IN BULK MILK AND APPARENT QUARTER INFECTIONS IN DAIRY HERDS

D. S. Postle
New York State Veterinary College
AND
R. P. Natzke and R. W. Everett
New York State College of Agriculture
Cornell University
Ithaca, New York 14850

(Received for publication June 24, 1971)

Abstract

Bulk tank milk samples and quarter samples from 15,982 cows were collected from 285 herds enrolled in the New York State Mastitis Control Program. Culture and strip-plate examination findings from quarter samples were correlated with bulk milk cell counts. A correlation of 0.50 was found between percent quarters yielding mastitis pathogens and bulk milk cell counts. A correlation of 0.51 was found between percent quarters producing abnormal secretions and bulk milk cell counts. These relationships indicated the bulk milk cell count was not a reliable measure of herd infection or prevalence of abnormal secretion. The most prominent trend in a relationship between pathogens and increased bulk milk cell count was found for *Streptococcus agalactiae* and a lesser relationship between staphylococci and increased bulk milk cell count. Herds classified as having low bulk milk cell counts produced an average of 1,759 lb. of milk more than herds with high bulk milk cell counts. Only three milking and management practices were found to be associated with bulk milk cell counts.

In *Current Concepts of Bovine Mastitis (5)*, it was stated that the presence of more than 500,000 leukocytes per milliliter of mixed herd milk strongly suggests a significant incidence of mastitis in a given herd. On this type of information the National Conference on Interstate Milk Shipments (NCIMS) prepared recommendations *(4)* which were approved by the United States Public Health Service (USPHS), that use the cell content of a bulk milk sample as an indicator of milk quality. Schalm et al. *(11, 12)* have shown that a pre-existing leukocytosis in the bovine udder may have a protective and therefore beneficial role.

Some factors believed to influence the cell numbers in bulk milk are: *(a)* age and stage of lactation; *(b)* trauma; *(c)* percent of quarters presently infected; this influence will be expected to vary according to infecting organism, as well as severity and duration of the infection; *(d)* percent quarters previously infected which continue to shed abnormal number of cells; *(e)* relative yield of milk from all quarters; and *(f)* milk withheld from the supply by the dairyman.

Milk produced by udders with clinical mastitis should be withheld from the market supply, however most udder infections are sub-clinical and probably are not recognized by dairymen.

Westgarth et al. *(14)*, using data from 30 herds, obtained a correlation of 0.52 between percent quarters infected and the log of the bulk milk cell count. They concluded that the bulk milk cell count provided an indifferent estimate of the infection level within a herd.

It has been suggested that the bulk milk cell count can be predicted by knowing the cell level of all quarters contributing to that supply *(13)*. If the cell content of quarter milk is directly related to infection, then a relationship should exist between percent quarters infected and bulk milk cell count. Milk withheld because of clinical mastitis may alter the degree of these relationships.

Several authors *(1, 8, 9)* have suggested that quarters with sub-clinical mastitis were the main contributors to the bulk milk cell count. Pearson et al. *(8)* stated that the bulk milk cell count could be a guide to the prevalence of udder damage within a herd. Frank and Pounden *(2)* reported that repeated examinations of bulk milk samples for abnormal secretions appear to reflect the mastitis status of herds. Postle *(10)* reported from a study of four herds that within each herd there was a significant relationship between the presence of either medium or high leukocyte content and the isolation of pathogenic bacteria. However, three of the four herds examined maintained low bulk milk screening test scores.

The purpose of the present study was to examine the following relationships: *(a)* bulk milk cell count and the isolation of mastitis pathogens from quarter samples within the same herd; *(b)* bulk milk cell count and incidence of abnormal milk as determined by a strip plate examination; and *(c)* bulk milk cell count and milk production.

Materials and Methods

Information was gathered from 285 dairy herds which were enrolled in the New York State Mastitis Control Program (NYSMCP). Only farms with bulk milk tanks were selected. Each of six laboratories operated by NYSMCP (5 regional and 1 central) contributed information to the study from approximately 25 herds which used Dairy Herd Improvement (DHI) records, and from approximately 25

herds without production records. The average herd contained 56.1 cows with a standard deviation of 21.7.

Each herd was visited once and the following were collected: *(a)* bulk tank milk sample; *(b)* quarter milk samples from all cows for bacteriological culture; *(c)* information from a strip plate examination of each lactating quarter; abnormal secretions (discolored milk, flakes, clots or pus) were recorded; *(d)* information on the efficiency of the mechanical milking equipment; *(e)* information on herd management; and *(f)* dairyman's identification of quarter production withheld from the bulk tank.

Milk samples were transported to the laboratory in iced containers and stored at approximately 4 C until processed. Samples were cultured on blood-aesculin agar (extract agar, Difco[1], aesculin, 0.1% and citrated whole bovine blood, 5%). Bacterial growth was recorded after 24- and 48-hr incubation at 37 C. Cultures were recorded as *Streptococcus agalactiae*, non-agalactiae streptococci, *alpha* or *beta* hemolytic staphylococci, or miscellaneous (coliform, psuedomonas, yeasts, molds, etc.).

Bulk milk samples were examined using the Modified Whiteside Test (MWT) and the Direct Microscopic Somatic Cell Count (DMSCC), *(6)*. Duplicate slides for DMSCC were prepared from bulk milk samples collected at the five regional laboratories. One was sent to the central laboratory for examination, and the other was examined in the laboratory in which it was prepared. All cell counts in the central laboratory were made by one technician. This procedure permitted a comparison between 2 counts on 199 slides.

Analytical Procedures

Data excluding observations from dry quarters and milk withheld from the bulk tank according to the dairyman's statement were analyzed by the following procedures:

1. Simple correlations between:
   a. DMSCC from regional laboratories and DMSCC from the central laboratory.
   b. Percent quarters showing abnormal secretions (PQAS) and percent quarters yielding pathogens (PQP).
   c. PQAS and bulk tank DMSCC.
2. Correlations among:
   a. MWT, DMSCC and PQP.
   b. Fat production, milk production and PQP.
   c. MWT, DMSCC, PQAS, and PQP within each of three bulk milk cell count categories: <500,000; 500,000 to 1,000,000 and >1,000,000 cells per milliliter.
3. Percent quarters yielding pathogens by genus or species stratified according to cell count in 2c above.
4. Average DHI production figures (fat and milk) were determined for herds in each cell count category in 2c above.
5. Average (DHI) production figures (fat and milk), MWT, and DMSCC were compared between herds which appeared to be free of S. *agalactiae* infection and herds which contained S. *agalactiae* infections.
6. Relationship between certain management factors and DMSCC.

RESULTS AND DISCUSSION

The National Mastitis Council Research Sub-committee suggested guidelines for comparing the accuracy and precision of DMSCC *(6)*. The counting error and the film component of variance could be expected to approximate 130% of the mean and 15% of the mean, respectively.

The variance of the counting error made on cell counts in the central laboratory on those films prepared by and counted by one technician in the central laboratory was 153% of the mean and the film component of variance was zero, with a mean count of 26.75 cells per strip. When considering the counting data of this same technician for all DMSCC films in this study (films prepared in six laboratories), the counting error was 136% of the mean and the film component of variance was 103% of the mean, with a mean count of 37.98 cells per strip.

Counts made in the central laboratory correlated 0.80 to counts from the five regional laboratories for 199 slides. Cell counts made in the central laboratory were used as the reference counts.

A correlation coefficient of 0.50 was found between PQP and PQAS. Two factors may explain this rather low correlation: *(a)* many sub-clinical infections are not apparent on strip plate examination; and *(b)* some secretions from clinically positive quarters yield no culture. There were 2,458 quarter samples which were classified as abnormal secretion following strip plate examination. Of these, 981 samples (40%) yielded no pathogens on a single culture attempt.

A similar correlation (0.51) was found between PQAS and bulk milk DMSCC. Since none of the milk classified as abnormal was claimed to have been withheld by the dairymen, this low correlation may be explained on the basis of a high dilution factor. In this study 3.8% of quarters from which milk was saved, produced milk classified as abnormal.

Correlations among MWT, DMSCC, and PQP are presented in Table 1. The correlation found between PQP and DMSCC of 0.44 compares favorably with that reported by Westgarth et al. *(14)*, reinforcing the suggestion that the bulk milk cell count is not an accurate indication of the amount of sub-clinical infection in a herd.

Correlations among fat production, milk production, and PQP are shown in Table 2. As anticipated, the fat and milk production figures were highly and positively correlated (0.87), while production was poorly and negatively correlated with PQP (fat, -0.28; milk, -0.18).

Data from all herds were divided according to three bulk milk cell categories: <500,000; 500,000 to 1,000,000; and >1,000,000. The average values and correlations within each cell category for MWT, DMSCC, PQAS, and PQP are shown in Table 3. The averages for each parameter showed an increase as the bulk cell count increased. The correlations within each cell count category were in every instance

[1]Difco Laboratories, Detroit, Michigan 48201.

TABLE 1. CORRELATIONS AMONG MWT DMSCC AND PERCENT QUARTERS YIELDING PATHOGENS IN 285 HERDS (15,982 COWS; 63,927 QUARTERS).

	2	3
1. MWR	0.65	0.50
2. DMSCC		0.44
3. Percent quarters yielding pathogens		

TABLE 2. CORRELATIONS AMONG FAT AND MILK PRODUCTION AND PERCENT QUARTERS YIELDING PATHOGENS IN 132 DHI HERDS (7,405 COWS).

	2	3
1. Fat	0.87	−0.28
2. Milk		−0.18
3. Percent quarters yielding pathogens		

TABLE 3. AVERAGE VALUES AND CORRELATIONS AMONG MWT DMSCC, PERCENT QUARTERS PRODUCING ABNORMAL SECRETIONS, AND PERCENT QUARTERS YIELDING PATHOGENS, DIVIDED ACCORDING TO THREE BULK MILK CELL COUNT CATEGORIES.

	$\overline{X}$	2	3	4
A. Bulk tank cell count <500,000 (n = 156 herds) (8,845 cows, 35,381 quarters)				
1. MWT	0.9	0.41	0.35	0.26
2. DMSCC	352,000		0.29	0.26
3. % Abnormal secretions	3%			0.25
4. % Yielding pathogens	19%			
B. Bulk tank cell count 500,000 to 1,000,000 (n = 80 herds (4,368 cows, 17,472 quarters				
1. MWT	1.6	0.38	0.15	0.30
2. DMSCC	661,000		0.11	0.18
3. % Abnormal secretions	5%			0.12
4. % Yielding pathogens	31%			
C. Bulk tank cell count >1,000,000 (n = 49 herds) (2,768 cows, 11,074 quarters)				
1. MWT	2.3	0.54	0.17	0.22
2. DMSCC	1,203,000		0.39	0.13
3. % Abnormal secretions	7%			0.25
4. % Yielding pathogens	36%			

lower than the correlations determined without stratifying according to cell count. There was a slightly higher correlation between DMSCC and PQAS than between DMSCC and PQP. It is interpreted from these data that the bulk milk cell count is not to be expected to accurately reflect either the percent of quarters infected within a herd, or the percent of quarters with abnormal secretions.

The percent of quarters yielding pathogens from 285 herds is presented in Table 4. The herds were divided by DMSCC as above and in Table 3. The percent of quarters which yielded pathogens was nearly equally distributed among the three DMSCC categories for all pathogens except S. *agalactiae*. The high cell count group average 16% quarters yielding S. *agalactiae* as compared to 3% in the low cell group.

Average production figures (fat and milk) were recorded for herds in each of the bulk milk cell count categories as above. The figures available for 132 herds using DHI records are presented in Table 5. Production of both fat and milk declined as bulk milk cell counts increased. The average production for the low cell count group was 65 lb. of fat and 1,759 lb. of milk greater than the high cell count group, and 21 lb. of fat and 468 lb. of milk greater than the middle cell count group. The average per cow production in New York state within herds on DHI test at the time these observations were made was 502 lb. of fat and 13,898 lb. of milk.

Average production figures for fat and milk as well as MWT and DMSCC values for 64 herds which appeared to be free of infection with S. *agalactiae* and for 68 herds which contained some quarters infected with S. *agalactiae* are presented in Table 6. The average milk production per cow in herds apparently free of the infection was 800 lb. greater than in infected herds, representing a 5.7% lower average production in infected herds. Hale et al. *(3)* reported a 14.8% increase in production in herds when the infection was eliminated, and a 10.2% decrease in milk production when an infection was discovered in previously uninfected herds. In the present study there was no attempt to compare the various management factors between farms which might relate to production. Bulk milk cell numbers were higher for infected herds, as indicated by both MWT and DMSCC scores.

Information dealing with management procedures was collected during each farm visit. Some of the information was collected in the form of objective measurements, but the majority was collected during an interview with the dairyman regarding his milk-

TABLE 4. PERCENT QUARTERS YIELDING CERTAIN PATHOGENS, LISTED ACCORDING TO BULK MILK CELL COUNT CATEGORIES.

Pathogen	<500,000	500,000 to 1,000,000	>1,000,000	All
S. *agalactiae*	3%	9%	16%	7%
Staphylococcus	5	7	8	6
Non-agalactiae streptococci	10	14	11	11
Coliform	1	1	1	1
Pseudomonas	0.01	0.001	0.1	0.02
Others	0.1	0.1	0.1	0.1

TABLE 5. AVERAGE PRODUCTION FIGURES (FAT AND MILK) FROM 132 HERDS[1], (7,405 COWS), DIVIDED ACCORDING TO THREE BULK MILK CELL COUNT CATEGORIES.

Cell Count Categories	<500,000	500,000 to 1,000,000	>1,000,000
n (herds)	82	37	13
Fat	515	494	450
Milk	13,951	13,483	12,192

[1]New York State average for DHI herds: 502 fat and 13,898 milk.

TABLE 6. AVERAGE PRODUCTION FIGURES FOR FAT AND MILK AND MWT AND DMSCC SCORES ON BULK MILK FOR 132 HERDS, AS RELATED TO HERD INFECTION WITH *S. agalactiae*.

	Not infected	Infected
n (herds)	64	68
Fat (lb.)	522 ± 7.6*	485 ± 9.7*
Milk (lb.)	14,059 ± 221*	13,259 ± 276*
MWT	1.0	1.47
DMSCC	400,000	664,000

*Std. error means.

TABLE 7. RELATIONSHIPS[1] BETWEEN CERTAIN MANAGEMENT FACTORS AND BULK MILK DMSCC, DIVIDED INTO THREE CATEGORIES

	Bulk milk DMSCC categories		
Management factors	<500,000	500,000 to 1,000,000	>1,000,000
Home-raised herd replacements	1.26	1.31	1.46
Post-milking teat dipping	1.27	1.46	1.51
Milking inflations boiled in lye prior to storage	1.63	1.68	1.77

[1]'Yes' answers were assigned a value of 1, 'no' answers a value of 2.

ing management practices. All information was in the form of a yes or no response, and the data were divided according to the three bulk milk cell count categories described above, so they could then be examined for any apparent relationships. Information of the following management items was collected: *(a)* source of herd replacements; *(b)* pre-milking udder preparation; *(c)* use of strip plate to examine fore-milk; *(d)* sanitizing teat cup cluster following use on each cow; *(e)* post-milking teat end sanitizing; *(f)* cleaning and storing of milking inflations; *(g)* milking machine operating vacuum level; *(h)* size of vacuum lines; *(i)* number of milking units used per man; and *(j)* condition of exercise yard. Three of the factors showed a trend toward increased use in herds with low bulk milk cell counts: *(a)* farm-raised herd replacements; *(b)* post-milking teat dipping and *(c)* treatment of milking inflations by boiling in lye solution prior to a storage period (Table 7). An inference of a direct cause and effect relationship between these management factors and the bulk milk cell count is not warranted. Other authors *(7, 15)* have suggested a relationship between vacuum reserve and prevalence of infection, but vacuum reserves were not measured in this study.

Difficulty was recognized in assigning an infection status to an udder or a quarter on the basis of a single culture. Results of cultures reported here could have been affected by: *(a)* the presence of contaminating organisms which were colonized in the streak canal and/or teat end; *(b)* incorrect identification of organisms in the laboratory; *(c)* presence of inhibitory substances in the milk following treatment; and *(d)* results of natural defense mechanisms within the cow, such as phagocytosis. It is expected, however, on the basis of subsequent work in progress, that an error of <5% would develop as a result of culturing only one sample from each quarter.

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GROWTH RATES AND FERMENTATION PATTERNS OF LACTIC ACID BACTERIA ASSOCIATED WITH THE SAUERKRAUT FERMENTATION[1]

J. R. Stamer, B. O. Stoyla, and B. A. Dunckel
New York State Agricultural Experiment Station
Department of Food Science and Technology
Cornell University
Geneva, New York 14456

(Received for publication May 24, 1971)

Abstract

The effects of pH values and NaCl concentrations on the growth rates of five species of lactic acid bacteria commonly associated with the sauerkraut fermentation were determined in filter-sterilized cabbage juice. Growth rates of all cultures, with the exception of *Pediococcus cerevisiae*, were retarded by addition of salt, lower pH, or interaction of both pH and salt. Based upon lag and generation times, *P. cerevisiae* was the culture most tolerant to the pH and salt concentration employed, whereas *Streptococcus faecalis* was the most sensitive species. Of the heterofermentative cultures, *Lactobacillus brevis* was less subject to growth inhibition than *Leuconostoc mesenteroides*.

Under conditions simulating those found during the initial phases of the sauerkraut fermentation (2.25% salt, pH 6.2), *L. mesenteroides* displayed the shortest lag and generation times of all cultures examined. This rapid growth rate coupled with a marked accelerated death rate may explain, in part, the reason this species is both the first to dominate and the first to die during the early phases of the sauerkraut fermentation. Although cabbage juice previously fermented by *L. mesenteroides* appears to inhibit growth of *P. cerevisiae*, it had no apparent inhibitory or stimulatory effects on the other cultures.

The sauerkraut fermentation is a complex microbiological process resulting essentially from the growth responses of heterofermentative and homofermentative lactic acid bacteria. During the course of this fermentation certain species display dominant growth characteristics. The resulting changes or shifts in the population produce an apparent sequence of predominant species *(1, 3, 4)*. This growth pattern or growth sequence invariably is initiated by *Leuconostoc mesenteroides* and is terminated by the succeeding associative actions of *Lactobacillus plantarum* and *Lactobacillus brevis*, with less frequent interaction by *Pediococcus cerevisiae* and *Streptococcus faecalis (6)*. It has been suggested *(7)*, that not only is *L mesenteroides* a vital species for producing a good quality kraut, but that this microorganism also serves as an essential microbial precursor for the induction of a proper growth sequence. The reason, however, for its early appearance during the initial phases of the cabbage fermentation and the nature of its possible inducing effects upon successive species in a pure culture system remain to be elucidated.

Under conditions simulating small scale commercial fermentations, it has been shown that growth and fermentation patterns are altered by such factors as variety of cabbage *(10)*, temperature, and salt concentrations *(5)*. These factors, coupled with the highly competitive microbial population found in a nonsterile, shredded cabbage medium, make it difficult to assess the maximum role each participating species may potentially contribute to the fermentation. In order to avoid some of the complexities of a nonsterile system, such as the uneven distribution of salt and concentration of hydrogen ions, and the interactions of an undefined microflora, we have used fresh cabbage juice for pure culture studies. This report describes the effects of hydrogen ions, and salt concentrations on the lag and generation times of the above species, the influence of *L. mesenteroides* on the growth rates of succeeding cultures, and the fermentation patterns (volatile and non-volatile acid values) produced in filter-sterilized cabbage juice.

Materials and Methods

Preparation of cabbage juice

Whole, fresh cabbage, 42.6 kg (variety Glory), was obtained from a local sauerkraut producer. Following removal of outer wrapper leaves, the stripped cabbage heads were quartered and passed through a Fitz mill equipped with a Number 4 stainless steel sieve. The pulp, containing 92.1% moisture, was pressed immediately in a hydraulic press equipped with press cloths to retain vegetable fibers. The fresh juice, 27.7 kg, was stored in polyethylene containers at −20 C. Prior to growth and fermentation studies, that amount of frozen juice required for experimental work was thawed, then centrifuged at 20,000 × *g* for 5 min. To study the effects of pH and salt concentrations on growth rates, cabbage serum (initial pH 6.2) was adjusted to pH 5.5, 5.0, and 4.5 with concentrated lactic acid. The salt levels, 2.25 and 3.50% (w/v), were achieved by adding granular NaCl to the above clear supernatant serum. The

[1]Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1883, May 20, 1971.

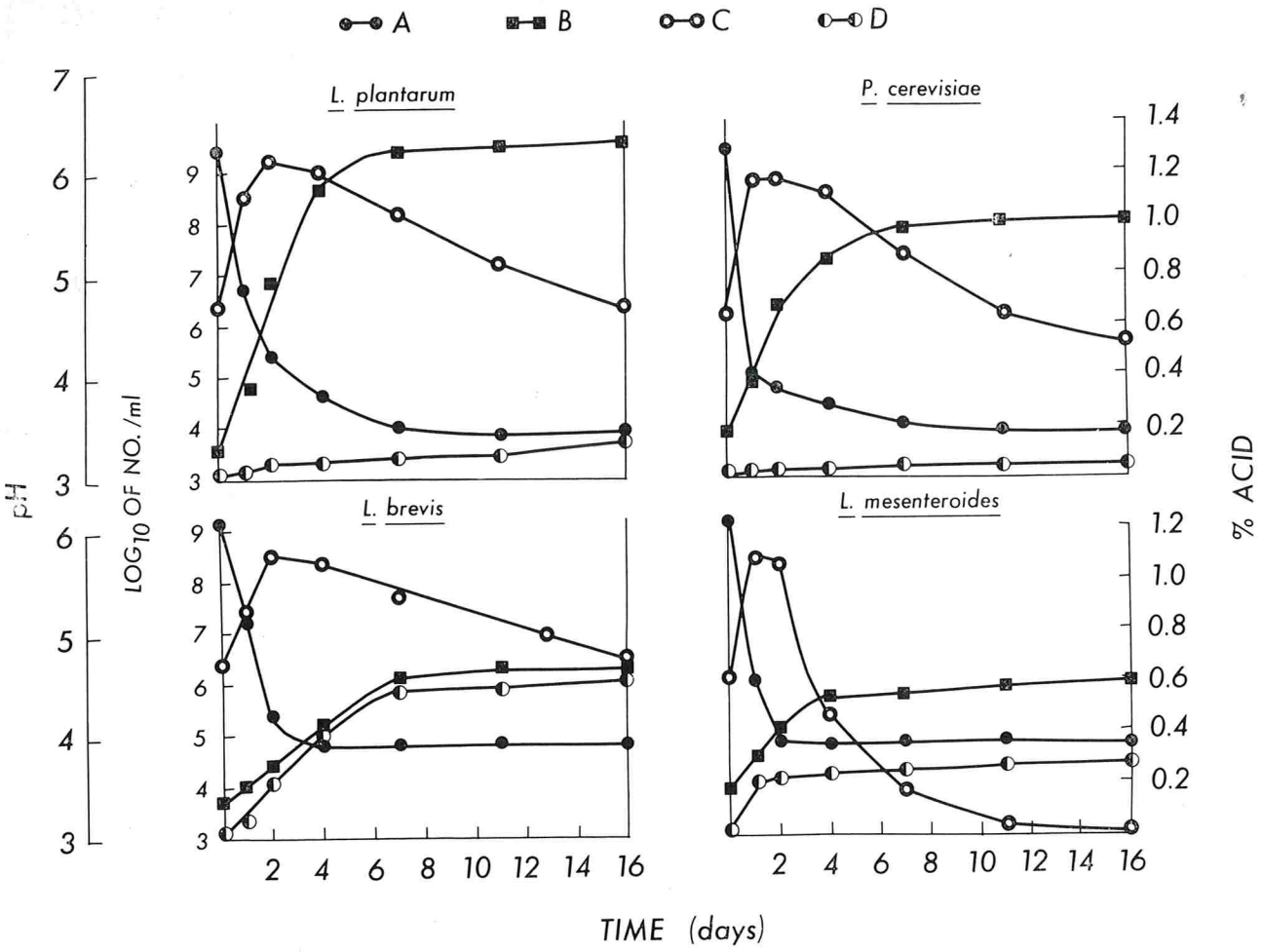

Figure 1. Fermentation patterns of lactic acid bacteria grown in filter-sterilized cabbage juice. A, pH; B, percent non-volatile acidity expressed as lactic acid; C, viable cell count; D, percent volatile acidity expressed as acetic acid. Incubation temperature 30 C.

sera, sterilized by membrane filtration in a glass assembly, were dispensed aseptically in 16 ml volumes (16 × 150 mm test tubes) for growth rate studies and in 500 ml volumes for end-product analyses.

Cultures

Leuconostoc mesenteroides (C33), *L. plantarum* (B246), *L. brevis* (B155), *P. cerevisiae* (E66), and *S. faecalis* (8043) were obtained from the culture collection of this department.

For growth rate determinations, each cabbage juice medium (16 ml volume) was inoculated with one loop of a 24 hr culture grown previously in cabbage juice. The inoculated test sera, initially containing about 5×10^5 cells per milliliter, were incubated in a water bath at 30 C. The viable cell counts were taken at 2 hr intervals during a 20 hr period. The viable count was estimated using total plate count agar (Difco) following 48 hr incubation at 32 C. The experiment was conducted in duplicate and average values reported. Viable cell counts per milliliter of medium were plotted on semilogarithm paper against time in hours. The lag and generation times were determined from the slope values of the above plots *(2)*.

Parabiotic growth chambers (Bellco Glass Inc., Vineland, New Jersey) were used initially for determining the effects of *L. mesenteroides* on growth rates of other lactic acid cultures. This method, however, was discontinued when the pH values of the inoculated chambers showed that fresh cabbage juice rapidly clogged the membrane and hindered exchange reactions. Therefore, the influence of *L. mesenteroides* on growth rates of the other species was determined by adding to the fresh juice, various amounts of juice previously fermented for 24 hr by the above culture.

The fermentation patterns were determined at 24-hr intervals during a 16 day period of incubation at 30 C. The total acid values were obtained by titrating 2.0 ml volumes of the media with a 0.02 N NaOH to pH 8.3. The volatile acids were recovered from 5.0 ml samples of the acidified brines by means of a Comes-Milano microsteam distillation apparatus by methods previously described *(8)*. The volatile acidity is expressed as percent acetic acid, whereas the percent lactic acid was calculated by difference.

Results and Discussion

Data on the growth and fermentation patterns, Fig. 1, indicate that fresh, filter-sterilized cabbage juice served as an excellent medium for supporting the growth of those lactic acid bacteria associated with the sauerkraut fermentation. A comparison of the individual growth responses (total counts and

TABLE 1. EFFECTS OF INITIAL pH AND SALT CONCENTRATIONS UPON THE GROWTH RATES OF LACTIC ACID BACTERIA CULTURED IN CABBAGE JUICE[1]

Culture	Percent NaCl added (w/v)	Initial pH[2] 6.2 L[3]	6.2 G[4]	5.5 L	5.5 G	5.0 L	5.0 G	4.5 L	4.5 G
L. mesenteroides C33	None	180	40	228	43	285	56	360	145
	2.25	250	43	320	51	360	86	390	154
	3.50	360	66	—	—	—	—	—	—
L. plantarum B246	None	330	43	350	47	370	47	420	53
	2.25	420	45	520	46	570	56	660	70
	3.50	540	72	—	—	—	—	—	—
L. brevis B155	None	215	83	240	81	255	83	260	84
	2.25	270	91	360	94	390	98	460	102
	3.50	360	110	—	—	—	—	—	—
P. cerevisiae E66	None	360	41	345	41	355	47	450	49
	2.25	400	43	480	45	540	45	750	49
	3.50	510	40	—	—	—	—	—	—
S. faecalis 8043	None	240	78	250	85	270	87		
	2.25	300	107	340	210	340	711		
	3.50	340	267	—	—	—	—	—	—

[1]Incubation temperature 30 C; [2]Fresh cabbage juice, pH 6.2, adjusted pH values obtained by acidification with concentrated lactic acid, [3]Lag time (min); [4]Generation time (min).

TABLE 2. THE FINAL ACID RATIOS AND THE EFFECTS OF SALT CONCENTRATIONS UPON MAXIMUM ACID RATES OF LACTIC ACID BACTERIA GROWN IN CABBAGE JUICE[1]

	Acid values[2] Percent NaCl added (w/v) 0	2.25	3.50	Percent acid[3] Total[4]	Non-volatile[4]	Volatile[5]
L. mesenteroides C33	0.87	0.56	0.20	1.04	0.68	0.25
L. plantarum B246	0.43	0.35	0.30	1.40	1.29	0.07
L. brevis B155	0.39	0.33	0.10	1.06	0.40	0.45
P. cerevisiae E66	0.60	0.55	0.50	0.90	0.87	0.02

[1]Initial pH 6.2; [2]Expressed as meq of titratable acid per 100 ml juice per hr as determined from the slope of the curve of titratable acid against time in hr; [3]Following 10 days incubation, without added salt, [4, 5]Expressed as lactic and acetic acids, respectively. All cultures incubated at 30 C.

TABLE 3. THE EFFECTS OF VARIOUS LEVELS OF CABBAGE JUICE PREVIOUSLY FERMENTED BY *L. mesenteroides*[1] UPON THE GROWTH RATES OF LACTIC ACID BACTERIA

	Percent supplementation (v/v) 0 L[2]	0 G[3]	10 L	10 G	25 L	25 G	50 L	50 G	75 L	75 G	90 L	90 G	100 L	100 G
L. mesenteroides	195	40	180	41	180	40	170	41	185	43	165	43	180	41
L. plantarum	336	44	345	47	330	47	360	43	345	43	340	47	345	41
L. brevis	190	81	210	82	225	31	225	83	205	80	230	83	230	84
P. cerevisiae	340	43	330	45	365	43	330	49	370	53	345	54	350	55

[1]Incubated at 30 C for 24 hr. The fermented juice was adjusted to pH 6.2 with concentrated NaOH, filter sterilized, and added to fresh juice to give above supplementations; [2, 3] Lag and generation times (min), respectively.

acid production) in the above sterile juice to that of the total growth activities in non-sterile shredded cabbage (*7*) indicated that filter-sterilized juice served as an excellent substitute for evaluating the roles of pure cultures found in the sauerkraut fermentation. It may be observed that *L. mesenteroids, P. cerevisiae,* and *L. plantarum* all reached populations of 3×10^8 cells per milliliter, within 24 hr, whereas *L. brevis* required an additional 24 hr incubation to reach this value. The extended time required by *L. brevis* to reach a similiar population also is reflected by its increased generation time (Table 1). Although the four cultures reached their maximum viable cell yields within 48 hr incubation, the onset of the phase of decline was most marked with *L. mesenteroides.* Following four days of incubation, the viable cell count of *L. mesenteroides* decreased nearly three log cycles, whereas the populations of the other cultures remained essentially unchanged. An additional seven days of incubation produced a recovery of less than 2×10^3 cells per milliliter, whereas the remaining three cultures provided viabilities of more than 5

$\times 10^5$ cells per milliliter following 16 days incubation. The short life span of *L. mesenteroides* apparently is not caused by the lack of essential nutrients since juice previously fermented by this species gave growth rates similar to the fresh juice (Table 3). The rapid death rate may result from the inability of the microorganism to survive at the lower pH values or its greater sensitivity to the more undissociated forms of lactic and/or acetic acids.

The maximum rate of acid production for each culture appears to be associated with the period of fastest growth. More than 80% of the total amount of acids produced arose during the logarithmic growth phase (Fig. 1). The types of acids formed, volatile and/or non-volatile, during the fermentation were species dependent and appeared to follow the assigned classical hetero- and homofermentative patterns. Following 10 days incubation, the homofermentative species, *L. plantarum* and *P. cerevisiae*, produced 1.40 and 0.90% titratable acidity, respectively. Less than 0.10% volatile acidity, expressed as acetic acid, was recovered from either species. Although the two heterofermentative species, *L. brevis* and *L. mesenteroides*, produced 1.06% and 1.04% total acid respectively, the final volatile acid values were quite dissimilar. Based on volatile and non-volatile acid recoveries, *L. brevis* produced nearly equal amounts of lactic and acetic acids (0.40% lactic, 0.45% acetic), whereas *L. mesenteroides* yielded a greater amount of lactic acid, 0.68%, and a lesser amount of acetic acid, 0.25%. These volatile acid compositions provided volatile to non-volatile acid ratios of 0.88 and 2.7, respectively, and had pronounced effects on flavor characteristics of fermented juices. Disregarding the possible effects of neutral compounds such as diacetyl and acetoin on flavor analyses, the juice fermented by *L. brevis* was harsh and vinegar-like, whereas that fermented by *L. mesenteroides* was mild and pleasantly aromatic as previously described *(9)*.

During the course of a "normal" kraut fermentation, the microbial and chemical environs are in a state of constant change. To simulate the chemical variables encountered in the early phases of the fermentation, effects of pH and salt interactions on growth rates, were investigated.

Of the five cultures studied, *L. mesenteroides* was the species most markedly inhibited by the decreasing pH values (Table 1). Reducing the initial pH of the juice to 5.0 increased both the lag and generation times nearly 50%. Lowering the latter pH an additional 0.5 unit extended the lag and generation times two- and three-fold, respectively. The influence of pH was less evident with the heterolactic *L. brevis* in that the generation times (81 to 84 min) remained quite constant throughout the range of pH values studied. The homofermentative species, with the exception of *S. faecalis*, displayed greater tolerances to lower pH values since their growth rates decreased only 20% at pH 4.5. *Streptococcus faecalis* when grown at the latter pH, yielded extreme variation in viable count recoveries and its rates therefore are not included. In addition to the influence of pH on generation times, it may be observed that the lag phases of all of the species were extended as a result of the increase in hydrogen ion concentration.

The influence of 2.25 and 3.50% NaCl on the rates of acid production during the logarithmic growth phases (maximum acid rate = meq of acid produced per 100 ml juice per hour during logarithmic growth phase) and the interactions of pH and salt concentrations on lag and generation times of each culture are shown in Tables 1 and 2. The heterolactic species, *L. brevis* and *L. mesenteroides*, when grown in cabbage juice containing no added salt, produced maximum acid rates of 0.39 and 0.87, respectively, whereas the homofermentative *L. plantarum* and *P. cerevisiae* yielded intermediate values of 0.43 and 0.60. The two-fold difference in acid values of the two heterofermentative species may be attributed, in part, to the 50% shorter generation time exhibited by *L. mesenteroides*. However, if the generation times influence the maximum acid values, such effects are less evident with the homofermentative species. The latter microorganisms possessed generation times of 43 and 41 min, values quite similar to that of the *L. mesenteroides*.

The addition of 2.25% NaCl, a concentration often used in commercial fermentations, reduced the maximum acid rates of all cultures tested. The most pronounced inhibition, 37%, was noted with *L. mesenteroides* and the least retardation, 8%, with *P. cerevisiae*.

Increasing the salt level to 3.5%, a level occasionally encountered in commercial practice because of over-salting or improper distribution of salt, produced a 90% reduction in the acid rates of the heterolactic species. Under similar conditions the acid values of the homofermentative species *L. plantarum* were reduced 30%, whereas *P. cerevisiae* was inhibited only 16%. Thus, salt concentrations in addition to influencing growth rates (to be discussed below) may significantly alter the rates of acid production—a condition which could lead to an undesirable imbalance of the proper acid ratios of the fermented product.

The addition of 2.25 or 3.5% salt to cabbage juice, pH 6.2, extended the lag phases from 1 to 4 hr. Once the cells had become acclimated to the 2.25% concen-

tration, generation times, with the exception of *S. faecalis*, were comparable to those observed in juice containing no added salt. However, increasing the salt level to 3.50% extended the lag phases of all organisms. Generation times of the cultures, with the exception of *P. cerevisiae*, likewise were extended. These data show that of the cultures examined, *P. cerevisiae* was the most salt tolerant. This salt and acid tolerance, as measured by generation times, affords a plausible explanation for the occurrence of pediococci in krauts treated with high levels of salt. These data also suggest that the reason *S. faecalis* plays only a minor, sporadic role in the fermentation is because of its sensitivity to lower pH values and higher salt concentrations. The inhibitory effects of the interactions of pH and salt were most evident with the species *L. mesenteroides.*

The role of *L. mesenteroides* as a possible agent for producing stimulatory or inhibitory effects on growth rates of lactic acid bacteria was evaluated by the supplementation method. The results (Table 3), indicate that the prefermented supplements, when supplied at concentrations ranging from 10 to 100%, had no major effects on the lag and generation times of *L. plantarum, L. brevis,* or itself. Although there was no discernable effect on the lag times of *P. cerevisiae,* there were inhibitory responses on its generation times. These suppressions were most apparent when the supplements were provided at levels in excess of 50% since the addition of 75, 90, and 100% (no fresh juice added) increased the generation times an additional 10 to 12 min. This suggests that the latter generation times increased as a function of supplement concentration. The reason for the inhibition, whether it be due to production of inhibitory substances or inducement of a condition where vital growth substances became limiting, is currently not known.

Results of this investigation with pure cultures of lactic acid bacteria grown in cabbage juice indicate that differences in lag and generation times and phases of decline of each singular species contribute to and may produce the apparent observable sequence of growth patterns so often associated with the sauerkraut fermentation. Differential plate counts and the isolation of catalase-negative, gram-positive cultures from eight freshly brined cabbage samples, collected at various intervals throughout the growing season, showed that such total populations vary from 8.0×10^5 to 8.1×10^8 (average 2.6×10^7) cells per milliliter of brine. Tentative identification showed that the lactic population was comprised of 30% heterolactic cocci, 38% heterofermentative rods, 30% homofermentative rods, and 2% homofermentative cocci. Based on these population distributions and the lag and generation times observed in juice containing 2.25% salt, it may be projected that in a mixed culture system, *L. mesenteroides,* although not the dominant species present at the time of shredding, will invariably initiate and predominate during the early phases of the fermentation. Under ideal conditions, this species should achieve a maximum cell population (excess of 10^8 cells per milliliter) following 12 to 14 hr incubation. An additional 10 to 22 hr incubation would produce the maximum populations for *L. plantarum* and *L. brevis,* respectively. The inhibitory effects of *L. mesenteroides* and the resulting lowering of pH values (below 4.0) would curtail growth activities of *P. cerevisiae. Streptococcus faecalis,* less tolerant to acid and salt, would not, in an actively growing competitive system involving the above microorganisms make a significant contribution to the sauerkraut fermentation.

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THE NEED FOR ENVIRONMENTAL LABORATORIES

PATRICK KNEAFSEY

Environmental Services Division
Health and Social Services Department
State of New Mexico
Santa Fe, New Mexico 87501

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ABSTRACT

The need for a regional environmental or ecological laboratory facility is explored. Inherent weakness of the traditional public health laboratory, from an environmentalist's viewpoint, are cited. Several concepts are listed on which this new facility should be based. Among these are: *(a)* the facility must be based upon "man *in* his environment;" *(b)* it must be capable of research activities; and *(c)* it must be available to the multitude of agencies concerned with a quality environment. This type of laboratory is described as multi-disciplined in composition, ecological in attitude, adaptable in organization, aware of yesterday, attuned to today, and anticipating tomorrow.

In his book, *Man Adapting*, Rene Dubos has as a major theme the concept that "states of health or disease are the expressions of the success or failure experienced by the organism in its effort to respond adaptively to environmental challenges." If this concept is true in its application to an organism, is it not equally true with regard to ecosystems? Is not the condition of an ecosystem an expression of its ability to cope with environmental challenges? And if the concept of "man *in* his environment" is more closely related to the truth than the concept of "man *and* his environment" we can more clearly see that not only does the environment present challenges to man (an organism), but that man in turn present challenges to his environment.

As the original definition challenges the human-oriented sciences to determine the nature and limits of the adaptation potentiality of modern man, enveloped in a panorama of environmental conditions, so too does the second definition challenge environmentalists to determine the nature and extent of the ability of an ecosystem to survive the impact of man. But most importantly, it defies mankind to perceive the biological and cultural consequences of its failure to grasp the significance of the adaptive processes inherent in the cyclic effects of man on his environment, and of his environment on man.

LIMITS OF ADAPTABILITY

Environment

Determination of the boundaries of the ability of the environment to adapt, as well as its present condition, is an obvious first step in the detailing of programs necessary to insure its continued existence, and thus the continued existence of man. Though essential, this step is both massive in scope, and somewhat frightening in that its pathways are uncharted. But its necessity becomes obvious when commentaries on the present environmental condition of the nation, and of the world, are reviewed. These range from despair to hope, from panic to promise. The discrepancy can only result from the lack of basic data of the long-range effects of pollution and population. Without baseline data, neither degradation nor recovery rates can be determined; and without reliable rates, the rational setting of program priorities is seriously hampered. Information on the effects of micro-insults, either singly or in combination, on both man and his environment are yet another void. Only limited work has been done on interrelationships of our air, water, and land resources as they occur in the "natural" state, and as they are affected by "progress." Little is known of the effects of a degraded environment on wildlife. The long-range effects of the simplistic concentrated ecosystems we call agriculture bear a great deal more study that has been done, especially in their interrelationships with a less than optimum climatic region, such as the semi-arid Southwest.

Man

In addition to the need for determining the effect of modern life on the adaptability of the environment, equally as much work remains to be done in determining the effects of the man-environment relationship on man himself. Being a non-specialized organism, man has shown a great ability in adapting himself to a wide variety of environmental conditions. If the prophets of hope are true in their predictions, man will continue to adapt, and thus survive, even in drastically degraded conditions. But the mechanisms of adaptation, the changes which will allow man to continue to exist, may not be of benefit either to the individual or to his society. The nature of the adaptation is not as yet predicable. The pathological destruction of some part of the body, so that the whole can survive, is but one form of change. Certain types of neuroses are

now thought to be defensive mechanisms having a protective influence for the organism as a whole. But is physical and mental disease the route we want to follow for survival?

An ironic feature of man's ability to adapt to a wide range of conditions lies in his very adaptability. By adjusting to less than optimum conditions he also may erase the values associated with the joy of living, values acquired through the countless generations of his existence. Millions of Americans have adapted so well to 20th century urban life that they are no longer concerned with having only an intermittant view of the smog-filtered sun; with having no open areas in which to roam in privacy; with living in and moving through treeless, artificial canyons; with contacting nature only in the form of botanical gardens and zoos. Biologically and culturally man can adapt to a less than quality environment but the price is high: pathological and mental changes, and a withering of the esthetic and moral values that distinguish man from other organisms.

Plants and animals

But man does not exist as the sole organism in his world. Plants and other animals cooperate with man and each other to make up the ecosystem which permits the survival of all. Unfortunately the adaptive capability of man is not widely shared by his co-inhabitants. The relationship of the increase of man with the decrease of other organisms is well documented, and closely related to man's increasing need for and use of land. Put another way, people destroy wildlife, not only in a deliberate manner, but by crowding them off the land. When man broke the prairie sod, drained the swamp, re-routed the watercourse, bulldozed the scrubland, mined the forest, and buried the rest; in other words, as man and his needs demanded more land, the natural ecosystems were systematically destroyed. Animals deprived of food and cover died; ground deprived of cover eroded; fish, unable to adapt to walking in the silt- and waste-laden rivers and streams, disappeared; man deprived of union with nature lost beauty, knowledge, opportunity for re-creation, and contact with his origins.

It is frightening to realize that this has happened, is happening, and will continue to happen unless the accelerating degradation of our surroundings can be reversed.

SEARCH FOR CORRECTIVE ACTION

The search for the essential data upon which corrective actions must be based requires a laboratory capacity unlike those now in existence. Traditionally, the envrionmentalists has been required to turn to the laboratory section of the public health organization. The main thrust of this section, however, has been directed toward needs of personal health and medicine. Environmental needs have often been considered as an additional workload at best, and as a nuisance at worst. With the increased popular concern over the environment and the resulting demand for protective services; and, with the growing realization of the extent of the panorama of factors encompassed within the framework of environmental protection, it is becoming increasingly clear that a redirection and reorganization of laboratory services is needed if identification and maintenance of a quality environment is to be accomplished. The rapid pace of technological and social change make it increasingly difficult for environmentalists engaged in programs such as waste management, evaluation of toxic environmental chemicals, product safety, wildlife management, soil quality, economic evaluations of environmental insults, and the host of other necessary, indeed mandatory, programs and studies, to communicate with laboratory personnel trained and directed toward the provision of services to clinicians and other members of the "health service team."

Indeed, the current organization and administration of such facilities has the tendency to reinforce the priority of medical needs over needs of environmentalists. Personnel are, by training and supervision, directed toward a skill and interest in medical technology. Equipment and instruments, reference journals and periodicals, reference texts, and even job descriptions are oriented toward the needs of medicine, rather than the multi-discipline approach to environmental and ecological problems. Thus, the laboratory, as presently organized is grossly deficient as a tool in ecological programming.

AN ECOLOGICAL LABORATORY

Furthermore, traditionally organized laboratories have neither the personnel nor the desire to tackle a project for which no procedure has yet been written. The need to preserve the environment of this large area for future generations, through programs based on the limits of nature's ability to convert wastes into resources make it essential that ecological laboratories be developed. It must be realized from the beginning that development of such facilities will be difficult. There are no patterns to follow. Such a laboratory must have the capability of providing services, and accomplishing research activities for an environmental program presently fragmented and administered by numerous agencies. It must have direction of sufficient breadth to comprehend the significance of a quality environment in all

its aspects, not simply the negative approach of identifying poor conditions.

This is not meant to degrade the importance of the medically-oriented or disease-oriented, laboratory as a tool in man's search for a better life, but rather to argue for development of a new component in this search – an ecological laboratory, one designed and directed toward the external environment of man and his society; an addition to the present facility which is organized and directed toward the internal environment of man as an individual.

As the realization of the importance of the relationship of man in his environment grows in the public mind, a parallel realization is developing in the mnids of administrators of programs directed toward this relationship that the lack of adequate ecological information has had and is having a harmful effect on the decision-making process. Without these data, the validity of program planning, implementation, and evaluation is suspect. Even a cursory review of the programming presently practiced by government agencies, on all levels, adds weight to this thesis. Data that are available are generally either national or local in scope, the national data often too general, and the local data too specific, for use on a problem-shed basis. This lack of usable data, plus administrative and economic pressures, have been greatly responsible for fragmentation of environmental programming – a trend directly opposed to the concept of the interrelated wholeness of the problems themselves.

The meaningful resolution of this ignorance cannot be accomplished on a national basis. Natural environments involved differ greatly from sector to sector of the country. It cannot be done on a local basis. The ecological systems involve watersheds, airsheds, mountain ranges, vegetative types, and wildlife, as well as the immense effects of natural physical activity, of a scope much greater than that found within the boundaries of a locality. Limits of the natural cleansing ability of a region's environment is the basic information upon which decisions concerning economic development and population growth for the area must be based.

In the past, standards and control programs have been based on the concept of control feasibility, rather than the concept of environmental maintenance. If technology could produce 99% control, the remaining 1% of pollutant was released to the environment with the feeling that a reduction of such magnitude was an accomplishment indeed. The more fundamental concept, that of determining the total amount of waste the natural system in the affected area could recycle, and basing control on this figure was ignored. A great part of this ignorance was due to just that – ignorance. Ignorance of the capability of the environment to absorb particulate matter from stacks of power generating plants; ignorance of the capability of the environment to absorb liquid and solid wastes of an increasing population; etc.

It is also becoming apparent that there is a sound potential in the use of the knowledge of ecology to predict environmental changes. However, much more data must be developed before this ability to forecast becomes a science rather than an art. An ecological laboratory is an essential tool in the development of information upon which the science of "prophecology" can be based. A laboratory emphasizing its role as a tool in clinical medicine, on the other hand, is not in a position to play more than an ancillary part in this development. Our ability to adapt to needs of a changing society, during its very process of adaptation, requires this new laboratory organization and direction.

Such a laboratory must be based in several concepts: *First,* it must be concerned with the "man *in* the environment" approach to environmental problems. *Second,* it must be regional in scope. *Third,* it must have the capability, not only to provide support in the day to day activities of a variety of agencies, but also to undertake research along the lines mentioned above. This includes work in the fields of economics, sociology, political science, and education, as well as the physical sciences.

Fourth, it must be available to all agencies within the region concerned with environmental programming; agencies such as:

(a) Health agencies
(b) Wildlife Conservation agencies
(c) Agricultural and Soil Conservation agencies
(d) Water Pollution agencies
(e) Air Pollution agencies
(f) Transportation agencies
(g) Planning agencies
(h) Economic Development
(i) Regional Government agencies
(j) Citizen organizations

Fifth, it must have as one of its prime missions identification of those components which make up a "quality environment" as well as identification of factors which result in environmental degradation.

We are thus talking about a laboratory that is multi-disciplined in composition, ecological in attitude, adaptable in organization, aware of yesterday, attuned to today, and anticipating tomorrow. Such a description fits not only the laboratory, but the program it serves – identification and restoration or conservation of a quality environment.

AN EVALUATION OF THE KOHMAN PROCEDURE AS APPLIED TO COMPOSITION CONTROL IN CONTINUOUS BUTTERMAKING[1]

VERNAL S. PACKARD, JR.
Department of Food Science and Industries
University of Minnesota
St. Paul 55101

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SUMMARY

The Kohman balance and another, more sensitive Torsion balance (Torbal ET-1), were compared and evaluated statistically to determine their applicability in composition control methods used in continuous buttermaking. On five replicate analyses of four supplies of butter at four different sample weights (10, 15, 20, and 25 g), the Torbal ET-1 balance proved somewhat more precise for moisture determinations. Precision tended to improve as sample weight increased, the improvement being more pronounced for the Kohman balance.

In replicate direct salt analyses of 1-, 5-, and 10-g samples, the best precision was noted with the more sensitive balance at a sample weight of 5 g. At all sample weights, results were positively biased compared to results obtained by the conventional method of salt analysis in butter.

Use of high volume, continuous buttermaking equipment has placed new demands on control methods traditionally used by industry. Monitoring a continuous stream of butter at production rates of over 4,000 lb. per hour requires fast, sensitive, precise control. Ideally, the product should be continuously monitored and automatic adjustments made when control limits are breached.

Current industry practice in Minnesota, where several continuous machines are in operation, involves use of a modified Kohman procedure. Moisture is determined by the standard Kohman method using a Kohman balance. Salt is analyzed directly in butter-water mixture on a 1- or 2-g sample weighed on the Kohman balance. Curd is assumed to be constant at 1.4%. Butterfat is obtained by difference. Thus, any errors inherent in moisture and salt analyses, or any variation in the assumed curd value, are reflected as error in butterfat content.

Some work has been reported on devices which may be used for continuously monitoring moisture content in a continuous flow of butter *(1, 2, 3)*. In general, it appears that the devices lack sensitivity or precision, or must be preset for one specific composition of butter. In this latter regard, the versatility to manufacture butters of varying composition is lost. However, precise continuous monitoring coupled with automatic machine adjustment must be the goal of research in this area.

At least two reports have been published *(4, 5)* which would indicate that curd content may not be constant in continuously manufactured butter. Both season of the year and pasteurization temperature were found to influence curd level. Highest average value (1.8%) was noted in May and July, lowest value (1.5%) in January through March. Curd content was observed to increase with increasing pasteurization temperature. Values of 1.45 to 1.71% curd were reported at pasteurization temperatures of between 85 and 90 C., and levels of 1.7 to 2.0% for temperatures ranging between 95 and 97 C.

In the second study, it was observed that double pasteurization of cream reduced the curd content of butter below the level found in single-pass pasteurized cream at any given temperature treatment.

In the work reported herein, an attempt has been made to evaluate some sources of error in the Kohman test procedure and to refine certain aspects of it.

MATERIALS AND METHODS

Kohman procedure

Four samples of butter in pound packages of quarter-pound prints were obtained from commercial processors. Two of the samples, 1 and 2, were of conventionally churned butter; two were from continuous churns, 3 and 4. Five replicate analyses were made on each sample at four different sample weights (10-, 15-, 20-, and 25-g) using both a Kohman balance (Type 1735, The Torsion Balance Co., New York) and a top-loading, direct reading Torsion balance (Torbal ET-1, The Torsion Balance Co., New York). The latter has 1 mg sensitivity over the range of its capacity.

Prior to weighing, a sufficient quantity of butter for five analyses was placed in a screw-cap glass jar, the lid was tightened solidly, and sample lots allowed to temper at 20 C in a constant temperature room. They were tempered overnight, or for at least 4 hr, prior to mixing with a spatula to a salvy consistency, and sampling. Each lot was mixed only once, initially, and not between extractions of analytical size portions. For all except the 25-g sample trials, a portion of a single quarter-pound print served as a sample lot. Since the primary objective of the research was concerned with a determination of analytical precision, no attempt was made to homogenize prints within the pound package.

On the 10- and 15-g samples, the USDA modified Kohman procedure *(7)* was used. In this modification, an electric hot plate is employed for moisture evaporation, three 50 ml

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TABLE 1. MEAN SQUARE VALUES[1] FROM AN ANALYSIS OF VARIANCE OF MOISTURE AND BUTTERFAT RESULTS BASED UPON FIVE REPLICATE ANALYSES OF FOUR COMMERCIAL BUTTER SAMPLES ANALYZED ON KOHMAN AND TORBAL ET-1 BALANCES AT FOUR DIFFERENT SAMPLE WEIGHTS (10, 15, 20, AND 25 GRAMS)

	Kohman				Torbal ET-1			
Component	Butter	Sample size	B × S[2]	Determination error	Butter	Sample size	B × S[2]	Determination error
Moisture	0.489	0.622	0.031	0.049	1.119	0.581	0.121	0.006
Butterfat	2.979	0.354	0.097	0.025	2.183	0.689	0.119	0.015

[1]The degrees of freedom were 3, 3, 9, and 64 for butter, sample size, B × S, and determination error, respectively.
[2]This value expresses the interaction between butter (B) and Sample Size (S).

TABLE 2. MEAN AND STANDARD DEVIATION VALUES OF FIVE REPLICATE MOISTURE ANALYSES ON FOUR COMMERCIAL BUTTER SAMPLES AT FOUR DIFFERENT SAMPLE WEIGHTS AS DETERMINED ON BOTH KOHMAN AND TORBAL ET-1 TORSION BALANCES

Sample size (grams)	Butter No. 1	Butter No. 2	Butter No. 3	Butter No. 4	Mean deviation All samples
			Kohman		
10	16.8 ± 0.16	16.5 ± 0.11	16.9 ± 0.19	16.4 ± 0.09	0.14
15	16.6 ± 0.19	16.3 ± 0.11	16.5 ± 0.15	16.4 ± 0.09	0.14
20	16.8 ± 0.09	16.4 ± 0.09	16.6 ± 0.08	16.5 ± 0.11	0.09
25	16.4 ± 0.13	16.1 ± 0.11	16.4 ± 0.08	16.2 ± 0.05	0.09
			Torbal ET-1		
10	16.98 ± 0.10	16.69 ± 0.11	16.74 ± 0.07	16.57 ± 0.06	0.09
15	17.18 ± 0.08	16.19 ± 0.10	16.45 ± 0.10	16.37 ± 0.04	0.08
20	17.82 ± 0.06	16.29 ± 0.04	16.50 ± 0.11	16.39 ± 0.04	0.06
25	16.48 ± 0.06	16.10 ± 0.09	16.47 ± 0.08	16.27 ± 0.05	0.07

fat extractions are made, and the entire residue of salt is titrated with 0.5 N silver nitrate using 10 drops of 5% potassium chromate solution as indicator. Curd is obtained by difference.

For 20- and 25-g samples, the method was varied as follows: butterfat was extracted three times using 75 ml petroleum ether for each extraction. The salt residue was washed into a 500 ml Erlenmeyer flask using two 75 ml water rinses, one hot (72 C) ane one cold (50 C) rinse. Fifteen drops of indicator were added and the salt titrated to a reddish brown end point with 1.711 N silver nitrate.

A 25 ml burette was used at all sample weights. Because the Kohman balance is designed for 10-g samples only, it was necessary to convert scale readings to grams in order to calculate moisture and butterfat percentages on larger samples.

Direct salt analysis

In the direct salt analysis, a known weight of butter is melted in warm distilled water and salt is titrated in the butter-water mixture. Standard industry practice is to weigh a 1- or 2-g sample on the Kohman balance, mix with 50-60 ml of warm water, and titrate with silver nitrate of a normality in which 1 ml is equivalent to 1% salt. Precision depends on the ability to make sensitive and precise weighings and to achieve precise end points in the titration. For this reason, an experiment was designed to compare the two balances at three different sample weights.

Five replicate determinations were made on each of the four butter samples using both balances and 1-, 5-, and 10-g samples. Samples were treated as follows:

(*a*) 1 g samples — The butter was mixed with 50-60 ml of warm water, 4 drops of 5% potassium chromate were added, and the mixture titrated with 0.1711 N silver nitrate to a reddish brown end point.

(*b*) 5 g samples — The butter was mixed with approximately 75 ml of warm water, 6 drops of indicator were added, and samples were titrated with 0.4277 N silver nitrate.

(*c*) 10 g samples — The butter was mixed with approximately 100 ml of warm water, 10 drops of indicator were added, and samples titrated with 0.8555 N silver nitrate.

In all instances, a 10 ml burette was used to dispense the silver nitrate. As a matter of note, 1 ml of the titrating solution in (*a*) above is equivalent to 1% salt. In (*b*) and (*c*), 2 ml of titrating solution are equivalent to 1% salt.

RESULTS AND DISCUSSION

The data obtained in this study were placed on punch cards and analyzed statistically by computer. Table 1 compares the Kohman and Torbal ET-1 Torsion balances in an analysis of variance of moisture and butterfat results, these two components being the ones which are determined gravimetrically and which can be influenced by balance sensitivity. Better consistency was noted for the Torbal ET-1, especially in moisture analysis. Since moisture represents a much smaller portion of the sample than butterfat, balance sensitivity would be expected to play a more important role in moisture determination. Also, it may be noted that sample size is a major source of variability in Kohman results, but is more important in moisture analysis than butterfat analysis. While sample size appears to influence results obtained on the Torbal ET-1, this influence is of approximately the same magnitude for both moisture and butterfat.

The results in Table 2 show mean values and standard deviation of moisture content of four commercial samples analyzed on both the Kohman and Torbal ET-1 balances. In general, standard deviation

TABLE 3. AVERAGE CURD CONTENT[1] OF FIVE REPLICATE ANALYSES OF FOUR COMMERCIAL BUTTER SAMPLES ANALYZED AT FOUR DIFFERENT SAMPLE WEIGHTS ON BOTH A KOHMAN AND TORBAL ET-1 TORSION BALANCES

Sample size (g ams)	Butter No. 1 (%)	Butter No. 2 (%)	Butter No. 3 (%)	Butter No. 4 (%)
		Kohman		
10	0.60	0.69	1.15	1.56
15	1.15	1.04	1.30	1.39
20	0.93	0.88	1.38	1.32
25	0.79	0.71	1.25	1.31
Grand Mean	0.87	0.83	1.27	1.40
		Torbal ET-1		
10	0.99	0.95	1.22	1.40
15	1.01	1.03	1.66	1.64
20	0.80	0.96	1.29	1.44
25	1.04	0.77	0.98	1.17
Grand Mean	0.96	0.93	1.29	1.41

[1]Curd content was measured by difference

values decrease as sample size increases, the magnitude of change being greater for the Kohman balance. In other words, some improvement in precision can be obtained by increasing sample size. For the Torbal ET-1 the improvement is slight, but sufficient, perhaps, to warrant consideration of samples larger than 10 g. On the Kohman balance, significantly better precision was noted up to a sample size of 20 g. Beyond 20 g little or no improvement was obtained and some difficulty was encountered in avoiding spattering loss during heating. Although wide discrepancies occur within the same sample (particularly in the conventional butters), it must be remembered that the samples represented only lots of churnings and no attempt was made to homogenize packages and prints from the same source.

One other fact is worthy of note. As sample size increased, there was an apparent decrease in moisture. In all probability this observed decrease is an artifact. It seems likely that an equivalent color change denoting the moisture end point occurs sooner at higher curd levels. For this reason, samples would be considered "ready" for reweighing prematurely; moisture content would appear lower.

In Table 3 are shown curd levels of the four butter samples as obtained by difference. Although the values vary considerably, as might be expected because of errors compiled in moisture, butterfat, and salt analyses, the grand averages likely come close to representing the true curd content. In the "continous" butters (samples 3 and 4), a difference of slightly more than 0.1% may be observed. Taken in total, the data suggest the potential for significant variation in curd from the assumed 1.4% values used commercially. On the other hand, the variations noted in replicate analyses would seem to preclude use of the Kohman procedure as a method for detecting such differences except where large numbers of samples are involved.

Results of direct salt analyses by balance and sam-

TABLE 4. MEAN AND STANDARD DEVIATION VALUES OF FIVE REPLICATE SALT ANALYSES[1] ON FOUR COMMERCIAL BUTTER SAMPLES AT THREE DIFFERENT SAMPLE WEIGHTS AS DETERMINED ON BOTH KOHMAN AND TORBAL ET-1 TORSION BALANCES

Sample size (grams)	Butter No. 1	Butter No. 2	Butter No. 3	Butter No. 4	Mean deviation All samples
			Kohman		
1	2.06 ± 0.07	2.12 ± 0.06	2.18 ± 0.03	2.24 ± 0.08	0.060
5	2.12 ± 0.03	2.15 ± 0.02	2.19 ± 0.02	2.25 ± 0.03	0.025
10	2.11 ± 0.02	2.14 ± 0.01	2.18 ± 0.03	2.24 ± 0.05	0.028
			Torbal ET-1		
1	2.12 ± 0.05	2.15 ± 0.00	2.20 ± 0.00	2.28 ± 0.05	0.022
5	2.14 ± 0.01	2.15 ± 0.00	2.20 ± 0.00	2.25 ± 0.00	0.003
10	2.18 ± 0.02	2.15 ± 0.00	2.20 ± 0.00	2.25 ± 0.00	0.005

[1]Salt was titrated in butter-water mixture

TABLE 5. MEAN VALUES OF SALT ANALYSES BY TWO METHODS, TWO DIFFERENT BALANCES, AND THREE SAMPLE WEIGHTS

Sample size	Direct-Titration method[1]		USDA method[2]		Difference[3]	
	Kohman (% salt)	Torbal ET-1 (% salt)	Kohman (% salt)	Torbal ET-1 (% salt)	Kohman (%)	Torbal ET-1 (%)
1 g	2.15	2.21	—	—	+.18	+.16
5 g	2.17	2.18	—	—	+.20	+.13
10 g	2.17	2.20	1.97	2.05	+.20	+.15

[1]Salt was titrated in butter-water mixture. Means represent five replicate analyses of four different butter samples.
[2]Salt was determined by the USDA modified Kohman method. Means represent five replicate determinations on four different butter samples.

[3]The difference is between direct titration and USDA results, each balance considered independently.

ple weight are summarized in Table 4. Standard deviation values are included to provide a basis for determining the most precise procedure-sample weight combination. At all sample weight levels the Torbal ET-1 balance provided better precision than the Kohman. For both balances, precision improved using 5-g samples compared to 1-g samples. No further improvement was noted at the 10-g level. Overall, the best precision was obtained using the Torbal ET-1 balance with 5-g samples of butter.

Data in Table 5 indicate that direct salt titration using potassium chromate as indicator yields higher readings than the conventional procedure, averaging 0.19 and 0.15% on the Kohman and Torbal ET-1 balances, respectively. Sample size did not appear to significantly alter the situation. Results remained high and at about the same magnitude. Further tests, to be reported later, involving larger numbers of samples and an improved end point gauge, indicate differences to range (positively) between 0.05 and 0.1%. This agrees closely with the findings of Skelton and Bryant *(6)*, and Weckel *(8)*, and indicates the desirability of using a more critical indicator.

This work would appear to indicate that a balance more sensitive than the Kohman has some potential for improving precision in analyses of butter components. Newer balances, such as the Torbal ET-1, are also speedier and have the additional advantage of allowing more than one complete analysis to be made at a time. The use of exactly 10 g of sample (as limited only to balance sensitivity), while requiring only slightly longer weighing time, makes for simpler, faster calculations for the unskilled employee who has no calculator at his disposal. Proximate weighings and/or larger sample weights might well be considered where appropriate skills and/or suitable calculating devices are available.

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PFIZER INC. AWARD GIVEN TO DR. N. F. OLSON

Dr. Norman F. Olson winner of the 1971 Pfizer Inc. Award was born in the town of Linden in Iowa County, Wisconsin in 1931. He entered the University of Wisconsin in 1949 as a Dairy and Food Industry major and earned his B.S. degree in 1953, M.S. in 1957 and Ph.D. in 1959. He joined the University of Wisconsin faculty in 1958 with assignments in research and teaching and was promoted to the rank of full professor in 1969. He is active in the Bethel Lutheran Church in Madison. Olson's research, both applied and fundamental, has been primarily in fermentations associated with cheese manufacture, mechanization of cheese making, control of cheese defects, and development of methods of analysis for cheese and cheese products. The recipient has authored or co-authored 51 papers in scientific journals during the past 10 years dealing with fermentations, cheese making, cheese quality and safety.

In addition to his research achievements, Olson is recognized as an outstanding teacher and for the economic value of the application of his basic research results to the cheese industry. He currently coaches the University Dairy Products Judging Team. The direct acidification procedures that were developed by him would reduce the manufacturing costs of Pizza, Mozzarella, and Blue Cheese. In addition, he has developed a continuous mechanized procedure for making Mozzarella cheese. Vacuum treatment for eliminating the openess defect in Cheddar cheese is now used on a commercial scale. Discovery of the causes of "pinking" in Italian cheese has solved one of the main economic losses to this industry. His recommendations for use of concentrated cultures have greatly benefitted the cheese industry. In the areas of process cheese and hydrogen peroxide treatment of milk in cheese making, his findings have solved many problems for the industry.

Professor Olson is widely known and respected nationally and internationally by the cheese industry and he is frequently consulted in his native state for solutions to various problems. He participates in a short course that is taught around the state of Wisconsin, is President of the Wisconsin section of the International Association of Milk, Food, and Environmental Sanitarians, and serves on the ADSA Committee on Flavor Nomenclature and Reference Standards. He was a lecturer and section chairman at the 1970 International Dairy Congress in Sydney, Australia.

BIOCHEMICAL CHANGES IN SHRIMP INOCULATED WITH *PSEUDOMONAS, BACILLUS* AND A CORYNEFORM BACTERIUM[1, 2]

B. F. Cobb III, and C. Vanderzant

Animal Science Department, Texas A&M University, College Station, Texas 77843

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Abstract

White shrimp *(Penaeus setiferus)* washed with ethanol and sterile water were inoculated with a fluorescent *Pseudomonas*, non-fluorescent *Pseudomonas*, *Bacillus*, and a coryneform bacterium. Washing reduced the microbial load but growth occurred on control samples during refrigerated storage. Samples inoculated with *Pseudomonas* became putrid 2-3 days sooner than their controls. Addition of coryneform bacteria delayed spoilage. Shrimp inoculated with *Bacillus* spoiled at the same time as the non-inoculated controls. Inoculation of shrimp with *Pseudomonas* species *(a)* retarded development of melanosis; *(b)* produced volatile nitrogen in the atmosphere surrounding shrimp but only after spoilage had taken place; *(c)* caused higher levels of water-soluble protein, non-protein nitrogen, and total volatile nitrogen than in their corresponding controls; and *(d)* reached higher pH levels sooner than the controls. No significant changes occurred in volatile reducing substances. Sterile shrimp juices exhibited more extensive melanosis than juices inoculated with *Pseudomonas*. No marked changes in amounts of soluble protein or non-protein nitrogen were noted upon storage of inoculated juices. Juices inoculated with *Pseudomonas* had higher levels of total volatile nitrogen after storage than comparable controls. Proteolysis by the fluorescent *Pseudomonas* was indicated by major changes in elution profiles on Sephadex G-100. Compared with sterile controls, levels of free amino acids decreased in juices inoculated with *Pseudomonas* or *Bacillus* and stored at 5 C.

Quality deterioration and subsequent spoilage of shrimp is caused by activities of tissue enzymes and microorganisms. Spoilage of shrimp is frequently accompanied by production of offensive volatile chemicals. A number of biochemical changes occur during shrimp spoilage, but there is considerable disagreement about their magnitude and significance. For example, Campbell and Williams *(10)* and Bethea and Ambrose *(7)* reported increases in trimethylamine of iced shrimp while they were still organoleptically acceptable. However, according to Fieger and Friloux *(19)* no increases in trimethylamine occurred until shrimp spoiled. Campbell and Williams *(10)* reported increases in amino nitrogen during storage, Fieger and Friloux *(19)* however reported decreases. Bailey et al. *(4)* indicated that shrimp with a pH greater than 7.95 were spoiled. On the other hand, Bethea and Ambrose *(7)* reported that shrimp were still acceptable until a pH of 8.20 was reached. Because bacterial counts usually increase greatly when spoilage of iced shrimp occurs, biochemical changes which accompany spoilage are usually associated with action of bacterial enzymes. According to Bailey et al. *(4)* the differences in biochemical changes reported by various investigators probably are caused by differences in the microbial flora of shrimp. Various bacterial species have been implicated in shrimp spoilage mainly by inference, i.e., they are part of the predominant flora on shrimp, hence they are responsible for spoilage. Little information is available about the role of individual bacterial species on the biochemical and organoleptical changes of shrimp. This study reports on the effect of species of *Pseudomonas*, *Bacillus*, and a coryneform bacterium on biochemical and organoleptical changes in shrimp. Biochemical parameters included were volatile N, volatile acid, volatile reducing substances (VRS), total volatile nitrogen (TVN), trimethylamine nitrogen (TMN), non-protein nitrogen (NPN), water-soluble proteins, salt-soluble proteins, free amino acids, and changes in size and/or shape of proteins as measured by Sephadex chromatography.

Materials and Methods

Bacteriological analysis

Cultures of *Pseudomonas*, *Bacillus*, and a coryneform bacterium were selected from predominant isolates from Gulf of Mexico shrimp *(26)*. Cultures for inoculation of shrimp and shrimp juice were grown on Plate Count Agar slants (Difco) at 25 C for 24 hr. The organisms were washed three times with 0.85% sterile saline. Dilutions were made to obtain a concentration of approximately 10^3 organisms per gram or milliliter.

White shrimp *(Penaeus setiferus)* were obtained live from Galveston Bay, Matagorda Bay, or Galveston Harbor. They were packed in ice for immediate transportation and use in the laboratory. Shrimp to be inoculated with individual bacterial species were headed and peeled aseptically. They were then rinsed in tap water (1 min), 70% ethanol (20 sec), and twice with sterile de-ionized distilled water (1 min). Sterile "shrimp juice" was prepared as follows. One hundred grams of peeled headed shrimp were blended for 2 min at high speed with 400 ml of sterile distilled water in a Waring blender. The homogenate was strained through a double layer of cheese cloth and centrifuged at 1000 × g for 20 min at 5 C. The supernatant fluid was sterilized by filtration through a Seitz filter. The filtrate was placed in

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100 ml aliquots in sterile screw-cap flasks.

Shrimp were inoculated by immersion in a bacterial suspension of approximately 10^3 organisms per milliliter. They were then placed in covered sterile 90 × 50 mm glass dishes with 5 mm glass beads on the bottom. A sterile 50 × 15 mm dish was placed in the center of the incubation chamber for biochemical test solutions. Shrimp juice was inoculated by addition of appropriate aliquots of the bacterial suspension. Inoculated shrimp, shrimp juice, and controls were stored at 5 C. Bacterial counts and organoleptic evaluations of the samples were made periodically until they were unacceptable. Organoleptic evaluations were based on appearance and odor. Bacterial counts were determined by the agar plate method *(2)* with plate incubation at 32 C for 2 days.

Biochemical methods

Volatile N was measured as follows: Five milliliters of 0.02 N H_2SO_4 containing mixed indicator *(12)* were placed into the center dish of the shrimp incubation chamber. At each sampling period, the center dishes from 3 incubation chambers with control samples and those from 3 incubation chambers with inoculated samples were removed. The dishes from the control chambers and from the test samples were placed in separate vacuum desiccators. The desiccators were subjected to a vacuum (aspirator) for 30 min to remove absorbed volatile acid. The unreacted H_2SO_4 was then titrated with 0.01 N NaOH. In measuring volatile acid, 5 ml 0.02 N NaOH with mixed indicator was placed in the center dish. By titrating to the endpoint of mixed indicator (pH 5.1, Conway indicator) the acidity contributed by carbonate, [apparent pK of 6.352; Bull *(9)*] was eliminated. In this manner the volatile acid value reflects mainly lactate and other organic acids produced. Titrations of solutions of sodium acetate, sodium lactate, and sodium carbonate of known concentrations indicated that approximately 60% of the acetate, 80% of the lactate, and negligible amounts of the carbonate were measured by this procedure. Other analysis made on shrimp extract were made as follows. Shrimp were weighed, minced, and comminuted in a Polytron homogenizer with three volumes of distilled water or buffer (pH 7.2) consisting of 0.45 *M* KCl, 0.0157 *M* Na_2HPO_4, and 0.0031 *M* KH_2PO_4 *(11)*. The resulting mixture was centrifuged and the supernatant decanted. The pellet was resuspended in 2 volumes of water or buffer, then centrifuged, and the supernatant added to the first extract. The protein content of the water extract (water-soluble protein) and buffer extract (salt-soluble protein) were measured by the biuret procedure of Snow *(25)*. TVN in the water and salt extracts was measured by the micro-diffusion method *(12)*. TMN was determined by the method of Beatty and Gibbons as described by Conway *(12)*. NPN was measured by deproteinating the water extract as described by De Wolf et al. *(13)*. The deproteinated extract was then analyzed by the microkjeldahl procedure *(3)* or by the procedure of Jacobs *(22)*. Hydrogen ion concentration in the water extract was measured with a Beckman Zeromatic pH meter. VRS production was measured by the procedure of Farber and Ferro *(15)*. Similar analyses were performed on shrimp juice. A Spinco model 120 C Amino Acid Analyzer was employed to determine the amino acids in the deproteinated shrimp juice. In addition, 1 ml of the inoculated and control shrimp juice was analyzed on a 1.5 × 90 cm Sephadex G-100 column on the first and last days of the experiment. The KCl-Na_2HPO_4-KH_2PO_4 buffer previously described was used. The protein content of the various fractions was determined by reading the optical density at 280 nm. Elution patterns were checked periodically with a mixture of bovine fibrinogen (I, Fig. 2), bovine albumin (II), and spermwhale myoglobin (III) to ascertain reproducibility of analyses.

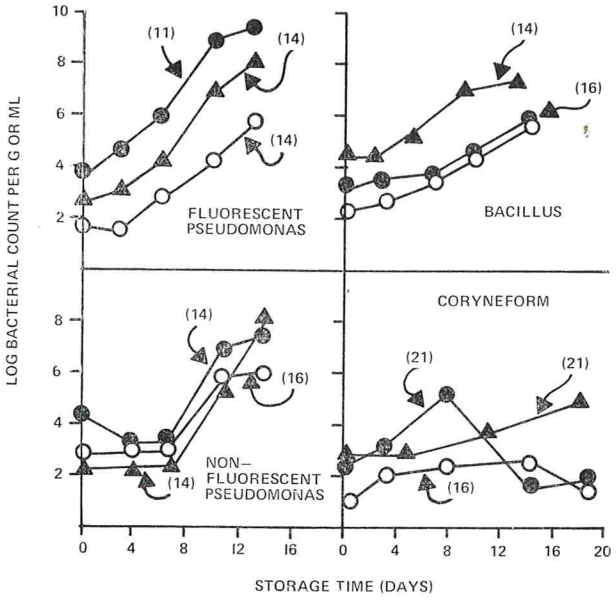

Figure 1. Bacterial counts of control shrimp (O-O), inoculated shrimp (●-●), and inoculated shrimp juice (▲-▲) during refrigerated storage. (Days) after which sample became unacceptable.

RESULTS

Shrimp

Changes in bacterial counts of inoculated shrimp, control shrimp, and inoculated shrimp juice during storage at 5 C are shown in Fig. 1. With the *Pseudomonas* and *Bacillus* species initial population levels of 10^2-10^4 per gram or milliliter developed to 10^8-10^9 during storage. Growth of the coryneform bacteria was not as profuse. The control shrimp (washed with ethanol and water) were not sterile. In 3 of the 4 trials, initial bacterial counts of 10^1-10^2 per gram increased in 13-14 days to approximately 10^5 per gram. At the end of the trial, the difference in population levels between samples inoculated with *Pseudomonas* species and their controls was 0.8-1.8 log; little difference in count existed between the controls and test samples inoculated with *Bacillus* or coryneform bacteria.

Samples inoculated with *Pseudomonas* species showed serious odor defects 2-3 days sooner than their controls. With the *Bacillus* species, rate of growth in the control was similar to that of the inoculated sample. Serious odor defects appeared in both samples at the same time. Shrimp inoculated with coryneform bacteria kept longer (5 days) than the control sample.

On the first day of the trials, control and inoculated

shrimp had a fresh clean odor and were without melanosis. The control samples from Galveston and Matagorda Bay developed moderate melanosis in 7 days and extensive melanosis in 10-13 days. Shrimp from Galveston Harbor, however, showed moderate melanosis in 1-2 days which became extensive in 5-7 days. Initial bacterial counts on these shrimp samples were similar. Incubation of the shrimp with the fluorescent *Pseudomonas* retarded the development of melanosis. A similar phenomenon, but to a lesser extent, was observed with the non-fluorescent *Pseudomonas*. The *Bacillus* species and coryneform bacterium did not influence development of melanosis.

To measure the role of volatile N and volatile acid in the volatile compounds produced by bacteria and/or tissue enzymes, shrimp were placed in incubation chambers and the volatile N and acid were trapped and measured. Only the *Pseudomonas* species produced significant amounts of volatile N but only after spoilage was organoleptically evident (Table 1). Differences also were evident in the odor of the shrimp. Shrimp inoculated with *Pseudomonas* had ammonia or amine-like odors on spoilage, whereas those inoculated with the *Bacillus* or the coryneform bacterium did not. Although some sour odors were detectable after storage for about one week, production of volatile acid was erratic. Both increases and decreases occurred in the controls and inoculated samples. With marked increases in volatile N production, volatile acid levels decreased probably because of absorbed volatile N. Volatile acid production measured in this manner would be mainly from the surface of the shrimp because of the basic pH of the interior (Table 2).

To measure proteolysis and other enzymatic changes caused by bacteria within shrimp, various chemical determinations (Table 2) were made on extracts from shrimp at time of inoculation and when the inoculated samples became unacceptable. Analyses were reproducible within trials, but values for individual characteristics varied from trial to trial. Only the fluorescent *Pseudomonas* caused major increases in water-soluble protein. At the end of the trial there was less salt-soluble protein in the sample inoculated with the fluorescent *Pseudomonas* than in the corresponding control. The increase in salt-soluble protein in the sample inoculated with coryneform bacteria was less than in the corresponding control. NPN levels increased markedly in the sample inoculated with the fluorescent *Pseudomonas*. NPN levels were not obtained for the samples with the non-fluorescent *Pseudomonas* because of an interference of an unknown substance in the shrimp with the Kjeldahl indicator. This phenomenon was encountered both with shrimp and fish from Matagorda Bay *(20)*. Because of this problem the method of NPN analysis was changed to the ninhydrin procedure of Jacobs *(22)*.

Shrimp inoculated with *Pseudomonas* species and stored for 11-14 days had higher levels of TVN than the controls. Upon storage, large increases in TVN were measured in the samples with the coryneform bacteria and its corresponding control. With a rela-

TABLE 1. PRODUCTION OF VOLATILE NITROGEN AND VOLATILE ACID BY CONTROL AND INOCULATED SHRIMP AT 5 C IN INCUBATION CHAMBERS.

Organism	Day	Volatile nitrogen[a]		Volatile acid[b]	
		Control	Inoculated	Control	Inoculated
Fluorescent *Pseudomonas*	4	0	0	0.03	0.04
	8	0	0	0.26	0.36
	11	0	0.15 (spoiled)	0.03	0.08
	14	0.73 (spoiled)	4.56	0	0
Non-fluorescent *Pseudomonas*	4	0	0	0.30	0.17
	7	0	0	0.17	0.22
	11	0	0.13	0.23	0.22
	14	0.13	0.97 (spoiled)	0.43	0
Bacillus	6	0	0	0.30	0.30
	9	0	0	0.33	0.46
	13	0	0	0.17	0.23
	16	0 (spoiled)	0 (spoiled)	0.60	0.40
Coryneform	7	0	0	0.35	0.22
	12	0	0	0.30	0.42
	16	0 (spoiled)	0	0.26	0.24
	21	0	0 (spoiled)	0.21	0.22

[a]mg N per 100 g of shrimp. [b]μeq per g of shrimp.

TABLE 2. BIOCHEMICAL CHANGES OF CONTROL (C) AND INOCULATED (I) SHRIMP STORED AT 5 C.

Organism	Day	Soluble protein[a] Water-soluble C	Water-soluble I	Salt-soluble C	Salt-soluble I	NPN[b] C	NPN[b] I	TVN[c] C	TVN[c] I	TMN[d] C	TMN[d] I	VRS[e] C	VRS[e] I	pH C	pH I
Fluorescent *Pseudomonas*	0	4.1		8.9		0.63		2.49		0.78		0.02		7.3	
	11	5.0	15.6	16.6	12.0	0.63	1.40	4.20	14.38	0.78	0.78	0.02	0.02	7.3	8.0
Non-fluorescent *Pseudomonas*	0	5.4		16.6		–		2.77		0		0.06		7.3	
	14	5.5	6.1	11.6	14.3	–	–	3.71	10.84	0	0	0.04	0.02	7.3	7.5
Bacillus	0	7.0		8.3		0.46		2.66		0		–		7.3	
	16	6.4	6.2	9.9	7.2	0.53	0.53	4.34	3.64	0	0	0.11	0.13	7.5	7.6
Coryneform	0	7.0		10.1		0.66		3.50		0.22		0.02		7.4	
	21	8.4	8.9	20.7	14.6	0.89	0.84	21.70	20.86	5.18	4.62	0.04	0.03	8.0	7.8

[a]g per 100 g of tissue. [b]g per 100 g of tissue. [c]mg per 100 g of tissue. [d]mg per 100 g of tissue. [e]meq per g of tissue.

tively low bacterial population (Figure 1) and lack of ammoniacal odor, the increase in TVN may have been caused by endogenous enzymes. Increases in TMN were encountered in the sample inoculated with the coryneform bacterium and its control. No major changes in VRS were observed in the control and inoculated samples.

Shrimp inoculated with the fluorescent *Pseudomonas* reached a high pH level (8.0) sooner than the other inoculated samples or controls. The presence of coryneform bacteria caused somewhat lower pH values than the corresponding controls. Shrimp became unacceptable in the pH range 7.5-8.0, but more frequently when the pH was near 8.0.

Shrimp juice

Considerable melanosis took place during the preparation of some shrimp juices. Shrimp from Galveston and Matagorda Bay gave a colorless juice which darkened slightly after 8-10 days. Juice prepared from shrimp harvested from Galveston Harbor frequently blackened overnight. Sterile juice exhibited more extensive melanosis than inoculated juice, indicating a possible inhibitory effect on melanin formation by the bacteria used in this study. This inhibitory effect was considerably more pronounced with both *Pseudomonas* species than with either the *Bacillus* or the coryneform bacterium. Except for the fluorescent *Pseudomonas,* the bacteria did not appear to reduce the level of melanin pigment once it had formed.

A putrid odor developed in the shrimp juices inoculated with *Pseudomonas* or *Bacillus* species, a slight fruity odor was noticed in the juice with coryneform bacteria. A slight amine-like odor developed in the sterile juices suggesting a possible role of endogenous enzymes in deterioration of shrimp.

No major differences in amounts of soluble proteins existed between sterile and inoculated juices stored at 5 C for 14-21 days (Table 3). Minor changes in soluble protein took place during storage both in the sterile and inoculated juices. The method of analysis (biuret) was able to discern only extensive protein synthesis or catabolism but not changes in size or shape. However, Sephadex chromatography indicated that marked changes occurred in molecular species III and IV (Figure 2) of the elution profile of samples inoculated with the fluorescent *Pseudomonas.* Precipitation on the column of proteins of juice inoculated with *Bacillus* also indicated that alterations in the protein had occurred. Changes in the elution profiles of the sterile controls were often evident but appeared to follow no particular pattern.

In most instances only minor changes in NPN were noted during storage of the sterile or inoculated juices. However, some increases in NPN occurred in the juice inoculated with *Bacillus* and the sterile control. No marked differences in NPN levels existed between inoculated juices and their corresponding

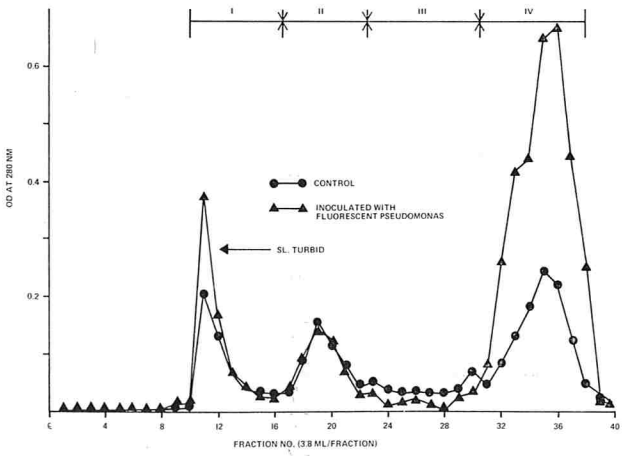

Figure 2. Elution profile from Sephadex G-100 column of control and inoculated shrimp juice.

controls. This observation was substantiated by the differences in NPN values calculated from amino acid chromatograms (Table 4). Net changes were usually of the magnitude 0.01-0.02 mg N per milliliter juice, values lower than the error of the method of analysis.

Large quantities of TVN were produced in the juice inoculated with the fluorescent and non-fluorescent *Pseudomonas*. Increases in their sterile controls were relatively small. Large increases in TVN were also observed in the juice inoculated with the *Bacillus* species and in the sterile control. After storage, differences in TVN between juices inoculated with *Bacillus* or coryneform bacteria and their respective controls were small. Subsequent measurement of ammonia with the amino acid analyzer (Table 4) indicated that in juices inoculated with *Pseudomonas* and *Bacillus* ammonia production was less than TVN. This suggests that a considerable amount of volatile N was not ammonia. Only the *Bacillus* species produced marked amounts of trimethylamine in the juice.

The amino acid levels of sterile and inoculated shrimp juice are presented in Table 4. All of the protein amino acids except cystine and tryptophan were present in measurable amounts. Glycine comprised almost one-half of the ninhydrin-positive material. High levels (>150μM/ml) of ornithine, ammonia, arginine, taurine, proline, and alanine were present. There was evidence in many samples of small amounts of citrulline. Considerable proteolytic and arginase activity was evident in the sterile controls. During 14 days storage of sterile controls significant increases were noted in ornithine, ammonia, lysine, aspartic acid, threonine, proline, glycine, alanine, and valine; and marked decreases in arginine and tyrosine. The decreases in arginine were much smaller than the increases in ornithine. Differences in amino acid levels of inoculated and control samples (Table 4) showed that decreases in nine amino acids occurred in the sample inoculated with the fluorescent *Pseudomonas*. Major decreases were noted in arginine, serine, and glutamic acid; large increases in alanine and ammonia. The decrease in arginine was not accompanied by an increase in ornithine indicating probable utilization in protein synthesis. There was evidence of arginase activity in the samples inoculated with the non-fluorescent *Pseudomonas* and *Bacillus* species. In both samples there was a decrease in arginine and increase in ornithine. Decreases in serine and glutamic acid and increases in ammonia also were noted in these samples.

The juice inoculated with coryneform bacteria showed increases in ammonia, glutamic acid, glycine, and alanine, and a decrease in serine.

DISCUSSION

To determine the effect of individual and mixed bacterial species on biochemical changes, shrimp free of the natural and acquired microbial flora

TABLE 3. BIOCHEMICAL CHANGES OF CONTROL (C) AND INOCULATED (I) SHRIMP JUICE STORED AT 5 C.

Organism	Day	Soluble protein[a]		NPN[b]		TVN[c]		TMN[d]		Molecular species (%)[e] I		II		III		IV	
		C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I
Fluorescent *Pseudomonas*	0	0.74	0.86	0.75	0.74	1.17		0.91		18.7		21.3		17.0		43.0	
	8					2.23	2.23	0.66	0								
	11					2.62	3.92	1.30	0.27								
	14	1.00	0.94	0.85	0.84	2.23	11.11	0	0	19.6	20.2	14.5	12.0	19.1	5.8	46.9	62.1
Non-fluorescent *Pseudomonas*	0	0.89	0.83	0.77	0.75	3.05		0.02		14.4		10.8		5.2		70.7	
	8					3.05	2.99	0.07	0.10								
	12					3.18	3.88	0	0.03								
	14	0.88	0.76	0.81	0.75	3.98	7.73	0.27	0.20	—[f]	11.5	—	11.8	—	7.4	—	69.3
Bacillus	0	0.78	0.79	0.60	0.71	2.63		0		14.0		4.5		20.2		61.3	
	5					3.40	3.81	0.03	0.14								
	9					16.53	18.28	0.38	0.31								
	14	0.91	0.95	0.94	1.07	21.56	23.31	0.81	1.41	18.5	ppt[g]	13.2	ppt	17.5	ppt	50.8	ppt
Coryneform	0	0.83	0.93	0.75	0.72	2.89		0	0	9.6		6.9		14.7		68.8	
	5					3.04	3.04	0.07	0								
	11					3.07	3.57	0	0								
	16					3.43	4.51	0	0.03								
	21	0.92	1.01	0.73	0.73	3.54	5.05	0.03	0.07	17.8	16.8	5.0	4.8	11.8	14.5	65.6	63.9

[a]g per 100 ml of juice. [b]mg per ml of juice. [c]mg per 100 ml of juice. [d]mg per 100 ml of juice. [e]Determined by chromatography on Sephadex G-100. [f]Laboratory accident. [g]Samples precipitated on column.

TABLE 4. CHANGES IN AMINO ACIDS (μMOLE/100 ML) OF CONTROL AND INOCULATED JUICE.

Amino Acid	Controls[a]		Inoculated-control[b]			
	Day 0	Day 14	Fluorescent *Pseudomonas*	Non-fluorescent *Pseudomonas*	*Bacilius*	*Coryneform*
Ornithine	178	310		+20	+128	
Ammonia	379	541	+216	+86	+ 20	+169
Lysine	30	41		+ 7	− 41	
Histidine	27	28	− 9			
Arginine	398	308	−237	−42	−234	
Taurine	286	308				
Aspartic Acid	27	35				
Threonine	39	47				
Serine	90	93	−106	−46	− 65	− 55
Glutamic Acid	130	125	− 70	−82	− 22	+ 19
Proline	440	478				+176
Glycine	2344	2500				
Alanine	441	528	+130			+ 85
Half Cystine	trace	trace				
Valine	40	50	− 12			
Methionine	32	30				
Isoleucine	25	27	− 10			
Leucine	42	40	− 15			
Tyrosine	27	20	− 8			
Phenylalanine	19	23	− 7			

[a]Average values of 4 samples
[b]Inoculated less control on the last day of the experiment (see Table 3)

would be the ideal substrate. Sterilization by heat causes extensive chemical changes in shrimp protein which would make any subsequent changes by microorganisms difficult to interpret. Although washing of peeled shrimp greatly reduced the bacterial load, extensive bacterial growth occurred in the controls during refrigerated storage. It is possible that the removal of a subsurface section of shrimp similar to the procedure employed for deep muscle tissue of beef muscle *(27)* may provide sterile shrimp tissue in future experiments. The use of shrimp juice was based on the report by Lerke et al. *(23)* in which the spoilage patterns of press juice of English sole were nearly identical to those of fillets.

Shrimp inoculated with *Pseudomonas* species developed a putrid amine-like odor and were sooner organoleptically unacceptable than those inoculated with *Bacillus* or coryneform bacteria. The latter became unacceptable because of a putrid odor which differed from that caused by *Pseudomonas* because of absence of amine-like substances. The differences in bacterial counts between shrimp inoculated with *Pseudomonas* and their controls probably represented primarily growth of the inoculum. Shrimp inoculated with coryneform bacteria had an acceptable odor for 5 days longer than their controls. This phenomenon could have been caused by an antagonistic effect of the coryneform bacteria on microbial species that survived the ethanol treatment and ordinarily produce organoleptically detectable defects. Antagonistic relations between bacteria through mechanisms such as competitive utilization of nutrients, production of antimicrobial substances, and changes in pH or redox potential are frequently observed in mixed microbial population of foods. Whether or not the taste of shrimp inoculated with coryneform bacteria would be acceptable for a longer period than that of the control has not been established. Lerke et al. *(23)* reported that *Pseudomonas* species from English sole spoiled sterile muscle press juice, whereas coryneform bacteria did not.

Of the various biochemical indicator tests, production of TVN probably gave the most consistent results. As quality of shrimp and juice deteriorated during storage, TVN increased particularly in the inoculated samples. In the atmosphere of the incubation chambers, volatile N did not increase markedly until spoilage had already occurred. The amount of volatile N in the atmosphere surrounding the shrimp in the chambers (Table 1) was not indicative of the TVN content of the shrimp (Table 2). In the sterile control juices progressive increases in TVN were noted indicating that endogenous enzymes also contribute to TVN production.

TVN and TMN are used to determine the limits of acceptability of shrimp in some sectors of the Australian and Japanese markets *(24)*. The limits are set at 5 mg TMN and/or 30 mg of TVN per 100 g of shrimp muscle. A much lower value of 1.5 mg TMN per 100 g shrimp has been suggested as indicative of spoilage of Gulf Coast shrimp *(19)*. In

the present study, the level of TMN could not be related to quality deterioration. Trimethylamine is generally considered to be the result of bacterial action on trimethylamine oxide *(16)*. The lack of TMN production in this study probably was caused by low levels of TMA oxide in the shrimp (Cobb, unpublished results). In addition, it has been reported that the TMA content of shrimp varies with the salinity of water *(28)* and with the species of shrimp *(21)*. These observations suggest that TMN production depends on many factors and that it may not be a reliable indicator of spoilage.

Nessler nitrogen values increased as spoilage of shrimp from the Gulf of Mexico begins *(18)*. Nessler nitrogen values remained at 2.5 mg per 100 g of shrimp until spoilage ensued. Whether or not these values represent true TVN is not known as the procedure was not given. TVN measurements in the present study were above 3.5 mg per 100 g for shrimp that were spoiled or near spoilage. These values are low compared with those accepted by the Japanese and Australians (<30 mg) and those reported by Iyengar et al. *(21)* for shrimp from India (about 15 mg TVN per 100 g of shrimp). It is possible that differences in origin, shrimp species, and level of organoleptic acceptability may have been involved. On the other hand, washing of the shrimp may have been responsible for low TVN values. This is supported by recent laboratory data (unpublished results) which indicate that unwashed Gulf Coast shrimp of borderline quality had about 15 mg TVN per 100 g. The level of VRS was considerably greater than that reported by Farber *(14)* for Pacific shrimp (*Pandalus* species). This may have been caused by residual ethanol from the washing procedure.

The fluorescent *Pseudomonas* sp. and the *Bacillus* sp. exhibited proteolytic activity, as evidenced by changes in the Sephadex chromatography patterns. However, this activity did not cause increased levels of free amino acids in the shrimp juice, suggesting that the enzymatic activity was mainly of the endopeptidase type or that the amino acids were rapidly metabolized by the bacteria. Experiments were concluded as soon as putrid odors developed in the juice. This may have been too soon for exopeptidase activity to develop. Adamcic et al. *(1)* reported that a pigmented *Pseudomonas* species did not initially change the levels of free amino acids in chicken skin, but produced marked increases in amino acids during the late log phase after off odor had developed. The effects of the fluorescent *Pseudomonas* on shrimp were similar to those reported for the effects of *Pseudomonas fragi* on porcine muscle by Borton et al. *(8)*. Salt-soluble protein nitrogen decreased while water-soluble protein nitrogen and NPN increased in the inoculated sample during storage.

Both proteolytic and arginase activity were discernable in the sterile shrimp juice. Increases in free amino acids suggest exopeptidase activity. The increase in ornithine without a corresponding decrease in arginine probably was caused by release of the latter by proteolysis with subsequent conversion to ornithine by arginase. The non-fluorescent *Pseudomonas* sp. and *Bacillus,* increased the conversion of arginine to ornithine suggesting that these organisms possess arginase activity. The production of ornithine from arginine should have resulted in the production of urea. No urea was detected indicating that the shrimp muscle possessed urease activity also.

The formation of melanin pigment in shrimp has been ascribed to the presence of enzymes in the shrimp *(5, 6, 17)*. Melanosis is an oxidative reaction with tyrosine as a precursor. The four bacterial cultures inhibited the formation of melanosis in shrimp juice, but only the *Pseudomonas* species reduced melanosis on the washed shrimp. Bacterial inhibition of melanosis could proceed through several mechanisms: (I) by utilization of the substrate tyrosine, (II) the formation of a reducing medium, (III) alteration of the enzymes responsible for melanin formation, and (IV) the metabolism of melanin. If bacterial inhibition proceeded through mechanisms I, III, and IV, melanosis should have been inhibited on the washed shrimp and in the shrimp juice. If, however, the inhibition were through mechanism II one could expect less inhibition on the surface of the shrimp than in the shrimp juice. The fluorescent *Pseudomonas* which strongly reduced melanin formation in both shrimp juice and shrimp, showed considerable proteolytic activity and could have inhibited melanosis through any of the suggested mechanisms. The non-fluorescent *Pseudomonas,* which reduced melanosis slightly in the whole shrimp and in shrimp juice did not decrease the level of tyrosine in the juice and did not exhibit measurable proteolytic activity. These observations suggest that with this culture reduction of melanosis was via either mechanism II or IV. Since the other bacteria reduced melanosis mainly in the juice and not on the peeled shrimp, the most logical mechanism was the formation of a reducing medium. Whether or not these bacteria could reduce to any extent melanin formation in intact shrimp tails is not known.

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3-A STUDIES STANDARDS FOR LARGE CAPACITY INSTALLATIONS

An amendment to the 3-A Fillers and Sealers Standard and a supplement to the Instrument Fittings Standard were officially adopted and authorized for publication at the Fall 3-A Sanitary Standards Committees meetings.

Meeting October 5-7 in Atlanta, the group reviewed drafts of tentative standards for scraped surface heat exchangers, uninsulated storage tanks and farm storage tanks. All three pieces of equipment incorporate critical design features and are needed by the changing dairy industry to cope with large capacity installations, both in plants and on farms. The drafts were returned to the Technical Committee of the Dairy and Food Industries Supply Assn. (DFISA) for revision.

Amendments were taken up in connection with the Fillers and Sealers, and the Fittings Standards; a complete revision of the Pump Standard was also reviewed by the industry segment of the Committees.

Joe Larson, President of DFISA, was keynote speaker for the biannual meeting. The Sparta Brush Company President pointed out the need for continued cooperation from all segments of the industry in advancing the 3-A program and other worthwhile projects.

E-3-A SANITARY STANDARDS FOR NON-COIL TYPE BATCH PASTEURIZERS

Serial #E-2400

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
United States Department of Agriculture
Institute of American Poultry Industries
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USPHS, USDA, IAPI, and DFISA in connection with the development of E-3-A Sanitary Standards, to allow and encourage full freedom for inventive genius or new developments. Batch Pasteurizer specifications heretofore and hereafter developed which so differ in technique, design, material and construction or otherwise, so as not to conform to the following standards but which are, in the opinion of the manufacturer or fabricator, equivalent or better, may be submitted at any time for consideration by IAMFES, USPHS, USDA, IAPI, and DFISA.

A. *SCOPE*

These standards cover sanitary aspects of non-coil type batch pasteurizers used to pasteurize liquid egg products, including those appurtenances necessary to meet pasteurization requirements. Batch pasteurizers may be either of the atmospheric or closed type. The latter may be operated at pressures from below to above that of the atmosphere. In order to conform with these E-3-A Sanitary Standards, Non-Coil Type Batch Pasteurizers shall comply with the following in design, material, and fabrication criteria.

B. *DEFINITIONS*

(1) *Product:* Shall mean liquid egg products.

(2) *Surfaces*:

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

(b) *Non-Product Contact Surfaces*: Shall mean all other exposed surfaces.

(3) *Lining*: Shall mean all surfaces used to contain the product including the ends, sides, bottom and top.

(4) *Shell*: Shall mean the material covering the exterior of the insulation and/or heat exchange jacket.

(5) *Breast*: Shall mean that portion of the metal used to join the top of the lining to the top of the shell on an atmospheric pasteurizer.

C. *MATERIALS*

(1) All product contact surfaces, including the breast, shall be of 18-8 stainless steel with a carbon content of not more than 0.12 percent, or equally corrosion resistant metal that is non-toxic and non-absorbent, except that:

(a) Rubber and rubber-like materials may be used for measuring devices (except measuring sticks), slinger or drip shields, agitator seals on vacuum and/or pressure pasteurizers, agitator bearings, protective caps for openings (other than manhole) and/or sanitary fittings, scraper blades, gaskets, seals and parts used in similar applications. These materials shall comply with the applicable provisions of the "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Egg Processing Equipment, Serial #E-1800".

(b) Plastic materials may be used for bearings, measuring devices (except measuring sticks), slinger or drip shields, agitator seals on vacuum and/or pressure pasteurizers, agitator bearings, protective caps for openings (other than manhole) and/or sanitary fittings, sight and light parts, scraper blades, gaskets, seals and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000," as amended.

(c) Where functional properties are required for specific applications, such as bearing surfaces and rotary seals where dissimilar materials are necessary, metal equal in corrosion resistance to 18-8 stainless steel with a carbon content of not more than 0.12 percent, carbon and ceramics may

be used. Ceramic materials shall be inert, non-absorbent, non-porous, non-toxic, insoluble, resistant to scratching, scoring, and distortion by the temperature, chemicals, and methods to which they are normally subjected in operation, or cleaning and bactericidal treatment.

(d) *Glass*: If a sight and/or light glass(s) is provided, it shall be of clear heat resistant glass.

(2) All non-product contact surfaces shall be of corrosion-resistant material, or material that is rendered corrosion-resistant. If painted, the paint used shall adhere. Non-product contact surfaces shall be relatively non-absorbent, cleanable and durable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D. *FABRICATION*

(1) All product contact surfaces, covers, fittings and appurtenances shall be easily accessible, and readily cleanable either when in operating position or when removed. Metallic surfaces shall be at least as smooth as No. 4 mill finish on stainless steel sheets or 120 grit finish properly applied. All permanent joints shall be welded, and all weld areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

(2) *Lining*: The lining shall remain in a relatively fixed position within the shell or body of the pasteurizer and shall be so constructed that it does not sag, buckle or become distorted in normal use. The bottom of the lining shall have a minimum pitch of 1/4 inch per foot toward the outlet. All corners in the lining having inside angles of less than 135 degrees shall have radii of not less than 1/2 inch. All corners in accessories, bridges, or appurtenances which are welded to the lining, and have inside angles of less than 135 degrees, shall have minimum radii of 1/4 inch. The design of the outlet shall conform to D. (9).

(3) *Shell*: All seams and openings in the shell shall be effectively sealed against moisture and vermin.

(4) *Breast*: The breast shall be integral with or welded to the lining, and shall be sloped so that drainage is away from the lining. The junction of the breast and the shell shall be welded or effectively sealed.

(5) *Main Covers and Bridges for Atmospheric Type Pasteurizers*:

(a) The main cover(s) shall be of a type which can be opened and maintained in an open position, shall be sufficiently rigid to prevent buckling, shall be self-draining in the closed position, shall be close fitting, and all edges shall have downward flanges of not less than 3/8 inch. The inside corners of the cover(s) shall have 1/4 inch minimum radii. The design shall be such that when raising the the cover(s), any liquid on the top will not enter the pasteurizer. When the cover(s) is in its fully opened position, the drops of condensate formed on the underside of the cover(s) shall not drain into the pasteurizer. The cover(s) shall be provided with an adequate, conveniently located and durable handle(s) of sanitary design, which is welded in place or formed into the cover material.

(b) The bridge(s) and/or the fixed cover(s) shall pitch to the outside edge(s) of the pasteurizer for complete drainage, and shall have a raised flange not less than 3/8 inch in height where the edge(s) meet the main cover(s). The bridge(s) and/or the fixed cover(s) shall be integral with or welded to the lining, and shall be installed so the underside is accessible for cleaning and inspection without completely entering the pasteurizer.

(6) *Manhole Covers for Closed Type Pasteurizers*: The cover for a manhole in the side wall shall be either the inside or outside swing type. If the cover swings inside, it shall also swing outside, away from the opening. Threads or ball joints employed to attach the manhole cover(s) and its appendages shall not be located within the lining. If mounted on top of the pasteurizer, the manhole cover(s) shall be of the outside swing type.

(7) *Gaskets and Gasket Grooves for Manhole Covers, Sight and Light Glasses*: Gaskets shall be removable or permanently bonded. Gasket retaining grooves for removable gaskets shall be no deeper than their width. The minimum radius of any internal angle in a gasket retaining groove shall be not less than 1/8 inch, except that a 3/32 inch radius is permissible where a standard 1/4 inch O-Ring is to be used.

Grooves in gaskets shall be no deeper than their width and the minimum radius of any internal angle shall be not less than 1/8 inch unless the gasket is readily reversible for cleaning.

(8) *Openings*: The edges of all openings in the top enclosure, main cover(s) or the bridge(s) shall be extended upwards at least 3/8 inch.

All openings into the lining not continually in use shall be provided with removable covers, which are designed to make close contact with the upper edges of the opening or cover surface, and when in the main cover, the removable covers shall remain in position when the main cover is in an open position.

(a) *Thermometer openings*: Connections and/or openings, which will accommodate indicating, recording and air space thermometers shall be provided. The connections and/or openings shall be located in the top enclosure, cover, bridge, or through the side wall. Thermometer wells may be used. When installed thru the side wall, the location shall be such that the thermometer(s) is easily readable. Thermometer connections and/or openings shall be located so that the thermometer is not influenced by the heating or cooling medium. All connections and/or openings shall conform to applicable parts of "E-3-A Sanitary Standards for Instrument Fittings and Connections Used on Egg Products Equipment, Serial #E-0900," as amended and supplements thereto.

(b) *Agitator openings*: The agitator shaft opening through the bridge or top enclosure shall have a minimum diameter of one inch on pasteurizers which require removal of the agitator shaft for cleaning, or be of a diameter that will provide a 1-inch minimum annular cleaning space between the agitator shaft and the inside surface of the flanged opening on pasteurizers which do not require removal of the agitator for cleaning. A shield that can be raised or dismantled, to permit the cleaning of all its surfaces, shall be provided to protect against the entrance of dust, oil, insects and other contaminants into the pasteurizer through the annular space around the agitator shaft.

(c) *Openings for Sanitary Piping*: The edges of the inlet openings shall extend upward at least 3/8 inch or be fitted with a permanently attached sanitary fitting conforming to "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Serial #E-0800." If fitted with a permanently attached sanitary fitting, the inlet opening may extend outward horizontally.

(d) *Manhole Opening*: The dimensions of a manhole opening shall not be less than 15" x 20" oval, 12" x 27" elliptical, or 18" diameter, except that pasteurizers with a capacity of 300 gallons or less may have top opening manholes having a diameter of not less than 16".

(e) *Sight and Light Glass Openings*: When provided, the opening(s) shall be of such design and construction that the inner surface of the glass will be relatively flush with the lining and the glass may be removed for cleaning. The diameter of the opening(s) into the lining shall be not less than 3 3/4 inches. The opening(s) shall be of such design and construction that there will be adequate agitation and circulation in all areas.

(f) *Air Space Heater Openings*: An air space heater opening(s) shall be provided and shall extend upward at least 3/8 inch or be fitted with a permanently attached sanitary fitting conforming to "E-3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Serial #E-0800."

(g) *Opening for Mechanical Cleaning*: An opening and connection(s) for mechanical cleaning shall be provided in pasteurizers, the inside height of which exceeds 96 inches. The opening shall extend upward at least 3/8 inch or be fitted with a permanently attached sanitary fitting conforming to "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Serial #E-0800."

(9) *Outlet and Outlet Valve*: The outlet, flare, and the outlet valve shall conform to the design and construction provisions of the "E-3-A Sanitary Standards for Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers,

Serial #E-1400."

The inside diameter of the outlet passage on pasteurizers with a capacity of 20-gallons or less shall not be less than the inside diameter of 1-inch sanitary pipe (.902 in.), or on pasteurizers of greater capacity, not less than 1.25 inch. The outlet opening shall be located at the lowest point of the lining.

The outlet and the outlet valve shall be so designed that either a single service or a multiple use gasket can be used.

The outlet valve shall be removable for cleaning. The valve shall be considered removable when secured by not more than four hex nuts.

(10) *Agitators*: The agitator shall be of sufficient size and powered to provide uniformity of composition and temperature throughout the product during the holding period to the extent that the simultaneous temperature difference between the product at the center of the pasteurizer and the coldest product in the pasteurizer will not exceed 1°F. at any time during the holding period. This shall be deemed to be satisfied if the agitator is so designed as to sweep the product current effectively through all zones occupied by the product, including the outlet flare, but excluding inlet pipes surrounded by product in the process of pasteurization and open to the pasteurizer at the bottom.

The inside angles of portions of the agitator having product contact surfaces shall have minimum radii of 1/4 inch.

The agitator shall be readily cleanable and shall be one of the following types:

(a) *Top Entering Non-Removable Type*: The top entering non-removable agitator shall be readily accessible and cleanable. There shall be at least a 1/2 inch space between the non-removable agitator and the bottom of the lining, unless the agitator is mounted on a hinged type cover.

(b) *Top Entering Removable or Demountable Type*: The top entering removable or demountable agitator shall be provided with an easily accessible, readily demountable coupling of either a sanitary type located within the lining or a coupling located outside of the lining provided that it is above the shield provided to protect the annular space around the shaft. All product contact surfaces of the agitator shall be visible when the agitator is removed. A bottom support or guide, if used, shall be welded to the lining, shall not interfere with drainage of the pasteurizer and the inside angles shall have minimum radii of 1/8 inch. When the agitator shaft has a bearing cavity, the diameter of the cavity shall be greater than the depth. The agitator shall be easily demountable for cleaning of the bearing and any shaft cavity.

(c) *Side or Bottom Entering*: The side or bottom agitator and shaft, including the complete seal, shall be readily demountable for cleaning. Non-removable parts having product contact surfaces shall be designed so that the product contact surfaces are readily cleanable from the inside of the pasteurizer.

Seals for the agitator shaft shall be of a packless type, sanitary in design, with all parts readily accessible for cleaning.

(11) *Agitator Mounting*: The driving mechanism shall be securely mounted in a position that will provide a minimum distance of 4 inches measured vertically downward from the bottom of the driving mechanism housing, excluding bearing bosses and mounting bosses, to the nearest surface of the pasteurizer; and in such a manner that all surfaces of the pasteurizer under or adjacent to the driving mechanism shall be readily accessible for cleaning and inspection.

(12) *Supports*: Adjustable legs, if provided, shall be of sufficient number and strength and so spaced that the filled pasteurizer will be adequately supported. Legs shall have closed bases. Exteriors of legs and leg sockets shall be readily cleanable. Supports shall be such that a 6 inch minimum clearance will be provided between the floor and the bottom of a pasteurizer 72 inches or less in diameter or width or an 8 inch minimum clearance for a pasteurizer more than 72 inches in diameter or width. On pasteurizers having bottom entering agitators, the clearance from the floor to the lowest point of the agitator or agitator drive shall conform to these dimensions.

(13) Non-product contact surfaces shall have a smooth finish, be free of pockets and crevices and be readily cleanable.

(14) Non-product contact surfaces to be painted shall be effectively prepared for painting.

(15) *Air Space Heater*: Means shall be provided for pasteurizers to keep the atmosphere above the

product at a temperature not less than 5°F. higher than the product temperature during the heating period, and not less than 5°F. higher than the required temperature of pasteurization during the holding period.

The air space heater(s) shall be mounted within the space between the top enclosure, cover or bridge of the pasteurizer and the level of the product when the pasteurizer is filled to its rated capacity.

The air space heater(s) shall be easily demountable for cleaning.

The air space heater(s) shall be installed through the cover, bridge, or top enclosure of the pasteurizer, and sanitary fittings conforming to the design and construction provisions of the "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Serial #E-0800," shall be used.

(16) A warning plate, furnished and permanently affixed by the manufacturer, shall be provided for closed-type pasteurizers which shall indicate the maximum operating pressure and/or vacuum under which the pasteurizer may be safely operated.

(17) Vessels made in conformance to these standards shall have a statement on the name plate or on the warning plate, or on a plate furnished and permanently affixed by the manufacturer that the vessel is a pasteurizer.

APPENDIX

A. *AIR SPACE HEATING FACILITIES*

Steam for Product Contact: Culinary steam should be provided for air space heaters.

The following procedures for providing steam of culinary quality are recommended:

SOURCE OF BOILER FEED WATER. Potable water or water supplies acceptable to the regulatory agency having jurisdiction shall be used. Water containing organic materials such as leaves, algae, detergents, etc., should not be used for boiler feed water without adequate pretreatment.

FEED WATER TREATMENT. Feed waters must be treated if necessary for proper boiler care and operation. Boiler feed water treatment and control should be under the supervision of trained personnel or a firm specializing in industrial water conditioning. Such personnel should be informed that the steam is to be used for culinary purposes. Pre-treatment of feed waters for boilers or steam generating systems to reduce water hardness before entering the boiler or steam generator by ion exchange or other acceptable procedures is preferable to addition of conditioning compounds to boiler waters.

A number of compounds are used to prevent corrosion and scale in boilers or to facilitate sludge removal. On October 15, 1969 a list of boiler water additives for the preparation of steam in contact with food was published in the Federal Register which conform to othe Food Additives Amendment of the Food, Drug and Cosmetic Act. The substances listed are:

- Acrylamide-sodium acrylate resin[1]
- Ammonium alginate
- Cobalt sulfate (as catalyst)
- Lignosulfonic acid
- Monobutyl ether of polyoxyethylene glycol[2]
- Monobutyl ether of polyoxypropylene glycol[2]
- Polyethylene glycol[3]
- Polyoxypropylene glycol[4]
- Potassium carbonate
- Sodium acetate
- Sodium alginate
- Sodium aluminate
- Sodium carbonate
- Sodium glucoheptonate[5]
- Sodium hexametaphosphate
- Sodium humate
- Sodium hydroxide
- Sodium lignosulfonate
- Sodium metasilicate
- Sodium nitrate
- Sodium phosphate (mono-, di-, tri-)
- Sodium polyacrylate
- Sodium polymethacrylate
- Sodium silicate
- Sodium sulfate
- Sodium sulfite (neutral or alkaline)
- Sodium tripolyphosphate
- Tannin (including quebracho extract)
- Tetrasodium EDTA
- Tetrasodium pyrophosphate

[1]Contains not more than 0.05 percent by weight of acrylamide monomer.
[2]Minimum molecular weight 1,500.
[3]Molecular weight 200-9,500.
[4]Minimum molecular weight 1,000.
[5]Less than 1 part per million cyanide in the sodium glucoheptonate.

No greater amount of the above boiler water treatment compounds should be used than the minimum necessary for controlling boiler scale or other boiler water treatment purposes and no greater amount of steam should be used than necessary.

Tannin is also frequently added to boiled water to facilitate sludge removal during boiler blow-down. This product, although included in the above list of approved boiler additives, has been reported to give rise to odor problems, and for this reason should be used with caution.

Boiler compounds containing cyclohexylamine, morpholine, octadecylamine, chromium and hydrazine are *not permitted* for use in steam in contact with liquid egg products.

BOILER OPERATION. A supply of clean, dry and saturated steam is necessary for proper equipment operation, therefore, boilers and steam generation equipment should be operated in such a manner as to prevent foaming, priming, carry-over and excessive entrainment of boiler water into the steam. Carry-over of boiler water additives can result in the

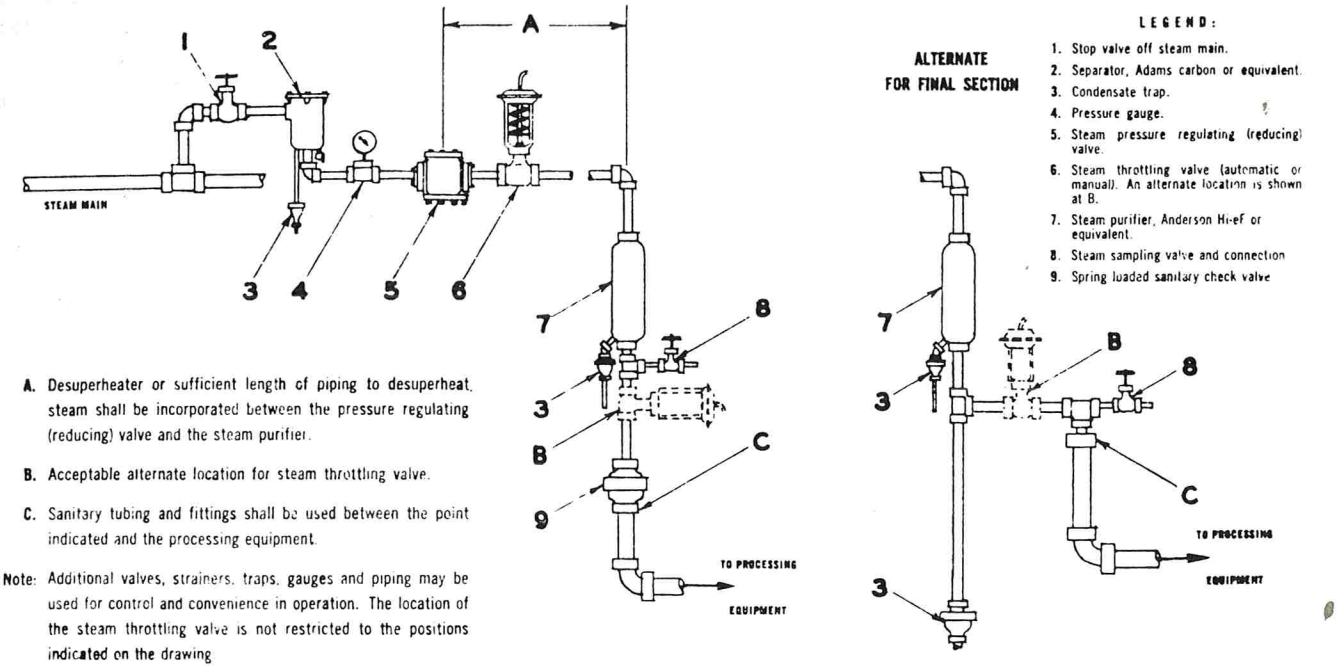

Figure 1.

production of product off-flavors. Manufacturers instructions regarding recommended water level and blow-down should be consulted and rigorously followed. The blow-down of the boiler should be carefully watched, so that over-concentration of the boiler water solids and foaming are avoided. It is recommended that periodic analyses be made of condensate samples. Such samples should be taken from the line between the final steam separating equipment and the point of the introduction of steam into the product.

CULINARY STEAM SUPPLY LINE. The steam pipe line between the steam main and the point of introduction of steam into the pasteurizer should be equipped with at least the following depicted units of adequate size for control and safety purposes as shown in Figure 1:

B. *THERMOMETERS*

(1) *Indicating Thermometers for Batch Pasteurizers*:

(a) Type-Mercury-actuated, direct-reading, contained in a corrosion-resistant case which protects against breakage and permits easy observation of column and scale; filling above mercury, nitrogen, or equally suitable gas.

(b) Magnification of Mercury Column-To apparent width of not less than one-sixteenth of an inch.

(c) Scale-Range, a double scale 30°F to 110°F and 128°F to 153°F or a single scale 30°F to 153°F, extension of scale on either side permitted; graduated in not more than 2 degree divisions between 30°F and 110°F and in 1 degree graduations between 128°F and 153°F with not more than 16 degrees per inch of scale between 128°F and 153°F; protected against damage at 220°F.

(d) Accuracy-Within 0.5°F, plus or minus.

(e) Stem Fitting—Fitting should fit one of the connections or fittings described in "E-3-A Sanitary Standards for Thermometer Fittings and Connections Used on Egg Products Equipment, Serial #E-0900".

(f) Bulb—Corning normal, or equally suitable thermometric glass.

(2) *Air Space Indicating Thermometers for Batch Pasteurizers*:

(a) Type-Mercury-actuated; direct-reading, contained in a corrosion resistant case which protects against breakage and permits easy observation of column and scale; bottom of bulb chamber not less than 2 inches, and not more than 3 1/2 inches, below under side of top enclosure, cover or bridge; filling above mercury, nitrogen, or equally suitable gas.

(b) Magnification of Mercury Column—To

apparent width of not less than one sixteenth of an inch.

(c) Scale—Span not less than 25°F, including 140°F plus or minus 5°F, graduated in not more than 1°F divisions, with not more than 16°F per inch of scale; protected against damage at 220°F.

(d) Accuracy—Within 1°F, plus or minus, throughout the specified scale range.

(e) Stem Fitting—Fitting should fit one of the connections described in "E-3-A Sanitary Standards for Thermometer Fittings and Connections Used on Egg Products Equipment, Serial #E-0900".

(f) Bulb—Corning normal or equally suitable thermometric glass.

(3) *Recording Thermometers for Batch Pasteurizers*:

(a) Case—Moisture-proof under operating conditions obtaining in pasteurization plants.

(b) Scale—Range 30°F to 150°F, extension of scale on either side permitted; graduated in temperature scale divisions of 1°F, spaced not less than one sixteenth of an inch apart between 130°F and 145°F; graduated in time scale divisions of not more than 10 minutes, having a chord or straight-line length of not less than one-quarter of an inch between 132°F and 140°F.

(c) Temperature Accuracy—Within 1°F, plus and minus, between 130°F and 145°F.

(d) Time Accuracy—The recorded elapsed time as indicated by the chart rotation, shall not exceed the true elapsed time, as shown by an accurate watch, over a period of at least 30-minutes at pasteurization temperature. Recorders for batch pasteurizers may be equipped with spring operated or electrically operated clocks.

(e) Pen-Arm Setting Device—Easily accessible; simple to adjust.

(f) Pen and Chart Paper—Designed to give a line not over one-fortieth of an inch wide; easy to maintain.

(g) Temperature Sensing Device—Protected against damage at temperature of 220°F.

(h) Stem Fitting—Should fit one of the connections or fittings described in the "E-3-A Sanitary Standards for Thermometers and Connections Used on Egg Products Equipment, Serial #E-0900".

(i) Chart Speed—A circular chart shall make one revolution in not more than 12 hours in 1 day. Two charts shall be used if operations extend beyond 12 hours in one day. Circular charts shall be graduated for a maximum record of 12 hours. Strip charts may show a continuous recording over a 24-hour period.

C. Means should be provided for access to the manhole and/or sight glass.

These standards shall become effective Jan. 22, 1972.

DR. JOSEPH TOBIAS RECEIVES THE MILK INDUSTRY FOUNDATION TEACHING AWARD

Dr. Joseph Tobias, recipient of the 1971 Milk Industry Foundation Teaching Award is described by a former student as "competent, logical and interesting—a very good teacher and adviser who doesn't try to solve your problems but helps you solve them yourself." What higher praise could a classroom teacher receive or what stronger testimony be given in support of a nominee for this particular award?

Tobias has always been devoted to quality teaching. He has brought relevant learning experiences to the student by developing multi-media teaching units. These have been made available to other institutions in this and other countries.

He has coached a dairy products judging team for 13 years. During this period his teams have placed first six times and second five times. In 1963, he was cited by Alpha Zeta as the outstanding teacher in his College of Agriculture. He has served as faculty advisor to the local chapter of Alpha Zeta and Agriculture Council. He has or is presently serving on the following committees of the American Dairy Science Association: Flavor Nomenclature and Reference Standards, Evaluation of Dairy Products and Student Affiliate Division Council. In addition, he is a member of the American Chemical Society, Chicago Dairy Technology Society and Central Illinois Dairy Technology Society.

Perhaps his greatest contribution to the dairy industry has been the outstanding record made by his students both in this and other countries. He has spent much of his personal time in non-credit short courses promoted by the milk industry of Chicago to educate and help its employees.

Tobias was born in Olomouc, Moravia, in 1920. He attended West Georgia College for one year and received the Bachelor of Science degree in 1942 from the University of Georgia. That same year he joined the U. S. Army as an enlisted man and rose to the rank of captain at the time of his discharge in 1946. He enrolled as a graduate student in dairy technology at the University of Illinois in 1946 and received the Master of Science degree in 1949 and the Ph.D. degree in 1952. He has served on the faculty at the University of Illinois since 1948, beginning as instructor and rising to his present rank of full professor in 1964.

SYMPOSIUM ON THE RESTORATION OF SUBLETHALLY IMPAIRED BACTERIAL CELLS IN FOODS[1]

I. INTRODUCTION

B. C. Hobbs
Food Hygiene Laboratory
Central Public Health Laboratory, Colindale Ave.
London N.W. 9, England
and
J. C. Olson, Jr.
Division of Microbiology
Bureau of Foods, Food and Drug Administration
Washington, D. C.

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Physical and chemical stresses to which microorganisms in foods have been subjected during food processing operations are of considerable significance quite apart from the importance of understanding the fundamental cellular processes and mechanisms involved in injury and repair. For example, it is increasingly evident that federal, state, and municipal regulatory agencies are looking to the establishment of microbiological criteria (standards or recommended limits) to serve as objective measures that will assist them in making judgments of the wholesomeness and safety of foods. Proper judgment can only be based on use of effective methods for determining bacterial populations in foods. Also, it is essential that such methods be uniformly applicable by different analysts in the same or different laboratories. These considerations are especially important because of the wide distribution, nationally as well as internationally, of food from a single manufacturer. An inadequate test that gives false or misleading results can mean destruction of a good product or shipment of a contaminated product—the former a wasteful process, the latter a serious danger to the public health.

Accordingly, stresses undergone by microbial cells that result in impaired metabolic and/or reproductive functions materially affect the ability of common analytical methods currently in use to qualitatively and quantitatively detect their presence in foods. This Symposium sponsored by the Committee on Food Microbiology and Hygiene, IAMS was an attempt to bring into focus certain of the problems associated with sublethally impaired microorganisms, cellular mechanisms affected, and factors significant in restoration of their viability. Presented below are summaries of the four papers presented.

The Committee acknowledges the cooperation of the Organizing Committee of the 10th International Congress of Microbiology in arranging meeting facilities, and expresses appreciation to the authors of the Symposium papers, Drs. Speck and Cowman, Nelson, Ordal and Sinskey, for their participation.

[1]Sponsored by the Committee on Food Microbiology and Hygiene, International Association of Microbiological Societies (IAMS) D.A.A. Mossel, *Chairman,* Laboratory of Microbiology, Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands, and presented at the 10th International Congress for Microbiology, August 8, 1970 at Mexico City.

II. INJURY AND RECOVERY OF FROZEN MICROORGANISMS

M. L. Speck and R. A. Cowman
Department of Food Science
North Carolina State University
Raleigh, N. C.

In measuring the viable population of microorganisms in a sample of food unrestrictive conditions are used. The medium and environmental factors are selected to promote growth of all viable microorganisms that may be present. When a specific type of microorganism is to be detected, others are depressed by use of one or more restrictive ingredients. Hopefully, the cultural conditions will detect all of the one type of microorganism to the exclusion of others.

In attempting to measure and detect viable microorganisms that may be present in a given product, microbiologists are becoming more aware that there are qualitative differences in the viability of cells. Increasing attention is being given to injury

of microorganisms caused by various environmental stresses. Many of these stresses are placed on microorganisms during processing of food. Increased use of semi-preservation processes designed to maintain flavor or other organoleptic qualities of food, usually results in survival of more organisms in a state of injury than when more extreme preservation processes are used.

Ability of injured cells to recover has focused on the importance of detection of such cells in analytical microbiology. For example, it has been shown that freeze-injured salmonellae were as pathogenic as noninjured populations. Also there is growing evidence suggesting that microorganisms can recover when foods are removed from protective storage conditions (such as the thawing of frozen foods) and that such recovery can promote unsuspected damage to the food or to other foods with which the thawed food is blended.

Studies were reported showing that when *Escherichia coli* was frozen in milk (−20 C) progressively fewer colonies developed on violet red bile agar (VRBA) as storage time increased. The inability of VRBA to detect cells surviving freezing was related to selective ingredients contained in the medium (i.e. bile salts and crystal violet). The previous nutrition of the culture before freezing and age of cells at the time of freezing also influenced detection of the cultures.

In order to detect injured bacteria with accuracy new procedures must be developed which will allow for prior recovery of such bacteria before exposure to selective media. Research on this currently is under way.

III. INFLUENCE OF THERMAL STRESS

Z. JOHN ORDAL
Department of Food Science and Microbiology
University of Illinois at Urbana-Champaign
Urbana, Illinois 61801

A variety of processing conditions used to manufacture and formulate food products cause stress or injury to the bacterial cells that might be present. Heat is not only used to achieve commercial sterilization but also in other functional capacities, where it may only exert a sublethal effect. The effect of a sublethal thermal treatment on vegetative cells, lesions produced, and conditions or requirements for cell repair or recovery was discussed in detail. In the model systems studied, emphasis was placed on quantitation; the direct microscopic count equal to the total viable count (the total of injured and noninjured cells), and special plating media to provide an estimate of the uninjured cells which in turn provided a basis for estimating and/or following the degree of injury or the extent of recovery from injury. The principal test organisms studied were the gram-positive, *Staphylococcus aureus* and the gram-negative, *Salmonella typhimurium*.

A sublethal heat treatment produces a variety of repairable lesions. In *S. aureus* the extent of injury may be estimated by plating treated cells on two different media; Trypticase Soy Agar (TSA) for the total viable count (injured as well as uninjured cells and on Trypticase Soy Agar containing an added 7.0% NaCl (TSAS) for the non-injured cell count. The difference in count between these two media is the injured cell count. For *S. typhimurium*, TSA Agar served for the total viable count but EMB agar containing 2.0% added NaCl (EMBS) was used to estimate the uninjured cells. When thermally injured cells are transferred to a suitable recovery medium, there is an extended lag period before cell division occurs. At the time of transfer there is a maximum difference between the TSA count and the TSAS count with *S. aureus* or between the TSA count and the EMBS count with *S. typhimurium*. As the incubation time is extended the two counts become equal; the lag period is ended, repair or recovery is essentially complete and the cells are ready for division.

Some of the thermal lesions produced may be summarized as follows:

(a) The cytoplasmic membrane is damaged or impaired. This permits leakage of pool material into the suspending solution.

(b) The metabolic capabilities of the cell are altered. With *S. aureus* there is a selective inactivation of cellular enzymes and a partial denaturation of cellular protein. In contrast the metabolic activity of thermally injured cells of *S. typhimurium* appears to be stimulated and there is a reduced denaturation of cellular protein.

(c) A prominent lesion is the degradation of the ribosomal RNA (rRNA). This is probably the result of activation of rRNA degrading enzymes in the model system being studied. Experiments are underway to further clarify this point.

Our information on the requirements for recovery from thermal injury help provide an understanding of the effect of the sublethal thermal treatment.

(a) With *S. aureus* the nutrient requirements for the injured cells to recover are an energy source (glucose), amino acids, and inorganic phosphate. The nutrient requirements are not as rigid as they are for cell division. Thermally stressed cells of *S. typhimurium* recover in the absence of added amino acids.

(b) Stressed cells will recover in a defined medium containing the protein inhibitor, 5-methyl tryptophan (5MT) but will not divide until the effect of the 5MT is counteracted by an excess of L-tryptophan.

(c) The degraded rRNA is reformed (resynthesized) during the lag period. This occurs before cell division can take place.

(d) The damage (malfunction) to the cytoplasmic membrane is, in all probability, rapidly repaired after the stressed cells are placed in a suitable recovery medium. Definitive experiments to substantiate these changes are underway.

(e) Stressed (injured) cells are more susceptible than unstressed or normal cells to a variety of conditions which may relate to food processing or distribution conditions. Such cells are more susceptible to temperature extremes or to perservatives such as salt, nitrite, benzoate, etc. If conditions are not conducive to repair or recovery, stressed cells die. Stressed cells are generally susceptible to the special ingredients commonly used in selective media; such media therefore loose their productivity.

IV. RESTORATION OF THE NORMAL PHYSIOLOGY IN SUBLETHALLY IMPAIRED CELLS OF BACTERIA IN FOODS. INFLUENCE OF pH AND INHIBITORS

F. EUGENE NELSON

Dairy and Food Sciences and
Microbiology and Medical Technology
The University of Arizona
Tucson, Arizona

Numbers of microorganisms, both "total" and of specific types, are assuming increasing importance in both regulatory and industrial situations, as standards are established or are explored for possible adoption. Changing processing and distribution patterns increasingly expose foods to conditions which impose sub-lethal physical stresses upon the microorganisms. Organisms subjected to these stresses have been shown to be more demanding in conditions which will permit them to initiate growth than are organisms which have not been subjected to these stresses. This discussion is concerned with the extent to which several variations in recovery procedures influence apparent survival of stressed microorganisms.

Studies on the influence of temperature of plate incubation upon results of enumeration of three representative species of non-sporulating bacteria showed this had no demonstrable effect on unstressed organisms over the range of 21 to 42 C. However, maximum counts were obtained at 32 C for all of the stressed organisms. Although prolonged incubation increased level of recovery of stressed organisms at all temperatures studied, the counts obtained at the extremes of the temperature scale never approached closely those at 32 C.

For the several gram-negative bacteria tested, pH 6.0 permitted maximum recovery following heat stress, whereas pH 7.0 was approximately optimum for representative gram-positive bacteria. The non-stressed bacteria of both types gave approximately the maximum viable count over relatively broad ranges of pH, whereas the viable counts on the stressed bacteria declined appreciably more rapidly at the extremes of pH. The viable counts on several strains of yeast stressed by sub-lethal heating were moderately to considerably less at pH 3.5 than at higher pH levels, the maximum recovery usually being achieved at approximately pH 8.0, whereas unstressed organisms were not affected similarly. This has considerable potential significance because most yeast and mold counts on food products are made by adjusting the pH of the medium to 3.5, to suppress development of other microorganisms, while still permitting many yeasts and molds to grow.

Using a culture of *Aerobacter cloacae* for test purposes, lead, mercury, nickel, cadmium, calcium, potassium, and sodium ions showed no greater effect upon stressed bacteria than on the unstressed. Cobalt at 4×10^{-6} molar had a distinctly stimulatory ef-

fect unequalled by a similar level of cobalt in cobalmin. This effect of cobalt could not be demonstrated on several other strains of coliform bacteria. Copper ions were definitely more inhibitory against heat-stressed bacteria than against the unstressed bacteria. The same was true of tyrothrycin, polymyxin B sulfate, dihydrostreptomycin, propriolactose, and monochloroacetic acid, although many other compounds did not behave in this manner.

Most of the selective media tested have been more inhibitory to the heat stressed bacteria than to the unstressed. Violet Red Bile Agar for coliform bacteria is a good example. Omission of neutral red, lactose, or peptone resulted in large increases in count. Omission of crystal violet or NaCl had a lesser effect. Selective media for enterococci are quite inhibitory to stressed organisms, sodium azide and sodium chloride being responsible agents. While sugars ordinarily are not looked upon as inhibitory agents, under certain circumstances their presence in recovery media may reduce apparent survival of stressed organisms considerably. At least under some circumstances, sucrose in the medium increases apparent survival of stressed bacteria, whereas glucose and lactose have the opposite effect.

Even within a quite well-defined species, not all strains of microoganisms react the same to modifications of recovery conditions. Therefore a test series should contain several strains, if at all possible. Certainly one must not assume that because several strains of unstressed bacteria grow well in a certain set of recovery conditions that those conditions will be equally satisfactory for stressed organisms such as those which have been sub-lethally heated, dried, frozen, or otherwise physically stressed.

V. CURRENT STATUS OF INJURY IN FREEZE-DRIED MICROOGANISMS

ANTHONY J. SINSKEY

Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Freeze-drying, as a method of food preservation, is a composite of the operations of freezing and drying under vacuum. This method of food preservation thus combines all of the food processing steps that are known to cause injury to surviving microorganisms. It is known that a portion of the contaminating microbial population which survives freeze-drying will even survive the rehydration process resulting in either a public health problem or a unique spoilage problem. It is thus of utmost importance to determine the behavior of survivors of freeze-drying and the significance of their presence.

Initial work in this area was conducted to determine whether freeze-drying, as applied in a commercial food processing sense, does indeed cause injury to bacterial cells. Freeze-drying injury was found to occur and could be monitored in various ways. For example, an increased sensitivity to salt was demonstrated for freeze-dried *Staphylococcus aureus (3).* For the gram-negative organisms, metabolic damage, as measured by the method of Straka and Stokes *(5),* has been demonstrated specificaly in *Escherichia coli* and *Salmonella typhimurium (1, 4).*

After finding that injury occurs in freeze-dried cells, considerable research effort has been directed towards determining the nature of this injury and the repair processes that the cells undergo *(2).* Research on freeze-dried *E. coli* has demonstrated that permeability alterations in the cells were important. For example, freeze-dried cells were susceptible to antibiotics normally ineffective against *E. coli,* and leakage of ribonucleic acid (RNA) occurred. However, the permeability alterations were reversible as shown by the observation that antibiotic susceptibility was a time-dependent process. Also, it was observed that during or immediately after recovery of cellular permeability, damage RNA, as well as the metabolic damage, was repaired *(2).*

Further studies on the mechanism of injury and the repair processes that freeze-dried cells undergo were done with *S. typhimurium (1).* As with *E. coli,* freeze-dried *S. typhimurium* cells undergo an altered membrane permeability as measured by increased sensitivity to antibiotics and to RNase, by changes in the proton permeability coefficient and by release of RNA, DNA, and ATP into the rehydration menstrum. The total quantities released were found to be dependent upon the rehydration temperature. After freeze-drying, resynthesis of the cell wall and cell membrane components, as well as reestablishment of transport properties, were found to be necessary before cellular growth took place.

Studies were next initiated to determine if, after freeze-drying, a certain portion of the *S. typhimurium* population requires specific metabolites for growth.

Three of the compounds, pyruvate, hematin, and menadione have received considerable attention. These compounds were found to be required by a significant fraction of the cells surviving freeze-drying and not by untreated cells or cells merely freeze-thawed. The effectiveness of the three compounds is additive (not synergistic) and strongly depends upon the rehydration temperature. The mechanism by which these compounds increased recovery of freeze-dried cells is still under investigation. Preliminary findings indicate that they in some way interact with energy generating systems of the cell with the cellular membrane and lead to reestablishment of the permeability barrier of the cell. It is felt that these investigations will lead to development of optimal recovery of organisms of public health significance in freeze-dried foods.

ACKNOWLEDGMENT—PART V

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BOOK REVIEW

BOVINE MASTITIS. *O. W. Schalm, E. J. Carroll and N. C. Jain.* Lea and Febiger, Philadelphia (1971), 360 pages.

Bovine mastitis is a disease of major economic importance to the entire dairy industry. Losses from mastitis are difficult to evaluate accurately, but this disease complex is claimed to be currently the most costly disease of dairy cattle. In addition to economic considerations, interest in improving milk quality through detection, prevention and control of mastitis, is evident in milk quality programs on local, state, and national levels. This book should be a helpful source of information for anyone concerned with the disease mastitis and its relationship to milk quality.

Each chapter is well supported by references from the English language literature, and a useful index is included after the last chapter. It is indicated in the preface that the book is not intended as an extensive review of the literature. The book includes many concepts developed and investigated by the senior author over one-third of a century.

The text is divided into 13 chapters. An excellent discussion of the entire mastitis complex makes up chapter 1. Chapters 2 and 3 deal with the origin, development, and structure of the bovine mammary gland. The chapter on the milking process, mechanical milking, and mastitis is especially practical and useful to the dairyman. This deals with an area of management that often is overlooked in attempts to control mastitis. A discussion on milk formation follows, and this chapter includes a consideration of the altered composition of milk from diseased udders. Chapters 6 and 7 contain information on the types of somatic cells in normal and mastitic milk, and a discussion on physical and chemical tests for the detection of mastitis.

Methods of detection and identification of pathogenic bacteria in milk are dealt with in chapter 8 followed by a chapter each on the streptococci and the staphylococci of bovine mastitis. These latter two chapters are thorough in their coverage, between discussion and references. Pathogens from these two genera are responsible for about 95% of all clinical mastitis, and warrant the attention of individual chapters.

There is a chapter devoted to the less common forms of mastitis. These account for about 5% of the clinical cases, but are frequently the most difficult to manage. The theoretical considerations of immunization against mastitis is dealt with in chapter 12, and this chapter concludes with a discussion of the practical application and limitations of this approach to mastitis control. In the last chapter there is a discussion of investigations into the pathogenesis of mastitis that reflects the research efforts of the senior author and his team at the University of California.

This book is readable, understandable, and well referenced. The dairyman who is seeking information on specific management practices for the prevention of mastitis, or specific recommendations for the treatment of clinical cases will not find easy answers here. This was not the intent of the authors. However the contents of this book do offer an understanding necessary as a basis for sound mastitis control programming.

DONALD S. POSTLE
Department of Large Animal Medicine, Obstetrics and Surgery
New York State Veterinary College
Cornell University
Ithaca, New York 14850

PERSONNEL SANITATION PROBLEMS IN NON-STERILE FOOD PROCESSING[1]

J. Orvin Mundt

Departments of Microbiology and Food Technology, The University of Tennessee, Knoxville 37916

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Abstract

Properties of the salmonellae are reviewed briefly to emphasize their potential for persistence in the environment, their widespread occurrence, and the ease with which they can be spread. These are related to observed undesirable practices by working personnel in foods processing plants. Stress is placed upon the role of management and its representatives, to exercise continuing alertness to situations, to continual supervision, and to a program of continuing education, to bring about compliance with good hygienic practices among the labor force.

It is the purpose of this paper to discuss some of our observations in the theory and the practice of personnel sanitation as these apply to one of our more widespread, perhaps the most insidious, disease problems associated with non-sterile foods today. It is a problem which is seemingly accepted as a way of life among a suffering populace, creating little consternation or even concern, thus differing from rarely occurring disease like the spectacular bubonic plague or the fearsome and often fatal botulism. It is a problem in which morbidity is estimated unofficially to be in the millions annually in North America alone.

Reasons for the Problem

It is a problem of peculiar reference to the foods processing industries, because of the infinitely small size and the elusive nature of the causative agent. That which cannot be seen cannot be comprehended; the biology of the unseen makes no impression upon the technically uneducated laity. It is unusual to find more than one or two persons among a group of employees being addressed who have seen a bacterium through the microscope. At the managerial level we find a lack of effective communication which precludes total performance in sanitation by personnel.

The disease is salmonellosis. About this disease, Dr. Milone *(4)* stated recently: "The most logical approach to the effective control of the salmonelloses is at the source, but this does not seem to be practical for some time to come. The next best means is to provide the protective barrier of cooking or pasteurizing of a potentially hazardous product to be consumed in the cooked state."

Dr. Milone is concerned with those raw food items which are potential vehicles for the salmonellae. There is an implication in his statement that cooked items are safe. Technically, if the item went directly from the cooker to the freezer or whatever its packaged destination, this would be true. Practically, all non-sterile items are subjected to various types of post-cooking handling, such as cooling, movement, temporary or even prolonged storage of components in open or closed containers, to measuring, metering, or portioning, to intimate handling in search for bones, given non-sterile additives, and to a variety of other treatments which expose these materials to the human.

These are the foods of the modern age, not only highly susceptible to contamination, but also prepared by a few persons in tremendous quantities in centralized plants, distributed over large geographic areas via our modern transportation systems, and many are capable of supporting growth of the salmonellae when held at suitable temperatures. It is not likely that the small initial numbers when present in foods, are capable of causing infections; rather, it is the mishandling of the foods prior to serving—the undercooking of tempered "heat and eat" dishes, for example—which results in multiplication of the bacteria to the point where their numbers exceed the minimum infecting dose.

Distribution and Durability of Salmonellae

Salmonella is a genus of bacteria with many hundreds of serospecies, all with the potential for establishing residence in man. Early studies with *Escherichia coli* did much to foster a concept that survival of salmonellae outside the host is brief, in the belief that all gram-negative intestinal bacteria followed the same pattern. It is axiomatic to say that "when bacteria cannot grow they die," but we have learned to add the corrollary, "but they do not die as rapidly as we would like to have them die."

[1]Presented at the Food Sanitation and Microbiological Conference sponsored by the Food and Drug Administration, the Tennessee Department of Agriculture, and the Institute of Food Technologists, Nashville, Tennessee, December 10, 1970.

Salmonella is a hardy bacterium, perhaps the hardiest of all the intestinal dwelling gram-negative rods. It has been recovered many miles below the outfall of a sewage treatment plant, travelling under ice all the way *(9)*. It has survived in clay and loam soils for 14 months, a complete cycle of summer heat and drought and winter cold and ice *(1)*. Surprizingly, it survives for weeks to months in quite acidic foods such as sauerkraut, pickles, cottage cheese, and beer *(8)*. At least one strain is exceedingly resistant to heat *(2)*. It can be spread from animal to animal, especially when these are crowded together *(3)*, from animal to man, and man to animal. Epidemiologists point to a cycle in which fish, contaminated by improperly treated human wastes and then consumed by migratory birds, gave rise to an outbreak extending over many hundreds of miles *(10)*. Both domestic and wild animals thus suffer and spread salmonellosis. Like man, they also become carriers and shed the bacteria over prolonged periods of time. *Salmonella* has been recovered from shells of eggs laid by experimentally infected birds for at least 35 days post-infection, although the birds were asymptomatic for at least 31 days *(6)*. Even snails and caterpillars may harbor them *(5)*.

As the result of spontaneous response to natural functions by animals, and unsuspecting carelessness, negligence, and the lack of knowledge on the part of man, salmonellae have become very widespread in our environment. Consequently, the human can encounter them from a great variety of unsuspected sources other than food. No person has positive assurance that he can or will escape it. Further, *Salmonella* spp. can become established in the body of man, apparently without producing detectable evidence of the disease.

Potential for Infection: The Asymptomatic Carrier

Several years ago, a large number of persons was examined during an outbreak of salmonellosis *(11)*. Of the 47 persons who were ill, 36 suffered from the species related to the outbreak, whereas 11 persons were suffering from other species, presumably acquired from outside sources. The primary agent was isolated from 62 other persons upon culture, and presumably none of these had been symptomatic, and other species of *Salmonella* were recovered from 49 persons who also apparently were asymptomatic. Thus, more than 100 persons had acquired salmonellae from their environment, in such manner that no evidence of illness became apparent.

During the study it was found that five individuals harbored salmonellae in the throat and nine individuals were voiding them with the urine. While these represent but a small per cent of the total number of persons carrying salmonellae, these modes of departure from the human body must be considered in any discussion of personnel sanitation.

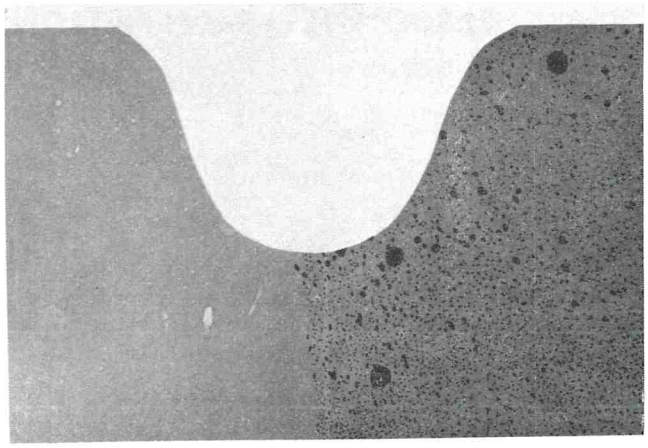

Figure 1. Urine spots on cardboard surrounding floor type urinal; inked on one side for emphasis.

Many line workers come from a stratum of life in which the rudimentary principles of good hygiene—particularly in relation to food handling—are foreign. They are able to scrupulously observe the law, where such may exist, but they are not able to extend their practices to those not specifically covered. We have seen personnel, dressed in whites, cough and spit on the floor of an otherwise well kept processing room. No posted law prohibited it! And, although not common, salmonellae have been recovered from lungs *(7)*.

Facilities themselves may become, unwittingly, a potential means for spread of pathogens. Figure 1 is the splash diagram on cardboard laid for a brief period around the base of a floor-type urinal. Undoubtedly many of us have noted urine spots on shoes and on the floor, and with this pictorial evidence, can it be denied that the floor type urinal is not a very satisfactory type of plumbing, or that its use does not contribute to the potential spread of salmonellae on shoes and clothing?

Other Observed Malpractices by Working Personnel

Failure to observe elementary principles of personal sanitation is not limited to any one group of persons. Several times we have noted that 12 or 13 men of 25 passing through the men's room on our floor of the Biology Building failed to wash their hands before departure. One of my female students has made the same observation in the ladies' room. If university students and faculty are not indoctrinated to spontaneous hand washing, how can we expect lesser educated personnel to comprehend the

significance, much less practice it? We have seen one plant in full compliance with the hand washing regulation; and until called to the attention of management, compliance consisted in wetting the hands with a few drops of water, a practice which does little more than to facilitate the transfer of bacteria from the hand to the food.

Some practices exist because management itself is less than astutely observant or even knowledgeable. Others creep in through laxity by intermediary supervisory personnel, or to only intermittent attention to sanitation; and still others, by unsuspecting actions brought in by new employees with changes in the labor force. Among these practices is the use of hands rather than paddles in scraping down plastic materials; the riffling of powdered materials, and the literal building of "sand castles" in such items; the caressing of walls, stationary surfaces, moving surfaces, and even food items on moving belts or in static situations, by personnel passing through but not assigned to the processing room, who seem to be under compulsion to touch things; utensils and hand tools moved without thought for use from one area to another, processing to nonprocessing and back again; unkept and unkempt floors in storage and feeder rooms, sometimes wet and sometimes dusty, from which foot prints and wheel tracks can be seen to establish infection threads into processing rooms. Employees have been seen who use clothing in lieu of hand washing or of towels, and in fact one needs only to observe streaks on clothing to recognize the practice as a habit. Employees have been seen to dash into side rooms and to thrust their hands spontaneously beneath outer clothing and downward to obtain sensory relief. Equipment has been restored to service without sanitization after repair by unclean (legitimately so!) mechanics. Obese personnel have been observed dripping perspiration from the face into the foods they were dispensing. These, plus other practices, may or may not have been observed by supervisors, and some have been more than tacitly condoned.

On first impression, many departures from ideal practices may appear to have no significance in contamination of foods. The many recorded instances in which bacteria have been recovered from a wide variety of raw and processed items belie the statement. Dr. Thatcher states it well in saying that "widespread salmonellosis can result, throughout the breadth of a continent, if a single source of contaminated raw material finds its way into the giant maw of the modern mass-producing convenience food industry" *(12)*.

NEED FOR PERCEPTION AND EDUCATION

It is an obvious conclusion that there is a failure in communication between management and its personnel. Perhaps employees are given credit for a sense of cleanliness which is not possessed; perhaps there is an expectation that proper sanitation and product protection can be achieved through a series of regulations, the "thou shalt's" and the "thou shalt not's" of personal conduct. If so, the approach is Pharasaical, or a compliance with the letter, but ignoring the spirit, of the law.

Our students are told that some day they will be management. As such, they must first of all be aware of, and become alert to, all possibilities of unwanted practices. They must know that regulations and laws are essential as a foundation, but that laws alone fail to achieve the desired objective. They must indoctrinate, and that unceasingly, a labor force originating largely in other strata of life, and one that is constantly changing. They are told that the objective of a sound product can be achieved only as there becomes instilled in the employee the concept of sanitation as a way of life, to be practiced in all areas of human activity, and not something to be left at the factory door at the end of the day. It is that extra step in communications which arouses in the individual a growing awareness of modes of contamination, leading to increasing effectiveness in the areas of food protection and safety.

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FIRST DAIRY RESEARCH AWARD GIVEN TO DR. C. H. AMUNDSON

Dr. C. H. Amundson, recipient of the first Dairy Research, Inc. Award is a pioneer. The quality and diversity of his research as well as his professional capacity as an educator attest to this. His career goes beyond scientific endeavors and has included humanitarian pursuits. His versatility and innovative ability is evidenced in numerous publications appearing in the *Journal of Dairy Science, the Journal of Milk and Food Technology,* and elsewhere.

Although his main concerns have been chemical and engineering problems related to concentration and dehydration of milk, milk constituents, and other foods, a large portion of Amundson's research has dealt with the operating principles of spray drying. Nationwide recognition of his authority in the concentration and dehydration of food won for him the honor of being a chairman of the 18th International Dairy Congress held in 1970 in Australia.

Amundson's ingenuity also has expressed itself in the ecological dimension. While society has been highly concerned with the ecosystem, he and his research group have taken action. A small non-edible fish was endangering the future productivity of other species of fish, particularly in Lake Michigan. Where other approaches failed, Amundson innovated and converted the problem fish into a high protein dried fish concentrate that can be used as a food supplement. Procedures have been developed to convert the oil portion into urethane foams. While this may seem irrelevant to the dairy industry, the same engineering principles used to produce the fish products may also be applied in developing new dairy products by specialized spray drying.

Several manufacturing plants in Wisconsin must be deeply indebted to him, for Amundson has developed a continuous fermentation and harvesting procedure for producing yeasts and reducing the BOD of whey. Now a high protein yeast concentrate that has been used extensively in the animal feed industry shows promise for use in human food.

While research is important, furthering the education of others is equally important. Amundson has been a fine teacher; he has directed the research training of a large number of graduate students.

He was born in Nekoosa, Wisconsin in 1927 and remained to receive his B.S. in Food Science from the University of Wisconsin in 1955. He followed up with his M.S. and Ph.D. in Food Science-Biochemistry in 1960. Amundson is now a Professor of Food Science at the University of Wisconsin. He is affiliated with many professional societies, including the AAAS, American Chemistry Society, Wisconsin Dairy Technology Society, International Association of Milk, Food, and Environmental Sanitarians, and Sigma Xi. He has been actively involved with the Institute of Food Technologists, the American Dairy Science Association and was a U.S. delegate and lecturer to the World Dairy Congress in 1970.

DR. F. E. NELSON GIVEN ADSA AWARD OF HONOR

Dr. F. E. Nelson, recipient of the 1971 Award of Honor, the highest tribute that the American Dairy Science Association bestows upon any of its members, is a distinguished scientist, teacher, editor, and leader. While he is a native of Iowa, he received his B.S. and M.S. degrees at the University of Minnesota and his Ph.D. at Iowa State University. During his 35-year membership in ADSA, he has served as Editor-in-Chief of the Journal of Dairy Science, Director, Vice President, and President. In addition, he has made extensive contributions through his committee activities. It was during his tenure as President that the Association developed and initiated the full time position of Executive Secretary, thereby enhancing its stability and stature. Nelson continues to serve his associates by his current committee membership.

He is author or co-author of more than 90 scientific papers in the areas of fundamental and applied science. His research has displayed unusual breadth and depth of perception and has contributed to fundamental knowledge of microbiology as well as to the benefit of the dairy industry. Recognition of his research was given when he received the Borden Award in Dairy Manufacturing. He also has co-authored the textbook *Dairy Microbiology* which has been published internationally, and translated into two foreign languages. He also has been a contributor to *Standard Methods for the Examination of Dairy Products.*

Perhaps the most important contributions that he has made to the Association and the Dairy Industry are reflected by his students. He has been the major professor for 31 students who have received the Ph.D. and 35 who have received the M.S. degree. These graduates have served as Associate Editors of the *Journal of Dairy Science,* committee members, officers of Divisions and directors of the Association and have been recipients of awards such as the DeLaval, Borden, and Pfizer-Paul Lewis. These men currently hold important academic and industrial positions. His students have been most complimentary of his abilities as a teacher.

His professional interests have extended beyond the American Dairy Science Association. He has been active in the American Society for Microbiology, American Chemical Society, Institute of Food Technologists, and the International Association of Milk, Food and Environmental Sanitarians. His memberships in honorary societies include Sigma Xi, Phi Kappa Phi and Alpha Zeta.

STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCAL FOOD INTOXICATIONS. A REVIEW

I. THE STAPHYLOCOCCI: CHARACTERISTICS, ISOLATION, AND BEHAVIOR IN ARTIFICIAL MEDIA [1, 2, 3]

T. E. Minor and E. H. Marth
Department of Food Science and The Food Research Institute
University of Wisconsin
Madison, Wisconsin 53706

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Abstract

Staphylococcal food intoxication ranked first among reported food poisoning outbreaks and third in number of cases during 1970. The nature of the staphylococci as they relate to food intoxications is reviewed in this paper. After considering the taxonomy and general characteristics of these gram-positive, facultative-anaerobic, mainly parasitic cocci; characteristics suggested as indices of enterotoxigenicity are discussed. The coagulase test is the most widely used of these indices; but, production of hemolysin, phosphatase, urease, deoxyribonuclease, and protein A; hydrolysis of gelatin; pigmentation; and mannitol fermentation also have been suggested. Consideration has been given to isolation of staphylococci. Virtually all selective and differential media used to isolate staphylococci, which include those employing tellurite, egg yolk, pyruvate, glycine, fibrinogen, sodium chloride, mannitol, polymyxin, and sodium azide, are somewhat inhibitory to growth of these organisms. Some studies are cited which compare the relative recovery efficiencies of these media. Finally, studies are discussed which relate to growth and inhibition of staphylococci in bacteriological media.

Staphylococcal food poisoning continues to be an important problem for food processors, food service workers, and consumers. Consequently, there has been a great deal of research activity in this area and many papers have been published in recent years. Reviews dealing with various aspects of the staphylococcus food poisoning problem have appeared in the literature in the last decade. Munch-Peterson reviewed broad aspects of the problem in 1960 (*233*) and phage-typing of staphylococci in 1963 (*234*). More recent reviews include those by Angelotti (*8*) on general topics of staphylococcal food intoxication and by Bergdoll (*18, 19*) on properties of the enterotoxins. Other reviews have been prepared by Bothwell (*29*), Bryan (*34*), and Galton and Steele (*117*).

Until recently, methods to detect the enterotoxins responsible for staphylococcal food intoxications were limited to biological assays which often were unreliable and, at best, lacked proper sensitivity. With the advent of improved methodology and, concurrently, greater insight into the nature of the enterotoxins, new vistas were opened by research in the area of staphylococcal food intoxications. As a result, information is now available which has not been summarized by other reviewers. It is the intent of this review to cover the subject in the broadest possible manner, highlighting historical as well as current topics. The review will be published in four parts: (*a*) nature of the staphylococci, (*b*) enterotoxins and epidemiology (*224*), (*c*) staphylococci in dairy foods (*225*), and (*d*) staphylococci in meat, bakery products, and other foods (*226*). The first part of this review will discuss: (*a*) taxonomy of staphylococci, (*b*) cultural and physiological characteristics of these organisms, (*c*) isolation of staphylococci, and (*d*) behavior of staphylococci in bacteriological media.

Some Historical Notes

The first recorded outbreak of staphylococcal food intoxication probably appeared in 1884; the same year in which Rosenbach described the type species for the genus *Staphylococcus* (*33*). In 1884, 300 cases of severe illness were reported in Michigan and were attributed to consumption of Cheddar cheese. Vaughn (*351*) ingested material he extracted from the cheese and contracted symptoms of the illness. Microscopic examination of the cheese revealed spherical bacteria. Vaughn concluded the bacteria produced a chemical poison responsible for the illness. The first well-documented report which clearly identified staphylococci as food-poisoning agents did not appear until 1914 when Barber (*15*) studied a milk-borne outbreak of illness in the Philippines and was able to consistently isolate staphylococci from the milk of a cow suffering from mastitis. The

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[2]Parts II, III, and IV will appear in subsequent issues of this Journal.
[3]References cited in all parts will be listed at the end of part IV.

significance of this work went unrecognized until 1930 when Dack et al. (*80*), working at the University of Chicago, isolated a yellow staphylococcus present in large numbers in a Christmas cake responsible for a food-poisoning incident. Sterile filtrates, prepared from broth in which the organism was grown, produced illness when swallowed by some human volunteers (*175*). This information was discussed in a paper by Jordan (*176*) and was compared to cases in his files and in the literature, leading him to suggest that staphylococci were responsible for a new type of food poisoning.

Stone (*316*), in a review published in 1943, tabulated 82 reported outbreaks of staphylococcal food poisoning for the period 1907-1939; 63 of these involved at least 4,123 individuals.

Staphylococcal food intoxications ranked first in incidence among reported outbreaks of foodborne diseases (27.5% of the total, or 102 outbreaks) during 1970 and third in incidence of reported cases (19.8% of the total, or 4,699 cases) *(236)*. The vehicles involved in these outbreaks are detailed in Table 1. Table 2 lists the places where food was mishandled in these outbreaks and the frequency of their involvement.

TABLE 1. VEHICLES ASSOCIATED WITH OUTBREAKS OF STAPHYLOCOCCAL FOOD INTOXICATIONS IN 1970 *(236)*.

Vehicle	No. of outbreaks caused by *S. aureus*	No. of outbreaks caused by All agents
Beef	9	60
Veal	0	3
Pork	20	37
Chicken	7	17
Turkey	7	29
Shellfish	7	13
Other fish	3	10
Other meat	1	8
Eggs	1	5
Milk	0	4
Cheese	0	2
Other dairy products	3	9
Bakery products	13	24
Fruits and vegetables	2	20
Chinese food	2	9
Multiple vehicles	6	8
Water	0	10
Other	5	27
Unknown	16	71
Total	102	366

TABLE 2. PLACE WHERE FOOD WAS MISHANDLED IN OUTBREAKS OF STAPHYLOCOCCUS FOOD POISONING DURING 1970 *(236)*.

Location	No. of outbreaks caused by *S. aureus*	No. of outbreaks caused by All agents
Food processing establishments	5	21
Food service establishments	45	115
Homes	13	42
Unknown - unspecified	39	185
Total	102	363

TAXONOMY OF STAPHYLOCOCCI

The word *Staphylococcus* is derived from the Greek nouns *staphyle* ("bunch of grapes") and *coccus* ("a grain, berry"). The genus name *Staphylococcus* thus describes the irregular clusters of spherical cells which this organism can form in liquid media (cells also occur singly, in pairs, or tetrads).

Taxonomically, staphylococci have been placed in the family *Micrococcaceae* along with the related genus *Micrococcus*. In 1965, a standard test was suggested to distinguish between staphylococci and micrococci (*163*): the genus *Staphylococcus* should contain the mainly parasitic, facultative-anaerobic, gram-positive cocci which produce acid from glucose under anaerobic conditions; and the genus *Micrococcus* should contain the mainly saprophytic, aerobic, gram-positive cocci that will produce acid from glucose aerobically but not anaerobically.

Bergey's Manual (*33*) describes the genus *Staphylococcus* as consisting of the species *Staphylococcus aureus* which ferments mannitol and is coagulase-positive and the species *Staphylococcus epidermidis* which does not ferment mannitol and is coagulase-negative.

Baird-Parker (*14*) divided the family *Micrococcaceae* into Group I (*Staphylococcus* Rosenbach) and Group II (*Micrococcus* Cohn). These groups were divided into a number of subgroups (*Staphylococcus* Rosenbach contains subgroups I-VI) on the basis of pigment production, coagulase reaction, phosphatase and acetoin production, and formation of acid from glucose (aerobic *versus* anaerobic conditions) and four other sugars.

Abd-El-Malek and Gibson (*1*) studied 799 cultures of staphylococci and micrococci isolated mainly from milk, and arranged the isolates according to their individual characteristics. They discovered that the strains yielded virtually a continuous series with gradually changing properties, the pathogenic staphylococci were at one extreme and thermoduric saprophytes at the other. They perceived three main groups: (*a*) the *Staphylococcus* group consisting of sugar fermentative organisms relatively sensitive to heat and mainly parasites of the animal body (four subgroups are recognized; bacteria in three of these form ammonia from arginine, and in two they form

acetoin from glucose), (*b*) an intermediate group in which the organisms are obligate aerobes which do not produce acid from sugars, and (*c*) the dairy micrococci, a group of thermoduric sugar fermenters.

The species *S. aureus* also includes organisms referred to in the literature at various times as *Micrococcus pyogenes* var. *aureus*, *Micrococcus pyogenes* var. *albus*, *Staphylococcus albus*, and *Staphylococcus pyogenes*.

CULTURAL AND PHYSIOLOGICAL CHARACTERISTICS OF STAPHYLOCOCCI AND THEIR POSSIBLE RELATIONSHIP WITH ENTEROTOXIGENICITY

General characteristics

Staphylococci, in addition to being facultative-anaerobic, gram-positive cocci, are strongly catalase-positive, non-motile, nonphotosynthetic, and do not form spores. The cocci are generally 0.8 to 1.0 μ in diameter (occasionally exceeding 2 μ in diameter). Many strains produce an orange or yellow pigment, particularly on media containing high levels of sodium chloride. Most strains form acetoin from glucose and ammonia from arginine, reduce nitrates (to nitrite), and ferment a variety of carbohydrates (producing acid but no gas). Indole is not formed. The organism can acidify litmus milk and may cause it to coagulate (*33*, *304*).

The optimum temperature for growth of *S. aureus* is 37 C. The organism also is able to grow at 10 and 45 C, but these temperatures are very near the minimum and maximum growth temperatures, respectively.

Members of the genus *Staphylococcus* require an organic source of nitrogen (amino acids) and two or more vitamins for growth in a synthetic medium. Mah et al. (*203*) determined that optimal growth of *S. aureus* strain S6 occurred with glucose as a carbon source only when the medium also contained 11 amino acids (glycine, valine, leucine, threonine, phenylalanine, tyrosine, cysteine, methionine, proline, arginine, and histidine) and three vitamins (thiamine, nicotinic acid, and biotin).

Characteristics suggested as indices of staphylococcal enterotoxigenicity

Several cultural and physiological characteristics have been suggested as indices of staphylococcal enterotoxigenicity. The most widely used is the coagulase reaction suggested by Chapman (*55*) in 1944. This test indicates the presence or absence of an enzyme able to clot citrated or oxalated rabbit or human blood plasma. Coagulase apparently is produced only during the exponential phase of growth (*249*); however, being a protein, active protein synthesis rather than growth *per se* is necessary for its production.

Besides correlating the coagulase reaction to enterotoxin production, many efforts have been directed at showing an association between coagulase production and other characteristics of staphylococci. Some time ago, Feldman (*105*) observed that staphylococci which produced coagulase, hemolysin, and a golden pigment were more likely to form enterotoxin. Gwatkin (*135*) found that mannitol was fermented by 190 of 275 strains of staphylococci which caused mastitis in cows. Mannitol fermentation was a reliable method for detecting coagulase-positive staphylococci in milk, according to studies by Joshi and Dale (*177*), provided anaerobic cultural conditions were maintained. Abd-El-Malek and Gibson (*1*) claimed a test for acetoin production defines those mannitol fermenting staphylococci which do not form coagulase. Urease production was noted by Joshi and Dale (*177*) to be a feature of most coagulase-positive staphylococci and β-hemolytic strains were predominant among hemolytic staphylococci isolated from milk.

Studies by Evans and Niven (*101*) and Evans et al. (*102*) implied that most, if not all, food-poisoning staphylococci are coagulase-positive. Furthermore, 22 of their strains which produced enterotoxin also fermented mannitol anaerobically, formed an orange or cream pigment on an agar medium containing 7.5% NaCl, hydrolyzed arginine, reduced nitrate, and were able to initiate growth at 10 and 45 C in broth with up to 20% NaCl.

Bergdoll et al. (*24*) found four coagulase-negative staphylococcus isolates which produced enterotoxin. Four coagulase-negative enterotoxigenic *S. aureus* isolates were also described by Thatcher and Simon (*342*).

Muth (*235*) studied a strain of *S. aureus* which produced large quantities of coagulase and discovered it also formed a white pigmented colony which was small and rough, and tenacious when probed with a needle. Further study led the author to advance the hypothesis that genetic markers responsible for the high coagulase production and the unique colonial morphology are linked and are extrachromosomal.

Nonenterotoxigenic variants were obtained by Markus (*204*) from a culture of *S. aureus* strain S6 (an enterotoxin-producer). In addition to losing toxin-producing ability, these mutants also lost their parent's ability to secrete coagulase, deoxyribonuclease, and hemolysin, and could not be typed with bacteriophages. After propagation of the variant in a casein hydrolysate medium, the supernatant fluid was found to contain a substance which inhibited coagulase, deoxyribonuclease, and enterotoxin pro-

duction. The inhibitor was dialyzable, heat-resistant, and unaffected by several proteolytic enzymes.

Lachica et al. (*192*) recently suggested that presence or absence of a heat-stable deoxyribonuclease could serve as an index of enterotoxigenicity. They noted that 95% of 251 enterotoxin-producing strains studied produced this enzyme, whereas 93% of the strains formed coagulase. In another study (*191*), these authors tested 150 enterotoxigenic, 55 methicillin-resistant, and 100 clinical isolates and determined all were positive for coagulase, thermostable nuclease, and lysozyme (except for two methicillin-resistant strains which did not form detectable lysozyme). Brandish and Willis (*31*) found 442 coagulase-positive staphylococci all produced deoxyribonuclease, whereas 594 of 596 coagulase-negative strains did not.

Production of protein A, an extracellular substance formed by staphylococci, was reported by Forsgren (*108*) to have a relationship to coagulase production. Of 700 coagulase-positive strains tested by the author, 692 (98.9%) produced protein A, whereas only two of 100 coagulase-negative strains excreted the substance. A test was devised by Jay (*170*) to estimate production of protein A. Of 585 coagulase-positive staphylococci evaluated by this procedure, 91% formed substantial quantities of the protein, while only 8.9% of 112 coagulase-negative strains produced equivalent amounts of the product.

Stone (*315*) suggested in 1935 that food-poisoning staphylococci would liquefy gelatin, whereas nonpoisoning strains would fail the test. While this idea rapidly found disfavor (*58*), it has received consideration in a number of papers.

Many staphylococci are susceptible to lysis by bacteriophages and can be grouped according to their sensitivity to a specific phage(s). Whereas most coagulase-positive staphylococci are lysogenic, phage typing cannot be employed to determine whether a given strain will produce enterotoxin.

According to Zemelman and Longeri (*377*), of 404 coagulase-positive staphylococci isolated from bovine raw milk, 96% produced a deep orange pigment, 92% fermented mannitol, 77% produced β-hemolysin, and 75% liquefied gelatin; whereas of 371 coagulase-negative strains, 16% fermented mannitol and liquefied gelatin, and none produced β-hemolysin. A similar study was made by Hussemann and Tanner (*160*) who found a good correlation between the coagulase test and enterotoxin production but essentially no correlation with pigment production, hemolysin, mannitol fermentation, and gelatin liquefication. Jay (*167*) recovered 67 coagulase-positive staphylococci from meats and found that 92% formed deoxyribonuclease, 88% formed pigments, 85% liquefied gelatin, and 22% produced β-hemolysin (while 91% formed α-hemolysin).

Clark et al. (*68*) tested correlation between coagulase production, mannitol fermentation, lipolytic and gelatinolytic action, bacteriophage and antibiotic sensitivity, gram stain and morphology, and pigmentation, and found it to be at a relatively low level. Pigment production, nitrate reduction, hemolysin production, action on milk, carbohydrate fermentation, and liquefication of gelatin could not be used by Kupchik (*189*) to distinguish a number of enterotoxigenic strains from nonenterotoxigenic strains. North (*242*) reported no correlation between hemolysin formation and enterotoxigenicity and determined proteolytic activity had no correlation, as well. The combination of coagulase and phosphatase production was studied by Thatcher and Simon (*342*) as an indicator of toxigenic strains but these two properties were not directly comparable.

No single physiological characteristic or combination of characteristics possessed by staphylococci has been found to be an absolutely reliable indicator of enterotoxigenicity.

ISOLATION OF STAPHYLOCOCCI

Media suggested for isolating staphylococci

Numerous selective and differential media have been employed to isolate staphylococci from sources containing a mixed microbial flora.

Several media are based on the ability of coagulase-positive staphylococci to aerobically reduce a tellurite salt to elemental tellurium, thus forming black colonies on an agar medium. A Tellurite-Glycine medium was developed by Zebovitz et al. (*374*) (glycine is required to form staphylococcal cell wall mucopeptide). A medium known as Vogel-Johnson agar is quite similar to Tellurite-Glycine agar but the former medium contains a pH indicator. An Egg Yolk-Tellurite-Glycine-Pyruvate agar medium was formulated by Baird-Parker (*13*). The use of egg yolk is based on an observation of Hopton (*153*) who reported that coagulase-positive staphylococci produced a zone of clearing in egg yolk emulsion.

Mannitol employed as the sole or major source of carbon in a medium along with a pH indicator can function as a differential agent. Commercially-available Mannitol Salt agar employs mannitol plus a selective level of NaCl. Chapman (*56*) suggested using a medium containing mannitol, gelatin, tryptone, lactose, yeast extract, and 7.5% NaCl for isolating coagulase-positive staphylococci. This medium subsequently came to be known as Staphylococcus Medium No. 110 or Chapman-Stone Medium.

Fibrinogen has also been used as a differential

agent (*197*). It is digested by coagulase-positive staphylococci, thus halos form around colonies on agar containing this agent. Sodium azide and the antibiotics polymyxin and neomycin have been employed as selective agents.

Virtually all selective and differential media used to isolate staphylococci are somewhat inhibitory to the growth of these organisms. This factor is a matter for concern whenever the staphylococci under study are likely to be in a debilitated state.

Suitability of isolation media

Moore and Nelson (*227*) plated 50 strains of coagulase-positive staphylococci on four different tellurite-glycine media and observed inhibition with each medium. Inhibition, however, could be minimized by increasing the concentration of or changing the nitrogen source, increasing the mannitol and yeast extract contents, and lowering the glycine concentration. Tellurite-Glycine agar was reported by Donnelly et al. (*93*) to be much more inhibitory for staphylococci than Staphylococcus Medium No. 110 or Mannitol Salt agar, and Staphylococcus Medium No. 110 was slightly more inhibitory than Mannitol Salt agar. Joshi and Dale (*177*) found Tellurite-Glycine agar to be unsatisfactory for selection of coagulase-positive staphylococci from milk. In another study, Joshi et al. (*178*) determined that eight of ten coagulase-negative staphylococci grew on Tellurite-Glycine agar in the presence of milk but failed to grow when the milk was diluted 1:32 or more.

The following data on recovery of staphylococci using five selective media were obtained by Sessoms and Mercuri (*295*): percent recovery (considering Plate Count agar results as 100%) was, (a) 91% for Tellurite-Polymyxin-Egg Yolk agar (BBL), (b) 81% for Vogel-Johnson agar, (c) 79% for Tellurite-Glycine agar, (d) 74% for Tellurite-Polymyxin-Egg Yolk agar (Difco), and (e) 69% for Staphylococcus Medium No. 110.

Aerobic growth on Mannitol Salt and Tellurite-Glycine agars was found by Jasper and Jain (*165*) to be unreliable for identification of coagulase-positive staphylococci, but Fibrinogen-Tellurite-Glycine agar was very useful for isolation of staphylococci from milk in the presence of other microorganisms.

Marshall et al. (*208*) found Tellurite-Polymyxin-Egg Yolk agar, when compared with Staphylococcus Medium No. 110 and Tellurite-Glycine agar, yielded the highest counts of coagulase-positive staphylococci for two-thirds of 40 strains tested. Highest counts of coagulase-negative staphylococci and micrococci were encountered with Staphylococcus Medium No. 110 for 9 to 12 cultures. Tellurite-Glycine agar gave the poorest recovery. Tellurite-Glycine and Vogel-Johnson agars were too inhibitory to be used for enumeration of staphylococci in dry milk.

According to Gilbert et al. (*128*), selectivity was especially poor on egg-yolk-sodium azide agar because contaminants were inadequately inhibited. Vogel-Johnson agar, Baird-Parker medium, Tellurite-Polymyxin-Egg Yolk agar, and Phenolphthalein agar with polymyxin suppressed the mixed flora of contaminating bacteria better than did Milk-Salt agar and Staphylococcus Medium No. 110.

Chou and Marth (*60*) showed that direct plating on Mannitol Salt agar and Staphylococcus Medium No. 110 was accompanied by a higher recovery of coagulase-positive staphylococci from naturally-contaminated samples of feed-grade meat products than was obtained with Tellurite-Polymyxin-Egg Yolk, Vogel-Johnson, and Tellurite-Glycine agars.

Many coagulase-negative cocci and some rods were observed by McDivitt and Jerome (*196*) to grow on Fibrinogen-Polymyxin agar and Staphylococcus Medium No. 110 used to isolate organisms from raw milk. In other work, McDivitt and Topp (*197*) found Polymyxin-B-Sulfate medium to be more inhibitory to staphylococci than Staphylococcus Medium No. 110-egg yolk medium, Polymyxin-B-Sulfate plus fibrinogen, Heart Infusion broth, and Blood agar base which in turn were more inhibitory than Trypticase Soy broth. Enrichment in brine and, to a lesser extent, in milk reduced maximal cell counts but rendered the cells better able to withstand the selective action of the various media.

Baer et al. (*12*) tested three enrichment broths and 18 plating media used for isolating staphylococci and determined that most of them significantly inhibited growth of one or more strains of *S. aureus*. Media with egg yolk were generally less inhibitory to staphylococci but were usually less efficient in restricting growth of other species.

Von Ruden and Olson (*353*) exposed *S. aureus* in milk to hydrogen peroxide and heat and found the time required for 99.9% destruction was significantly longer when survivors were enumerated on Plate Count agar rather than on Staphylococcus Medium No. 110, Staphylococcus Medium No. 110-egg yolk agar, and Trypticase Soy agar.

Tests by Stiles and Witter (*313*) showed that only 1% *S. aureus* cells surviving a heat treatment (as determined by plating on Trypticase Soy agar) were able to grow Trypticase Soy agar plus 7.5% NaCl.

Data by Busta and Jeseski (*36*) indicated that numbers of *S. aureus* surviving heat treatment in whole milk (60 C) were lower when determined with Staphylococcus Medium No. 110 rather than

with Plate Count agar. Lowering the salt content improved recovery with the 110 medium.

Methods suggested for isolating staphylococci

The Food Research Institute (University of Wisconsin) recommends a method detailed by Foster et al. (*109*) for quantitating staphylococci in foods. It consists of surface-plating dilutions of a food sample on Egg Yolk-Pyruvate-Tellurite-Glycine agar and incubating 24-30 hr at 35 C. Typical black colonies surrounded by a clear zone of proteolysis (zones may become opaque after 48 hr because of lipase activity) are inoculated into either Brain Heart Infusion or Trypticase Soy broths and incubated overnight at 35 C. The coagulase test is performed on the cultures.

The Food and Drug Administration recommends surface plating dilutions of a food sample on Vogel-Johnson agar and incubating 40-48 hr at 35-37 C. Typical black colonies are inoculated into Brain Heart Infusion broth and the cultures are tested for coagulase activity after 18 hr of incubation at 35-37 C (*350*).

Five methods are presented by Thatcher and Clark (*339*) for enumerating staphylococci in foods. They include techniques similar to those mentioned above, but employ Baird-Parker, Egg Yolk-Azide, Milk Salt, or Tellurite-Polymyxin-Egg Yolk agars and one method uses an enrichment in Trypticase Soy broth (plus 10% NaCl) followed by plating on Vogel-Johnson agar.

Standard Methods for the Examination of Dairy Products (*361*) should be consulted for sampling and sample preparation procedures.

Rammell and Howick (*262*) used a method for isolating coagulase-positive staphylococci from cheese which consisted of plating dilutions of samples emulsified in 1% sodium citrate solution onto the surface of Bovine Blood agar and counting the number of hemolytic colonies (of over 4,500 such colonies tested, <3% were found to be coagulase-negative).

A rapid method was developed by Wilson et al. (*369*) for isolating staphylococci from foods. It involved blending 20 g of sample in 99 ml of phosphate buffer followed by inoculation of 0.5 ml of this mixture into 1 ml of Brain Heart Infusion broth containing 2% mannitol and 7.5% NaCl and incubating at 35 C with shaking for 4-6 hr. The broth was examined for gram-positive organisms and a coagulase test (1- 3 hr) was performed.

A rather unique method was introduced by Chesbro and Auborn (*57*) based on the observation that staphylococci produce a nuclease under any conditions allowing growth of the organisms. The nuclease is extracted from a food sample and measured quantitatively (requiring about 3 hr).

BEHAVIOR OF STAPHYLOCOCCI IN BACTERIOLOGICAL MEDIA

Chemical and physical inhibition of growth

Staphylococci, as noted previously, are highly tolerant of high salt concentrations. Nunheimer and Fabian (*243*) observed NaCl was inhibitory at concentrations of 15-20% in broth at optimum growth temperatures, whereas a 20-25% level was definitely germicidal. Good growth of *S. aureus* was reported by Genigeorgis and Sadler (*120*) at pH 6.9 and 16% NaCl in Brain Heart Infusion broth at 37 C but no cells survived after 10 days at pH 5.1 and 16% salt. According to Hojvat and Jackson (*150*), staphylococci multiplied in 12% NaCl in broth cultures at temperatures between 20 and 35 C.

Tests by Parfentjer and Catelli (*248*) showed that staphylococci grew in all concentrations of NaCl up to saturation when incubation was at 37 C for 4 days and the medium was Tryptose Phosphate broth. Growth in the saturated solution was less prolific than in media containing 10% or less salt. On the other hand, NaCl in distilled water was injurious and 100% mortality occurred after 1 hr at all salt concentrations with the exception of a 2% survival in an 0.85% salt solution. When as little as 0.003% dehydrated Tryptose Phosphate broth was added to the 0.85% salt solution, all added staphylococci survived.

Data by Iandolo et al. (*161*) showed that the lag phase and growth rate of staphylococci increased at 37 C when 4% NaCl was added to Trypticase Soy broth. At a salt concentration of 8%, an additional increase in the lag phase occurred but the growth rate was lower than in the control.

Staphylococci also are resistant to high sugar concentrations. Hucker and Haynes (*159*) noted growth of the organisms was vigorous in solutions up to 50% sucrose during the first 24 hr but growth was checked after that, in part because acid developed in the medium. Nunheimer and Fabian (*243*) reported 50-60% sucrose was required to inhibit growth and 60-70% for germicidal action, whereas 30-40% dextrose solutions were inhibitory and 40-60% were germicidal.

Staphylococci also have been noted to be relatively sensitive to the presence of acids in the medium. The following acids were found by Nunheimer and Fabian (*243*) to be germicidal (listed in decreasing order of effectiveness): acetic > citric > lactic > tartaric > HCl. Jay (*169*) found 95% of 235 coagulase-positive staphylococci were partially or completely inhibited by boric acid or its salt, whereas 11% of 57 coagulase-negative staphylococci were in-

hibited.

While staphylococci are able to grow in or on a suitable nutrient medium at temperatures up to 45 C, they are relatively heat sensitive and do not normally survive the heat treatment employed in pasteurization of milk. Allwood and Russell (*3*) found a direct relationship between death of *S. aureus* and leakage of intracellular constituents from the cell when stored in water at up to 50 C. Total leakage at 60 C was less than at 50 C, but there was greater membrane damage at the higher temperature. A sucrose solution (1*M*) almost completely prevented leakage at 37 and 50 C but not at 60 C. Sodium alginate was noted by Scott and Strong (*289*) to protect *S. aureus* cells suspended in several diluents during mild heating (55-65 C). Cells survived heating to a greater extent in peptone water than in phosphate buffer or physiological saline.

The behavior of *S. aureus* following frozen storage (−11, −21, and −31 C) was studied by Woodburn and Strong (*370*). Viability of cells stored in 0.0003*M* phosphate buffer was increased by adding 4% (by dry weight) corn syrup, cooked waxy rice flour, egg white, or sodium alginate.

Biological inhibition of growth

Staphylococci are often controlled by a natural process in mixed flora through the activity of other microorganisms. Peterson et al. (*252*) noted, from studies performed in bacteriological media, definite repressive effects on the growth of *S. aureus* by a mixture of saprophytic, psychrotrophic bacterial species. This repression became more pronounced as the relative proportion of the bacterial population which was staphylococcal became smaller. Repression also was extremely effective at or below room temperature. In another study (*253*), these workers found that staphylococci when in the presence of a saprophytic bacterial population could only multiply appreciably in the pH range of 6 to 8 and at temperatures above 20 C, whereas the competitors were able to grow at pH 5 and 9. At salt concentrations above 3.5%, inhibition at and above 20 C was not as marked as in the controls, although normal numbers were never reached. Saprophytic growth was inhibited more as the salt concentration was increased. Staphylococci were able to dominate the population at 30 and 37 C in 5.5% salt and at 20, 30, and 37 C in 9.5% salt. In a third study (*254*), the authors observed sucrose was more inhibitory than lactose or dextrose to a saprophytic population than to staphylococci and dextrose was the least inhibitory to saprophytes. They further found (*255*) growth of staphylococci increased only slightly when whole egg was added to the medium but growth was greatly enhanced in the presence of saprophytes when whole egg plus sucrose were added. Incorporation of a 4% concentration of corn oil repressed growth of saprophytes (but only at 37 C), allowing the staphylococci to dominate the population. Buffering media of high carbohydrate content greatly increased growth of staphylococci.

Troller and Frazier (*346*) observed that maximum inhibition of *S. aureus* growing in the presence of seven food bacteria occurred after 8 or 10 hr, mostly at 20-25 C, and in the pH range 6.2-7.4. As the ratio of other organisms to *S. aureus* was increased, there was a marked increase in the inhibitory effect. Even when *S. aureus* cells outnumbered the other bacterium by a ratio of 10:1, growth was inhibited by a factor of 10 or more after 24 hr. In other tests, the authors (*347*) reported that *Bacillus cereus, Proteus vulgaris, Escherichia coli, Enterobacter aerogenes,* and *Achromobacter* sp. inhibited staphylococci by means of antibiotic substances which were Seitz-filterable, dialyzable, and stable at 90 C for 10 min. *Staphylococcus aureus* did not develop resistance to filtrates of these organisms after six consecutive transfers. *Serratia marcescens,* and a pseudomonad appeared to inhibit *S. aureus* by outcompeting it for amino acids. Changes in pH, oxidation-reduction potential, or production of peroxide or fatty acids did not appear to contribute to the inhibition. Oberhofer and Frazier (*244*) tested the effect of 66 food microorganisms on *S. aureus* by a spot-plate procedure and found the most consistently inhibitory cultures were *Streptococcus faecium, Streptococcus faecalis, S. faecalis* var. *liquifaciens,* a nisin-producing *Streptococcus lactis,* and several lactobacilli associated with meats. *Streptococcus faecalis, S. faecium, Lactobacillus lactis, Lactobacillus brevis,* and *Leuconostoc mesenteroides* were noted by Kao and Frazier (*180*) to stimulate growth of *S. aureus* during early hours of growth, especially at higher temperatures of incubation, but most cultures were inhibitory. Graves and Frazier (*131*) screened 870 cultures of microorganisms isolated from market samples of foods and found 438 which influenced the growth of *S. aureus.* Over one-half of these cultures inhibited staphylococcal growth and < one-half were stimulatory. Inhibition was also found to be more common than stimulation by McCoy and Faber (*195*) who studied 44 microorganisms. Seminiano and Frazier (*294*) found that most of 28 psychrotrophic food bacteria which were tested inhibited *S. aureus* in broth but not on spot plates. Inhibition in broth became greater as the population of *S. aureus* in the inoculum or the temperature of incubation decreased.

Culture filtrates of *Lactobacillus lactis* and *Lactobacillus bulgaricus* were found by Dahiya and Speck

(*81*) to contain hydrogen peroxide which was bacteriostatic to *S. aureus* at levels of 6 μg per milliliter or greater depending on the strain of staphylococcus involved. Iandola et al. (*162*) observed that *S. aureus* growth was suppressed when grown in association with *Streptococcus diacetilactis* and other lactic streptococci. Their data indicated that the initial proportion of *S. aureus* present in the medium was of less importance than depletion of vital nutrients. Further investigation revealed factors present in yeast nitrogen base medium that reversed the inhibition; the major one of which was nicotinamide. Filtrates from skimmilks fermented by two strains of *Leuconostoc citrovorum* were observed by Marth and Hussong (*210*) to regularly inhibit a strain of *S. aureus* in a disk assay procedure.

Truby and Bennett (*348*) reported *Proteus mirabilis*, *Salmonella schottmuelleri*, and *Enterobacter aerogenes* in mixed culture with *S. aureus* can protect the staphylococcus from the effect of 2,4,6-trichlorophenol in sodium borate. There was a direct correlation between this protective effect and the quantity of total lipid extracted from the gram-negative organisms.

IN CONCLUSION

This review included only that material believed to be necessary to understand staphylococci as they relate to the general problem of staphylococcal food intoxications. The subject of epidemiology has been deferred for discussion in the context of the next part of the review (*223*) which will entail a treatment of the enterotoxins and various aspects of staphylococcal intoxications.

ASSOCIATION AFFAIRS

RESOLUTIONS ADOPTED BY IAMFES AT FIFTY-EIGHTH ANNUAL MEETING

The following resolutions were adopted by the members of IAMFES who attended the business meeting on August 18, 1971. The business session was held in conjunction with the annual meeting of IAMFES in San Diego on August 15-19, 1971.

RESOLUTION NO. 1

WHEREAS: The California Association of Dairy and Milk Sanitarians and the California Fieldmen's Conference hosted the 58th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc. held at the Sheration Harbor Inn, August 15-19, 1971; and

WHEREAS: The Local Arrangement Committee, so effectively chaired by Harold Y. Heiskell, and the management of the Sheraton Harbor Inn provided excellent facilities in beautiful surroundings for the conduct of the meeting as well as exceptionally enjoyable entertainment for the members and their families; and

WHEREAS: The contributors who have so generously financed ladies tours, entertainment, and social gatherings; and

WHEREAS: The California Fieldmen's Conference and K999 Yellow-Dog Association of Los Angeles have sponsored the tour and barbecue;

THEREFORE, BE IT RESOLVED: That we, the members of the International Association of Milk, Food and Environmental Sanitarians, express our gratitude to the California Sanitarians and the California Fieldmen's associations, officers, committees, members, and friends who have contributed so unselfishly of their time, financial contributions, and individual efforts to make this Annual Meeting an outstanding success; and

BE IT FURTHER RESOLVED: That copies of this resolution be forwarded to the Chairman of the California Fieldmen's Conference, the President of the California Affiliate, and to the Chairman of the Local Arrangements Committee.

RESOLUTION NO. 2

WHEREAS: The Food and Drug Administration has undertaken a revision of the *Grade "A" Pasteurized Milk Ordinance - 1965 Recommendations of the United States Public Health Service*; and

WHEREAS: This Ordinance is the standard of sanitation requirements for the Cooperative State-U.S. Public Health Service Interstate Milk Shippers Program; and

WHEREAS: This Ordinance has been widely adopted by state and local governments as the legal basis for regulating the production, processing, and sale of Grade "A" milk and milk products;

THEREFORE, BE IT RESOLVED: That the International Association of Milk, Food, and Environmental Sanitarians, Inc. go on record as endorsing this revision; and

BE IT FURTHER RESOLVED: That this Association urge the Food and Drug Administration to retain those features and format which have made this Ordinance so acceptable and widely used; and

BE IT FURTHER RESOLVED: That changes be made only to include new technology and to clarify certain items that are open to interpretation; and

BE IT FURTHER RESOLVED: That copies of this resolution be forwarded to the Commissioner of the Food and Drug Administration.

RESOLUTION NO. 3

WHEREAS: The Federal Import Milk Act is based upon herd health requirements, bacteriological and farm, and processing plant sanitation standards current in this country many years ago; and

WHEREAS: Since that time several revisions of the *Grade "A" Pasteurized Milk Ordinance - 1965 Recommendations of the United States Public Health Service* have been widely accepted by federal agencies, many states, cities, and local communities;

THEREFORE, BE IT RESOLVED: That the International Association of Milk, Food, and Environmental Sanitarians, Inc. go on record as urging that action be taken to revise the requirements of the Federal Import Milk Act to provide for the equivalent protection to the health of the consumer as the present Ordinance provides; and

BE IT FURTHER RESOLVED: That copies of this resolution be forwarded to the Commissioner of Food and Drug Administration.

RESOLUTION NO. 4

WHEREAS: The International Association of Milk, Food, and Environmental Sanitarians, Inc. is and has been basically engaged in the improvement of the total environment; and

WHEREAS: The Environmental Protection Agency, recently established by the President of the United States, will coordinate and set the general guidelines for all environmental control activities; and

WHEREAS: These activities and efforts are interrelated with our interests;

THEREFORE, BE IT RESOLVED: That the International Association of Milk, Food, and Environmental Sanitarians, Inc. pledge its assistance and support to the Environmental Protection Agency and that a copy of this resolution be forwarded to the Director of the Environmental Protection Agency.

ENVIRONMENTAL QUALITY—THEME OF 1971 PENNSYLVANIA DAIRY FIELDMEN'S CONFERENCE

Prepared by SIDNEY E. BARNARD
Extension Dairy Specialist
Pennsylvania State University

Agriculture is the strongest weapon in the United States arsenal and new technology will further strengthen this dynamic industry, Dr. Russell E. Larson, dean of The Pennsylvania State University College of Agriculture, declared at the recent 29th annual Pennsylvania Dairy Fieldmen's Conference held on the University Park campus.

Dr. Larson, one of the featured conference speakers, vividly described the progress in the College's three divisions—Research, Extension, and Resident Education—by using a three-screen illustrated slide presentation. He placed emphasis on environmental quality, the theme of the two-day meeting.

More than 320 people from the Northeast and Canada participated in the sessions.

Dr. W. J. Moroz, Director of the Center for Air Environment Studies, stressed that there will be increasing emphasis on control of pollution from all of man's activities in the densely populated Northeast. "We must learn to anticipate problems of air, water, and soil pollution in advance," Dr. Moroz said, "We can expect more regulations, particularly as they relate to large production units. Success in environmental pollution control will come through recovery, reuse, and recycling of products now considered disposable."

As part of the conference, a panel reviewed milk sample collection and hauler responsibility. Dr. C. W. Livak, Penn Dairies, stressed the proper procedure for collecting farm milk. Mr. M. Smoker emphasized the importance of trained, dedicated haulers from the viewpoint of a contract hauler. The requirements of state and federal agencies were covered by Mr. Harry Behney, who stressed proper collection and handling of quality test samples.

Mr. James Reeves, Southland Corporation, predicted changes in milk procurement during the next 10 years. Producers and cooperatives will become large and processors will demand their supply over only a four-day period each week, he stated.

New dairy products were discussed by Mr. George Weir, Dairy Development Inc., who provided samples of the milk-orange juice beverage. The majority of the people attending the conference liked the taste and felt it would be acceptable in the marketplace.

Another conference speaker, Mr. Daryl Evans, Girton, described a method for bulkheading storage tanks for large farms. Together with plates or precoolers, these are recommended when capacities above 1,000 are required or milking rates are excessive.

A session featuring two manufacturers of CIP

equipment, Mr. Donald Holdren and Mr. Lloyd Bender, answered fieldmen's questions. They discussed some of the operational problems such as vibration which loosens fittings, short cycling, and the effect of temperature and abrasives.

Dr. Edward Glass, from Pennsylvania State University, demonstrated a truck-mounted metering system for measuring milk from farm tanks. "It meets all present calibration citeria and is more accurate than the dip stick," he said.

Mr. G. W. Fouse, of the Pennsylvania Department of Agriculture summarized the IMS Conference and reviewed changes in Pennsylvania regulations. He emphasized the importance of preventing pollution on farms and in plants. Mr. Fouse emphasized that more meaningful tests for determination of milk quality should be adopted and promised more checking of store handling practices.

Two other panels discussed problems faced by fieldmen. Regulatory, industry and producer representatives reviewed the complex problems posed by large herds including waste disposal, feeding, herd health, and milking equipment and practices.

Solving high somatic cell counts was a topic which stimulated considerable discussion following a presentation by a three-member panel including a fieldman, veterinarian, and milking machine manufacturer. There was agreement that a team approach was necessary to correct problems with equipment, feeding, milking practices and housing.

Mr. Sidney Barnard, Penn State Extension dairy specialist, discussed the protection of fluid milk quality, emphasizing the importance of fresh raw milk; processing equipment sterilization; containers which block out light; and proper product handling by stores and consumers—especially 40°F refrigeration.

Dr. John W. Oswald, president of The Pennsylvania State University, was main banquet speaker. He related the problems and some solutions to our educational problems.

The 1972 confernce will be held June 6-7 in the Keller Conference Center on the Penn State campus.

LETTER TO THE EDITOR

Dear Fellow Sanitarians:

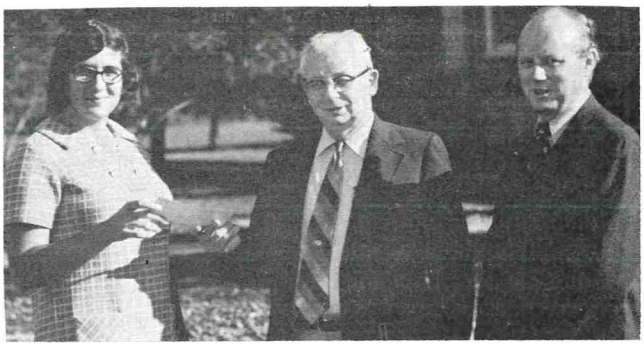
After attending year after year the annual meeting of the International Association of Milk, Food and Environmental Sanitarians, you miss the many friends you have made. Needless to say I was very disappointed that I was unable to be in San Diego.

As you know "mother nature" decided I should spend a short vacation in the hospital.

The card signed by all of you was greatly appreciated and definitely help my spirits toward a quick recovery.

Thanks to you all.

A. K. "Kelly" Saunders



Miss Susan Mitchell receives a scholarship check for $500.00 from Dr. R. M. Parry and Dr. William Ullmann on behalf of the Connecticut Association of Dairy and Food Sanitarians. Miss Mitchell is a sophomore, at St. Joseph College, West Hartford, Conn., majoring in nutrition.

CHANGES IN THE EDITORIAL BOARD

The following have agreed to accept appointment to the Editorial Board of the *Journal of Milk and Food Technology.*

S. E. GILLILAND, Assistant Professor, Department of Food Science, North Carolina State University, Raleigh, North Carolina.

R. V. LECHOWICH, Professor and Head, Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

H. PIVNICK, Chief, Microbiology Division, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada.

Two long-time members of the Editorial Board have retired from professional activities and have resigned from the Board. They are: Mr. C. A. Abele, formerly with the Diversey Company, and Dr. W. S. Mueller, formerly with the University of Massachusetts. Thanks go to both for the help they have given to the Journal and to countless authors whose papers they reviewed.

NEWS & EVENTS

COMING EVENTS

FLORIDA DAIRY FOOD INDUSTRIES CONFERENCE

"'Partners in Quality' will be the theme for the second Annual Florida Dairy Food Industries Conference to be held at the Park Plaza Hotel, Orlando, Florida on January 26. This event, sponsored cooperatively by the University of Florida Dairy Science Department and the Florida Dairy Products Association, will feature presentations on guarding the quality of dairy food products at the retail, institutional, and processing plant levels. In addition, information pertaining to the handling of dairy products in Florida restaurants will be shared with the conferees.

Speakers for the conference include: Dr. Max Gregory, North Carolina State University, Raleigh, North Carolina; Professor Sidney Barnard, Pennsylvania State University, State College, Pennsylvania; Mr. Robert Johnson, Convenient Industries of America, Louisville, Kentucky; Mr. Forrest Johnson, C. P. - St. Regis, Nashville, Tennessee; Mr. Harry Daume, Marschall Division of Miles Laboratories, Madison, Wisconsin; Mr. Fred Greiner, Evaporated Milk Association, Washington, D. C.

For additional information, please contact Dr. C. Bronson Lane, 203 Dairy Science Building, University of Florida, Gainesville, Florida 32601."

February 16-17, 1972—Dairy Industry Conference, Center for Tomorrow, The Ohio State University, Columbus, Ohio. Contact: John Lindamood, Department of Food Science and Nutrition, The Ohio State University, Columbus, Ohio 43210.

March 20-24, 1971—Mid-West Workshop in Environmental Science, Center for Tomorrow, The Ohio State University, Columbus, Ohio. Contact: John Lindamood, Department of Food Science and Nutrition, The Ohio State University, Columbus, Ohio 43210.

February 22-23, 1971 - 1972 National Dairy and Food Engineering Conference. Kellogg Center for Continuing Education. Michigan State University. Contact D. R. Heldman, Agricultural Engineering Department, Michigan State University, East Lansing, Michigan 48823, for additional details.

KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS EDUCATIONAL CONFERENCE

The 1972 Educational Conference for Fieldmen and Sanitarians will be held, February 22-23, 1972 at the Executive Inn Motor Hotel, Louisville, Kentucky.

All county and state health department personnel (sanitarians, administrators and health officers) and milk and food industry fieldmen, plant managers and related service companies and university personnel are invited to attend.

The conference is sponsored by the Kentucky Association of Milk, Food and Environmental Sanitarians with assistance from the Kentucky State Health Department's Division of Environmental Service, the Kentucky Dairy Products Association and the Kentucky Dairy Association.

The program will be broken into general sessions, food and environmental sanitarians section, and milk sections.

Also, an awards luncheon will be held at the close of the meeting at which time awards will be presented to the Outstanding Sanitarian, Outstanding Fieldman and Outstanding Service Award.

Program topics to be included are as follows: Warehouse Sanitarian, N S F Equipment Standards, Camping Facilities, Retail Food Problems, Lead Poisoning, Revision of Pasteurized Milk Ordinance, Abnormal Milk, 3-A Equipment Standards, Manufacturing Milk Program, Kentucky State Health Dept., Milk Control Program, Ecology, Status report of Kentucky State Health Department, Division of Environmental Services, Public Relations. Contact: Leon Townsend, Secretary- Treasurer, 110 Tecumseh Trail, Frankfort, Kentucky 40601.

FOOD MICROBIOLOGY

The Cincinnati Facility of the Food and Drug Administration is offering a course in Food Microbiology in Cincinnati, Ohio, March 27 to April 6, 1972.

The course presents advanced technical information for laboratory and supervisory personnel actively involved with the bacteriological examination of food. It is designed to enable the trainee to participate in surveillance programs to determine the sanitary quality of foods and to examine food implicated in foodborne disease episodes. Instruction prepares the trainee to perform selected analyses and to interpret the results of such analyses.

The laboratory work includes Aerobic Plate Count, Coliform Group MPN, *E. coli* MPN, Salmonellae, Staphylococci, *C. perfringens*, *C. botulinum* including mouse assay for toxin, and Examination of sus-

pect food sample. Emphasis is on the detection, isolation and enumeration of these organisms from foods.

In addition to lectures on each organism studied in the laboratory, other topics include Sampling procedures and sample preparation, Staphylococal enterotoxins, Time-temperature relationships in food processing, Relationships among *C. botulinum* types, Viruses in foods, Surveillance sampling and marine versus botulinal toxins.

The course is limited to 20 students. All applications must be received by December 1. There are no registration or course fees. Send applications to: O. W. Kaufmann, Ph.D. Senior Microbiologist, Cincinnati Training Facility, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

EGG PRODUCERS, HANDLERS FACING NEW REGULATIONS

A meeting to inform egg producers and shell egg handlers about proposed new shell egg requirements to become effective next year is being planned by the Maryland State Egg Council.

Those interested in participating in the meeting should notify the State Board of Agriculture's Department of Markets, Rm. 362, Symons Hall, Univ. of Md., College Park 20742, telephone (301) 454-4319.

The proposed regulations, under the Federal Egg Products Inspection Act, are scheduled for publication soon—and those concerned will have only 90 days after publication to comment before the proposed regulations become law.

Egg Council President Murray McHenry emphasizes that it is important for egg producers and handlers to know the regulations and be prepared to comment on them.

Date and place of the Shell Egg Meeting will be announced as soon as the regulations are published. Raymond Greenfield, Head, Shell Egg Section, USDA Poultry Division, and other USDA personnel will explain the proposed regulations and answer questions at the meeting.

All egg producers and handlers from Maryland and nearby states are urged by Egg Council President McHenry to participate in the meeting.

NATIONAL DAIRY HOUSING CONFERENCE

NOTICE
and
CALL FOR PAPERS
February 6-8, 1973
Kellogg Center
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OBJECTIVE

To exchange the latest technical information concerning dairy cattle housing for the entire dairy herd from birth through milking. Emphasis will be placed on innovative ideas and *practical* solutions to layout construction and management problems.

TENTATIVE CONFERENCE TOPICS

Housing Systems
Feeding Systems
Milking Systems
Construction Systems and Building Materials
Environmental Control
Regulations
Manure and Waste Management
Economic Considerations
Sanitary Considerations
Herd Health

COOPERATING ORGANIZATIONS

American Dairy Science Association
American Society of Farm Managers and Rural Appraisers (considering cooperating)
American Veterinary Medical Association (considering cooperating)
Canadian Society of Agricultural Engineering
Farmstead Equipment Association
Milking Machine Manufacturers Council
International Association of Milk, Food and Environmental Sanitarians
USDA - Agricultural Research Service
USDA - Extension Service

DEADLINES FOR SUBMISSION OF PAPERS

November 30, 1971—Abstract (200 words) or proposed paper.
Send abstract to Program Committee Chairman: Professor T. J. Brevik, Extension Agricultural Engineer, Agricultural Eng. Department, University of Wisconsin, Madison, Wi. 53706.

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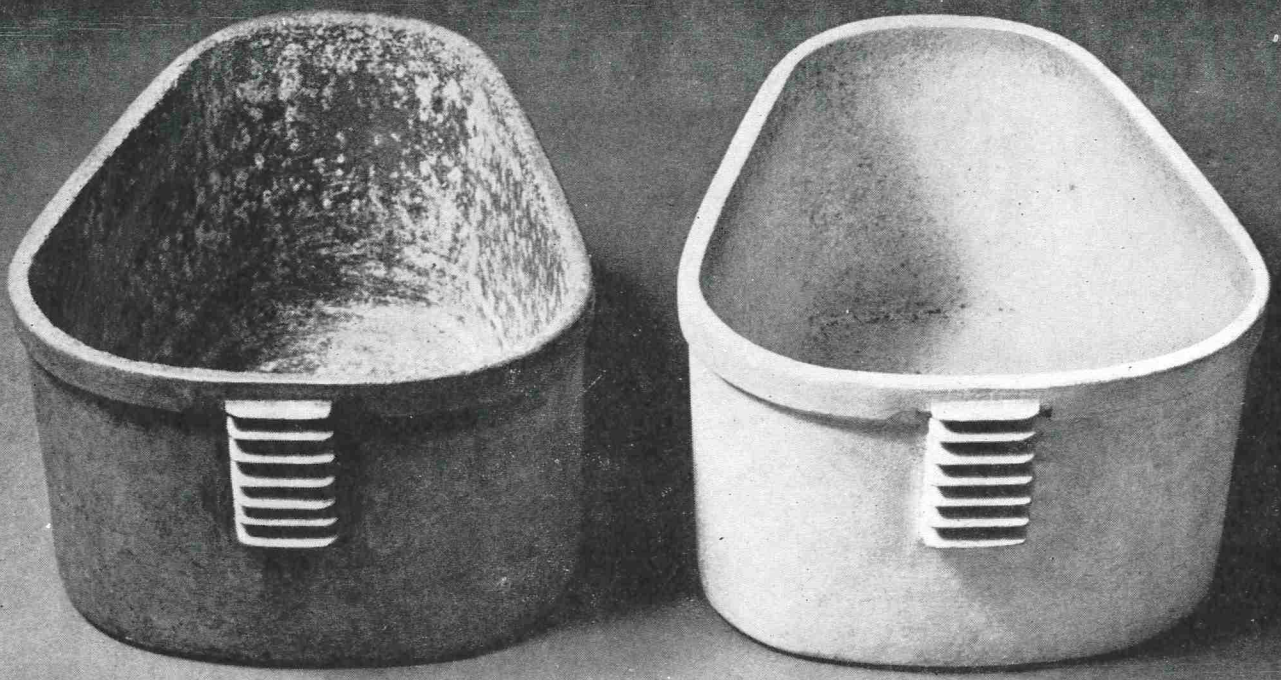

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